ISSN 0142-0843

ҚАРАҒАНДЫ УНИВЕРСИТЕТІНІҢ ХАБАРШЫСЫ

ВЕСТНИК КАРАГАНДИНСКОГО УНИВЕРСИТЕТА

БИОЛОГИЯ. МЕДИЦИНА. ГЕОГРАФИЯ сериясы
ДИАБЕТТІК шығарылым

№ 4(72)/2013

Серия БИОЛОГИЯ. МЕДИЦИНА. ГЕОГРАФИЯ
ДИАБЕТИЧЕСКИЙ выпуск

Қазан–қараша–желтоқсан
26 желтоқсан 2013 ж.

1996 жылдан бастап шығады
Жылына 4 рет шығады

Октябрь–ноябрь–декабрь
26 декабря 2013 г.

Издается с 1996 года
Выходит 4 раза в год

Собственник РГП **Карагандинский государственный университет имени академика Е.А.Букетова**

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Басуға 24.12.2013 ж. қол қойылды.
Пішімі 60×84 1/8.
Офсеттік қағазы.
Көлемі 12,0 б.т.
Таралымы 300 дана.
Бағасы келісім бойынша.
Тапсырыс № 1014.

Подписано в печать 24.12.2013 г.
Формат 60×84 1/8.
Бумага офсетная.
Объем 12,0 п.л. Тираж 300 экз.
Цена договорная. Заказ № 1014.

Отпечатано в типографии издательства КарГУ им. Е.А.Букетова

Зарегистрирован Министерством культуры и информации Республики Казахстан.
Регистрационное свидетельство № 13106–Ж от 23.10.2012 г.

ISSN 0142-0843

BULLETIN

OF THE KARAGANDA UNIVERSITY

BIOLOGY. MEDICINE. GEOGRAPHY Series

№ 4(72)/2013

DIABETES Issue

October–November–December
December, 26, 2013

Founded in 1996.
4 issues for year

Proprietary RSE **Academician Ye.A.Buketov Karaganda State University**

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Kazakh language Editor
Zh.T.Nurmukhanova
Russian language Editor
I.D.Rozhnova
Technical Editor
V.V.Butyaikin

Publishing House
of Academician Ye.A.Buketov
Karaganda State University
38, Gogol Str.,
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e-mail: izd_kargu@mail.ru

Signed in print 24.12.2013 y.
Format 60×84 1/8.
Offset paper.
Volume 12,0 p.sh.
Circulation 300 copies.
Price upon request. Order № 1014.

Printed in the Publishing House
of Academician Ye.A.Buketov
Karaganda State University

Registered by the Ministry of Culture and Information of the Republic of Kazakhstan.
Registration certificate No. 13106–Zh from 23.10.2012.

М А З М Ұ Н Ы

C O N T E N T S

Preface

Diabetes mellitus is a severe disease, and its prevalence is dramatically increasing world-wide. The complications associated with the disease include cardiovascular disease, blindness, amputations, end-stage renal disease, kidney dialysis, and kidney transplantations and present major public-health problems. Furthermore, diabetes costs are exceeding meanwhile billions of dollars annually and put tremendous burden on national health care systems. Recent years have seen a significant progress in basic knowledge on diabetes due to enormous research efforts, making possible the development of new technology and therapeutics for diabetes management and care.

It was just diabetes research, when Dr. G.G.Meyramov from the Karaganda State University joined me in 1977 in Karlsburg and started out to study how tryptophan metabolites could induce diabetes in animals. Since then we look back on a very fruitful cooperation between the Karaganda State University and the Institute of Diabetes Karlsburg.

It was a landmark decision of the Faculty of Biology of the University and the Publishing House of this journal to issue a volume focusing on problems in diabetes. Of note, this is the first time in the history of Kazakhstan and Central Asia that there is a special issue of this distinguished journal, exclusively devoted to diabetes research. The Editors of this journal, by inviting contributions from international as well as national diabetes experts, made an important step in enhancing and disseminating knowledge about diabetes but, beyond that, lay emphasis on care and management of diabetes in Kazakhstan.

We trust that readers will welcome the present issue and benefit from the contributions herein provided by experts in the field.

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BIOLOGY

UDC 613.2:612.359

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Optimizing diabetes management: Comprehensive analysis of glucose monitorring data and use of better metrics for glycemic control

Current diabetes treatment relies primarily on hemoglobin A1C measurement to assess quality of glycemic control and to adjust therapy. Recent studies have revealed that A1C has some important limitations, and it conveys a rather complex message. This has to be taken into consideration for adjustment of diabetes therapy. To significantly improve diabetes treatment, both key metrics for glycemic control on a day-to-day basis and more advanced monitoring methods are needed. In addition to traditional discontinuous monitoring methods, continuous glucose sensing has become an indispensable tool to reveal insufficient glycemic management in patients with complicated diabetes. Several continuous glucose monitoring (CGM) systems, which have shown usefulness in clinical practice, are currently on the market. The widespread clinical application of CGM is still hampered by the lack of generally accepted measures for assessment of glucose profiles and standardized reporting of glucose data.

Key words: Diabetes, continuous glucose monitoring, short- and long-term markers of glycemic control, hyperglycemia, hypoglycemia, measures of glycemic variability, standardization, quality of diabetes control, therapy adjustment, computer-assisted decision support systems.

Since landmark studies have provided evidence that hemoglobin A1C (A1C) is linked to vascular complications of diabetes [1, 2], current glycemic management is mainly based on measurement of A1C. Optimal diabetes control aims to restore levels of A1C to as normal as possible to reduce or prevent diabetic complications. However, recent studies have revealed that A1C has some important limitations, and represents a rather complex measure of glucose metabolism. A1C is a marker for overall glucose exposure and integrates both fasting as well as postprandial hyperglycemia but their relative contribution varies with the quality of glycemic control [3]. The increased cardiovascular risk observed in patients with type 2 diabetes is only partly explained by traditional cardiovascular risk factors. It is well known that chronic sustained hyperglycemia increases the risk for microvascular complications in type 1 diabetes and the cardiovascular risk in type 2 diabetes. Especially postprandial hyperglycemia, independent of A1C or fasting glucose, has been associated with cardiovascular disease [4], and this could be confirmed very recently in a post-hoc analysis of the «Effects of prandial versus fasting glycemia on cardiovascular outcomes in type 2 diabetes (HEART2D)» study [5].

It is generally accepted, and as laid down in the American Diabetes Association and IDF guidelines, that strict glycemic control, implicating a comprehensive diabetes evaluation, is needed to prevent or delay diabetes complications. The outcomes of the ACCORD [6] and ADVANCE [7] trials have taught us that A1C levels should be tailored to the patients´ health status extensive comorbid conditions require less stringent targets. Now, time has come to accomplish measurement of A1C by other markers of glycemic control, allowing for assessment of shorter-time changes in glycemia.

Although self-monitoring of blood glucose (SMBG) is still the predominant mode of glucose monitoring, the use of advanced technology such as continuous glucose monitoring (CGM) has shown remarkable

benefits and expanded signifycantly during recent years. One major problem in utilization of CGM is appropriate evaluation of the great amount of data provided by CGM systems and the lack of standardization.

The purpose of the present review article is to give an insight into the problems of choosing the most relevant markers of glycemic control and how to eva- luate CGM data properly to optimize management of diabetes.

Markers of glycemic control

Several markers of glycemic control have been used in routine practice as well as in clinical trials to guide therapy and to investigate the efficacy of therapeutic agents on patients´ glycemic control. A comparison of validated markers is shown in Table 1.

Table 1

Long- and short- term markers of glycemic control

Marker	Glycemic control period	Reference
Hemoglobin A1	1–3 months	Cohen [8]
Glycated serum proteins	2–3 weeks	Takahashi et al. [14]
1,5-Anhydroglucitol	1–2 weeks	Dungan et al. [17]
Glycemic variability indices	24–72 hours	Rodbard [32]
Mean glucose	24–72 hours	Rodbard [32]

Hemoglobin A1C

Among these markers, hemoglobin A1C (A1C) has been accepted as the fundamental biomarker and clinical surrogate endpoint in diabetes management and was used for the last three decades. It is well documented that in both type 1 and type 2 diabetes, A1C is predictive for the occurrence of diabetes complications many years later. However, deeper insight into the pathogenesis of diabetes has disclosed important limitations of A1C measurement. Early analyses recognized that upon comparing average glucose levels in patients with diabetes can result in different average glucose concentrations at a given A1C value. In a minority of patients such mismatch might partly be explained by unequal temporal distribution of glucose sampling, but more importantly, there are studies to provide evidence that this observation is due to changes in intracellular glycation rates [8]. Other known conditions that could interfere with A1C measurement, causing erroneous values, are high red cell turnover, anemia, blood transfusion, chronic renal or liver disease [9], and drug treatment. The most important limitation of A1C, as a marker of glycemic control over the previous 2–3 months, is its inability to capture shorter-term changes of glycemia. In well-controlled patients with type 2 diabetes, we have previously shown that A1C is mainly determined by chronic sustained hyperglycemia and glycemic fluctuations go undetected [10]. However, this is critical for safe and timely adjusted insulin administration and clinical decision making. Therefore, researchers tried to introduce additional markers for better characterizing glycemic control during shorter periods of time. These markers, however, have specific characteristics and are not equally suited for diabetes management.

Glycated albumin

In recent years, serum glycated proteins with shorter half-lives (17–20 days) than hemoglobin have been evaluated as markers of intermediate glycemia. The fructosamine assay is used to measures glycation of serum proteins, principally albumin [11]. Glycated albumin (GA) has been reported to be a useful marker of glycemic control in diabetes [12]. It is a more rapidly responding indicator than hemoglobin, although the glycation rate for both proteins is comparable [13]. Since glycated albumin was shown to be an independent variable of maximum glucose levels, it appears to be a more sensitive marker than A1C for glycemic excursion, as they occur during postprandial times [14]. This is important because postprandial glucose excursions are known risk factors for diabetic micro- and macrovascular complications. More recently, it was found that serum GA levels are higher in relation to A1C in diabetes patients with reduced basal pancreatic ß-cell function [15]. If in the state of postprandial hyperglycemia, indicating postprandial ß-cell dysfunction, serum GA were found to be increased, then it could be a useful surrogate marker for cardiovascular risk. This has not yet been confirmed by clinical trials, although the finding of elevated GA, but not A1C levels in patient with coronary artery stenosis points out such a relationship [16].

1,5-Anhydroglucitol

Another analyte, 1,5-anhydroglucitol (1,5-AG), has been suggested for use as intermediate marker of glycemia to complement A1C measurements [17]. It is a naturally occurring polyol that competes with glucose for tubular re-absorption and can thus not be used as a marker for glycemic control in patients with impaired kidney function. Furthermore, it should be noted that glucose levels exceeding the renal threshold for glycosuria, i.e. 10 mmol/L (180 mg/dL), lead to a rapid reduction in serum concentration of 1,5-AG [18]. Poor glycemic control, indicated by high A1C values, is therefore associated with lower instead of higher 1,5-AG levels. Although this marker responds sensitively and rapidly to daily glucose excursions in patients with near or at goal A1C levels [19], it can not identify hypoglycemia. Dungan et al. [20] have reported that 1,5-AG varied markedly in diabetes patients despite similar A1C and showed that this was mainly attributable to different postprandial glucose excursions. This makes 1,5-AG superior compared to A1C or GA (serum fructosamine) measurements as a marker for identifying postprandial hyperglycemia. Consequently, 1,5-AG has been used to evaluate drug strategies on postprandial glycemia. Studies, including exenatide [21], sitagliptin [22] or biphasic insulin [23], for example, support the usefulness of 1,5-AG as a marker to identify treatment effects on postprandial glycemic excursions that would have otherwise been missed.

Measures of glycemic variability

It is a well-known clinical observation that glucose profiles can greatly differ at similar or even identical A1C values. While some patients have small or moderate glucose excursions and rare hypoglycemia, others have marked postprandial increases with frequent hypoglycemic episodes. These ups and downs in glucose levels over time, either measured within 24 hours or from day to day at the same time point, reflect glycemic variability (GV) classified as within-day and between-day variability, respectively [24]. It remains controversial whether GV is an independent causative or contributing factor to diabetes complications. However, there are data demonstrating close associations between GV and carotid intima-media thickness [25] and microvascular complications [26]. The findings and observations that GV more than sustained chronic hyperglycemia induces increased oxidative stress [27] provide strong indications that GV is involved in the development of vascular disease. In clinical practice, minimizing GV is important to achieve acceptable glycemic control without hypoglycemia [28–30].

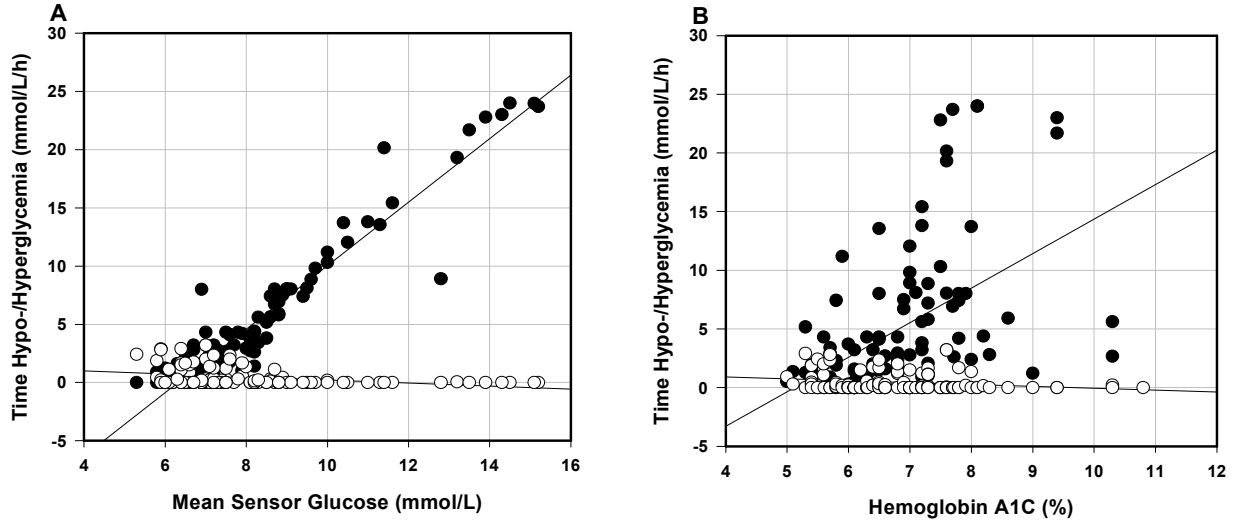
With the advent of CGM various indices of GV gained considerable clinical importance [31]. Currently, numerous indices are available, which have been carefully characterized by Rodbard [32] and Cameron et al. [33] for evaluation of various aspects of GV. Although they can principally be calculated from frequently sampled SMBG data, it is most suitable to use CGM datasets, because capturing all glucose peaks and nadirs requires sampling frequencies of 1–5 minutes. Furthermore, it is very important to clearly differentiate between indices of GV and indices of the quality of glycemic control. Measures of GV quantify short-term changes in glycemia and are suitable for different and specific aspects of glycemic control but should not be interchanged. Validated indices such as mean amplitude of glycemic excursions (MAGE), mean of daily difference (MODD), continuous overall net glycemic action (CONGA) are often used in clinical research, but they are not easy to calculate and computer programs have been developed for better handling of sampled glucose data. We have recently developed a computer prog- ram to calculate MAGE [34], and meanwhile, there is other software available, such as GlyCulator [35] and EasyGV [available at www.easygv.co.uk] for computing glycemic variability indices. Most recently, an expert panel of diabetes specialists recommended for the ease of use, familiarity, and correlation with other factors of glycemic control, the following three measures of GV: SD around the mean glucose (SD), coefficient of variation (CV), and interquartile range (IQR) [36]. Especially, if CGM data are collected, IQR is the most reliable aggregate measure of GV, as the panel announced.

In addition to evaluation of GV by the aforementioned metrics, various indices have been developed to estimate the quality of glycemic control to complement clinical assessment of diabetes treatment, such as the average daily risk ratio (ADDR), including the high (HBGI) and the low blood glucose index (LBGI) [37] and the glycemic risk assessment diabetes equation (GRADE) [38]. These metrics are calculated by converting glucose values into risk scores, i.e. they quantify the risk for glycemic extremes.

Mean glucose

Mean glucose is a metric that is equally understood by patients and clinicians. Although not crucial for therapeutic decisions, it is useful to indicate glucose exposure during specified time periods and could help

determine effects of food, exercise or diabetes medications [39]. Especially when CGM data is being reviewed, an A1C level derived from mean or average glucose [40] provides an option for reporting glucose exposure during a defined time period. However, as reported by Kilpatrick et al. [41], the relationship between mean glucose and A1C may differ between different treatment groups. As demonstrated in Fig. 1, our results obtained from a cohort of type 2 diabetes patients (n = 114) treated with diet and oral antidiabetes drugs, further revealed that mean glucose derived from CGM measurements was more strongly correlated with time spent in hyper-/hypoglycemia than with A1C. Correlation coefficients for mean glucose *vs.* hyper- and hypoglycemia were $r = 0.965$ and –0.345 (Fig. 1A) and for A1C $r = 0.508$ and –0.226, respectively (Fig. 1B). Correlation coefficients of similar magnitude were reported by Nielsen et al. [42] for type 1 diabetes patients between A1C and fraction of time during hyper-/hypoglycemia, expressed as area under the CGM curve (AUC).



The hypoglycemic range is disproportionally compressed by *y*-axis scaling

Figure 1. Correlation between time spent in hypoglycemia (open circles) / hyperglycemia (filled circles) and (A) mean sensor glucose ($r = –0.345$ and 0.965) and (B) A1C ($r = –0.226$ and 0.508, $P < 0.001$ for all)

As our previous data from the cohort of type 2 diabetes patients demonstrated, % CV is one of the GV metrics, which is closely correlated with the risk of hypoglycemia ($r = 0.554$, $P < 0.001$). Even though significant, the correlation between A1C and hypoglycemia shown in Fig. 1B is weak. Multiple logistic regression analysis further revealed that the odds ratio for % CV was higher than for mean sensor glucose: 1.25; 95 % confidence interval (CI), 1.14–1.37 *vs.* 0.41; 95 % CI: 0.21–0.61 ($P < 0.001$ for both), while A1C was not a significant predictor (unpublished data). Overall, this clearly shows that A1C provides no reflection of hypoglycemia exposure.

It has also been discussed whether postprandial glucose should become a marker of glycemic control. As suggested by Avogaro [43], postprandial glucose may rather represent a surrogate of metabolic events occurring in the postprandial phase. On the other hand, we found a close correlation between mean glucose and postprandial glucose in our cohort of type 2 diabetes patients ($r = 0.630$, $P < 0.001$). This indicates that changes in postprandial glucose levels are adequately reflected by mean glucose values.

Glucose monitoring

The development of hand-held blood glucose meters some decades ago made it possible for diabetes patients to monitor their own blood glucose levels at any time in a convenient way and enabled adjustment of therapy. With the universal availability of glucose meters, SMBG found broad application for management of glycemic control. However, this traditional monitoring usually measures single glucose values at any time point, which is determined by the user. Thus, it provides only a snapshot of the whole glucose picture and rapid changes occurring between single measurements escape detection. The development of the CGM technology presented a great step forward toward modern diabetes management, because it overcomes limitations of traditional SMBG by producing glucose profiles instead of distinct measurements over several days, real-time glucose values, glucose trends and warnings when glucose values approach dangerously low or high levels.

As demonstrated in Figure 2, CGM recordings also, provided evidence that diurnal glucose patterns may considerably differ in individual patients, even at identical A1C levels — a fact overlooked in the past.

The figure shows individual average CGM profiles from a subsample of type 2 diabetes patients with an A1C value of precisely 6.5 %. Although this is an accep- table A1C value and indicative of good metabolic control, the CGM profiles are quite different in that: (1) most of them exceed the target range and (2) they show marked glycemic excursions. It is conceivable that frequent use of CGM and careful pattern analysis is able to improve glycemic control by uncovering such trouble points.

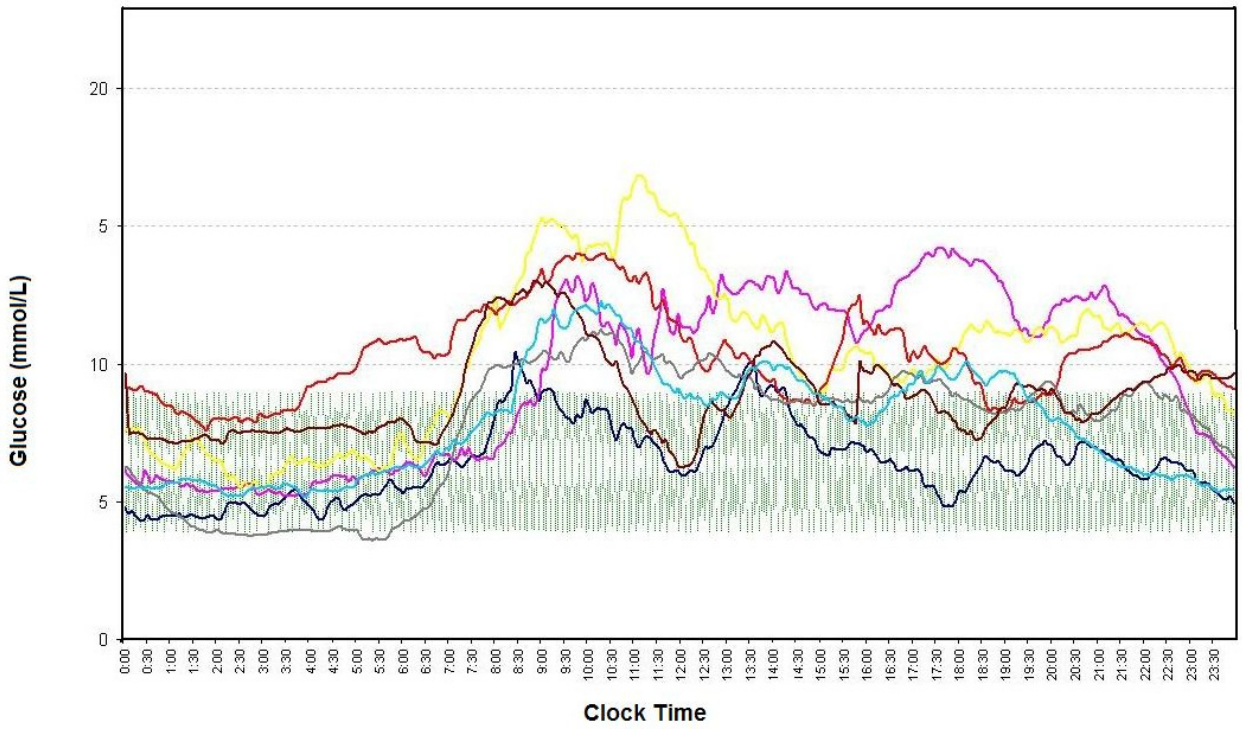


Figure 2. Continuous glucose monitoring tracings from seven patients with type 2 diabetes treated with oral antidiabetes drugs. A1C value was 6.5 % for all. Average 24-h glucose profiles are shown. For glycemic and metabolic characteristics of these patients, see Table 3.

Clinical study outcomes and data obtained from every-day diabetes management have shown that the use of CGM can consistently improve glycemic control [44]. Although especially those with unstable diabetes who are prone to hypoglycemia and hypoglycemia unawareness will benefit most, the majority of diabetes patients can achieve their glucose targets when using CGM [45]. Two variants of CGM based on sensor technology are available: retrospective and real-time glucose monitoring [46–48]. While CGM systems such as CGMS Gold, Guardian T, Glucoday, and iPro2 were mainly designed as a tool for health care providers to collect glucose data over a sensing period of 3–7 days during which the data were masked to patients, provide real-time sensors (Guardian RT, Dexcom Seven Plus and Navigator) real-time glucose values, trends, and alarms if glucose levels become high or low. The latter systems enable immediate therapy adjustment and correction of glucose levels, but require training experience for both health care practitioners and patients. Even though use of CGM has convincingly demonstrated improvement of glycemic control, i.e. reduction of time spent in hypo-/hyperglycemia, reduced glucose variability, and improvement of A1C levels, this technology is still underutilized in diabetes management for a number of reasons [36]. All the commercially available CGM devices have similar but somewhat different software to analyze the data and provide reports. However, the main problem is the lack of standardized metrics and a more user-friendly presentation of data.

There are currently several well-established clinical and research measures that have shown to be useful in analyzing and characterizing CGM profiles.

Table 2 summarizes measures of glycemic control often used in clinic and research for which normative values are available. Of the metrics shown (Table 2), it should be noted that the aforementioned expert panel identified time in range (TIR) as one of the key metrics for guiding diabetes treatment [36]. This metric can

be expressed either as «% of glucose readings» or «hours per day». As the default target range, 70–180 mg/dL (3.9–10.0 mmol/L) was selected. This is not a «normal» range, but commonly used in clinical practice. Individual targets closer to the ideal range can be defined, depending on age, comorbidities or patient compliance.

Table 2

Measures of glycemia derived from continuous glucose monitoring

Measure	Definition
Measures of sensor glucose	
Mean SG (mmol/L)	Mean of all sensor glucose values
Max SG (mmol/L)	Maximum sensor glucose value
Min SG (mmol/L)	Minimum sensor glucose value
Measures of glycemia	
Percentage above target range	% of readings >7.8[1]; >10.0[2] mmol/L
Percentage in target range	% of readings within 3.9–10.0[2] mmol/L
Percentage below target range	% of readings <3.9[1, 2] mmol/L
Measures of glycemic variability	
% CV	100 x SD/Mean
SD (mmol/L)	SD around mean glucose
IQR (mmol/L)	Interquartile range (25^{th}–75^{th} percentile)
MAGE (mmol/L)	Glucose fluctuations (nadir to peak or peak to nadir >1SD)
CONGAn (mmol/L)	Difference between glucose values at different set intervals (n x 60 min ago)
MODD (mmol/L)	Mean difference of glucose values at the same time point on two consecutive days
Measures of glucose complexity	
DFA (scaling exponent α)	Dynamic measure indicative of glucoregulation, not related to magnitude of glucose fluctuation

Note. [1]International Diabetes Federation Guidelines; [2]Guidelines of the American Diabetes Association; CONGAn, continuous overlapping net glycemic action; CV, coefficient of variation; DFA, detrended fluctuation analysis; MAGE, mean amplitude of glycemic excursions; MODD, mean of daily differences; SD, standard deviation; SG, sensor glucose.

Standardized glucose reporting

One major barrier for broader application of CGM is certainly the existence of multiple indices and parameters for measurement of glycemic control and glycemic variability. Clinicians must interpret these parameters to extract the information they need to guide management of their diabetes patients. Some of the parameters, which we use for evaluation of glycemic control and guiding patients treatment are displayed in Table 3.

The data in Table 3 demonstrate how the magnitude of glycemic measures may individually differ even in rather well-controlled type 2 diabetes patients and compared to healthy subjects. In our random sample (n = 7) all patients were treated with oral antidiabetes drugs and, even though well-controlled, it should be noted that the maximum time spent in hypoglycemia was roughly 49 min/day. Among the parameters shown, the detrended fluctuation analysis (DFA) scaling exponent can not be used for adjustment of therapy or glycemic management but rather in clinical research for assessment of the impaired complexity of glucoregulation.

In view of the various metrics used to characterize glycemic variability and quality of glycemic control, an integrated approach is required. To ease analysis of CGM and SMBG data, Rodbard [52] has presented a practical approach to definition of reference values for measures of quality of glycemic control and measures of glycemic variability. He calculated quartiles (minimum, 25^{th}, 50^{th}, 75^{th} percentiles and maximum) for six measures of glycemic variability as well as measures of glycemic control from a reference population. So, by using such a score sheet, one can utilize the areas above and below the curves for the percentiles in relation to A1C levels to define glycemic control as Excellent, Good, Fair, and Poor. We used a somewhat different

approach to evaluate the quality of glycemic control from CGM profiles and developed a Quality score (Q-score) [53]. This score is a composite metric related to A1C, integrating mean glucose, time hypo-/hyperglycemic, range of glucose values, and MODD. After calculating the score, one can classify the quality of glycemic control into five categories; Excellent, Good, Fair, Poor, and Unsatisfactory.

Table 3

Variations in the magnitude of characteristic glycemic and metabolic measures in well-controlled type 2 diabetes patients with A1C value of 6.5 % and normative values in patients without diabetes

Measure	Type 2 diabetes (range)	Normative values (mean ± SD)
Glucose[a]		
Mean glucose (mmol/L)	6.4–10.4	5.8 ± 0.6
Max glucose (mmol/L)	12.7–18.1	8.0 ± 1.3
Min glucose (mmol/L)	3.0–8.0	4.3 ± 0.7
Max PP glucose (mmol/L)	8.9–14.4	8.0 ± 1.6
Percentage time (%) at		
Glucose ≥ 10.0 mmol/L	3.0–23.2	0.7 ± 0.8
Glucose ≤ 3.9 mmol/L	0.0–3.4	0.2 ± 0.3[b]
Glycemic variability[c]		
SD	1.8–3.6	1.5 ± 0.7
% CV	20.6–38.1	16.6 ± 3.4[b]
IQR	2.5–4.3	1.2 ± 0.4[b]
MAGE	4.1–7.1	1.4 ± 0.5
ADRR	14.0–24.4	0.4 ± 4.5
GRADE	3.2–17.9	0.4 ± 2.0
Metabolic parameters		
IS (Matsuda index)	1.9–16.7	15.6 ± 2.0 [48]
PP beta-cell function (10^{-9}/min)[d]	21.7 (17.3–42.5)	74.8 (58.7–106.2) [49]

Note. Normative values [a]adapted from Zhou et al. [50], [b]own data, [c]data from Hill et al. [51]. [d]Values presented as median (25^{th}–75^{th}). IS, insulin sensitivity; PP, postprandial. See footnote of Table 2 for further abbreviations.

With the goal to translate glycemic variability measures into the clinic, Rawlings et al. [54] created a user-friendly Continuous Glucose Monitoring User Interface for Diabetes Evaluation (CGM-GUIDE©). This interface calculates and displays multiple measures derived from CGM data. It allows for user-defined thresholds for hyper- and hypoglycemia and calculates the glucose variability measures SD, MAGE, CONGAn, and MODD in conjunction with glycemic statistics, i.e. time spent in target range, time spent in hyper-/hypoglycemia, areas under the CGM curve (AUC-CGM), and mean glucose.

Only recently, the International Diabetes Center (Minneapolis, USA) has developed the data analysis software program (capture AGP™) called Ambulatory Glucose Profile AGP «Dashboard» and issued recommendations for standardizing glucose reporting and analysis to optimize clinical decision making [36].

Computer-assisted decision support systems for diabetes management

With the growing number of diabetes patients worldwide, the expanding classes of diabetes medications and variety of treatment modalities, it becomes more and more difficult for primary care providers to assess the quality of glycemic control and keep abreast with recent developments. As a consequence, the portion of patients not achieving their treatment goals remains irresponsibly high.

In type 1 diabetes, software to adjust insulin dosage and adopt treatment regimens was successfully introduced [55, 56], but to generate computer-assisted decision support programs for type 2 diabetes has been difficult, because of its complex pathophysiology. Only as of 2007, the Karlsburg Diabetes Management System (KADIS®), developed by a team of researchers at the Institute of Diabetes Karlsburg, Germany, became available as a computer-based decision support for management of type 2 diabetes, using input of CGM data for glycemic control and optimized diabetes therapy. A mathematical description of the KADIS® model can be found at [57] and more details will be given in a following article of this journal volume.

A randomized study performed in outpatients with type 2 diabetes over three months, utilizing KADIS®, demonstrated a net reduction in A1C of 0.6 % and curtailed time spent in hyperglycemia by 22 % without increasing hypoglycemia [58].

In 2011, Rodbard and Vigersky [59] developed a computer-assisted decision support (CADS) for primary care providers to improve diabetes management in type 2 diabetes patients. This system is based on the input of SMBG data, including clinical information (diagnosis, comorbidities, medication history, history of adverse events, and laboratory data), rules for dosing individual medications, adding or discontinuing medications; and rules for individualizing targets for A1C and glucose levels by time of day. Various outputs are provided, such as analysis and display of SMBG data, therapy recommendations, several therapy options; and educational information for care professionals and patients. CADS can interact with other systems to collect glucose meter data via the Metr*i*Link device and with the comprehensive diabetes management program (CDMP). As the authors stated, the system is currently implemented in a clinical research setting and adaptation to other health care systems are being intended [59]. Even if the outputs of KADIS® and CADS are similar, the underlying algorithms appear to be different. KADIS® is able to generate a virtual copy of glucose metabolism and allows interactive simulation of various therapeutic regimens in optimizing glycemic control of individual patients.

Conclusions

During recent years, many new tools and metrics have been developed. The time has come to use CGM more widely in diabetes management and to introduce, in addition to A1C, metrics that allow assessment of continuous glucose sensing for better glycemic control on a day-to-day basis. An important step in this direction would be standardization of glucose metrics and glucose reporting.

Data analysis software (capture AGP™) as well as computer-assisted decision support systems (KADIS®, CADS) has the potential to optimize clinical decision making and diabetes management to the benefit of our patients.

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К.-Д.Конерт, П.Хайнке, Л.Фогт, Ғ.Ғ.Мейрамов, Э.Зальцидер

Диабетті басқаруды оңтайландыру: глюкоза деңгейі мониторингіне жан-жақты талдау және гликемиялық бақылау үшін таңдаулы әдістерді қолдану

Қант диабетін емдеу қан құрамындағы глюкоза деңгейін бақылау мен A1C гемоглобинді анықтаумен қатар жүреді. Емдеу барысында A1C бастапқы көрсеткіште кейбір маңызды шектеулерге ие болатынын ескеру кажет. Емдеу нәтижесі жақсы шығуы үшін, әрдайым гликемиялық қадағалау екі көрсеткіште жүргізілуі тиіс. Қазіргі кезде глюкоза CGM мониторингін бақылау бірнеше үздіксіз әдістермен жүзеге асады. Олардың кең қолданысы клиникада болмауымен қоса беріледі.

К.-Д.Конерт, П.Хайнке, Л.Фогт, Г.Г.Мейрамов, Э.Зальцидер

Оптимизация управлением диабета: всесторонний анализ мониторинга уровня глюкозы и использование лучших методов для гликемического контроля

Лечение сахарного диабета сопровождается определением гемоглобина A1C в сочетании с контролем уровня глюкозы крови. Ранее было показано, что A1C имеет некоторые важные ограничения, что необходимо учитывать в процессе лечения, для улучшения результатов которого необходимы оба показателя регулярного гликемического контроля. В дополнение к традиционным периодическим методам мониторинга, непрерывный контроль уровня глюкозы является необходимым для пациентов с осложнениями диабета. Авторы утверждают, что сегодня используются несколько непрерывных методов мониторинга глюкозы (CGM), которые недостаточно информативны в клинической практике.

UDC 613.2:612.359:616–036

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Diabetogenic derivatives of 8-oxyquinolin (mechanisms of action and principles for prevention developing of diabetes caused by them)

Alteration and destruction of pancreatic B-cells caused by chemicals are one of numerous causes of developing of diabetes mellitus. Authors demonstrated results of investigation of mechanisms developing of diabetes caused by some of 18 diabetogenic derivatives of 8-oxyquinolin including chemicals formed in Human as result of disturbances of metaboilsm of aminoacids. Authors proposed a few ways for prevention of diabetes developed as result of their action and proposed for possible using of method inhibition of endogene synthesis of diabetogenic metabolites of Tryptophan as more suitable way for prevention developing of diabetes.

Key words: experimental diabetes, pancreatic B-cells, insulin, diabetogenic derivatives of 8-oxyquinolin, diabetogenic metabolits of tryptophan.

Today there are more than 30 chemicals which are able to induce experimental diabetes mellitus by selective destruction of B-cells. More than 20 of their possess chelat diabetogenic properties. 18 from them belong for derivatives of 8-oxyquinolin.

More than 70 years ago Scott and Fischer were separated insulin from the native pancreas as insulin-Zn complex and supposed that the presence of Zn-ions determined physiological activity of insulin [1]. Interest to this problem was increased after reporting by these authors in 1938 that in pancreas of death diabetic patients total amount of Zn is not more than 50 % in compared with non diabetic men [2]. They found 0,07 mg of Zn per 1 g of pancreas tissue of diabetic patients comparatively with 0,14 mg per 1 g pancreas of healthy persons. Analogical result was obtained by Eisenbrandt and coll. [3]. A large amount of Zn were found in human pancreas of healthy men. In 1942–1943 K.Okamoto discovered in pancreatic B-cells a large amount of Zn [4–12]. It is supposed today the important role of Zn-ions in processes of storage of insulin in B-cells [13]. There are proportional dependence between content of Zn-ions in B-cells and in cytoplasm. Decreasing of content of deposited insulin accompanied by decreasing of Zn-ions in B-cells [12, 14]. It is known that Zn-ions reacted in processes of synthesis as in cristallization of insulin [4]. It was showed that pancreas of mammals-animals, birds and in earth-water animals contained a large amount of Zn-ions.

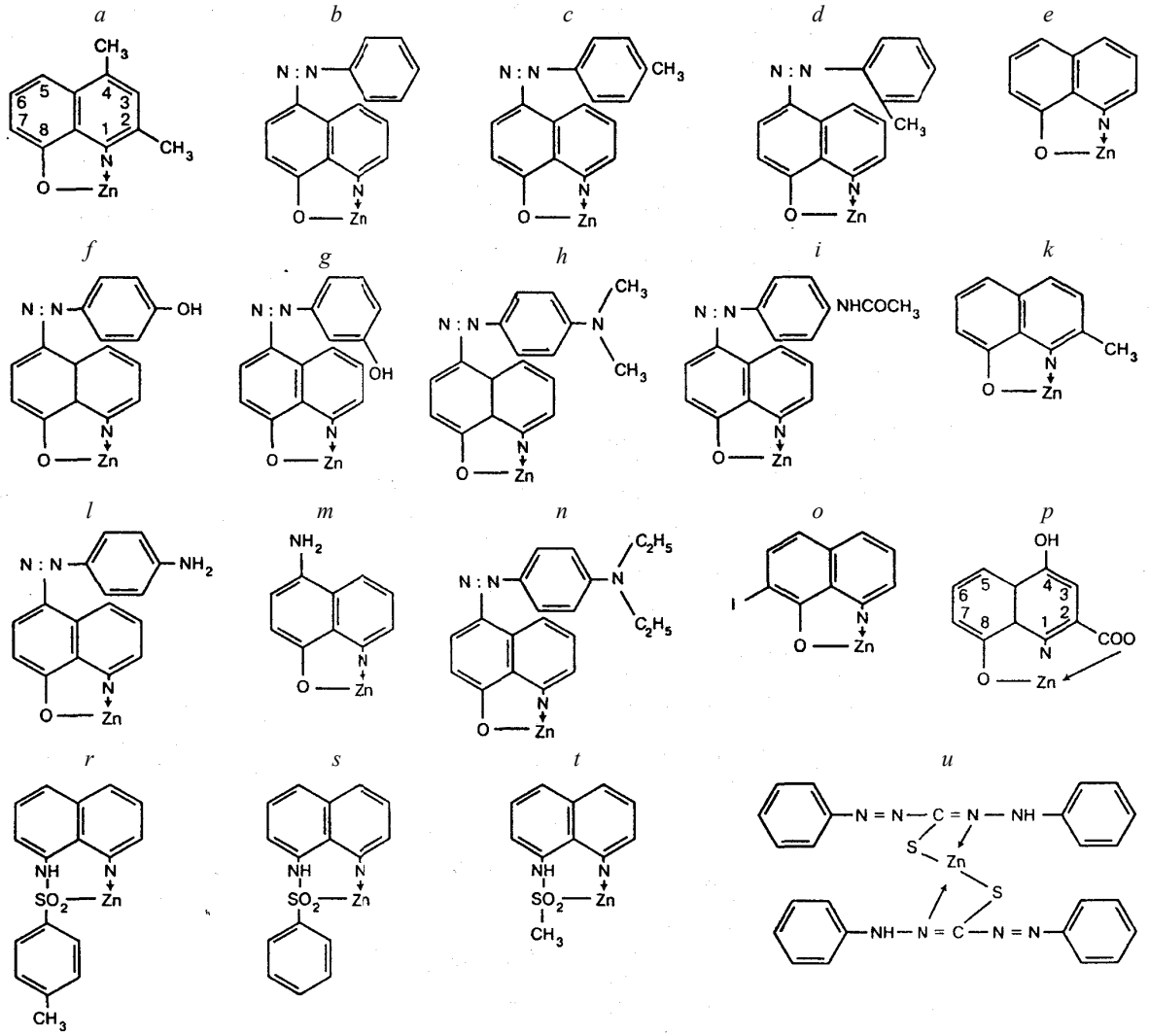
The amount of Zn is evidently decreased in experimental diabetes induced by any causes [9, 10, 14–16]. Zn-ions are able be accumulated in pancreas tissue. Administration of Zn in organism outside accompanied by increasing of total amount in pancreas in 4–20 times [17]. 0,3 % of Zn administrated in organism was accumulated in pancreas of alloxan diabetic rats comparatively with 2,6 % in healthy animals [18]. H.Kawanishi and K.Okamoto confirmed [19, 20] by electron histochemical microscopy that in B-cells Zn-ions are located in B-granules, a deposited form of insulin and that Zn is concentrated in central part of B-granules, in periphery and partly in cover of granules.

Zn-ions contained in cytoplasm of B-cells have the coordinate number (chemical coordinate number) 4 and 6 and interacted with chemicals which formed with Zn-ions chelat salts in which atom of Zn is fixed between a few other atoms [21]. The affinity of Zn-ions to formation of chelats is evidently more high comparatively with other metals of main group.

1. Diabetogenic derivatives of 8-oxyquinolin

In 1947 A.Albert reported that 8-oxyquinolin which usually is not toxic substance, is very toxic for cells in the presence of metals and for the first time of Zn-ions. It was showed that this fact determined by ability of 8-oxyquinolin to form with metals the chelat metal-complexes which are toxic for B-cells [21]. Studying of toxicity of 8-oxyquinolin for B-cells Okamoto K. [7, 20, 22] reported that injection of it to animals accompanied by developing of experimental diabetes. Later it was showed that injection of 18 derivatives of 8-oxyquinolin and of 8-oxyquinaldin accompanied by rapid developing of heavy diabetes in animals [7].

It was noted that all these chemicals have in position 8 of quinolin ring OH^- group or any other radical contained atom of S or atom of O. Six isomers of 8-oxyquinolin which not contained in position 8 of the active group are not able to form chelat complexes with Zn-ions and not induced experimental diabetes. Experimental diabetes is induced by derivatives: 8-para(toluenesulphonylamino)quinolin /8PTSQ/, 8-para(benzolsulphonylamino)quinolin /8PBSQ/, 8-para(methansulphonylamino)quinolin /8PMSQ/, 5-para(acetaminophenylaso)-8-oxyquinolin /5A8OX/, 8-hydroxyquinaldin, 5-amino-8-hydroxyquinolin and others (Fig. 1). It was demonstrated by Okamoto K. and Kadota I. that injection of these derivatives result strongly selective necrosis of B-cells and developing of diabetes. Injection of these chemicals in doses of 30–100 mg/kg accompanied by developing within a few days of heavy diabetes with marked degenerative changes in islets [23–28].



a) 2,4-dimethyl-8-oxyquinolin, 35 mg/kg; b) 5-phenylaso-8-oxyquinolin, 20 mg/kg; c) 5-para(toluene)-8-oxyquinolin, 20 mg/kg; d) 5-orto(toluene)-8-oxyquinolin, 40 mg/kg; e) 8-oxyquinolin, 50–60 mg/kg; f) 5-para(diethylaminophenylaso)-8-oxyquinolin, 20 mg/kg; g) 5-meta(hydroxyphenylaso)-8-oxyquinolin, 30 mg/kg; h) 5-para(dimethylaminophenylaso)-8-oxyquinolin, 45 mg/kg; i) 5-para(acetylaminophenylaso)-8-oxyquinolin, 50 mg/kg; к) 8-oxyquinaldin, 10 mg/kg; l) 5-para(aminophenylaso)-8-oxyquinolin, 10 mg/kg; m) 5-amino-8-oxyquinolin, 30 mg/kg; n) 5-para(diethylaminophenylaso)-8-oxyquinolin, 40 mg/kg; o) 9-oxy-7-jodoqui- nolin, 50–60 mg/kg; p) 4,8–dihydroxyquinolin-2-carboxylic acid (xanthurenic acid); r) 8-para(toluenesul- phonylamino)quinolin, 30–50 mg/kg; s) 8-para(benzolsulphonylamino)quinolin, 30–100 mg/kg; t) 8-para(metansulphonylamino)quinolin, 40–81 mg/kg; u) diphenylthiocarbazone (dithizon), 45–50 mg/ kg

Figure 1. Complex salts of Diabetogenic zincbinding chelat active chemicals with Zn-ions and its diabetogenic doses

It is known that most stable complexes are formed in case if atom of Zn is fixed between 2 atom of N, S and O of molecule of chelator. Later it was reported that only derivatives of 8-oxyquinolin contained in position 8 of quinolin ring of the hydroxyl or other radical contained atoms of S, N or O possess diabetogenic properties. Atom of Zn is fixed between atoms of S and O in position 8 and between atoms of N and O in position 1 or 2.

It was reported, what is more, that extraction of these radicals from position 8 accompanied by complete disappearing of diabetogenic properties of chelators [29]. Formation of chelats by atoms of O and N of chelator result usually forming of pentagonal or hexagonal rings [21] (Fig. 1). Pentagonal rings are more stable. The most stable are quadrangular complexes with atom of S. It is known that derivatives of 8-oxyquinolin formed quadragonal complexes with atom of S often. Electrons of indivisible pair are displaced from donor-atom of N in position 1 to Zn atom.

On the base of data obtained by A.Albert, G.Zentmyer supposed that toxic effect of 8-oxyquinolin is determined by its ability to bind and eliminate ions of metal from B-cells [30]. But later this hypothesis was not confirmed: it was showed that long time prolonged elimination of Zn ions from B-cells result any effect on the state of histostructure and function of B-cells [31]. Finally, S.Rubbo and A.Albert established that toxic effect of 8-oxyquinolin determined by its ability to form in cells toxic complexes with metals [32] that many times was confirmed later. It was showed that presence of chelat a short time in cytoplasm of B-cells accompanied by alteration of cells. In experiences with using derivatives of 8-oxyquinolin — a various isomers of the azaoxyquinolin (azaoxyn) — it was demonstrated dependence: most toxic are isomers formed chelats 1:1 with metal and with logarifm of constant of stability as 7.6 and more high, until 9.4. Meanwhile toxicity of chelats of other isomers of azaoxyn with constant of stability 5.8–6.7 was clearly more less [21]. It was showed that very toxic chelats of derivatives of 8-oxyquinolin with Zn-ions have a more high logarifm of constant of stability as 8.5. Weitzel G. and coll. showed that complex 1:1 contained 1 molecule of 8-oxyquinolin and 1 atom of ion of Zn is most toxic for cells [33].

Stability of formed complexes 2:1 is depended not only of affinity of chelator to metal but in added — of 2 properties of chelator and metal: 1) presence of additional radicals in para-positions molecule of chelator, especially — in zones contacted with part of molecule, reacted with ions of metal conduce to forming of the steric effect; as result, two molecules of the chelator are not able to approach for to put atom of metal in stable ring; 2) size of diameter of atom; in case if atom of metal have a small diameter, ring may be not formed; atom of Zn have radius as 0,74 nm between Berillium (0,31 nm) and Rubidium (1,49 nm). A high stability of the complex Zn-Dithizon is determined by stretch form of molecule of Dithizon and by location of 2 phenol rings on the 2 ends of molecule. That is why atom of N and S are easy approach to atom of Zn. More over, atom of Zn is fixed between atoms of N and S. Meanwhile it is known that affinity of Zn to N and S is more high comparatively with affinity of Zn to O. In added, complex is formed by two molecule of Dithizon each of two have a great number of double couplings.

Mechanisms of diabetogenic action of derivatives of 8-oxyquinolin and Dithizon were investigated since 1967. For the first it was showed that injection of diabetogenic doses of derivatives of 8-oxyquinolin (D8OX) 1–2 min past injection accompanied by complete binding of all amount of Zn-ions in cytoplasm of B-cells. 1.5–2 h later this complex is dissociated and same amount of Zn-ions in B-cells is reveal as before injection [14, 34–36].

Stability of complexes 1:1 formed by derivatives of 8-oxyquinolin is determined by: 1) great number of double coupling in molecule of chelator; 2) forming of quadragonal ring; 3) derivatives of 8-arensulphonylaminoquinoline formed chelat-complex by aid of atom of S. More high stability of the complex Zn-Xanturenic Acid is determined by additional fixation of the atom of Zn between 2 atom of O.

Later it was showed that 8PTSQ, a derivative of 8-oxyquinolin, formed with Zn ions toxic chelats which in UV-light have specific intensive green fluorescence. This fact was used for elaboration of high specific and very high sensitive fluorescent method of revealing of Zn ions [13, 37, 38].

This amount of Zn is able to form a new chelat-complexes with new portion of diabetogenic substance again. Extraction of complexes Zn-D8OX from B-cells by $CHCl_3$ or by CCl_4 result completely negative fluorescent reaction for Zn-ions in B-cells. This complex dissociated within 1–2 h and Zn-ions are able again to react with chelator [14, 35].

2. On the mechanisms of Diabetogenic activity of chelat active substances

In 1949 K.Okamoto first induced experimental diabetes by injection of Dithizon [39]. Dithizon are able to form red chelat complexes with 18 metals; Zn-ions only contained in pancreatic B-cells of some animals

and human. Dithizon possess very high affinity to Zn-ions and rapidly reacted with forming complex DZ-Zn 2:1. Dithizon is not synthesed in organism as of animals as human. Later Maske [40] proposed vital method of colour detection of Zn-ions in B-cells based on ability of Dithizon to form purple granules of Zn-dithizonat past injection of Dithizon solution. It was noted that diabetes induced by DZ accompanied by formation of red granules of chelat Zn-DZ in B-cells. Diabetes never developed in case if red granules are not formed in cytoplasm of B-cells. By aid of this method Zn-ions were discovered in islets of rabbits, human, rats, pigs, mice, dogs, horses, pigeons, frogs, some sorts of fish and other animals, excluding guinea pig only which not contained Zn in B-cells [22, 41–47]. As it was showed later Dithizon not formed red granules in B-cells of guinea pig and diabetes in this case not developed [34]. Later it was confirmed by spectral analysis that spectrum of absorbance of purple granules formed in B-cells past injection of Dithizon exactly correspond to spectrum of absorbance of pure synthetic Zn-DZ chelat [34].

K.Okamoto supposed that diabetogenic action of Dithizon determined by its ability to form in B-cells of chelat complexes with Zn-ions. He concluded finally: the binding of Zn-ions in B-cells by DZ is main cause of developing of diabetes. This suggestion was confirmed many times later. For the first it was confirmed that diabetes developed past injection of DZ in case if purple granules of Zn-DZ is formed in cytoplasm of B-cells only. Meanwhile according conception of Okamoto K. is not possible to understand what are mechanisms of diabetogenic action of Dithizon on B-cells as of chelator.

There are important question: does this complex Zn-DZ is eliminated from B-cells or this complex is dissociated in B-cells and Zn-ions are preserved in islets and are able to interact again with DZ?

It was established that 30 min past injection the total amount of red granules of Zn-DZ in cytoplasm of B-cells is evidently decreased and 1 h past injection (mices) and 1,5–2 h (rabbits) red granules disappeared completely from islets. In opposite, parallelly at the same time the amount of free Zn-ions in B-cells is increased and 1,5–2 h past injection concentration of Zn-ions in cytoplasm of B-cells is same as before injection of Dithizon [34, 35]. Meanwhile it was showed that injection of diabetogenic dose of Dithizon result a complete binding of all amount of Zn-ions contained in B-cells: past extraction from cytoplasm of B-cells of all amount of complex DZ-Zn by $CHCl_3$ free Zn-ions were not revealed in cytoplasm of B-cells by absolutely and high specifical for Zn-ions fluorescent method. However, 1–1,5 h later concentration of free Zn-ions in cytoplasm of B-cells was maximally high as before injection of Dithizon. Meanwhile it was not possible to have same result in case if Zn-ions were eliminated from cytoplasm of B-cells.

Thus, it was confirmed that injection of Dithizon accompanied by forming of chelat Zn-DZ in cytoplasm of B-cells, which is dissociated in cells within 1–2 h past injection of chelator and free Zn-ions are able to form chelat complexes again with chelator in cytoplasm of B-cells.

Later it was showed that in animals at +36 ºC without circulation of blood dissociation of complex DZ-Zn is markely delayed: 3 h past injection of Dithizon 50–60 % of granules contained in cytoplasm of B-cells are not dissociated yet [34, 35].

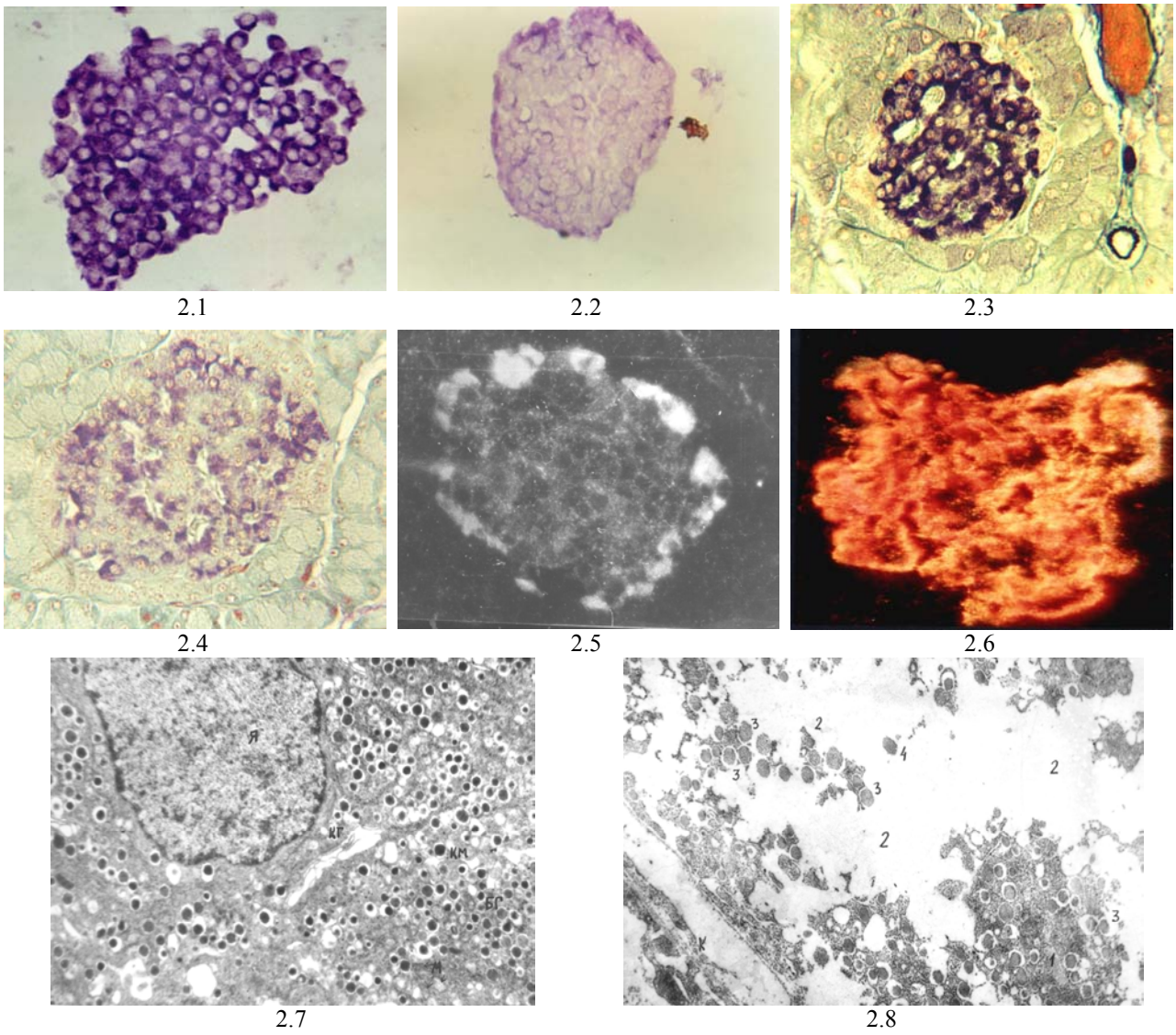
However later it was supposed that injection of chelator result binding of Zn-ions in Zn-contained enzymes that result inactivation of enzymes and as final — disturbances of metabolism in cells and developing of histological changes. But this conception was not confirmed later. It is known that metal-contained enzymes usually fixed strongly atom of metal in structure of molecule enzyme and chelators are not able to form chelat-metal complexes. According other conception, complete elimination of Zn-ions from B-cells protect cells of formation of toxic complexes Zn-diabetogenic chelator but in this same time result disturbances of processes of synthesis and storage of insulin in B-cells. But this hypothesis was not confirmed too, because it was showed, that complete binding of Zn-ions in B-cells by Zn-diabetogenic chelator not accompanied by elimination of complex. As it was evidently established, this complex is dissociated in cytoplasm of B-cells within 1–2 h and Zn-ions are not eliminated from cytoplasm of B-cells.

In opposite, it was showed, that complete elimination of Zn-ions from cytoplasm of B-cells by Glibenclamide not accompanied by changes of structure and function of B-cells [48]. Injection of Dithizon to animals pretreated by Glibenclamide not accompanied by forming of complex Zn-DZ in cytoplasm of B-cells and diabetes is not developed.

As is known, Zn-ions contained in rethina of eye and injection of DZ to rabbit accompanied by forming of chelat-complex Zn-DZ in retina which result blindness [21, 45]. Meanwhile, displacing of Dithizon from complex Zn-DZ by non-diabetogenic chelators within first 5 min. past forming of complex accompanied by prevention developing of diabetes in 95 % of animals [48] whereas analogical displacing of Dithizon 15–20 min past forming of complex Zn-DZ result developing of diabetes in 95–97 % of animals and diabetes was prevented in 3–5 % animals only.

2.1. Investigation of toxic action of complex «Zn-chelat active metal» on histostructure and ultrastructures of pancreatic islets

It was showed that first changes in cytoplasm of B-cells appeared 5 min past injection of DZ as small zones of destruction of cytoplasm. More detail analysis by aid of transmission electron microscopy showed that process of destruction of B-cells started by destruction of B-granules [49] (Fig. 2.7, 2.8).



2.1 — Isolated intact islet. Aldehyde fucshine; ×280; *2.2* — Destruction of isolated islet past 6 min prolonged action of 8-para(toluenesulphonylamino)quinolin. Aldehyde fucshine; ×280; *2.3* — Section of pancreas of intact rat; Aldehydefucshine; ×280; *2.4* — Section of rat's pancreas. Destruction of B-cells caused by Dithizone; Aldehydefucshine; ×280; *2.5* — Islet of intact rabbit; ×280; *2.6* — Red granules of Zn-DZ complex in B-cells of rabbit. Total destruction of B-cells followed 5 min past formation of complex in cytoplasm of B-cells; ×280; *2.7* — Transmission electron microscopy of intact Rabbit's pancreatic B-cell. Ultrastructure withot changes. Multiple B-grenules contained Zn-insulin complex; ×4800; 2.8 — Transmission electron microscopy of Rabbit's pancreatic B-cell. Diabetes induced by Dithizon. Destruction of main part of cell matrix. Destroying of B-granules; ×4950

Figure 2

For the first, the 2–4 B-granules are destructed with forming of small zones of destruction of cytoplasm of B-cells [7, 49, 50], not more than 3–5 % of total surface of section of B-cells. 15–20 min later the sizes of these zones rapidly increased until 30–40 % of surface of B-cells and 1–2 h past injection almost all cell's matrix, 80–90 % of section's surface, is destroyed completely [7, 49, 50]. We showed that these changes are

not visible on light microscopy but very well discovered by transmission electron microscopy. Destructive histological changes developed a few days later — are secondary changes as result of not visible destroying of B-cells within first 15–20 min past forming of chelat complex in cytoplasm of B-cells.

Thus, it was concluded that destruction of B-cells past injection of chelators is determined by destructive action of red complex Zn-DZ on structures, for the first — B-granules, of B-cells within first 15–20 min. past forming of complex in cytoplasm of B-cells (Fig. 2, 3).

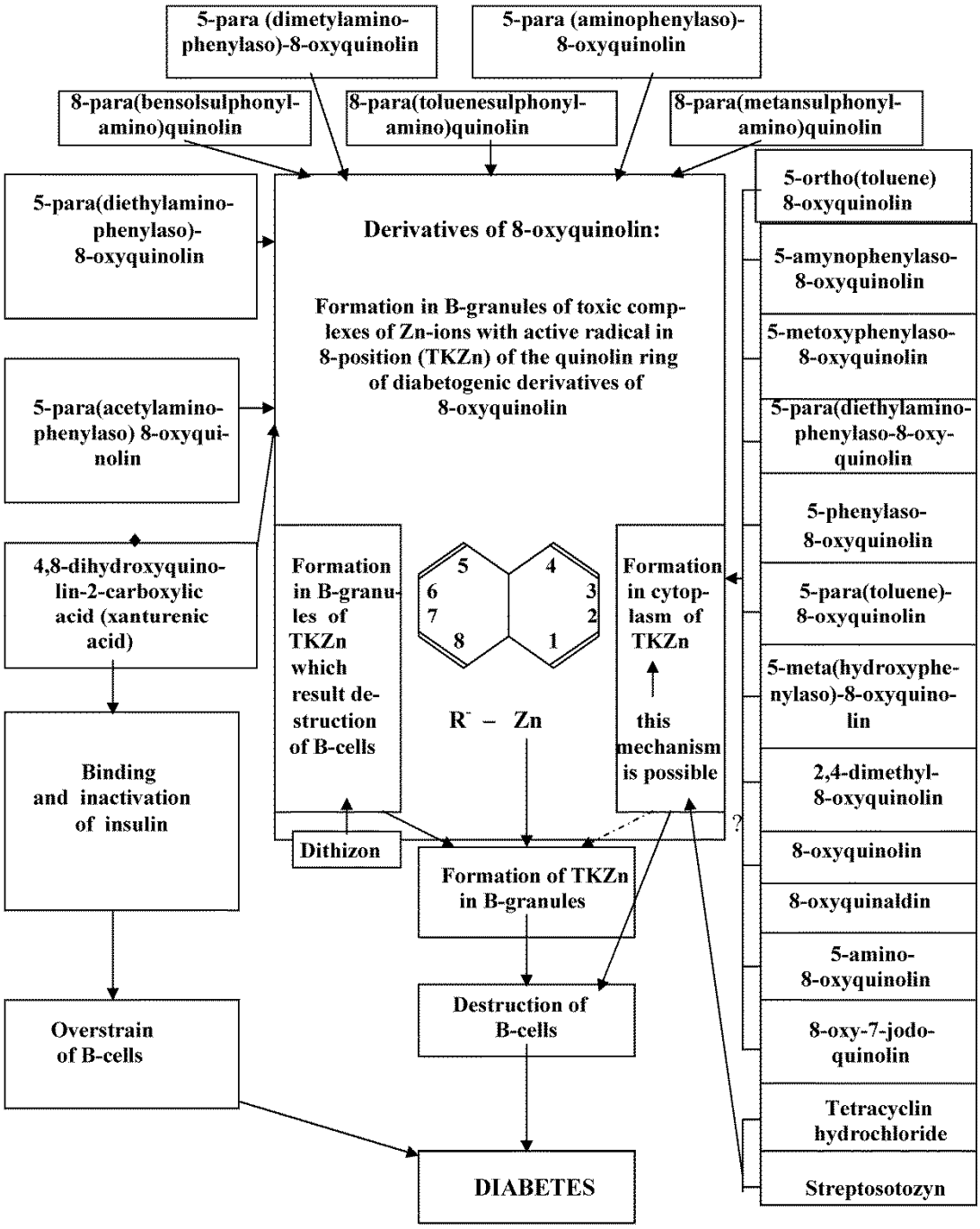


Figure 3. Mechanismes of action of the Diabetogenic Chelat Active Chemicals (♦ — formed in human)

Using of transmission electron microscopy method we showed that 2 h past injection of Dithizon a strongly marked destruction of B-cells was developed: total devastation of cytoplasm of cell's matrix, destruction of mitochondria, endoplasmatic reticulum and B-granules were discovered in the most parts of cells with remained matrix [49, 50]. Same results were obtained 1 h past injection. Meanwhile, 15 min past injec-

tion in the contrary to 2 h cell's matrix was remained on 60–70 % of B-cell's surface but 30–40 % appeared as zone free of matrix or zone of complete destruction of ultrastructures of B-cells [49]. Minimal alterations were revealed 5 min past injection: sometimes a few destructed B-granules formed very small zones of destruction of cells. Detail analysis showed that process of destruction of B-cells was begun by destruction of B-granules and forming of zones of destruction of cytoplasm [49]. Investigation of state of ultrastructure of B-cells 5 min past injection have important role for revealing first initial changes in B-cells which are important for understanding mechanismes of diabetogenic action of substance. Then it was confirmed that start destruction of B-cells by destruction of B-granules is determined by forming toxic chelat complexes of diabetogenic chelat chemical with Zn-ions located in B-granules. From the granules process of destruction is spread on other parts of cells. Meanwhile, it is reason to note, that in B-granules is locaited all amount of Zn-ions contained in B-cells.

3. Dabetes induced by Xanturenic Acid, a abnormal metabolit of Tryptophan

L.Musajo in 1935 reported about fact of synthesis of Xanturenic Acid (XA). This substance was separated from the urine of experimental animals and confirmed as 4,8-dihydroxyquinolin-2-carboxylic acid [50], $C_{10}H_7NO_4$.

S.Lepkovsky and coll. interested by this substance [51]. There are intensification of synthesis of XA in organism when abundance of fat acids was accumulated in organism in combination with vitamin B6 deficiency. These changes were accompanied by developing of symptoms of diabetes [52–57].

XA is product of disturbance of tryptophan metabolism. Usually tryptophan is metabolised by serotonin or kynurenine metabolic ways which completed by forming of 5-oxyindolacetic acid and NADF correspondly. The deficiency of Pyridoxal-5-Phosphate (P-5-P) induced by deficiency of vitamin B6 result inhibition of 5-oxytryptophandecarboxylase and kynureninase which result inhibition of both metabolic ways. As result 4 substances are formed: XA and 8-oxyquinaldine from 3-oxykynurenine and kynurenic acid and oxykynurenic acid from kynurenine (Fig. 4). Meanwhile it was showed that vitamin B6 (Pyridoxin) possess ability to inhibite endogene synthesis of the XA [58–62].

The synthesis of XA determined by main enzymes kynureninaminotransferase (KAT) and oxytryptophandecarboxylase (OTD) with co-enzym as P-5-P [60, 63]. XA is formed from the 3-oxykynurenine by action of KAT. The deficiency of P-5-P result inhibition of synthesis of serotonin and, in the contrary, synthesis of XA and kynurenine are increased [32, 51]. But here we have contradiction: why deficiency of P-5-P accompanied by inhibition of serotonin way and by stimulation synthesis of XA? On the one hand this is determined by fact that pyridoxal enzymes reacted differently for the P-5-P deficiency: activity of kynureninase decreased for 83 % and of KAT — for 42 % only [64]. On the other hand as it was established, KAT are localized as in mitochondries as in soluble part of cells while kynureninase — in soluble part of cells only. Deficiency of P-5-P accompanied by decreasing of content of both enzymes in soluble part of cells and content of KAT-mitochondrial not decreased [65]. This is because elimination of XA with urine is increased. For first time increasing of elimination of XA was discovered in urine of rats contained on diet enriched by tryptophan in combination of vitamin B6 deficiency. XA is disappeared from the urine when vitamin B6 was is added in diet [58, 61, 66]. But aggravation B6 deficiency accompanied by decreasing activity of KAT that result increasing of elimination of XA by urine [32]. More later XA was discovered in the urine of rats, dogs, guinea pigs and human [52, 59, 67–71]. The high concentrations of XA discovered in urine of diabetic patients in middle and old age [72] as more high concentrations of kynurenic acid. Additional administration of Pyridoxine accompanied by decreasing of XA in urine but without complete normalization [72] especially in organism of old persons. XA is eliminated from organism by kidneys. The middle concentrations of XA in the urine of healthy persons in 24 h portion of urine are equalled 2.1–8.9 mg [61].

Deficiency of P-5-P in organism is result of deficiency of vitamin B6 in diet or related with disturbances of synthesis of P-5-P from vitamin B6. Synthesis of XA is intensified by diet enriched by fat acids and casein. It is known 2 enzym systems which determined byosynthesis of P-5-P: pyridoxinphosphateoxydase (PPO) and pyridoxinkynase (PK). Enriched of diet by fat acids result inhibition of activity of PPO in the liver [73] and may be restored by administration of vitamin B2, a co-enzym of PPO. It was showed that in neonatal period — first 3 days — derivatives of kynurenine metabolic ways are not discovered in the urine [74]. In period between 5th and 20th days minimal concentrations of XA are present in urine of babies contained on milk of mother [75, 76]. Addition of α-Tryptophan accompanied by increasing concentration of XA in the urine of babies taked off mother milk as in children in age 4–6 years. In elderly human in age 70 and more synthesis of kynurenine is active. Addition of 100 mg per 1 kg of α-tryptophan in diet accompanied by inten-

sive elimination of XA with urine from organism of old men in age of 70 and more [77–79]. It is possible to normalyse elimination of XA by administration of Pyridoxin [80]. Administration of Pyridoxin accompanied by normalization [21, 80–86] of concentration of xanturenic acid in the urine excluding patients with strong deficiency of vit. B6 [62, 83] due to reduction activity of kynureninaminotransferase. Administration of 100 mg per 1 kg of α-tryptophan in organism of pregnant women accompanied by abnormally high elimination of XA during all time of pregnancy as of kynurenic acid for 3–4 months [82]. It was showed deficiency of vit. B6 in diabetic patients with high concentration of xanturenic acid in the urine [87, 88]. Usually disturbances of tryptophan metabolism accompanied by forming of abundance of XA as of other abnormal products in result of deficiency of P-5-P in organism [47].

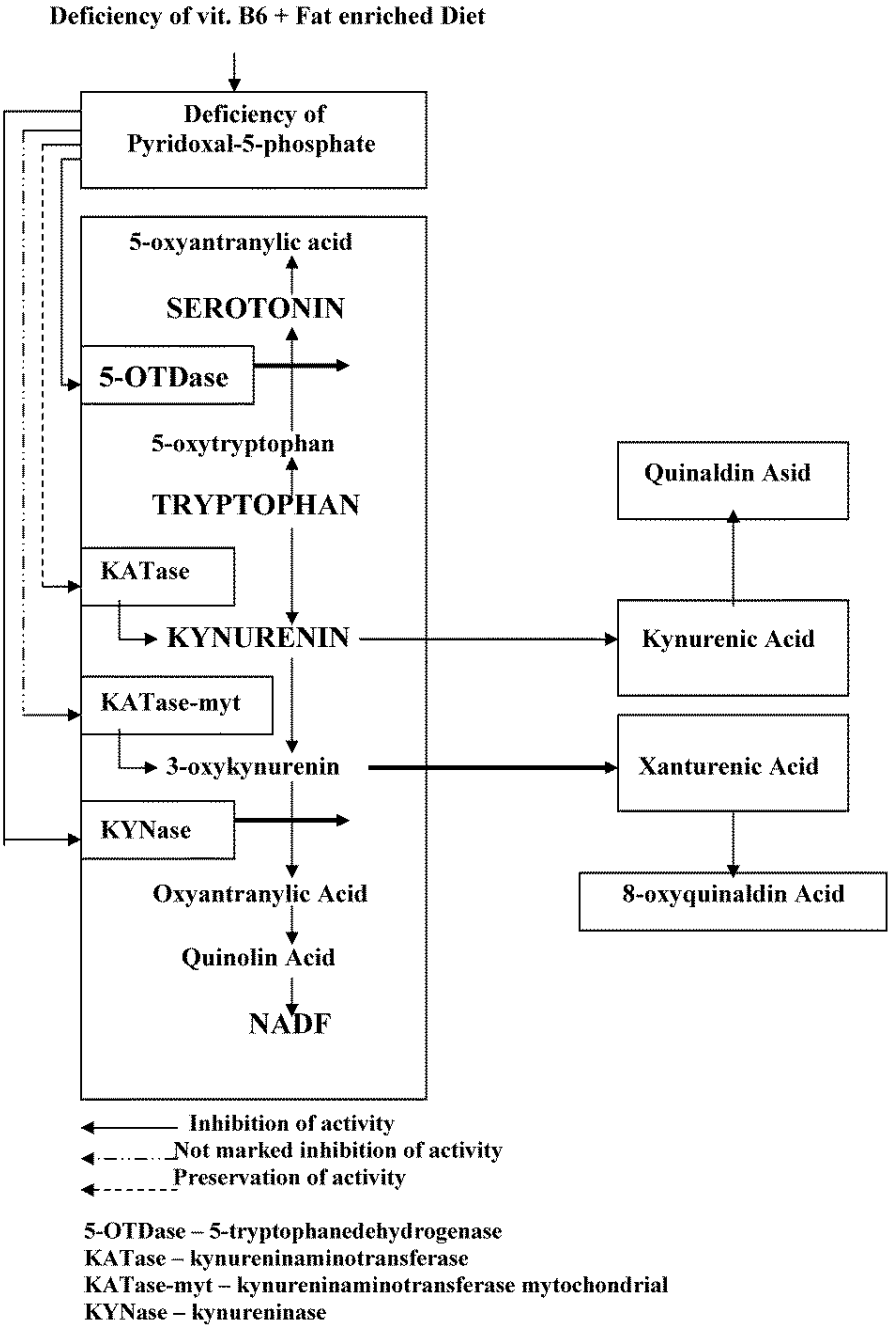


Figure 4. Disturbances of Tryptophan metabolism

Y.Kotake and coll. in 1957 investigated processes of forming and elimination of XA from organism. He used various Na-salts of fat acids and tryptophan which were administrated intraperitoneally in organism of rats. The most large amount of XA was formed and eliminated (elimination — 10,49 mg of XA per 24 h) by

urine after using of mixture «tryptophan+oleic acid» and the most less effect — 1,6 mg per 24 h, after administration of tryptophan only. Effects of other mixtures: «tryptophan+acetic acid» — 5,37 mg of XA per 24 h, «tryptophan+propyonic acid» — 8,79 mg of XA per 24 h, «tryptophan+oleic acid» — 9,87 mg of XA per 24 h, «tryptophan+valeryanic acid» — 9,64 mg of XA per 24 h, «tryptophan+palmytin acid» — 9,61 mg of XA per 24 h and «tryptophan+stearynic acid» — 8,57 mg of XA per 24 h. On the base of its experiences Y.Kotake recommended to include in diet a followed products for stimulation of synthesis of XA: casein, salt mixture of McColumn, agar-agar, sugar, saturated oleum, yeast and starch. It was showed that biosynthesis of P-5-P is depended of containing of fat or fat acids in diet. It was concluded: fats and fat acids stimulated decreasing activity of pyridoxinaminotransferase in the liver of rats [73]. It was reported that stress stimulated acceleration accumulation of diabetogenic metabolits of tryptophan [88, 89].

Meanwhile injection of 10,0 mg of vitamin B6 in experimental conditions accompanied by decreasing of elimination of XA till 2,03 mg per 24 h [14] in compared with 8,42 mg per 24 h in control. Y.Kotake in 1968 established that fat acids stimulated inhibition of synthesis of P-5-P from the vitamin B6. As result — increasing of forming of XA. Intraperitoneal injection of 200 mg per 1 kg of endogene synthezed XA to mouse accompanied by developing of diabetes [89] and by temporary hyperglycemia in rabbits [90]. But injection of synthetic XA in dose of 200 mg per 1 kg not accompanied by diabetes after administration to dogs and to rabbits [33]. Combination of injection of synthetic XA and using of diet enriched by large amount of fat accompanied, in the contrary, by hyperglicemia and developing of histological changes in islets typical for diabetes [60, 91–94]. Meanwhile diabetes was not developed in conditions: injection of synthetic XA+diet free of vitamin B6 [95].

Using of diet «10 mg/kg tryptophan+deficiency of vitamin B2» accompanied by hyperglycemia and xanturenuria [53]. Same effect was obtained after using of diet «tryptophan+deficiency of vitamin B6». Using of diet enriched by fats by Y.Kotake accompanied in added by increasing of weight of animals on average from 140 g till 220–260 g and by developing of obesity [94]. The excretion of XA was equalled to 2–3 mg per 24 h [96]. Using of histology it was showed developing of evident changes in B-cells: degranulation of B-cells, vacuolisation of cytoplasm, destruction of cells, developing of hydropic degeneration and changes in the nuclei [29, 53, 54, 97–101]. Not only XA but kynurenic acid induced hyperglicemia. It is transformed in quinaldic acid. XA is metabolized in final product — 8-oxyquinaldic acid which possessed diabetogenic properties.

Theoretically this substance may be transformed in diabetogenic agent 8-oxyquinaldin but we have not found information about forming 8-oxyquinaldin in organism of animals or in human organism as result of disturbances of tryptophan metabolism. It is known that diabetogenic effect of 8-oxyquinaldin is not so intensive as effect of other derivatives of 8-oxyquinolin described above. XA as quinaldic acid and kynurenic acid possess insulin releasing activity [66, 102] and stimulated intensive insulin releasing from isolated pancreatic islets in the first 30 min after start of incubation. Later activity is decreased. The quinaldic acid inhibite completely 2nd phase of insulin releasing [103, 104] and is more active comparatively with 8-oxyquinaldic acid [105]. Incubation of insulin and XA accompanied by forming of stable complex XA-insulin separated on the Sephadex [96, 106]. It was showed by using fluorimetric method that 2 moles of XA are binded with 1 mole of insulin. Activity of this complex is as 49 % of activity of pure insulin.

Not only XA but kynurenic acid induced hyperglicemia too [107]. XA is transformed in quinaldic acid [113] and later is metabolized in final product — 8-oxyquinaldic acid [66] which possessed diabetogenic properties. Activity of native insulin [96, 108] is increased after administration of Zn-ions in incubation medium [106, 109, 110]. It was showed that Zn-ions force out molecule of insulin from the complex XA-insulin and formed new complex XA-Zn. Meanwhile the role of this chelat complex was not investigated.

E.Murakami reported [97, 111–116] that incubation of XA with insulin result forming of 2 sorts of complexes which were separated and purified: complex XA-insulin 1:1 and complex XA-insulin 1:1,5. Activity of both complexes is as 50 % of activity of native insulin only [66, 108]. He supposed that same complex may be formed in human organism. In the blood XA is easy binding with insulin and not transformed chemical structure of it. This complex is stable [96] and chemical connection is formed via atom of Zn and imidazol group of histydin in molecule of insulin [96, 98]. XA possess especial affinity to Zn-ions [114]. Addition of Zn-ions to the serum of blood contained complex XA-insulin stimulated restoration activity of insulin [115]. Not only deficiency of vitamin B6 induced active synthesis of XA in organism as of other abnormal metabolits of tryptophan. The biosynthesis of P-5-P in human organism is inhibited by some drugs for treating of patients with tuberculosis due to its ability to block both enzyme systems of P-5-P [48]. Treatment by Hydraside of Isonicotine acid accompanied by deficiency of P-5-P [116, 117] and as result by

xanturenuria. Isoniasid is antagonist of P-5-P [118] and of kynureninase [119]. Treatement by Isoniasid accompanied by xanturenuria and kynurenuria [76]. It was established in added that tuberculosis accompanied by deficiency of vitamin B6 in organism which stimulated aggravation of deficiency of P-5-P.

Other state — pregnancy — accompanied by xanturenuria too. Intensity of xanturenuria is decreased by administration of vitamin B6 [50]. Total amount of XA eliminated with urine from organism of patients with pregnancy aggravated by toxycosis and reached 190 mg per day in compared with 0,3–13 mg of XA per day in control group without pregnancy and toxycosis [50]. It is showed that frequency of diabetes is depended of number of pregnancy during woman's life: 2,7 % among women with 1 pregnancy, 5,2 % — 2 pregnancies, 7 % — 3 and more pregnancies [120]. Now are not cleared causes of xanturenuria in pregnancy. There are suppositions that pregnancy induced acceleration of desintegration of tryptophan by activation of tryptophanoxygenase [21] but it is evidently that more high frequency of diabetes in patients with tuberculosis and pregnancy have relation with endogene synthesis of large amount of XA in organism.

Thus, noted above data about diabetogenic properties of XA are especially interested due to fact that in the contrary to many other diabetogenic chemicals, including all investigated previously diabetogenic derivatives of 8-oxyquinolin, XA only may be formed in organism of animals and human in result of simple disturbances of diet accompanied by deficiency of vitamin B6.

It is necessary to turn attention on 4 coincidences: 1. Xanturenuria is often discovered in the urine of diabetic patiens. 2. Xanturenuria is often discovered in organism of old men. Meanwhile it is known that diabetes of 2nd type is developed more often among elderly persons. 3. Very often deficiency of vitamin B6 is discovered in the group of elderly and old men. 4. XA as chemical is belong to derivatives of 8-oxyquinolin which have in position 8 active group as all other diabetogenic derivatives of 8-oxyquinolin which diabetogenic properties were investigated previously. 5. Extraction of active group from position 8 of molecule of XA accompanied by complete disappearing of its diabetogenic properties as of some other diabetogenic derivatives of 8-oxyquinolin. Returning back of this group in position 8 accommpanied by restoration of diabetogenic properties of XA. 6. Contrary to all other 18 diabetogenic derivatives of 8-oxyquinolin XA is formed in human organism.

4. On the mechanisms of diabetogenic action of tryptophan's metabolits

More than 40 years ago Y.Kotake was fixed attention on fact that chemical structure of XA is very similar with structures of other diabetogenic derivatives of 8-oxyquinolin. He supposed that its diabetogenic properties determined by the presence of active OH-group in position 8 of quinolin ring [121, 122]. In 1957 Y.Kotake and M.Kato were confirmed fact that XA may to induce diabetes only in case if in position 8 is fixed OH-group. Extraction of this group from molecule accompanied by complete disappearing of diabetogenic properties of XA [29, 122].

G.Weitzel and coll. and S.Ikeda and coll. [33, 114] confirmed that XA formed with Zn-ions complex 1:1 and atom of Zn is fixed between hydroxyl and carboxyl groups of quinolin ring. As it is known this sort of complex of 8-oxyquinolin derivatives (1:1) is most toxic for cells. E.Murakami and Y.Kotake were investigated interaction between insulin and XA. They confirmed that xanturenic acid in vitro formed complex XA-insulin [112].

On the base of obtained data Kotake Y. and Ueda T. were proposed a followed point of view on the understanding of mechanismes of diabetogenic action of XA [108, 112, 115] **(**Fig. 5, left part**)**.

Meanwhile authors in passing showed that after dissociation of complex XA-insulin, xanturenic acid again formed new complex with Zn-ions as XA-Zn. But it was make not attention to this fact and this chelat-complex was not investigated. Howerer, more later it was showed in vitro that XA binded Zn-ions in B-cells [123–131] and that presence of this complex in cytoplasm of B-cells within short time result alteration and destruction of cells [132–139].

Deficiency of vitamin B6 stimulated forming not only of XA but additionally kynurenic acid and quinaldic acid. These acids stimulated releasing of insulin from the isolated islets [102–104]. On the other side these metabolits are inhibite forming of B-granules [104] in result of blocking of Zn-ions in cells. 8-oxyquinaldic acid inhibite in added synthesis of proinsulin [122]. More over XA inhibite synthesis of insulin by bindng of insulin with Zn-ions [124].

As result of disturbances of Tryptophan metabolism the 8-oxyquinaldin may be accumulated. Meanwhile 8-oxyquinaldin, a derivative of 8-oxyquinolin, is diabetogenic substance which are able to induce hyperglycemia and degenerative changes in B-cells [33]. However XA is eliminated from the organism with

urine and now there are not reported facts that XA is transformed in 8-oxyquinaldin in organism. Nevertheless, we cannot to exclude this possibility.

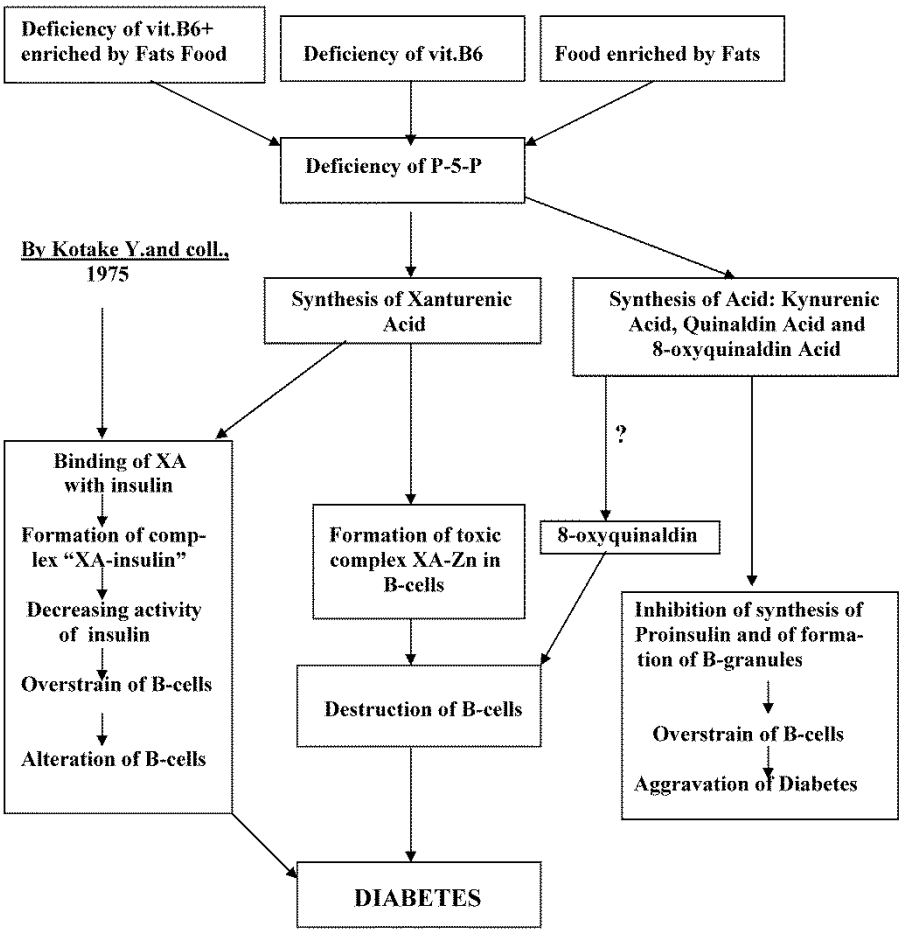


Figure 5. Mechanisms of Diabetogenic Action of Xanturenic Acid

Diabetes induced by derivatives of 8-oxyquinolin may be prevented in experimental conditions by preliminary preventive binding of Zn-ions in B-cells by not diabetogenic chelat active substances as Dietyldithiocarbamate of sodium which are able to protect B-cells in 95–100 % animals for 24 h [48] of destruction. The other, 2nd way: preventive almost complete elimination of Zn-ions from B-cells before administration of diabetogenic chelat active chemicals. And 3rd way: preventive concurent interseption of diabetogenic chelator in nutria media by Zn-ions delivered in solution outside as ZnSO4. But this way is valid for experimental defence of B-cells of isolated pancreatic islets. The advantage of this method: reaction passed not into cytoplasm of B-cells, but outside of cells.

These methods protection of B-cells which we have used in process of investigation of mechanisms of developing of diabetes induced by chelat active chemicals, not have perspective of practical using because it is not possible and not expediently to keep Zn-ions in B-cells permanently connected with not diabetogenic substances or to eliminate permanently Zn-ions from the cytoplasm of B-cells and to keep cells free of Zn-ions permanently.

Thus, despite of fact that by aid of both methods it is possible to prevent developing of experimental diabetes in 95–100 %, these methods are not suitable for prevention developing of XA-diabetes on aspect of human diabetes.

However it is known that synthesis of XA in organism may be prevented by administration of vitamin B6. This way of preventinion of xanturenic diabetes is, as we think, more perspective. Besides this method not need additional investigations and scientifical reasons concerning elaboration related with practical using of vitamin B6.

Injection of other diabetogenic derivatives of 8-oxyquinolin result 2–3 days later developing of heavy diabetes 1 type. Diabetes induced by XA in the contrary look like diabetes of 2 type. It is explayned probably

by a followed circumstances. Other diabetogenic derivatives of 8-oxyquinolin were used as one injection of 100 % diabetogenic doses of substance. More less amount of XA is formed in organism more slowly day by day permanently in result of changes of diet or changes of tryptophan metabolism especially in old organism.

Interest to diabetes induced by XA is increased due to followed factors: 1. XA in the contrary to other diabetogenic derivatives of 8-oxyquinolin is formed in human organism as result of simple changes of diet. 2. A large amount of XA is discovered in the urine not only of diabetic patients in middle or old age, but in the urine of persons in same age without diagnosis of diabetes. 3. Deficiency of vitamin B6 is discovered in organism of old persons with registrated diagnosis of diabetes or without it.

Previous our investigations of mechanismes of diabetogenic action of derivatives of 8-oxyquinolin, which cannot be synthezed in organism or to come into organism outside, have theoretical significance only. However data obtained during these experiences let us to understand more profoundly mechanisms of diabetogenic action of XA. XA due to noted above data make us to call our attention on this substance which may to have some significance in pathogenesis of human diabetes.

On the base of data obtained by other investigators and by us I propose a followed point of view on the mechanisms of diabetes induced by XA **(**Fig. 5).

Thus, noted above data showed a potential role of diabetogenic metabolits of tryptophan in pathogenesis of human diabetes. From the presented data it is possible to conclude that main role among a few metabolits — XA, kynurenic acid, oxyquinaldic acid, 8-oxyquinaldic acid and 8-oxyquinaldin — are belong to XA. Kynurenic acid and oxyquinaldic acid not contained, in the contrary to XA, in position 8 of quinolin ring of active chemical group and not induced diabetes. Both these chemicals activate insulin releasing from B-cells.

Thus, now it is possible to suppose that main role is belong to XA as to diabetogenic chemical. Other metabolits are able to aggravate diabetes induced by XA.

5. On the possible ways for prevention developing of diabetes caused by chelators

What is minimal period of the presence of chelat complexes in B-cells for to induce diabetes? As it was showed previously this period is 15–20 min. For to confirm this fact the experiences with non diabetogenic chelator-Na salt of Diethyldithiocarbamic acid (NaDDC) were conducted. NaDDC have more high affinity to Zn-ions and able to displace Dithizon from chelat complex Zn-DZ as of derivatives of 8-oxyquinolin from its chelats with Zn-ions. Analogical properties possess Dimethyldithiocarbamic acid and its derivatives. EDTA as chelator possess more high affinity to Zn and constant of stability of its chelats with Zn is 13.1 meanwhile with ions of Mg^{+2}, Ca^{+2} and Fe^{+3} correspondly 5.4, 7.3, and 10.9 [15]. It was showed that EDTA prevent diabetogenic action of streptosotozin by binding of Zn ions [131]. More detail investigation of processes of interaction of Zn-ions contained in B-cells with NaDDC showed that injection of 250 mg/kg of NaDDC result binding of part amount of Zn-ions inlocated in cytoplasm of B-cells, and of 500 and 1000 mg/kg — a complete binding of all amount of Zn-ions. Dissociation of complex is passed more slowly: 10–12 h past injection dissociated less part of complex and 24–48 h past injection — all amount of Zn-ions are free [34, 35, 49]. Injection of NaDDC 5 min — 6 h before diabetogenic chelator result complete prevention of diabetes in 100 % of animals for 8–10 h. Injection of DZ within this period not accompanied by formation of red stained granules of Zn-DZ complex in cytoplasm of B-cells and diabetes is not developed [41, 48]. Injection of NaDDC 15 min past injection of DZ accompanied by complete displacing of DZ from complex Zn-DZ and by formation of not diabetogenic complex Zn-NaDDC but diabetes developed in 95–96 % of animals and was prevented in 5 % of animals only. Injection of NaDDC 5 min past DZ accompanied also by complete displacing of DZ from complex Zn-DZ but diabetes was prevented completely in 95 % of animals and was developed in 5 % of animals only (49). Injection of NaDDC 2 h past injection of DZ accompanied by developing of diabetes in 100 % of animals [48]. Thus, by these experiences it was confirmed that the presence of toxic chelat complexes of DZ and diabetogenic derivatives of 8-oxyquinolin in B-cells within first 15 min after its forming result not visible for the first a few hours incorrigible destructive changes in B-cells. Formed more later degenerative histological changes in islets is result of action of chelators in the first 15 min.

The aminoacids Cystein and Glutathion formed not toxic chelats with atoms of heavy metals due to sulfhydril radicals which have high affinity to ions of Zn^{+2}, Pb^{+2}, Cd^{+2} and Hg^{+2}. It is suggested that by these radicals aminoacids formed not toxic chelats with Zn-ions. The constant of stability of complex Zn-Cystein is very high — 17.1–18.2 [48].

Aminoacid Hystidin formed with Zn-ions high stable complex 2:1 which logarifm is 12. Contrary to other aminoacids chelat activity of Hystidin is determined by the presence in molecule of the imidazol ring [21].

Injection of Cystein 1000 mg/kg prevent formation in B-cells of toxic chelat Zn-DZ an completely protect of diabetes all animals within 6 h; 12 h past injection diabetes was prevented in 6 animals from 8 and 24 h past injection of Cystein — in 2 animals from 4. Cystein protect B-cells of destruction caused by diabetogenic derivatives of 8-oxyquinolin. Aminoacid Serin, which contained hydroxyl radical in molecule instead of sulfhydril radical in molecule of Cystein, not possess diabetogenic properties.

Diabetes is prevented by Restored Gluthation. Preventive injection of it protect B-cells of destruction and of developing of diabetes in all animals: normoglycemia and B-cells — without changes. Oxydation of Restored Gluthation result: two molecules of Restored Gluthation formed one molecule with forming of disulfide connection. Injection to animals of 1000 mg/kg of the Gluthation — oxyd not accompanied by prevention of diabetes in all experimental animals.

Thus, inactivation or change of Sulfhydril radicals in molecules of Cystein and Gluthation result complete disappearing of diabetogenic properties of these both substances [28]. Injection to animals of 1000 mg/kg of the Hystidin Hydrochloride (HH) result complete prevention of diabetes past injection of Dithizon followed 5 min past injection of HH and — in half of total number of animals injected of Dithizon 0,5–1 h past injection of HH [28].

Prevention of binding of Zn-ions in pancreatic islets by diabetogenic chelators may be realized by other way — preliminary complete elimination off B-cells zinc-insulin complex by derivatives of Sulphonurea [31]. Injection of Dithizon or derivatives of 8-oxyquinolin past maximal complete elimination of Zn-ions from B-cells not result forming of toxic copmlexes «Zn-chelator» in cytoplasm of B-cells and diabetes not developed in 100 % of animals. This method of prevention of diabetes is effective and suitable in cases when administration of diabetogenic dose of chelator is expected. In opposite, this method it is not suitable when low doses of diabetogenic chelators are formed in organism during long period.

Dehydroascorbic Acid (DA) which is formed in organism as result of metabolisation of Ascorbic Acid, possess diabetogenic properties and result of direct alterative effect on B-cells [120]. Concentration of DA in organism of diabetic patients is evidently increased in opposite to decreasing concentration of Ascorbic Acid [123].

It is known that Streptozotocin possess chelat properties and have high affinity to Zn-ions. Alterative action of Streptozotocyn may be prevented or reduced by preventive action of EDTA [137].

Investigation of diabetogenic properties of Dithizon and derivatives of 8-oxyquinolin have theoretical significance because these chemicals are not formed in human and really not delivered in human organism outside. In added, peroral administration of its is not effective because they are not soluble and not absorbed in intestinum. Parenteral injection of diabetogenic chelators result developing of diabetes only. Meanwhile solutions of all these chelators are not stable and only injection of the fresh prepared solutions (ex tempore) result diabetogenic effect.

Among 18 diabetogenic derivatives of 8-oxyquinolin the Xanturenic Acid (XA) only is formed in elderly humans. It is known that XA is accumulated in organism of old human as result of disturbances of Tryptophan metabolism. Low doses of the XA accumulated in human gradually. May be that is why diabetes caused by XA developed gradually as type 2 contrary to type 1 diabetes caused by injection of diabetogenic doses of other chelators. Mechanisms of diabetogenic activity of chelat active chemicals and way of prevention development of diabetes caused by them were studied by us before [140–194].

The most perspective way for prevention of diabetes caused by XA is, of course, prevention of endogene synthesis of XA in human in disturbances of Tryptophan metabolism.

Last decades the number of diabetogenic chelators human have contacts is very increased. As example, Tetracycline hydrochloride is active chelator which have high affinity to Zn-ions and formed with it complex 1:1 and 2:1 with high constant of stability as 9,0 [21]. Direct action on B-cells of high doses of tetracycline result hyperplasia and degeneration of cells. Isoniazid, a drug for treatment of tuberculosis, formed pentagonal stable chelats with Zn-ions. May be more high frequency of diabetes among patients treated by Isoniazid determined by this fact? This interest is increased taking into consideration fact that in this case concentration of the Xanturenic acid in urine is high because Isoniazid in antagonist of Pyridoxal-5-Phosphate [63].

Diuretics as derivatives of Benzothiodiazine possess chelating properties and treatment during long time accompanied by developing of diabetes sometimes. Treatment by Chlorthiazid accompanied by hyperglycemia and glucosuria.

It is known that chelators which formed with Zn-ions tetragonal or pentagonal rings possess diabetogenic properties. Chelators contained in molecule as least 4 or 5 double chemical connections possess diabetogenic properties also in opposite to chelators contained 1–2 or not contained its which not possess analogical properties. As example — derivatives of Diethyldithiocarbamic Acid of Dimethyldithiocarbamic acid, aminoacids Cystein, Gluthation and Hystidin. Complexes formed by noted above protectors not contained in molecule tetragonal or pentagonal rings and not contained or contained minimal number (1–2) of double connections. Administration of large amount of these chelators not result destruction of B-cells and protect, in opposite, B-cells of destruction caused by diabetogenic chelators.

Noted above data put us to look on these chemicals as on one possible factor in ethiology of human diabetes. The significance of this possibility is increased taking consideration fact that human pancreas contained a large amount of Zn-ions possess to form chelat complexes with diabetogenic chelators.

On the base of presented above our experimental data as data obtained by other investigators we propose a followed point of view on the role of Zn^{+2}-ions in the pathogenesis of diabetes mellitus caused by diabetogenic derivatives of 8-oxyquinolin as followed main steps:

1. Diabetes induced by chelators is determined by formation in cytoplasm of B-cells of the chelat complexes «Zn-chelator» only and developed as IDDM. Any mechanisms prevention synthesis of this chelats-complexes in cytoplasm of B-cells protect completely cells of destruction and of developing of diabetes.

2. Destruction of B-cells caused by direct action of chelat complexes on histoctructures of B-cells — is main mechanism of developing of these models of diabetes. Inevitable destructive changes in cytoplasm of B-cells developed within first 15–20 min past forming of chelats in cells. Changes developed within first 4–5 min are not significant and displacing of chelator from complex «Zn-chelator» by not diabetogenic chelators that result prevention of destruction of B-cells and developing of diabetes. Incorrigible histological changes in B-cells developed a few days later, are as result of noted above changes developed within first 15–20 min. past formation of toxic chelat complexes in B-cells.

3. Diabetes induced by chelators may be prevented by prevention formation of complexes «Zn-chelator» by 4 ways: a) preliminary complete elimination of Zn-ions off cytoplasm of B-cells; b) by preliminary binding of Zn-ions in B-cells by not diabetogenic chelators have more high affinity to Zn-ions comparatively with diabetogenic chelators; 3) by concurent interseption of chelator in liquid media by Zn-ions delivered outside with Zn-contained salts; 4) by prevention of the synthesis in organism (including human) of diabetogenic chelators.

4. Inhibition of endogene synthesis in cytoplasm of B-cells of diabetogenic metabolits of tryptopan using of Pyridoxine is most preferable and perspective way for prevention developing of diabetes in animals and Human.

Next years be investigated possibilities for prevention developing of diabetes caused by 4,8-dihydroxyquinolin-2-carboxylic acid (XA) by partial or almost complete inhibition of endogenous synthesis in animals and human of the XA, a product of abnormal tryptophan metaboilsm.

Acknowlegement

This work proceeding 41 years was supported since 1977 by reagents, research and travel grants from Prof. K.-D.Kohnert, Institute of Diabetes «Gerhardt Katsch» (Karlsburg, Germany); by H.-A.Langisch, J.Watzek «Boehringer Mannheim» (Germany), H.Niedderer, E.Horn «SERVA» (Germany), H.Ritzel «HOECHST» (Germany), G.Putlik «SARTORIUS» (Germany), W.Manneck «MERCK» (Germany), «BACHEM» (Germany), O.E.Grundemann «FERAK» (Germany), B.Alimbekova «SERVIER» (France), Ch.Studer «CALBIOCHEM» (Switzerland), R.Thunberg «Pharmacia Fine Chemicals» (Sweden), «Yanssen Chimica» (Belgium), P.Felch, R.Schilly «Boehringer Mannheim Wien» (Austria), V.Riedl (Austria) by research and travel grants from vice-president of International Diabetes Federation, Prof. John R.Turtle (Sydney, Australia), by vice-president of International Diabetes Federation Dr. Lorna Mellor (Sydney, Australia), by President of the American Diabetes Association, Prof. H.Rifkin (USA), Prof. B.Tuch (Sydney, Australia), Prof. Y.A.Lasaris and Prof. I.A.Serebrovska, Karaganda by Prof. F.Wohlrab (Germany) and Prof. E.Murakami (Japan). Scientific project and main financial supporting of work — by families of Prof. G.A.Meyramov and Prof. G.G.Meyramov since 1972.

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8-Оксихинолиннің диабетогендік өнімдері (әсер етуінің механизмдері және олардың әсерінен туындатын диабет дамуының алдын алу жолдары)

Қант диабеті дамуының себептерінің біріне ұйқыбездің В-жасушаларының зақымдануы мен ыдырауы жатады. Авторлар 8-оксихинолин туындыларының әсер ету механизмдерін зерттеген, оның ішінде заттектердің алмасуы бұзылған кезде адам ағзасында өндірілетін өнімдері де бар. Ауру дамудың алдын алуының бірнеше жолдары зерттелген. Адам ағзасында өндірілетін 8-оксихинолин туындылары туындататын диабет дамуының алдын алуының жолына организмде олардың эндогенді түзілуін тежеу мүмкіндігі көрсетілген.

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Диабетогенные производные 8-оксихинолина (механизмы действия и пути предотвращения развития диабета, вызываемого ими)

Одна из причин развития сахарного диабета — повреждение и разрушение В-клеток поджелудочной железы химическими веществами. Авторами изучены характер и механизмы действия некоторых из них, включая и те, что синтезируются в организме человека при нарушениях обмена веществ. Исследовано несколько способов предотвращения развития заболевания, вызываемого ими. Показано, что наиболее приемлемым способом предотвращения развития диабета производными 8-оксихинолина является возможность подавления их эндогенного синтеза в организме.

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UDC 613.2:612.359:616–036

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Decision support and e-health services for diabetes management

In this article, we will introduce and discuss new options by using personal decision support and e-health services in diabetes management. Usage of the KADIS® system for personal decision support provides opportunities to identify improved therapy options in accordance with the guidelines of the professional societies. This enables the best treatment modality for the individual patient to be determined in a very short time and, individual circumstances of the patient as well as the practical feasibility of treatment recommendations are taken into account. Furthermore, the telemedicine platform TeleDIAB® together with the embedded KADIS® simulation are suitable to make knowledge and experience globally available to treat diabetes.

Key words: diabetes, personal decision support, telemedicine, in silico model, e-health service, diabetes management.

Introduction

According to data, recently published by the International Diabetes Federation (IDF), an estimated 371 million persons worldwide have diabetes mellitus. Four out of 5 diabetes patients live in developing and emerging countries. Especially in Asia and in countries of the Gulf region with a high population growth rate and drastical changes in living conditions, the disease has reached an epidemic dimension. The diabetes prevalence in these regions already exceeds 20 % [1–3]. Prevalence figures also rise, even though not to the same extent, in developed countries, like the U.S. and Germany; however, other factors, such as an aging society, overnutrition, and lack of physical activity may play a role. In contrast to developing and emerging countries, significantly larger healthcare budgets are available in highly-developed countries for diabetes treatment. Furthermore, within the latter healthcare systems extensive know-how and sophisticated treatment strategies have been established for management of diabetes. This includes research, established structures of diabetes care, and special programs aiming to prevent diabetes.

Thus, general practitioners as well as diabetes specialists can take advantage of a wide variety of pharmaceutical and technical resources available for diabetes treatment. Guidelines have been established by the ADA and EASD in order to standardize diabetes care [1, 4]. With data provided by blood glucose self-monitoring, continuous glucose monitoring systems or collection of self-control data by the patient, and numerous diagnostic parameters needed for treatment decisions, the doctor is faced with a huge amount of information. For the evaluation of glucose variability, for example, alone over 30 different metrics have been published until now [5–11].

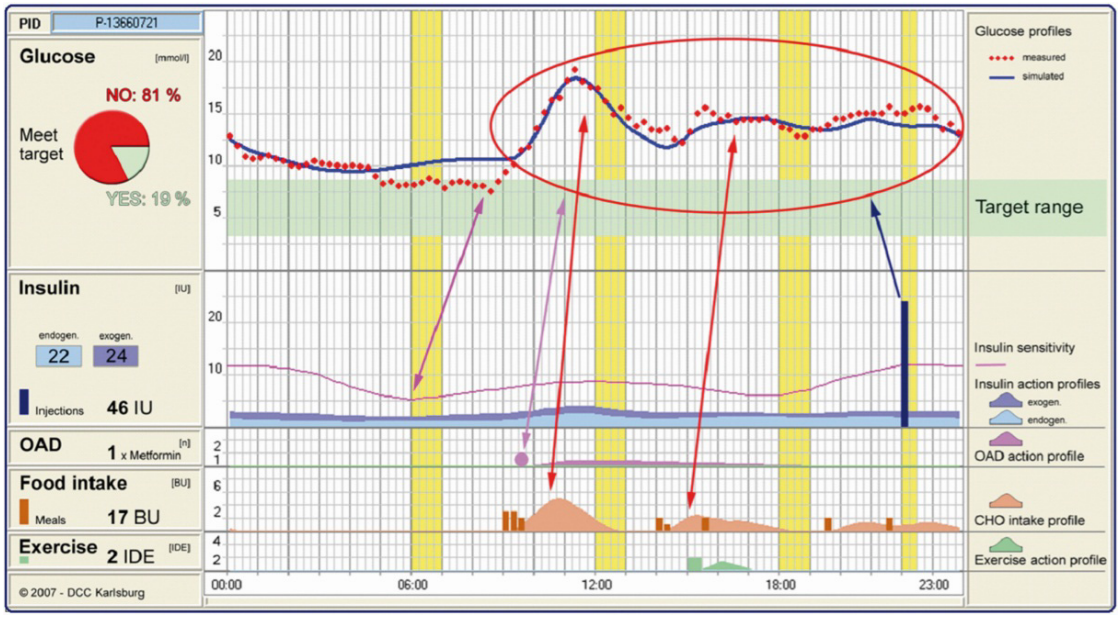
The increasing prevaalence of diabetes, the great variety of treatment options, and the flood of data raise the necessity of appropriate and effective strategies applicable to find a timely therapeutic approach tailored to the individual needs of the patient. As our previous outcomes showed, the telemedicine-based Karlsburg DIiabetes management System KADIS® could play an important role [5, 6] in achieving this goal. In addition, telemedicine solutions are suitable to make knowledge and experience globally available to treat diabetes.

Karlsburg Diabetes Management System, KADIS®

The metabolic status is as different as a fingerprint for each person and clearly shows an individual rhythm reflected in the 24-h blood glucose curve of a patient. By means of KADIS®, a so called in silico copy of the metabolic behavior of diabetic patients can be created for the first time worldwide. The KADIS® system itself is protected by patents, but its application was already published in peer-reviewed journals [12, 13].

To map the individual metabolic behavior in silico, i.e. in a computerbased model, the KADIS® system requires data, which can be easily collected under everyday conditions. This includes blood glucose readings,

patient's self-control data, such as glycemic therapy (insulin, oral antidiabetics), food intake as well as physical activities (sports) and demographic data (e.g., age, diabetes type, disease duration, body weight and height). After entering all these data, they will be automatically analyzed by the KADIS® system. Upon information on dose and insulin formulations or oral drugs, the corresponding activity profiles are calculated, absorption profiles for food intake be determined, and sports activities are converted into insulin action equivalents. Importantly, the insulin responsiveness during the day can be visualized. Moreover, in the case of Type 2 diabetes, the daily profile of endogenous insulin can be determined and simultaneously displayed with the insulin responsiveness by means of the KADIS® model. During the subsequent iteration process, blood glucose curves will be simulated on the basis of the available data. The specific parameters of the KADIS® model are gradually adapted based on a patented mathematical method until defined termination conditions are reached. As a result of this procedure, one obtains a setting of the KADIS® system, where the blood glucose tracings of the patient derived from glucose monitorings is best reflected by the simulated glucose profile. The in silico mapping of the individual metabolic behavior on the PC is referred to as personal «Metabolic fingerprint» of the patient (Fig. 1).



Blood glucose tracing obtained from 24-hour glucose monitoring (red dotted line); 24-hour insulin activity profiles (total insulin available) exogenous insulin (blue area) and endogenous insulin (light blue area); insulin sensitivity throughout the day (pink curve); 24-hour resorption profile of meals (light brown area), insulin and oral drugs intake (time, dose, type); meals (food, quantity, time)

Figure 1. Presentation of the «metabolic fingerprint» by means of KADIS®

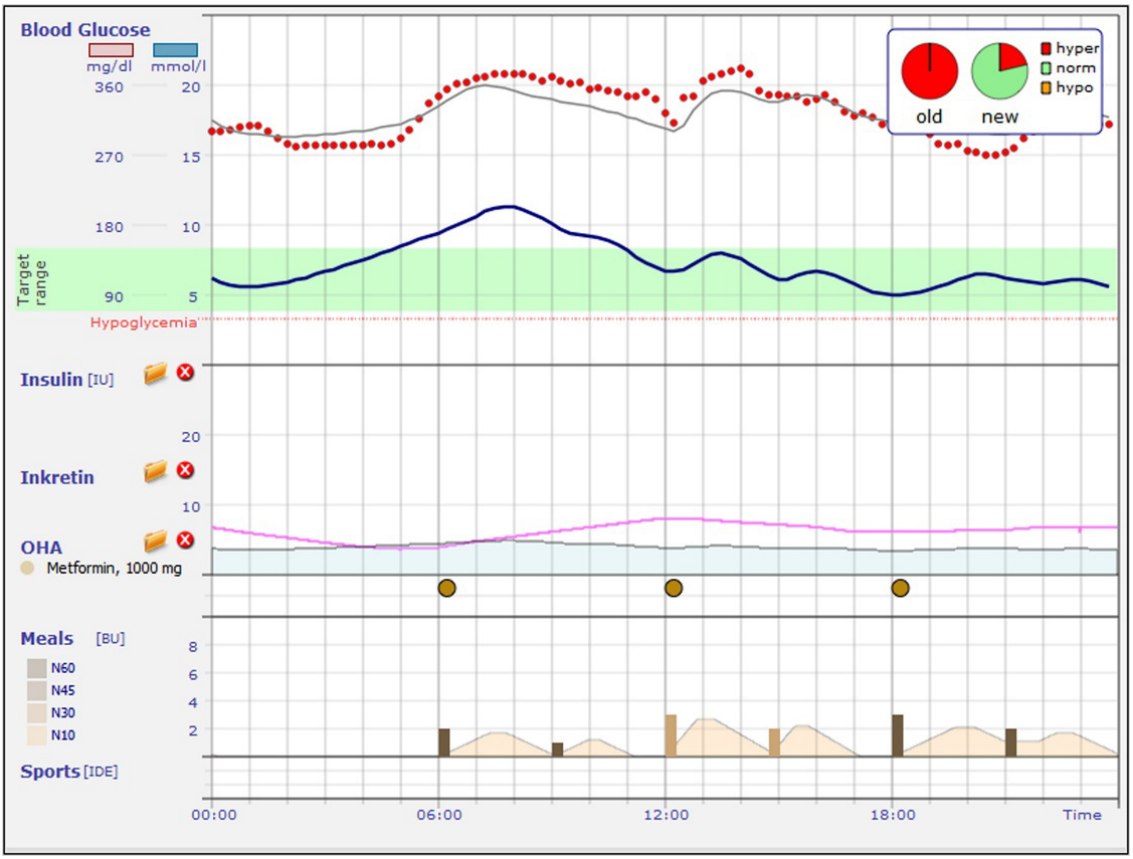
Weak-point Analysis

Weak points, such as hypo- or hyperglycemia can be read directly in the course of blood glucose changes from the «metabolic fingerprint» (Fig. 1). Using KADIS®; however, a more sophisticated analysis of potential weak points can be performed. A causal relationship of hyperglycemia/hypoglycemia with current therapy (action profiles, time regime), with food intake (carbohydrate units), with physical activity and special events can be established. For example, rising blood glucose levels in the morning, before the first meal, could be explained by a significantly reduced insulin sensitivity and/or insufficient administration of exogenous insulin. Such a specific analysis of weak points induced by such factors is another unique feature of KADIS®.

Decision support, testing therapy recommendations

Based on the in silico mapping and weak point analysis, modelbased simulations of the blood glucose curve could be performed in terms of a decision support. Therapy changes envisaged by the diabetes care giver will be entered into the KADIS® system, which then simulates immediately the corresponding glucose curves for the entered data. Thus, the usual time-consuming and tedious «trail and error» approach can be

circumvented. Strategies and therapeutic options recommended by guidelines of professional societies [1] can quickly and safely be tested in an interactive dialogue with the KADIS® system. This enables the best treatment modality for the individual patient to be determined in a very short time and, individual circumstances of the patient as well as the practical feasibility of treatment recommendations are taken into account. The results of this testing and individual treatment options are automatically documented in the KADIS® Report. This report is available either online or as download for use by the attending physician to adjust the patient´s glycemic therapy (Fig. 2).



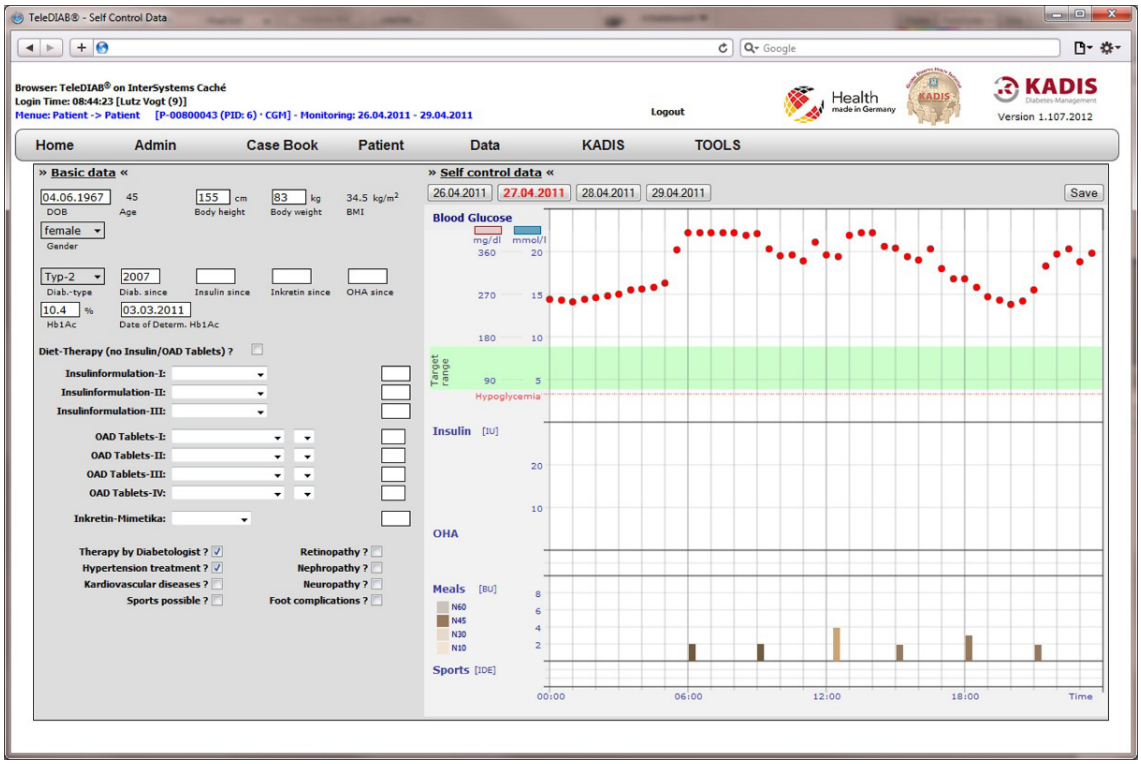
The test shown in the figure demonstrates significant improvement of the blood glucose curve and the glycemic control to be expected (blue curve vs the original gray and red represented blood sugar curve). The percentage of time, where the patient is hyperglycemic, drops down from 100 % to 18 % (see «quality eye» in the upper right corner).

Figure 2. In this example of inadequate glycemic control with diet, switching to an OHA monotherapy with metformin is recommended, according to the guidelines of the German Diabetes Association

TeleDIAB® — E-Health Platform

To give GP's as well as Diabetologists full access to the KADIS® functionality described above, the e-health platform «TeleDIAB®» was developed. TeleDIAB® is based on a high-performance secured database and is provided as a browser-based solution via the Internet. The Diabetes Service Center Karlsburg, as operator of the e-health platform, provides appropriate access accounts on the basis of licenses. In addition to the KADIS® simulation, the required routines for the acquisition of basic and self-control data are integrated in the TeleDIAB® platform (Fig. 3).

As soon as all necessary data has been collected, the KADIS® simulation component is ready to simulate glucose profiles. Depending on the design of the access to the TeleDIAB® platform, recommendations of diabetes experts can be provided and added to the start settings of the KADIS® system (KADIS® Identification). Up to 7 variants of tested therapy options can be stored simultaneously in TeleDIAB®. A brief note may be entered, explaining each simulation in order to allow better identification (Fig. 4).



The schedule representing the basic data is shown on the left, the right contains the Graphical User Interface (GUI) for recording blood glucose readings from glucose meter measurements and the corresponding self-control data, including therapy modalities and meals. In the case that CGM systems are used for recording of blood glucose, the glucose data will be uploaded to the TeleDIAB® platform.

Figure 3. Collecting data required for KADIS® application

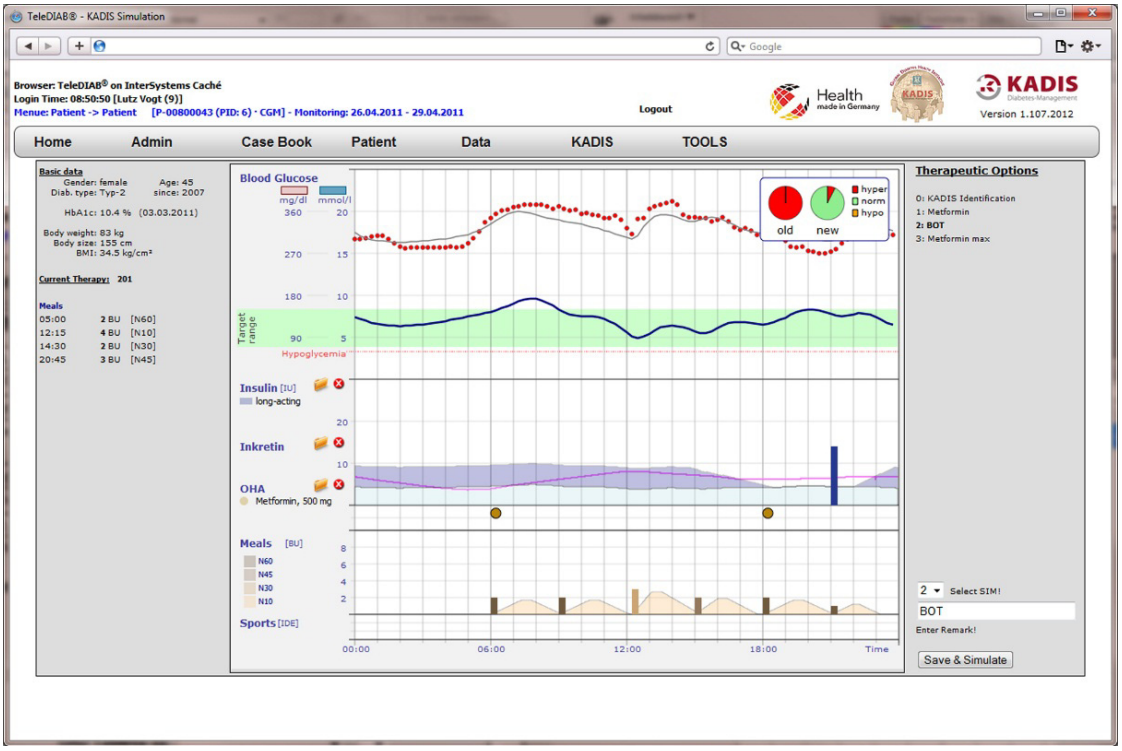


Figure 4. Screen of the KADIS® simulation which is embedded in the TeleDIAB® platform

The KADIS® Report summarizes the analysis of blood glucose monitoring, outcome of the KADIS® identification, and results of the simulation for recommendations of treatment options. The report is created automatically and can be read either online or downloaded as a PDF file (Fig. 5).

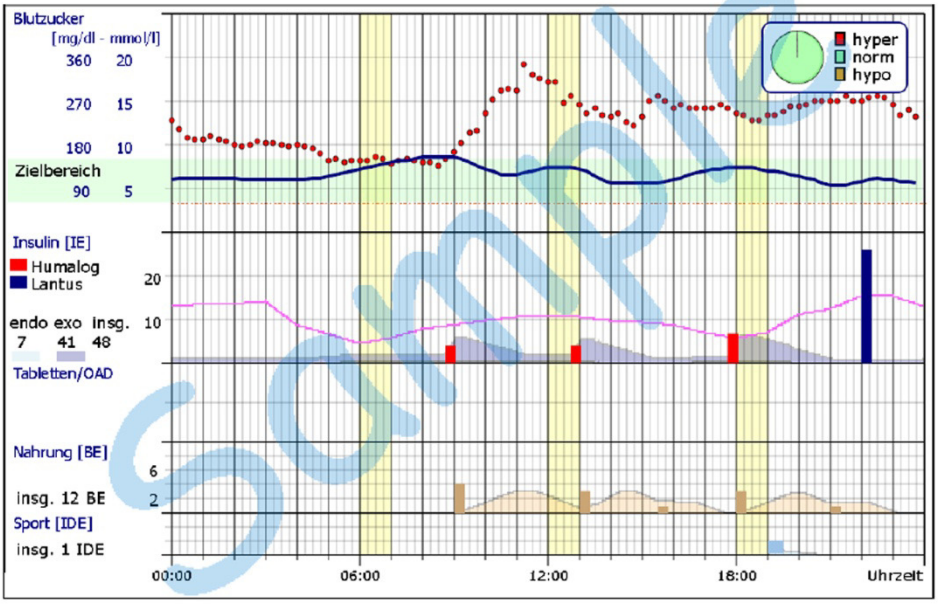
Proposal (2): Adjustment to an intensive insulin therapy (ICT)

Insulin
- adjustment to an ICT as indicated in the table

Food
- adjustment of eating habits as indicated in the table
- if not already taken place, the participation on a dietary training course is recommended

Sport
- an evening walk of approx. 30 mins. duration

Blood glucose self-monitoring
in the course of the Diabetiva Program, self-monitoring of blood glucose (SMBG) should be performed according to the guidelines of the German Diabetes Association (DDG) on an empty stomach and before the administration of each insulin bolus.



	breakfast	snack	lunch	snack	dinner	supper	total.
Meals (BU**)	**4** [09:00]	-	**3** [13:00]	**1** [15:30]	**3** [18:00]	**1** [21:00]	**12**
Insulin (IU)							
Humalog	**4** [08:45]	-	**4** [12:45]	-	**7** [17:45]	-	**15**
Lantus	-	-	-	-	-	**26** [22:00]	**26**
Incretin							
OHA							
Exercise - IDE***	-	-	-	-	**1** [19:00]	-	**1**

** BU - Bread Units (10-12 g CHO); *** IDE - calculated Insulin action equivalent for Exercise

Figure 5. KADIS® report

Benefit analysis

The value benefit analysis will be released, provided that, as in the past, only the attending physician is to decide the treatment modalities. The main differences to generally accepted treatment practice are:

1. Predictability of the therapy efficacy (e.g., avoidance of hypoglycemia, percentage of blood glucose values in target range) is achieved with KADIS® online immediately (within seconds), without loosing time

until the next patient´s physician consultation for evaluating therapy outcome. This significant time saving helps, especially those patients with complicated diabetes.

2. As patients can be involved via KADIS® Online in the process of optimization, the clear and visible identification of weak points in glycemic control on the KADIS® report, stimulates the interest in taking an active part in the improvement of metabolic control and makes the therapeutic approach better understandable.

3. By bringing all important data for glycemic control on a single print sheet together, the doctor in attendance is able, at a glance, to assess individual metabolic characteristics of the patient and the efficacy of the therapeutic approach in question.

4. The doctor can also consider alternative therapeutic approaches and is thus supported in its competence to find out the optimal treatment for his patient.

Finally, the benefit through improved metabolic control in gaining personal quality of life and reducing the risk of late diabetes complications, as has been shown by the large diabetes studies DCCT and UKPDS, cannot be overemphasized.

Conclusions

Diabetes mellitus is a global problem of epidemic dimension. The determinants of the increasing prevalence of diabetes are different in each country. For several reasons, there are also considerable differences in the level of diabetes care. Telemedicine is a tool to make knowledge and experience in the treatment of diabetes globally available. The use of the KADIS® system, the world's unique computer program for diabetes management, enables care givers to examine personalized treatment recommendations and quickly and safely to find out the best treatment strategy for optimizing a patient`s glycemic control. With the embedding of KADIS® functionality in the telemedicine information system TeleDIAB®, essential conditions are met for using KADIS®, particularly in regions of the world where there is a need for evidence-based personalized medicine.

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Диабетті басқаруда KADIS-жүйесімен бірге телемедицинаны қатар қолдануды негіздеу

Диабет ауруының өсуі емдеудің тиімді жолдарын іздеу қажеттілігін негіздейді. Авторлар жеке қолданыста KADIS жүйесін пайдалануды ұсынып отыр, бұл өз кезегінде кәсіби ассоциация талаптарына сай емдеудің сапасын айтарлықтай жақсартуға мүмкіндік береді. Жүйе уақыт тапшылығы және емделушілерге қатысты басқа да себептері болған жағдайда емдеудің ең жақсы дербес мүмкіндіктерін береді. Сонымен қатар енгізілген KADIS жүйесімен қатар телемедициналық TeleDIAB негізі диабетті емдеу кезінде қолда бар ғаламдық ақпаратты қолдану үшін қажет.

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Обоснование использования системы телемедицины в сочетании с KADIS-системой в управлении диабетом

Рост заболеваемости диабетом обосновывает необходимость поиска эффективной стратегии лечения. Авторами предложено использование системы KADIS® для индивидуального применения, что помогает значительно улучшить качество лечения в соответствии с требованиями профессиональных ассоциаций, расширяет возможности индивидуального лечения пациентов в условиях нехватки времени и других факторов, касающихся пациентов. Кроме того, телемедицинская основа TeleDIAB® и внедренная система KADIS® пригодны для использования имеющейся глобальной информации для лечения диабета.

UDC 613.2:612.359

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Vascular changes in pancreas in diabetes caused by abnormal metabolits of tryptophan aggravate developing of diabetes

Contrary to many models of experimental diabetes caused by chemicals, diabetes induced by endogene synthesis of xanturenic acid (XA), a metabolit of abnormal Tryptophan metabolism, approached to human diabetes. Meanwhile in these conditions of natural developing of diabetes not in result of artificial injection of diabetogenic substances, are not investigated yet state of blood vessels and blood circulation as in exocrine pancreas tissue as in pancreatic islets. Authors showed developing in experimental XA-diabetes of numerous destructive changes of blood vessels of a pancreas, fibrinoid changes of parenchyma of pancreas tissue, dystrophya and necrosis of exocrine and endocrine pancreas tissue; necrotic changes of endothelium of arteries. In pancreatic islets: necrosis of endothelium and cells in pericapillar. Authors conclude that described changes can result aggravation of developing of diabetes.

Key words: diabetes, pancreas, exocrine tissue, B-cells, vascular changes.

The main cause for mortality of patients with type 2 diabetes are cardiovascular complications [1, 2]. The leading role in development of these complications belongs to a hyperglycemia which is a cause of a number of pathological processes such as endothelial dysfunction, oxydative stress, changes of rheological properties of blood in macro- and microvessels [3]. It was reported that thickening of basal membranes is developed in capillaries as result of fixation on the endothelium of vessels of amorphous material consisting mainly of mucopolysaccharides [4]. It is known that microcirculation in diabetes accompanied by aggregation of blood cells and damage an endothelium. Sclerosis, inflammation and destruction of vessels result developing of heavy blood circulation [5].

Research objective: to study state of histostructure of blood and of stroma of tissue of pancreas in experimental Xanthurenic acid induced experimental diabetes.

Materials and methods

Diabetes in animals caused by containing of animals on diet by Y.Kotake [6] stimulated endogene synthesis of 4,8-dihydroxyquinolin-2-carboxylic acid (Xanthurenic acid, XA) which possess diabetogenic properties due to direct selective destruction of B-cells as to binding and inactivation of insulin [6]. 72 white rats Vistar 160–240 g. body wheight were used. Animals were distributed for 5 groups. Rats of Groups 1, 2 and 3 were contained 60, 90 and 120 days respectively on a diet stimulated endogene synthesis of XA. The diet components included starch, butter, sugar, casein, yeast and salt additives. Group 4 (diet+vit. B6), investigation of blood Glucose concentration and of Xanturenyria excluding histological and histochemical analysis of pancreas tissue: animals were treated within period of containing on diet by injections of water solution of vit. B6 8,7 mg/kg per day. Group 5 (control 2) — intact animals. Blood Glucose control-weekly by Glucose oxydase method. Concentration of Xanturenic acid in urine [7] was measured monthly and body weigh in the beginning and at the end of experience.

Histology. Samples of pancreas tissue fixed in Bouin liquid, carried out in alcohols 70º, 80º,90º and 100º, filled in paraffin. Leica 2125 rotation microtome used for preparing sections 4–5 mcm. For survey microscopy of tissue of a pancreas staining technology was applied using hemalaoun of Mayer and eosin [8] as hemathein of Mayer [9].

Histochemical methods: Method by Gomori, a differential staining of β-and α-cells by Aldehyde fucshine and Helmi's mix [10, 11]. Deposited form of insulin [12] revealed as violet granules in cytoplasm of B-cells. Kikui Y. and coll. method [13] using reagent Victoria 4R with floxyn, phosphorum-volfram acid and the light green was used for differential staining of β- and α-cells. Immunohistochemical method [14] staining of insulin by kits from DAKO (Denmark) was used with photometrical measuring intensity of staining of B-cells [15]. Parameter K was calculated as AB1/AB2. AB1 — light absorbtion of B-cells; AB2 — light absorbtion of exocrine tissue.

Results

Blood Glucose level. 60 days containing of rats on diet: increasing of blood Glucose level for 1,5–1,8 times in majority of number of animals excluding 6 rats have kept normal value. On average level of a glycemia is 6,91 ± 0,36 mmol/l ($p \leq 0,05$) in compared with initial 4,20 ± 0,11 mmol/l (Fig. 1). On 90th day containing on diet blood Glucose level is increased for 1,9 times comparatively with initial ($p \leq 0,001$). In some animals increasing for 2,5–3 times was observed. 4 rats have not changes of blood Glucose level. We observed till 90th day decreasing of body weight of experimental animals on the average from 216,84 ± 4,07 g to 183,20 ± 4,06 g ($p \leq 0,001$).

At 120th day of experience concentration of blood Glucose level was increased for 2,8–3 times on average, until 11,81 ± 0,56 mmol/l ($p \leq 0,001$) comparatively with initial 4,11 ± 0,19 mmol/l by 2,8–3 times; $p \leq 0,001$. The body weight was decreased for 23–25 %; ($p \leq 0,001$) comparatively with initial.

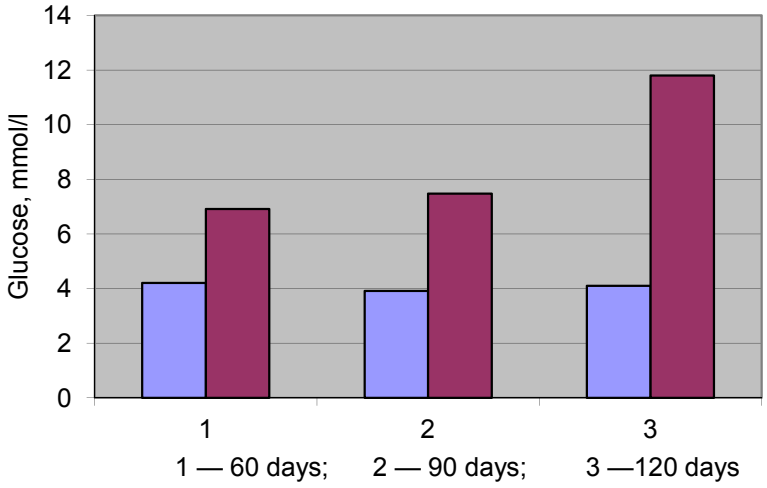
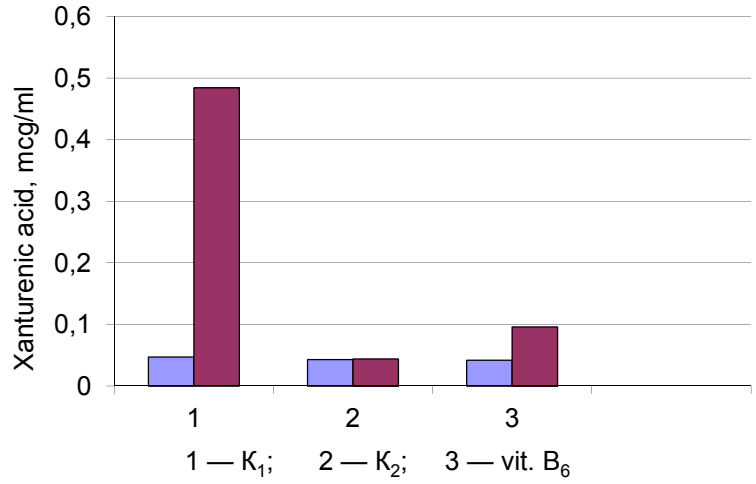


Figure 1. Blood Glucose concentration in animals contained on diet



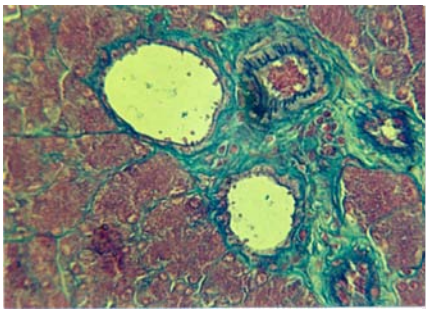
K_1 — animals contained on diet; $p \leq 0,001$; K_2 — intact animals (control);
blue column — before diet; red column — 120 days on diet

Figure 2. Concentration of Xanturenic acid in the urine of rats contained on diet and diet+vit. B6

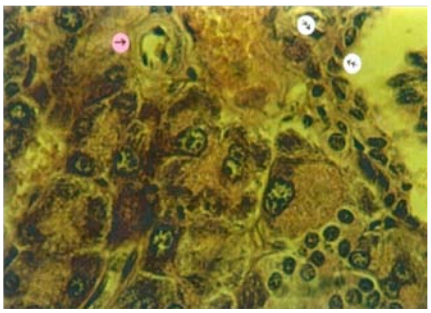
The analysis of level of xanturenuria of rats contained on a diet for 120 days showed reliable increase in compared with control for 9–10 time ($p < 0,001$) (Fig. 2). Thus, the maintenance of animals on diet accompanied by development of marked hyperglycemia reaching the maximum till 120 day.

Morphological researches

30th days containing on diet result developing of: disturbances of blood circulation; fibrinoid changes of intraglobular arteries of exocrine tissue; necrosis of veins, destroying of vessel's wall, hemostasis, lysis and infiltration of eritrocytes in tissue (Fig. 3.1); distribution of fibrinoid processes to parenchyma.



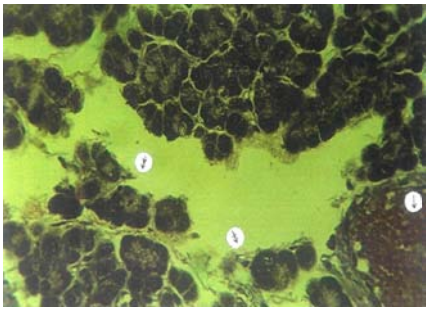
3.1



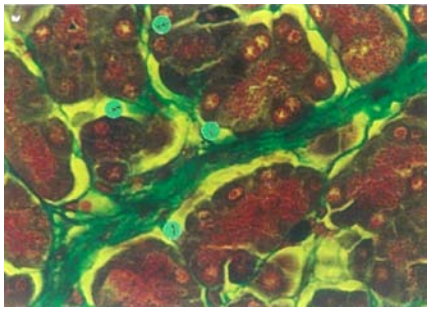
3.2

3.1 — Pancreas tissue. Stasis and hemolysis in interglobular arteries (→). Destruction and necrosis of connecting tissue of blood vessels (→→). 30 days on diet. Staining by Victoria 4R; ×280;

3.2 — Pancreas tissue. Fibrinoid changes of arteries (→). Necrosis of veins (→→) and distrophya of ductus (→→→). 60 days on diet. Staining by Hemalaoune and eosin; ×280;



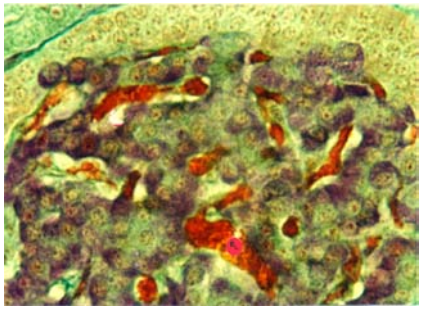
3.3



3.4

3.3 — Pancreas tissue. Stasis and hemolysis in interglobular vein (→). Fibrinoid chanhes, destruction of acinuses and atrophya of exocrine parenchyma (→→). 60 days on diet. Staining by Hemalaoune and eosin; ×280;

3.4 — Exocrine tissue of pancreas. Gwoving of collagen fibers in globules (→). Hypostasis in inter acinuses spaces and collagen fibers around acinuses (→→). 60 on diet. Staining by Victoria 4R; ×900;



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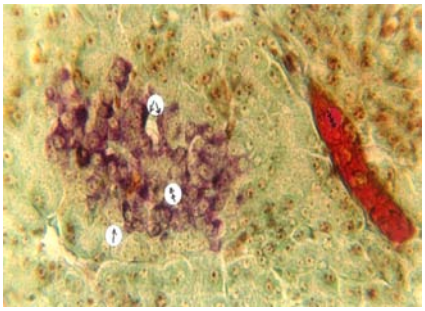
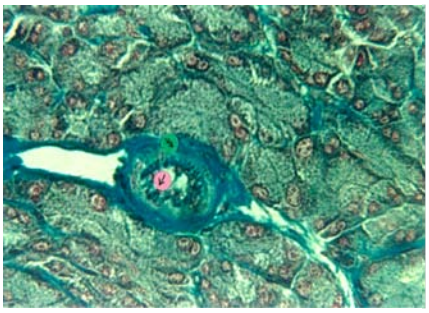


3.6

3.5 — Pancreatic islet. Marked hyperemia and lysis of eritrocytes in capillaries (→). Degranulation and destruction of B-cells (→→). 60 days on diet. Staining by Aldenyde Fucshine and Helmi; ×700;

3.6 — Pancreatic islet. Degranulation of B-cells (→). Distrophya and necrosis of B-cells (→→). Thrombosis of veins (→→→). 100days on diet. Staining by Aldenyde Fucshine and Helmi; ×280;



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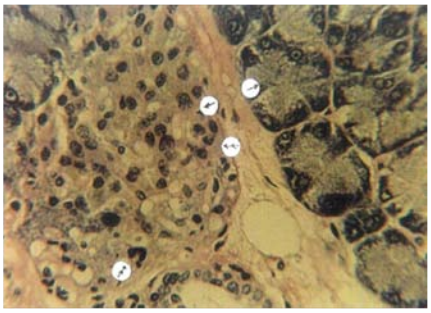

3.8

3.7 — Pancreatic islet. Destruction of internal capsule (→) and hyalinosis on middle and external capsules of arteries (→→). 120 days on diet. Staining by Victoria 4R; ×280;

3.8 — Pancreas tissue. Dissociation of acinuses (→). Destruction of islets, necrosis of B-cells (→→). 120 days on diet. Staining by Hemalaoune and eosin; ×280

Figure 3. State of pancreas tissue and vascular changes in exocrine and endocrine tissues

60th days containing on diet accompanied by: developing of destructive changes in parenchyma (Fig. 3.2); hyperemia and destruction of capillaries; growing of collagen fibers accompanied by necrosis of adventicium of vessels; necrosis of veins with infiltration of eritrocytes in tissue; fibrinoid changes in parenchyma of tissue, fat infiltration and fat necrosis of the gland's cells; intraglobular fibrillogenesis; infiltration of parenchyma by collagen fibers (Fig. 3.4); hemostasis and lysis of eritrocytes in capillaries of islets (Fig. 3.5). Arterioles: fibrinoid changes, thickening of walls. Venules: necrosis, destruction, infiltration of eritrocytes in tissue. Fibrinoid changes of stroma of pancreas, growing of fat tissue in interseptal spaces;

pressing of acinuces by fat tissue and forming of necrosis centers in gland's tissue; developing of intraglobular fibrillogenesis that accompanied by thickening of intersticium of pancreas tissue. Infiltration of parenchyma by collagen fibers, dissociation of parenchyma for little gland segments consisting of a few acinuses (Fig. 3.4).

90 days containing on diet result: hemorrhagic necrosis of exocrine tissue, sclerosis of wall of arteries; growth of fibrous structures; sclerosis of capillaries. Hyperemia in veins and in capillaries; fibrinoid changes of arterioles; thickening of basal membrane of endothelium; stagnant hyperemia in vessels of venous collector; alteration of arterial endothelium, destruction of endothelial layer in arteries and in interglobular veins, proliferation of facile muscle cells; destruction and distrophya of walls of vessels, infiltration in tissue of components of blood; sclerosis and inflammation, concentration of leucocytes in the gleam of vessels and infiltration of parenchyma of tis- sue; growing of fat tissue in islets with degranulation, distrophya and necrosis of B-cells, stasis and hemolysis in capillaries of islets (Fig. 3.6); infiltration of lymphocytes and leucocytes outside of vessels, homogenization of blood cells in vessels; thrombosis of veins and capillaries; developing of hyalinosis in arteries (Fig. 3.7); destruct- tion, distrophya and incapsulation of acinuses; agglomeration of fibroblasts, lymphocytes and collagen fibers nearest destroyed acinuses. Near vascular bunches, islands and of acinuses; the wide cavities, filled by homogeneous consistence liquid near vascular bunches as near island (Fig. 3.8).

100–120 days containing on diet. Inflammation of arteries and veins, infiltration of leukocytes outside of arteries and veins parenchyma; necrosis of parenchyma, growing of fat tissue accompanied by intraglobular lipomathosis; islets: marked hyperemia, stasis in capillaries, degranulation and necrosis of B-cells (Fig. 3.6); marked hyperemia in veins in combination with infiltration of lymphocytes into the wall of vessels; gomogenisation of collagen fibers in adventicium of arteries, concentration of fibroblasts between collagen fibers.

Thus, disturbances of metabolism in animals contained on diabetogenic diet result marked destructive changes in arteries, veins and capillaries as in islets as in exocrine tissue of pancreas that accompanied by destruction of walls of vessels and fibrinoid changes of stroma. Noted above changes accompanied by disturbances of circulation of blood in vessels and by hemostasis which is estimated as sign of acute pathological process [3.3].

Stasis is a frequent effect in disturbances of cardiovascular system and of blood circulation caused by external causes [16]. J.Andersen and coll. [17] supposed that accumulation in their wall of fibronectin, of type 4 collagen, hyaluronic acid and calcium result damage of blood vessels. Dysfunction an endotelium accompanied by angiospasm, thrombosis and tendence for developing of atherosclerosis [18, 19]. Insulin resistance is estimated as one of cause of destruction of blood vessels [20]. On 30^{th} day containing on diet we observed accumulation of fats in wall of interglobular arteries and developing of lipomathosis in globules. Diabetes accompanied by marked forms of this processes as by fibrosis and lipomathosis of intraglobular spaces [21].

Dysfuction of endothelium is shown by angiospasm, tendencies for formation of thrombs and developing of atherosclerosis [19].

Thus, at first week of experience disorders of blood circulation and destructive changes of vessels were developed and accompanied by fibrinoid changes, fibrosis and lipomathosis in intraglobular spaces. Stagnation and long time prolonged hemostasis result destruction of vessel's wall and exit of eritrocytes in exocrine tissue. Formation of blood clots is a symptom of chronic process. Vascular changes, result developing of necrosis in acinuses and of atrophya of exocrine tissue of pancreatic islets. Proliferation of epithelial tissue and periductal sclerosis of gland's ductus as hemorrhagic sclerosis of exocrine tissue cells with sclerosis of capillaries walls are estimated as structure symptoms diabetes mellitus [16, 21].

As it was observed in pancreas sections of rats contained on a diet from the 30th till 120th days, sclerotic changes underwent some stages of development: from plasmatic infiltration and fibrinoid changes of a wall of vessels to hyalinosis. Hyalinosis of small arteries and the capillaries, developing as result of plasmatic infiltration is widespread at diabetes and most expressed in a brain, a kidney, in retina and islets.

Thus, analysis of results of research of series of experience showed accruing suppression of function of the B-cells, accompanied by degenerative changes and decreasing of insulin content in cytoplasm of B-cells for 76 % caused by XA.

Developed multiple wascular changes in blood vessels as in pancreatic islets as in exocrine tissue result developing of fibrinoid changes, of sclerosis of stroma including hyalinosis of arteries and sclerosis of capillaries and veins. These changes aggravate developed diabetes in spite of the fact that are not its direct cause.

Acknowlegement

Authors are thankful to Prof. G.G.Meyramov, head of Diabetes Research Centre, Karaganda, for supporting of this investigation.

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Триптофанның диабетогенді метаболиттерінің ағзаға әсерінен қан айналымының бұзылуы және оның тәжірибелік қант диабетінің өтуін қиындатуы

Авторлар эксперименталды ксантурендік диабет кезінде ұйқыбез қантамырларының деструкциясы, оның паренхиматоздық тінінің фибриноидті өзгерістеріне себеп болғанын байқаған. Бұл өзгерістермен экзо- және эндокриндік бөлімдердің дистрофиясы мен некрозы ере жүрген. Ұйқыбез аралшықтарында капиллярлар эндотелийінің деструкциясы және капиллярлар маңындағы В-жасушалардың некроздық өзгерістері айқындалған. Авторлар ұйқыбездің инсулярлы аппаратының жетіспеушілігі, диабетогендік емдом әсерінен көмірсулар мен майлардың алмасуының бұзылыстарына, сондай-ақ қан

айналымның бұзылыстарына тәуелді екенін болжайды. Сондай-ақ зат алмасу процестердің бұзылу нәтижесінде пайда болған қантамырлардың өзгерістері В-жасушалардың жаңадан деструктивтік өзгерістеріне себеп болып диабет барысын күшейте түспек.

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Нарушения кровообращения при воздействии на организм диабетогенных метаболитов триптофана, усугубляющие течение экспериментального сахарного диабета

При экспериментальном ксантуреновом диабете, который по характеру развития и течения приближается к диабету у человека, авторы исследовали развивающиеся деструктивные изменения кровеносных сосудов поджелудочной железы. Они сопровождаются фибриноидными изменениями паренхиматозной ткани, те, в свою очередь, дистрофией и некрозом экзо- и эндокринной ткани. В панкреатических островках выявлены деструкция эндотелия капилляров и некротические изменения перикапиллярных В-клеток. Авторы полагают, что сосудистые изменения, не являясь прямой причиной диабета, могут значительно утяжелять его течение.

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UDC 613.2:612.359:616–012

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Victoria-4 histochemical method staining of insulin

Results using of histochemical Victoria-4 method staining of insulin in pancreatic B-cells are presented by authors comparatively with results of using other histochemical methods: immunofluorescent, immunohistochemical, pseudoisocyanine and aldehyde fucshine. By authors it is shown that this method being specific concerning insulin revealing in B-cells, is not high precise for quantitative measuring of insulin content in cells because not only B-cells but blood vessels and connecting tissue of islets are painted in dark colour registered by a photometer as well as blue colour of B-cells. Using of Victoria-4 method is possible for stain of various histostructures of islets that gives possibility to estimate the state of histostructure of islets not only insulin content.

Key words: insulin contents, immunohistochemical, immunofluorescent, pseudoisocyanine and aldehyde fuchsine methods, B-cells.

The are a few histochemical and immunohistochemical methods staining of insulin in pancreastic B-cells: immunofluorescent (IF), immunohistochemical (IG), aldehydefucshine (AF) [1], Victoria-4 methods [2–8] and diethylpseudoisocyanine (PS) [7, 9].

Advantage of fluorescent IF and PS methods determined by more high sensivity of fluorescent stainings: minimal concentration of substances as 10^{-7}–10^{-8} maybe revealed using these methods. Meanwhile histological sections past staining maybe used for microscopy within short time as 0,5–1 h. Using fluorescent methods not possible to estimate state of histostructure of pancreatic islets. Immunohisto-chemical method as IF is more high specific for staining of insulin comparatively with all other methuds and now widely are used in the world.

Aim of work: to compare results of insulin staining in sections of rat's and rabit's pancreas tissue using staining by Victoria-4 method [V4] comparatively with immunohistochemical [IG], aldehydefucshine [AF] and pseudoisocyanine [PS] technologies.

Materials and methods

Pancreas tissue of 12 rats Vistar and 2 rabbits were used. Fixation in Bouin 24 h. Paraffin sections 4 mcm were used. Staining methods for insulin revealing in B-cells: Victoria-4 [V4], immunohistochemical [IG], aldehydefucshine [AF], pseudoisocyanine [PS] and staining by Dithizon [DZ].

The reagent Victoria blue 4R, diphenylnaphtylmethane derivative, mol. wt. 520 ($C_{34}H_{34}N_3Cl$), color Index 42563, «MERCK» (Germany) was used for staining of insulin in B-cells [3]. We have used fixation of pancreas tissue in Bouin, permanganate oxidation of sections prior staining using V4 as a 0.05 % acid alcoholic solution [4, 5].

Mixture for oxidation: 0,3 % aqueous potassium permanganate — 50 ml, 0,3 % Sulpuhric acid — 50 ml. Victoria Blue 4R main solution: 96º alcohol — 100 ml, Victoria Blue 4R — 1 g. Victoria Blue 4R staining solution: Victoria Blue 4R main solution — 25 ml, 96º alcohol — 100 ml, Glycerin — 300 ml, 1 % acetic acid glacial — 25 ml [2].

Maximum light absorbtion of V4 solutions depended on the concentration of the reagent. It is suggested that V4 forms molecular aggregates with maximum of absorbtion at 593 nm which could represent monometric dye particles and minimum absorbtion at 540 nm dimeric dye molecules [6]. The nature of the fixation in Bouin on staining of B-cells is not clear. Perhaps this is dependent on the formation of waterinsoluble insulin picrate [4]. Without Bouin fixation crystalline bovine insulin stains by V4 only after oxidation [4, 5].

Insulin content in B-cells was estimated by measuring of absorbance by photometry of B-cells located in central part of islets. Parameter K was calculated. For Pseudoisocyanine and Immunofluorescent methods:

K=IN1/IN2; IN1-intensity of fluorescence of B-cells, IN2-intensity of fluorescence of exocrine tissue. For calculation direct dependence was used: increasing of amount of insulin in cells accompanied by higher intensity of fluorescence. For Immunohistochemical, Aldehydefucshine and Victoria-4 methods: K2=AB1/AB2; AB1-absorbance of light by exocrine tissue, AB2-absorbance of light by B-cells. For calculation inverse relationship is used: more intensive staining of cells for insulin result reduction of light amount accepted by photometer. By each method 20–25 pairs measurements endocrine/exocrine tissue was used. The average values of K1 and K2 parameters for exocrine tissue was accepted for 1.00.

Results

Insulin in pancreatic B-cells was identified using of all histochemical methods (Table 1, Fig. 1).

Table 1

Intensity staining of B-cells and of exocrine tissue using various methods and insulin content (parameter K) in pancreatic B-cells

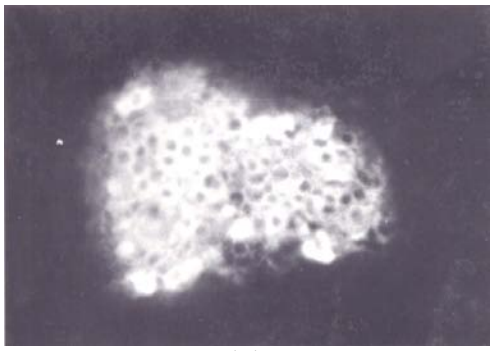
№	Pancreas tissue	Staining technologies				
		AF	IG	PS	V4	IF
1	Rats: B-cells; exocrine tissue; results staining of histostructure of islets	1.94±0,05 1.00±0,02 high	1.79±0,04 1.00±0,03 insufficient	2.02±0,11 1.00±0,08 insufficient	1.83±0,08 1.00±0,04 high	2.08±0,12 1.00±0,02 insufficient
2	Rabbits: B-cells; exocrine tissue	1.98±0,04 1.00±0,04	– –	2.07±0,12 1.00±0,07	1.96±0,05 1.00±0,03	– –
3	Rats; diabetes: B-cells; exocrine tissue	 1.07±0,07 1.00±0,06	 1.04±0,04 1.00±0,03	 1.02±0,03 1.00±0,07	 1.16±0,09 1.00±0,05	 1.09±0,03 1.00±0,04
4	Density staining of exocr. tissue V4/AF, V4/IG	1.14±0,04	1.07±0,03	–	–	–

Table 2

Practical characteristics methods of insulin staining in B-cells

№	Characteristics of methods	Staining technologies				
		AF	IG	PS	V4	IF
1	Specifity for insulin staining	Specifity for staining a few hormones; for B-cells specific for insulin	Absolute specifity for insulin staining in any tissues	High specifity for staining of A-chain of insulin	High specifity for staining of A-chain of insulin	Absolute specifity for insulin staining
2	Staining of histostructure of is lets and of exocrine tissue	Staining in detail; suitable for detail analysis of state of histostructure	Staining not in detail; not suitable for detail analysis of state of histostructure	Not staining of histostrusture; not suitable for analysis of state of histostructure	Staining in de tail; suit able for detail anaysis of state of by histostruccture	Not staining of histostrusture; not suitable for analysis of state of histost ructure
3	Reagents	Reagents for staining are produced by many firms	Kits for insulin staining are produced by many firms; method is widely used as uni versal for staining many hormones	Diethylpseudoisocyanine for staining is produced only by SERVA	V4 is produced by a few firms	Anticorps are produced by a few firms as by research laboratories
4	Practical using	Long time stored histological paraffin sections of tissue	Long time stored histological slides	Slides stored a short time (0,5–1 h)	Long time stored histologycal slides	Slides stored a short time (0,5–1 h)

Fluorescent methods showed more high deviations of value of parameter K that can be explained by more wide fluctuation intensity of fluorescence in various islets. The highest value of parameter K was obtained at measurement intensity of staining in B-cells of complex zinc-insulin by Dithizon slides (Fig. 1.7).



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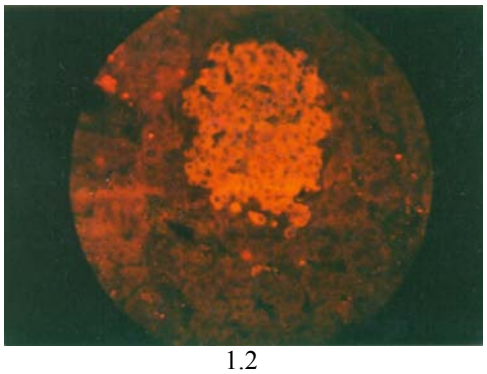

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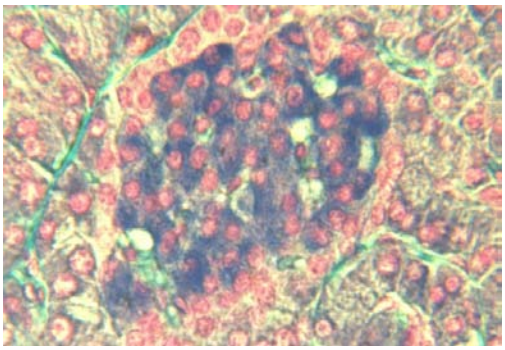

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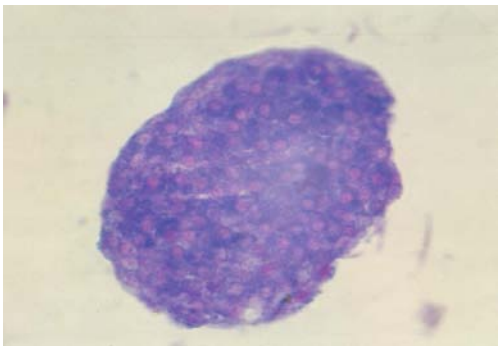

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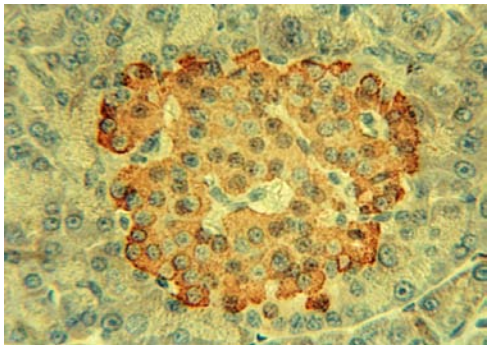

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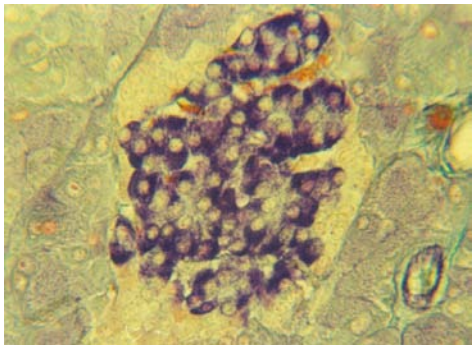

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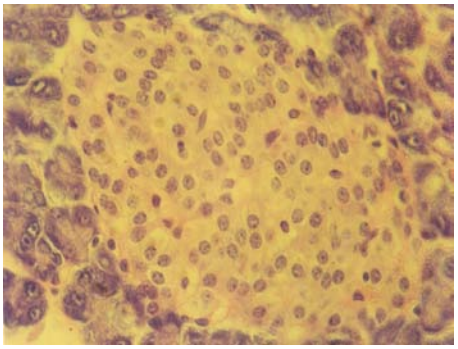
1.7



1.8

1.1 Intact pancreatic islet. Insulin. Intensive fluorescence. Immunofluorescent staining method; 5×40;
1.2 Intact pancreatic islet. Insulin. Red fluorescence of A-chair of molecule of insulin. Diethylpseudoisocyanine staining method; 3×40;
1.3 Intact pancreatic islet. Insulin. Dark blue color of insulin.Victoria-4 staining method; 7×40;
1.4 Isolated intact pancreatic islet. Dark blue color of insulin.Victoria-4 staining method; 7×40;
1.5 Intact pancreatic islet. Insulin. Brown color of insulin Immunohistochemical staining method; 7×40;
1.6 Intact pancreatic islet. Insulin. Dark violet color of hormone. Aldehyde fucshin staining method; 7×40;
1.7 Intact rabbit's pancreatic islet. Insulin-Zn^{+2} red coloring complex. Staining by Dithizon; 7×40;
1.8 Intact pancreatic islet. Insulin. Hematoxylin and eosin staining method; 7×40.

Figure 1

High fluctuations of absorption in sections painted by Victoria-4 comparatively with AF and IG methods are obviously caused besides the following reasons: 1) evidently more intensive staining of other structures of pancreatic islets as wall of blood vessels, nucleus, connecting tissue and of exocrine tissue that result more intensive absorbtion of comparatively with IG and AF methods; 2) density of staining of exocrine tissue is more high too comparatively with AF and IG methods and approximately same, as well as using staining by Hematoxyline and Eosin (Table 1; Fig. 1.3–1.6, 1.8).

It was reported that a possible explanation for the ability of B-granules to react with V4 past oxidation only determined by structure of insulin. Oxidation result dissociation of disulfide bonds between two chains of molecule of insulin. It is suggested that reactivity of B-cells with V4 is dependent on the staining of oxidised A-chain of insulin. The sulphonic acid groups in the A-chain give conditions favourrable for staining by V4 [10].

Analysis of characteristics of methods of insulin staining (Table 2) showed that V4 and AF methods are more preferable for estimate as of insulin content as state of histostrusture of pancreatic islets and exocrine tissue. Chemicals specific of V4 method is more high comparatively with AF method.

Immunofluorescent staining method [IF]. We have obtained same results of staining by IF as using of sections of pancreas tissue (Fig. 1.1–1.3). IF is high specific method for revealing of Insulin in B-cells. Decreasing of Insulin content in B-cells of islets past action direct action of Streptosotozin was evidently demonstrated by this method (Fig. 1.3).

Diethylpseudoisocyanine chloride fluorescent method [PS], a high specific for revealing A-chair of molecule of Insulin, showed same result comparatively using of sections of Pancreas tissue (Fig. 1.4–1.6). Time for staining of sections in 0,4 % solution of Diethylpseudoisocyanine was reduced from 20 min till 15 min as was reduced time for washing of sections past staining procedures. This method showed marked decreasing of Insulin content in damaged B-cells (Fig. 1.5, 1.6) in com pared with intacts.

Aldehydefucshine method showed analogical results. A significant differences are revealed of state of histostructure as of Insulin content in damaged isolated islets comparatively with intacts (Fig. 1.8). Aldehydefucshine method [AF] contrary to IF and PS is not belong to high specific because colours other hormones too. But for pancreatic B-cells not contained other hormones AF is specific for Insulin.

Discussion

Analysis of results showed that using of histological and histochemical methods for staining of sections of isolated pancreatic islets have similar or equal to similar results obtained in pancreas tissue past staining by same methods. Fluorescent histochemical methods as Immunofluorescent reaction for Insulin as method using of Diethylpseudoisocyanine are more sensitive and identify the very low concentrations of investigated substances as 10^{-7}–10^{-8}, that has been confirmed by our results. Meanwhile both these methods have a common fault: histological sections past completing of staining procedures are not permanent and must be investigated within short time. Both methods are belong to high specific for staining of Insulin or of A-chair of molecule of Insulin. These methods are more precise for measuring intensity of insulin staining in B-cells because no other structure of islets are stained.

More suitable for practical using is Aldehydefucshine technic. Histological sections of pancreas tissue as of isolated islets stained by this method are permanent and can be stored for a long time. Aldehydfucshine method is not belong to high specific for Insulin staining. It is known that some pituitary hormones can also be stained by Aldehydefucshine method. Meanwhile for pancreatic islet's B-cells this method you can be measured as specific for insulin because the other hormones in B-cells are not synthesized. Method Victoria 4R is high specific for Insulin and as Aldehydefucshine technic gives an opportunity to obtain permanent histological sections. Quantitative estimation of insulin content in stained sections is based on measuring of absorbed by B-cells of light. However, both of these methods are belong to histological methods too and result staining not only of Insulin, but also other structures of B-cells which absorbed light as Insulin. Therefore, results of estimation of Insulin content in the B-cells by measuring of absorbance is not so precise as using fluorescent histochemical methods for Insulin staining.

We used significantly reduced time for fixation of Islets in Bouin from 24 h for pieces of pancreas tissue up to 15–30 min for isolated Islets. Time for staining of sections of isolated islets by Diethylpseudoisocyanine was reduced to 15 min comparatively with 20 min for sections of pancreas tissue.

Conclusions

1. V4 and AF methods evidently are more suitable for to estimate state of histostructure of pancreatic islets and of exocrine tissue, not only the content of insulin in B-cells.

2. V4 method is more specific for staining of insulin comparatively with AF method.

3. V4 method is more precise for quantitative estimate of the insulin content in B-cells comparatively with Pseudoisocyanine and Immunofluorescent methods and less precise in compared with Immunohistochemical and Aldehyde-fucshine methods.

Acknowlegement

Authors are thankful to Prof. G.G.Meyramov, Prof F.Wohlrab (Leipzig, Germany), Dr. O.E.Grundemann (“FERAK”, West Berlin), to Mr. K.Niethammer and Mr. W.Manneck (“MERCK”, Darmstadt, Germany) for free substance Dimethylnaphtylmethan (Victoria Blue, c.i.42563).

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Инсулин бояғышының «Виктория-4» гистохимиялық әдісі

Мақалада «Victoria-4» гистохимиялық әдіс (мәліметтер) деректері иммуннофлюоресцентті, иммунногистохимиялық, жаңа псевдоизоцианил және альдегидфуксинды басқа гистохимиялық әдістер қолдану нәтижелерімен салыстырмалы тұрғыда берілген. Авторлардың көрсетуімен бұл әдіс жоғарыдағы әдістердің болу тығыздығының сандық бағасының дәлдігін беруінде жеткіліксіз болғанымен, инсулинге қатысында спецификалық артықшылығымен ерекшеленеді. Бұл әдістің құндылығы панкреатит аралшарының әр түрлі құрылымдарын сапалы бояу негізінде В-жасушалардағы инсулинді сапалы анықтаумен қатар, аралшалардың гистоқұрылымдық күйін бағалау мүмкіндігін береді.

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«Виктория-4» гистохимический метод окраски инсулина

В работе приведены данные использования гистохимического метода Victoria-4 в сравнении с результатами применения других гистохимических методов: иммунофлюоресцентного, иммуногистохимического, псевдоизоциани нового и альдегидфуксинового. Авторами показано, что данный метод, являясь специфичным в отношении инсулина, уступает по точности при количественной оценке плотности окраски перечисленным выше методам, но выигрывает в стоимостном отношении. Его преимуществом также является достаточно качественная окраска различных структур панкреатических островков, что дает возможность точнее оценивать состояние гистоструктуры островков, помимо определения содержания в В-клетках депонированного инсулина.

MEDICINE

UDC 613.2:612.362.616–002

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Statistical analysis of diabetes in land Karaganda in 2008–2012

On the basis of statistical analysis of dynamics indicators of incidence, morbidity by diabetes among population of the Land Karaganda from 2008 for 2012 was investigated. The average annual indicator of incidence of diabetes in area is 187,2 ± 19,4 on 100 000 population, morbidity — 1500,7 ± 169,2 on 100 000 population. There are tendency for increasing numbers of incidences of diabetes as of 1 and 2 types. Indicators of diabetes incidence and morbidity were authentically more higher among urban population comparatively with country people especially is more high in compared with in compared with territory of absolute prevalence of Kazakh population.

Key words: 1st and 2nd type of diabetes, indicators of diabetes, incidence and morbidity.

Relevance of diabetes is caused by metabolic changes, a high risk of development of atherosclerosis, vascular disorders, mental disorders, a frequent invalidism of patients, including children, teenagers, decrease in quality of life [1–6]. The Diabetes (D) is included into group of the diseases, including also cardiovascular diseases, malignant new growths, a chronic obstructive illness of lungs which is at the bottom of about 80 % of all cases of death from noninfectious diseases. According to WHO forecasts, in 2030 diabetes becomes the seventh cause of death on the importance [6, 7].

Actuality is determined by possibility of prevention developing of type 2 diabetes, making 80 % of all patients with diabetes, in patients with prediabetes. In Kazakhstan is organized some activity for early identification and prevention of development of diabetes: routine medical examinations of target groups of the population for the purpose of identification of socially significant diseases, including diabetes, at early stages, are carried out within the guaranteed volume of free medical care (order of Minister of Health of Kazakhstan dated November 10, 2009 No. 685 «About the approval of Rules of carrying out routine medical examinations of target groups of the population»). Considering that consequences of diabetes suffer an irreplaceable loss to the state, for the purpose of decrease in an incidence by diabetes, reduction of number of complications, increases in average life expectancy of patients of diabetes in Kazakhstan is allocated in the category of socially significant diseases demanding system decisions and measures of the state reaction. Thus, studying in developing of indicators of an epidemiological situation not only gives valuable information on a condition of a problem, but also is a basis for rational planning of the actions directed on improvement of results and quality of treatment of diabetes.

Now the territorial system of Kazakhstan include 14 Lands among which the Land Karaganda is the largest in KZ with more than 8 % of all population of the Kazakhstan.

Aim of work: to study main statistics data regarding diabetes in the Karaganda region, studying of incidence and morbidity of diabetes among the population of the Karaganda region from 2008 until 2012 were a research objective.

Material and research methods

Data of annual forms No.12 in 5 years (2008–2012) containing data of number of patients with for the first time established diagnosis of diabetes and patients, consisting on the account depending on diabetes

type were a material for research. Forms of account included the patients living in 9 cities and 9 rural regions of area. Data on average annual population are submitted by Department of statistics of the Karaganda region. Statistical processing was carried out with Excell 10.0 program use.

Intensive indicators of incidence, morbidity on 100 000 population were calculated for the studied period, the average annual rate of increasing, check of a temporary row on a trend using of criterion of Coke and Stewart. Reliability was estimated by means of coefficient of Dickson and Mud. In the analysis of indicators recommendations L.Sachs [8] were considered.

Results

Results showed that indicators of incidence of diabetes in area from 2008 for 2012 increased by 22,3 %, growth of incidence by diabetes of 1 and 2 types — for 20,6 % and 25,6 % respectively (Fig. 1) was thus noted. Trends of incidence of diabetes of 1 and 2 types had increasing character ($p<0,03$). In structure for the first time the revealed patients with diabetes SD 2 types which specific weight averaged 95,1±3,15 % prevailed.

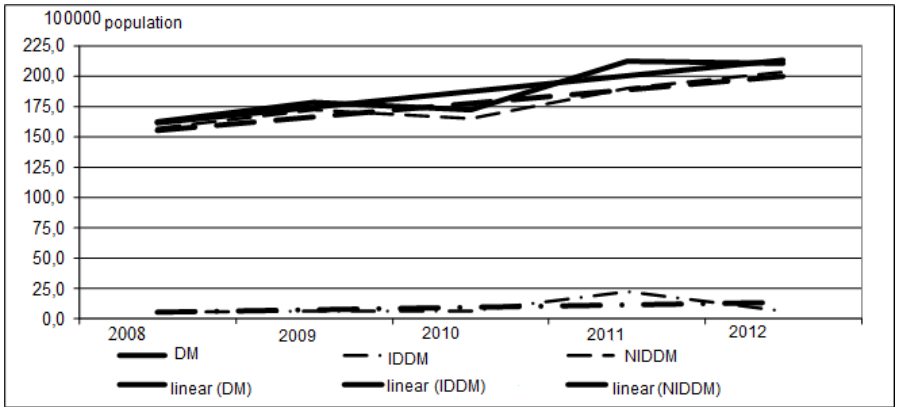

Figure 1. Dynamics of Indicator of Incidence of Diabetes, 1st and 2nd types of diabetes of the population of the Karaganda region from 2008 for 2012

The number of revealed new patients with other forms of diabetes was for the first time small and hesitated from 0 to 4 patients per year (only 9 patients for 5 years were revealed). In group of other specific forms of diabetes there were mainly patients with diseases of the exocrine tissue of pancreas and of gestational diabetes.

Results of studying of epidemioliogy showed growth of number of incidence of diabetes as in city was noted, and villages (Table 1). Thus, incidence of urban population in 2012 increased by 25,5 % in comparison with an indicator of 2008, among country people — for 10,4 %. Due to faster rate of a gain of incidence among residents in 2012 the indicator of incidence of diabetes of the persons living in the city exceeded a similar indicator of rural areas for 27,4 %, while in 2008 — for 12,7 % ($p<0,01$). The comparative analysis of an incidence of 1 type and 2 types showed (Table 1) that incidence indicators during the whole period were authentically higher among the persons living in the city in comparison with country people: 1 type — for 61,8 %, 2 type — for 29 %.

Now the Land Karaganda includes 9 rural areas: 1) Aktogaysky with the center in the village Aktogay; 2) Abaysky with the center in the city of Abay; 3) Bukhar-Zhyrausky with the center in Botakara settlement; 4) Karkaralinsky with the center in the city of Karkaralinsk, 5) Nurinsky with the center in Kievka settlement; 6) Osakarovsky with the center in Osakarovka settlement; 7) Zhanaarkinsky with the center in Atasu settlement; 8) Ulytausky with the center in Ulytau village, 9) Shetsky with the center in Aksu-Ayuli village and also 9 cities of regional submission. Biggest of them — the regional center the city of Karaganda. The second — Temirtau then followed Zhezkazgan, Balkhash, Shakhtinsk, Satpayev, Saran, Abay, Priozersk.

The comparative analysis of incidence in the certain cities and regions of area showed existence of essential distinctions on certain territories. Fluctuations of average annual indicators of incidence of diabetes last 5 years within 63,8 on 100000 population in the city of Priozersk to 277,6,0 on 100000 population in the city of Balkhash (Fig. 2). Distribution of areas depending on an incidence of diabetes showed that in 4 cities (Karaganda, Temirtau, Zhezkazgan and Balkhash) is noted rather high incidence (higher than 197,0 on 100000 population), middle — in the city of Shakhtinsk and in Bukhar-Zhyrausky area — average, and in

4 cities and 8 regions of area the average annual indicator of incidence of diabetes made less than 177,5 for 100 thousand population.

Table 1

Indicators incidence of diabetes among urban and country people of the Karaganda region in 2008–2012 years

Year	Incidence of urban population (on 100 000 pop.)				Incidence of country people (on 100000 pop.)			
	diabetes	1st type	2nd type	other forms	diabetes	1st type	2nd type	other forms
2008	167,8	5,80	162,0	0	146,5	4,29	142,2	0
2009	196,0	7,60	188,4	0	122,9	2,48	119,8	0,62
2010	185,0	6,85	178,2	0	131,3	4,89	125,2	1,22
2011	236,7	28,0	208,5	0,29	136,6	4,61	132,0	0
2012	225,1	7,44	217,7	0	163,5	4,92	158,6	0
M ± m	202,1 ± 23,0	11,1 ± 6,74	190,9 ± 17,7	0,06 ± 0,09	140,2 ± 11,9*	4,24 ± 0,70*	135,6 ± 11,9*	0,37 ± 0,44
СТП	8,56	64,1	158,7	–	13,9	13,9	109,5	–

Note. *p < 0,01 — reliability of distinctions indicators of incidence of diabetes, 1st type, 2nd types among country people in comparison with urban population.

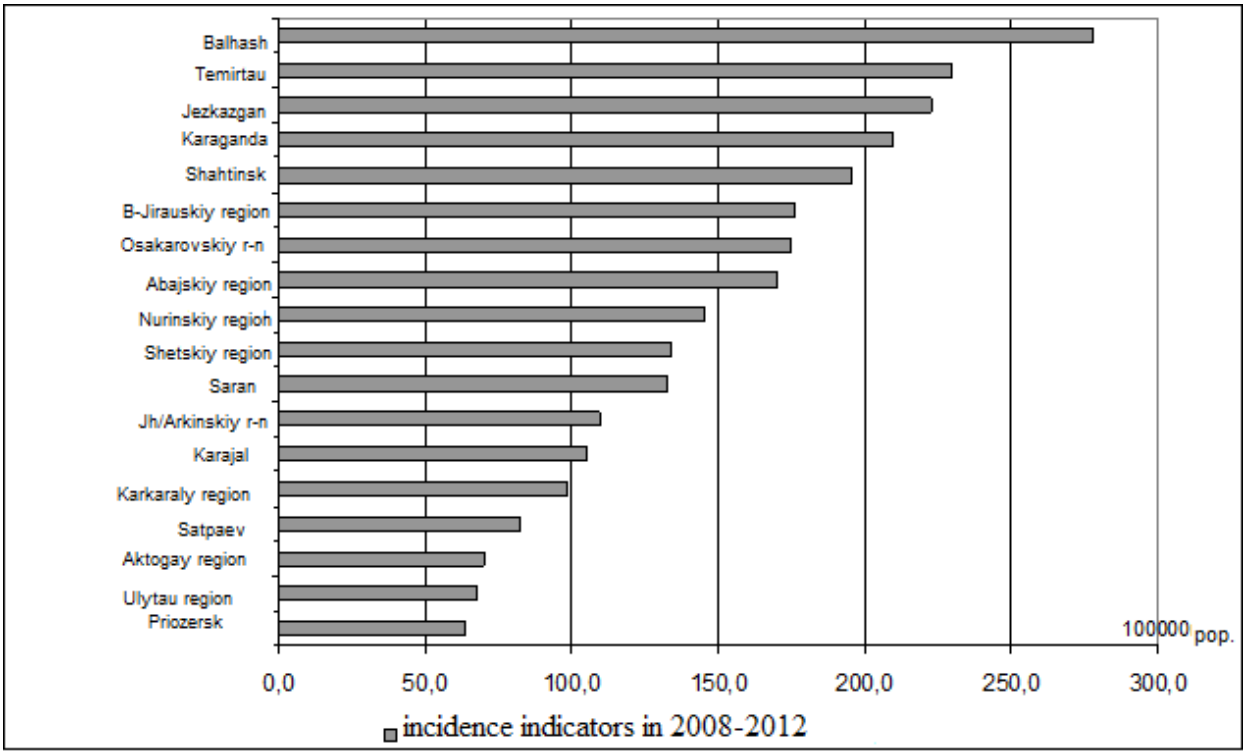

Figure 2. Distribution of the cities and regions of the Land Karaganda depending on level of average annual indicator (2009–2012) of incidence of diabetes

It is known that formation of epidemiological indicators is difficult process and these indicators formed in conditions of influence of various endogenous factors and factors of environment [9]. In view of multiple-factor nature of formation of epidemiological indicators, is of interest that in 64,3 % of regions with a low incidence of diabetes there were no specialists endocrinologists.

Studying of the contingent of patients with diabetes registered in Diabetes Re gistre showed that the number of patients with diabetes in area increased from 1,29 % in 2008 to 1,71 % in 2012, thus accumulation of patients with diabetes first of all determined by growth number of patients with diabetes 2 types (Fig. 3). Number of registered patients with 2 types in 2008 in Land Karaganda was increased from 93,9 % in 2008 until 94,7 % in 2012. In Karazhal, Priozersk, Aktogaysky, Bukhar-Zhirausky, Jean-Arkinsky, Nurinsky areas for 2–3 years, and in the Ulytausky area throughout all analyzed period it isn't registered any case of diabetes of 1 type.

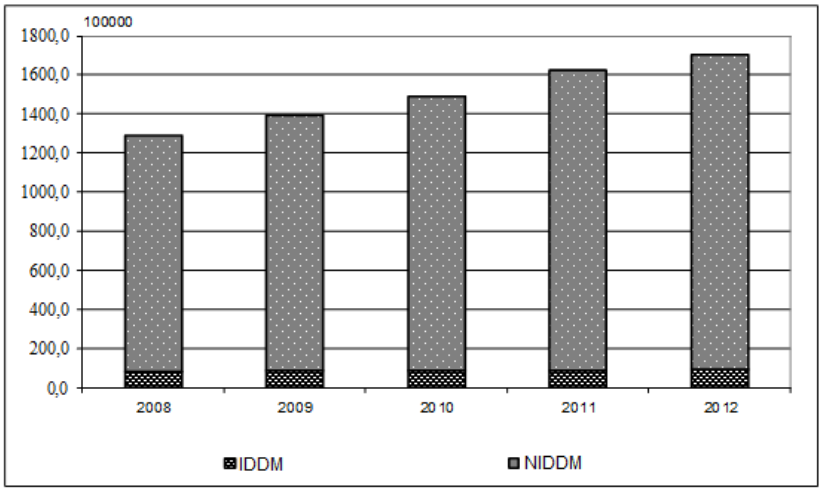

Figure 3. Indicators of morbidity diabetes of 1 type and 2 types in 2008–2012

Thus, this is negative tendence for increasing number of new patients with type 1 diabetes in Ulytausky, and Bukhar-Zhirausky areas as in the cities Priozersk, Karazhal, and positive tendence — in Zhezkazgan, Karaganda, Temirtau, Saran, Satpayev, Shakhtinsk, Abaysky, Osakarovsky and Shetsky areas. In the contrary other tendence developing of dynamics indicator of morbidity of 2 type diabetes: in all cities and regions of area during period 2008–2012 years we observe increasing number of patients, varying from 6,0 % in the Karkaralinsky area to 46,9 % in the Nurinsky area is observed.

It is obviously that indicators of morbidity are reliable more high among urban population comparatively with population of rural areas. The comparative analysis of indicators of morbidity between the various cities and areas showed existence of essential distinctions not only between the population of city and rural areas, but also between the certain cities and regions of area (Table 2). Among cities of the Land Karaganda region the highest rate of morbidity diabetes both 1, and 2 types is noted in Karaganda, Zhezkazgan and Shakhtinsk, the lowest — in Priozersk. Among areas more high morbidity is in Abaysky and Osakarovsky area, the lowest indicators of morbidity are noted in Aktogaysky, Karkaralinsky and Ulytausky areas.

For planning organization of medical service for patients important significance have information about absolute number of patients in each area and in cities. Among 9 cities of area 64,4 % of city patients concentrated in 3 cites: Karaganda, Temirtau, and Shahtinsk.

Average annual indicators of morbidity for 2008–2012 (on 100 000 population), average annual number of patients for 2008–2012 and predicted number of patients for 2014 by diabetes 1 and 2 types depending of the place of residence in the Karaganda region are concentrated in Table 2.

Now the Land Karaganda includes 9 rural areas: Aktogaysky with the center in the village Aktogay; 2) Abaysky with the center in the city of Abay; 3) Bukhar-Zhyrausky with the center in Botakara settlement; 4) Karkaralinsky with the center in the city of Karkaralinsk; 5) Nurinsky with the center in Kievka settlement; 6) Osakarovsky with the center in Osakarovka settlement; 7) Zhanaarkinsky with the center in Atasu settlement; 8) Ulytausky with the center in Ulytau village; 9) Shetsky with the center in Aksu-Ayuli village and also 9 cities of regional submission. Biggest of them — the regional center the city of Karaganda. The second — Temirtau then followed Zhezkazgan, Balkhash, Shakhtinsk, Satpayev, Saran, Abay, Priozersk.

Among 9 cities of area of 64,4 % of city patients are concentrated in 3 industrial cities — Karaganda, Zhezkazgan and Shakhtinsk, from 9 rural regions 58,3 % of pa- tients live in 3 areas — Abaysky, Bukhar-Zhirausky and Osakarovsky. The percent of patients with diabetes of 1 type in population by the end of noted above period varies from 0,02 % in the Ulytausky area to 0,11 % in Karaganda, Zhezkazgan, Shakhtinsk and Osakarovsky area, diabetes 2 types — from 0,58 % in the city of Priozersk and the Aktogaysky area to 2,17 % in the city of Zhezkazgan. The absolute number of patients with diabetes in area by 2012 reached 23 198 people, from them 21976-with diabetes 2 types. On the base of today's tendence of growing of incidence the predicted number of patients in 2014 in Land Karaganda will increase by 34,3 % in comparison with 2008 and will average 26292,8 people.

Table 2

Average annual indicators of morbidity for 2008–2012 (on 100 000 population), average annual number of patients for 2008–2012 and predicted number of patients for 2014 by diabetes 1 and 2 types depending of the place of residence in the Land Karaganda

	1st type diabetes				2nd type diabetes			
Place of residence (city, area)	% patients with 1 type among the population on 2012	Morbidity on 100000 for 2008–2012 ($M \pm m$)	Number of patients in 2008–2012 ($M \pm m$)	Predicted number of patients for 2014 ($M \pm m$)	% of patients among population 2012	Morbidity on 100000 for 2008–2012 ($M \pm m$)	Number of patients in 2008–2012 ($M \pm m$)	Predicted number of patients for 2014 ($M \pm m$)
Balkhash	0,08	86,2±1,49	65,6±0,89	66,0±0,56	2,02	1677,6±287,6	1279,0±236,2	1874,6±238,6
Zhezkazgan	0,11	97,2±9,02	89,8±5,81	100,2±4,84	2,17	1781,7±286,9	1642,6±204,5	2151,0±204,7
Karaganda	0,11	106,4±7,01	500,2±38,6	597,4±38,9	1,89	1689,7±180,2	7950,0±937,3	10278,8±937,8
Karazhal	0,09	89,7±12,3	17,2±2,28	14,8±1,58	0,79	647,6±95,2	124,2±18,6	170,2±18,6
Priozersk	0,05	56,5±9,53	7,60±1,34	6,0±0,96	0,58	459,4±103,1	61,8±13,9	96,6±14,0
Saran	0,08	74,9±7,74	38,0±4,06	48,0±4,04	1,10	1053,0±37,0	534,0±20,9	584,8±20,6
Satpayev	0,09	79,0±9,55	55,4±6,43	71,0±6,34	1,09	954,0±101,7	669,4±69,3	842,2±69,5
Temirtau	0,08	74,9±2,62	133,0±6,89	158,2±6,92	1,74	1520,7±190,4	2704,2±381,8	3662,6±384,5
Shakhtinsk	0,11	107,3±4,0	60,6±2,19	63,0±1,53	1,91	1749,6±187,0	988,0±102,9	1240,8±102,2
Totally in cities	0,10	94,2±5,25	967,4±59,3	1116,6±59,8	1,77	1553,6±182,4	15953,2±1962,1	20901,6±1981,9
Abaysky	0,07	72,7±4,88	39,2±2,39	42,8±1,85	1,63	1488,9±109,5	802,6±52,3	932,6±52,3
Aktogaysky	0,04	39,4±4,36	7,20±0,84	7,6±0,52	0,58	505,0±51,3	92,4±11,1	119,6±11,0
Bukhar-Zhyrausky	0,05	51,4±5,80	31,8±2,59	37,8±2,48	1,21	1013,0±140,1	629,6±102,0	886,8±103,0
Zhanaarkinsky	0,05	45,5±2,80	13,8±1,64	17,4±1,52	0,84	714,7±76,0	216,8±32,4	294,0±31,6
Karkaralinsky	0,03	35,4±3,41	14,4±1,52	12,4±1,12	0,73	695,9±20,2	283,0±7,11	296,6±6,12
Nurinsky	0,06	52,0±8,64	13,8±1,64	16,2±1,26	1,15	968,5±155,7	256,8±22,8	313,2±22,7
Osakarovsky	0,11	91,8±15,5	31,6±4,56	42,8±4,53	1,50	1280,8±173,6	441,4±48,7	563,8±49,1
Ulytausky	0,02	44,8±20,9	6,0±2,71	0,50±2,54	0,71	535,2±152,4	72,4±21,8	125,2±21,5
Shetsky	0,06	52,0±6,41	23,6±2,70	30,0±2,6	1,07	870,6±144,6	395,2±62,0	550,0±62,2
Totally in areas	0,06	55,7±1,91	181,4±6,39	195,0±5,83	1,13	980,3±108,5	3190,2±354,5	4081,8±357,5
Totally in Lands	0,09	85,0±4,44	1148,8±64,9	1311,6±65,3	1,62	1415,7±164,7	19143,4±2313,0	24983,4±233,8

Discussion

Last more than 20 years there are in the World a intensive increasing of number of diabetic patients till 366 millions in 2011. In 1992 diabetes is confirmed by WHO as «Non infectious Epidemy of the 20th Century» and later was confirmed as «Threat for the world». Land Karaganda is not an exception despite of fact that morbidity in Kazakhstan is not so high comparatively with majority of European countries and many countries in Asia and America. The incidence of diabetes in Land Karaganda exceeds similar indicator for Kazakhstan for 15,1–25,3 % from 2009 for 2011. The risk of development of diabetes depending of type is determined by various factors among which important value has age, sex, the comparative analysis of intensive indicators of incidence and morbidity not always reflects because age, sexual structure of population as other factors of other Lands of Kazakhstan are very different comparatively with Land Karaganda.

In Lands with high birth rate and prevalence of young population intensive indicators can be lower in comparison with other areas. It is known that the greatest peak of development of diabetes of 1 type is the share of age group till 15 years — from 30 to 50 % of all cases, diabetes 2 types, on the contrary, developed at adult population. Number of diabetic patients among of adult population (over 15 years) in Land Karaganda region for 3–4 % higher in compared with other Lands of KZ. In other countries according report of International Diabetes Federation, incidence of diabetes is very various: in Russia in 2011 — 10,02 %; France — 7,3 %; Finland — 8,7 %; Germany — 8 %; Austria — 9,1 %; Italy — 7,8 %; Poland — 10,6 %; Sweden — 5,7 %; Mexico — 18 %; Caribbean countries — from 12,8 % in Antigua until 16,4 % in Guyana; a dramatic increasing of diabetes in Persian Gulf countries last 10–15 years: in Saudi Arabia — 16,2 %; Kuwait — 15,9 %; Qatar — 14,1 %; UAE — 12,6 %; Jamaica — 15,9 %; China — 9,3 %; India — 8,3 %; Malaysia — 11,7 %; Singapore — 11,1 %; USA — 10,8 % [10]; Uzbekistan — 0,44 %; Kyrgyzstan — 0,61 % and in the World on average — 8,5 % [11].

The prevalence of 2 types diabetes in the general structure of a disease is noted in all countries. Existence of areas in which within several years it is not revealed any cases of diabetes of 1 type, can be caused by features of epidemiology of this disease. Nevertheless it should be noted that important value for the accounting of incidence, in particular diabetes of 1 type has improvement of monitoring and registration of new patients. The analysis of registers shows that in age from 19 till 20 years the incidence of teenagers decreases; one of the reasons is loss of a certain number of patients by transfer for making diabetes care from children's endocrinologists to adult endocrinologists [1].

A high incidence of diabetes 2 types among urban population in comparison with the rural is noted by other researchers [3]. Decreasing of physical activity, obesity, fat and carbohydrates enriched food, intensive using last 10–15 years European food traditions excluding rural areas (90–100 % of ethnical Kazakh people) where population strongly keep national tradition of nutrition, alcohol, smoking — are well known factors as widely in the World. Smoking, development of obesity are undoubted determinants in development of violations of a carbohydrate exchange. The great attention of researchers is drawn by influence of ecological factors on emergence and diabetes development. It was reported about increasing of resistance to insulin partially as result possible influence of polluted air, compounds of nitrogen-nitrates, nitrites, nitrosamines, influence of chemicals of environment diabetogenny and contributing to obesity [12].

From 1934, year of found, Karaganda is developed as industrial centre only where in concentrated 100 % of black metallurgy production of KZ, coal industry. Now the main pollution of the air environment of area is emissions of contaminations from stationary sources of pollution despite evident decreasing of its total volume due to using of new technologies by industrial plants, is increased by transport. The most polluted industrial centers of area there are cities of Temirtau and Balkhash to which share in 2010 62,6 % of all emissions of polluting substances, then Zhezkazgan and Karaganda [13].

Among rural areas the greatest amount of emissions in the atmosphere is observed in the Abaysky area. We keep attention that in the cities of industrial areas the are a highest rates of incidence and morbidity of SD.

Identification of factors of the environment promoting development of diabetes, has huge value as possibilities for prevention of development of diseases are created.

In 2012 1,72 % of the population of the Land are a registered diabetic patients, from them 8,5 % have disability, 5,3 % have a diabetic nephropathy, from them 0,7 % are on a program hemodialysis, 15,1 % have a retinopathy, from them 1,1 % blindness. According today's tendencies number of incidence and morbidity the number of patients in 2 years will increase more than for 30 %. In this regard it should be noted special relevance of early diagnostics of diabetes and tolerance to glucose. The number of patients with the estab-

lished diagnosis of diabetes does not exactly correspond to real number of diabetic patients. As example, in 2010 the number of diabetic patients in the city of Alma-Ata made 24 821. Meanwhile in result of carrying out purposeful screening it was established that in population of adult population of Almaty (total population 1,475,000 in 2012) prevalence of diabetes makes 15 %, in group of risk — 39 % [2].

Conclusions

Results of investigation showed that in Land Karaganda the reliable growth of incidence of diabetes which has reached in 2012 of 210,4 on 100 000 population is over the last 5 years; in structure of diabetes prevalence of 2 types — 95,1±3,15 %. Incidence and morbidity indicators during the whole period were authentically higher among the residents of cities in comparison with country people: diabets of 1 type — for 61,8 % and 40,9 %, and diabetes 2 types — for 29 % and 36,9 % according to ($p<0,05$). According today's tendencies, incidence and morbidity in 2014 number of patients with SD will increase on average by 34,3 % in comparison with 2008 and will reach 26292,8. The highest incidence and morbidities of diabetes is noted in Karaganda, Zhezkazgan, Shakhtinsk, among rural areas — in Abaysky and Osakarovsky areas.

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2008–2012 жылдарда Қарағанды облысындағы қант диабеті бойынша статистикалық көрсеткіштерінің динамикасы

2008–2012 жылдар аралағында Қарағанды облысы тұрғындарының арасында 5 жыл ішінде аралығында № 12 жылдық есеп беру формалары мәліметтерінің статистикалық сараптамасы негізінде қант диабетімен сырқаттану, аурушаңдық көрсеткіштері зерттелді. Облыс бойынша орташа жылдық қант диабетімен сырқаттану 100 000 тұрғынға шаққанда 187,2±19,4, аурушаңдық 1500,7±169,2 құрады. Қант диабетімен және диабеттің 1 және 2 типтерімен сырқаттылық заңдылықтары үдемелі сипатта болды ($p<0,03$). Ауыл тұрғындарымен салыстырғанда қала адамдарының арасында сырқаттылық және аурушаңдық көрсеткіштері дәлелді түрде жоғары екендігі анықталды.

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Динамика статистических показателей сахарного диабета в Карагандинской области в 2008–2012 гг.

На основании статистического анализа данных годовых отчетных форм № 12 за 5 лет изучена динамика показателей заболеваемости, болезненности сахарным диабетом среди населения Карагандинской области за период с 2008 по 2012 годы. Среднегодовой показатель заболеваемости сахарным диабетом в области составил 187,2±19,4 на 100 000 населения, болезненности — 1500,7±169,2 на 100 000 населения. Тенденции заболеваемости сахарным диабетом и диабетом 1 и 2 типов носили возрастающий характер ($p<0,03$). Показатели заболеваемости и болезненности диабетом были достоверно выше у городского населения по сравнению с сельским населением ($p<0,03$).

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UDC 616.12-005.4/.379-008.64

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Clinical features and risk factors of development and coronary heart disease progressing in patients with diabetes 2 type

At patients diabetes 2 types noted the high frequency of bezbolevy ischemia of a myocardium (17,4 %), atypical symptoms of coronary heart disease (59,4 %), chronic warm insufficiency (60,9 %) and early development of coronary heart disease (63,8 %). Risk factors of development of coronary heart disease at patients with diabetes 2 types are the long course of diabetes, an arterial hypertension, a heavy decompensation of a carbohydrate exchange, violation of the lipidic exchange, the raised level of uric acid.

Key words: type 2 of diabetes, ishemia heart disease, arterial hypertension, nephropathy, coronary heart disease, risk factors of diabetes, vascular complications and developing of atherosclerosis, biochemical risk factors.

Diabetes mellitus (DM) is one of the most widespread endocrine diseases. The forecast of a current of DM, life expectancy of patients are defined by development and progressing of vascular complications: arterial hypertension (AH), nephropathy, coronary heart disease (CHD), cerebral atherosclerosis [1, 2]. At DM 2 type the risk of development of ishemia heart disease (IHD) increases by 2–4 times in comparison with population without DM. Cardiovascular diseases (CVD-SSZ) serve as a cause of death at 50–60 % of all patients suffering from DM [1]. High frequency of IHD is caused by that DM 2 types and are one of the most important risk factors of atherosclerosis as often combined with other risk factors (AH, a dyslipidemia, obesity), strengthening their adverse action [2–6].

Despite the sufficient volume of data on risk factors of development of IHD in DM 2 types, studying of clinical features of a current, risk factors of development and coronary heart disease progressing at diabetes 2 types is important problem for [3, 4] researchers.

Thus, a research objective of this work: investigation of clinical features of a current and of clinical and biochemical risk factors which are taking part in development and progressing of IHD in 2 type DM.

Materials and methods

118 patients with NIDDM which were divided for 2 groups: the first group included 69 patients with IHD, second group — 49 patients without IHD. Groups were comparable on a sex, age: 28 men (middle age of 54,4±3,3 years) and 41 women (middle age of 53,0±4,9 years) in the first group and in second — 15 men (middle age of 52,6±4,03 years) and 34 women (middle age of 51,9±4,4 years). Criteria of inclusion in research: patients aged from 45 till 60 years with IHD or without IHD. Criteria of an exception of research: the age of the patient till 45 years also is more than 60 years; existence in patients of chronic kidney insufficiency, accompanying chronic diseases, a purulent — necrotic complications. Verification of the diagnosis carried out according to criteria of Committee of WHO experts on MD (1999). For definition of the IBS form used the WHO classification (MKB — 10. T.1. P.1. C. 487): 120. Peroral sulforea and biguanides were used for treatment of 64 patients. Insulin therapy used for 54 patients (45,8 %). The average dose of insulin — 28,7±11,5 PIECES/days.

Clinical and biochemical investigation: profile of a glycemia, HbA1c, cholesterol, low density lipoproteides (LPNP), high density lipoproteides (LPVP), triglycerides (TG)), a daily proteinuria, microalbuminuriya, calculation of body weight (BWI), an index a waist circle to a circle of hips (OT/OB). All laboratory indicators were determined by the standard techniques [7, 8]. For confirmation or first time revealing of IHD was used Holterov monitoring of an electrocardiogram using «Markett HELLIGE» with computer processing of results by means of Mars 8000 system and a tredmilmetry on «HELLIGE CardioSoft V3.0». For an assessment of vegetative changes a standard autonomous electrocardiography (electrocardiograms) tests were used: test with deep operated breath (respiratory test), Valsalva's test.

Statistical analysis of obtained data is carried using computer with application of packages of the applied programs «Biostats» and «Statistica 6.0». All data are presented as arithmetic averages and their standard deviation (M±SD). Reliability of distinctions was estimated by means of t-criterion of Student at normal

distribution of a sign and in other cases — using of Mann's nonparametric method Whitney. For the analysis of qualitative signs were used Fischer's exact criterion and χ^2. Reliability of coefficients of distinctions accepted at value $p < 0,05$. For establishment of interrelation of signs carried out the correlation analysis according to Pearson (at normal distribution of a sign) or across Spirmen (at distribution of a sign, excellent from normal).

Results

Among patients with NIDDM the IHD following forms were revealed: tension stenocardia — 60,9 % (42 patients); previous infarct of myocardium — 60,9 % (42 patients); ischemia of a myocardium — 17,4 % (12 patients). At 13 (18,9 %) (9 men, middle age of 53,6±3,9 years; 4 women, middle age of 54,8±2,9 years) IHD was verified on average for 3,5±1,6 earlier, than diabetes was distinguished. From them at 6 (46,1 %) IHD was diagnosed aged from 45 till 50 years and at 7 (53,9 %) patients are more over 50 years. In 3 (4,3 %) (2 men, average age — 57,5±2,1 years; 1 woman — age of 46 years) cases of NIDDM and IBS were diagnosed at the same time. At 53 (76,8 %) patients (15 men, middle age of 54,2±2,8 years; 33 women, middle age of 52,8±5,1 years) NIDDM was diagnosed for 7,3±3,5 years before IHD development. From them in 9 (17 %) patients IHD was revealed aged till 45 years, in 27 (50,9 %) — from 45 to 50 years and in 17 (32,1 %) patients are diagnosed over age as 50 years.

In 12 (17,4 %) patients (4 men, middle age of 56,7±2,6 years; 8 women, middle age of 52,6±4,8 years) painless form of ischemia of myocardium is revealed. From them at 7 patients have diagnosis as stenocardia of tension. Duration of a disease of NIDDM at patients with painless form ischemia of myocardium proceedes 5,5±4,9 years.

Among patients with NIDDN and IHD at 42 (60,9 %) age 53,7±5,5 years had previously IM: 18 men (middle age 54,0±3,2 years) and 24 women (middle age 52,5±5,1 years). Duration of NIDDM in patients with myocardial infarction preceding it — 10,2±7,4 years.

In patients with NIDDM+IHD 23 persons (33,3 %, 10 men and 13 women) 3 persons had the expressed heartaches developed after physical exercises. 41 patients (59,4 %) in were found atypical symptoms, from them 27 people (9 men and 18 women) have aching heartaches accompanied by fatigue and weakness and 14 people (7 men and 7 women) have heartaches not connected with physical activity.

Results of Holterov monitoring: in 5 patients painless ischemia of a myocardium was revealed, and in 7 patients — stenocardia of tension noted bezbolevy episodes of ischemia of a myocardium. In 22 patients with atypical symptoms a shift of a segment of ST according ischemic type was registered.

The cardiovascular form of the diabetic autonomous neiropathy (DAN) was revealed in patients of 1 group with existence of atypical symptoms of IHD in 92,7 % cases, from them — among patients with painless ischemia of a myocardium in 91,7 % cases, among patients with typical with typical pain behind a breast — in 82,6 % cases (Fig. 1). Respiratory coefficient and values Valsalva's coefficient had no reliable differences depending on a clinical current of IHD (6,0±3,9 and 1,00±0,09; 5,0±4,2 and 1,02±0,09; 6,1±5,6 and 1,03±0,09 respectively, $p > 0,05$).

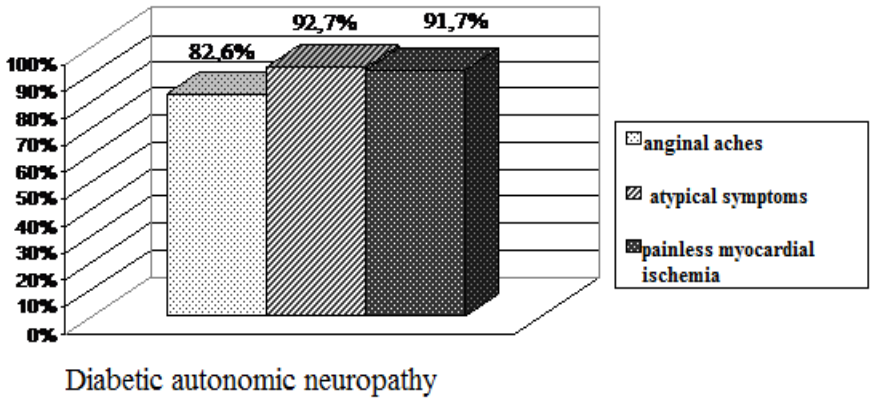


Figure 1. Frequency of autonomy neiropathy in patients with NIDDM depending of clinical symptoms of IHD

In patients with NIDDM + IM Diabetic Autonomy Neuropathy (DAN) was found in 92,9 %. In patients with IM and without as without it the average values of coefficient of Valsalv'a and test with breath authentically didn't differ (1,02±0,09 and 5,6±4,3; 1,02±0,09 and 5,5±4,5 respectively, $p > 0,05$).

As result of clinical investigation of 20 patients (9 men and 11 women) with NIDDM with IHD is revealed the chronic heart insufficiency (CHI) of the I functional class (FC) according classification of the New York association of the heart (NYHA), the II FC — in 18 persons (6 men and 12 women) and III FC — in 4 patients (2 men and 2 women). Average age of sick SD 2 types with CHI FC I made 54,8±4,4 years, with the II FC — 54,0±3,7 years, with the III FC — 57,0±2,7 years. The interrelation of duration of a current of NIDDM, AG, IHD and FC CHI are investigated. Thus, patients with the III FC CHI had higher duration of IHD. So at the I FC IHD duration of IHD was prolonged 3,9±2,5 years, in the II FC — 6,4±3,2 years, and in patients with III FC HSN — 9,0±7,1 years (p <0,05).

One of research problems was investigation of clinical and biochemical risk factors which are taking part in development and progressing of IBS at sick SD 2 types. By results of this research Duration of NIDDM prevailed at patients with IHD comparatively with patients without it (8,7±5,9 and 6,5±5,2 years respectively, $p < 0,05$). Women with IHD had duration of NIDDM authentically above, than at patients is a male which made 10,3±5,7 and 7,5±5,3 years respectively, ($p < 0,01$). Duration NIDDM at women with IHD authentically prevailed in comparison with patients without it (10,2±6,4 and 5,8±5,1 years respectively, $p < 0,001$).

Prevalence of AG at patients with NIDDM made 96,6 %. AG duration in patients with NIDDM and IHD made 10,3±5,7 years and in patients with NIDDM without IHD 7,5±5,3 of years ($p < 0,05$). AG duration at women with NIDDM and IHD authentically prevailed comparatively with women without IBS which made 11,6±6,2 and 6,9±5,6 years, respectively ($p < 0,01$). Among patients with NIDDM and IHD according to MOAG (1999) WHO classification 2nd degree of AG is revealed in 28,9 % of patients, the 3rd degree of AG in 71,1 % cases. In patients without IBS 2nd degree of AG is established in 30,6 %, the 3rd degree of 61,2 % cases and at 4 (8,2 %) patients weren't noted arterial pressure (AP) increase (($\chi^2 = 6,068$, $p = 0,048$). Patients with IHD had systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) indicators authentically above than in patients without IHD.

Tobacco smoking frequency in patients with NIDDM made 46,6 % and in patients with NIDDM and IHD (55,1 %) in comparison with in patients with NIDDM and without IBS (34,7 %) ($\chi^2 = 3,998$, $p = 0,046$). Among patients with IHD the percent of smoking men made 96,4 %, and among patients without IHD — 86,7 % ($\chi_2 = 0,324$; $p = 0,569$). Female patients with NIDDM with IBS smoked in 26,8 % cases, women with NIDDM without IHD in 11,8 % cases ($\chi^2 = 1,779$, $p = 0,182$).

High prevalence of IBS and other diseases of cardiovascular system at SD is defined by also such factors as a hyperglycemia, insulin resistance and giperinsulinemya, obesity, low physical activity, a dislipidemya.

The Body Weight Index (BWI) was raised in both groups. At patients of SD 2 types with IHDS BWI on average made 33,1±5,5 kg/sq.m, in patients without IHD — 30,8±6,8 kg/sq.m ($p > 0,05$). Irrespective of existence or abscence of IHD, a high values of an index «a waist circle to a circle of hips (OT/OB)» are revealed. Among men with NIDDM and IHD and without IHD the abdominal type of obesity is established 82,1 % and 73,3 % cases correspondingly ($\chi^2 = 0,080$, $p = 0,777$), among women in 92,7 % and 88,2 % cases respectively ($\chi^2 = 0,068$, $p = 0,794$).

When studying indicators of a carbohydrate exchange at sick SD 2 types with IBS in comparison with patients of SD 2 types without IBS are revealed. A higher levels of morning glycemia (p<0,001), a postprandial glycemia ($p < 0,001$), an average daily glycemia ($p < 0,001$) and HbA1c ($p < 0,01$) were revealed in patients with NIDDM +IHD omparatively with NIDDM only (Table 1).

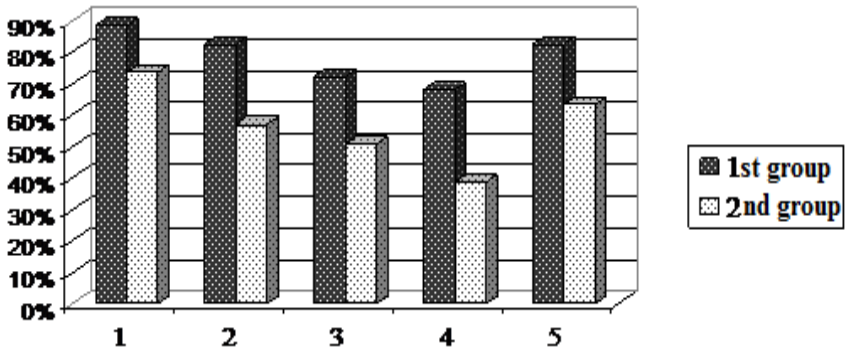
Table 1

Parameters of carbohydrate metabolism at patients with NIDDM with IHD and without IHD

Parameters	IDDM with IHD, $n = 69$	NIDDM without IHD, $n = 49$	p
Fasting glycemia, mmol/l	9,3±1,8	7,2±1,4	0,001
Postprandial glycemia, mmol/l	11,2±1,9	8,7±2,04	0,001
Daily average glycemia, mmol/l	9,8±1,7	7,7±1,4	0,001
HbA1c, %	10,2±1,9	8,9±2,2	0,002

Lipid metabolism disorders. According to criteria of European Diabetes Policy Group (1998–1999) patients with NIDDM and IHD the hypercholesterolemia (>4,8 mmol/l) is revealed in 88,4 %, in patients without IBS in 73,5 % ($\chi^2 = 3,408$, $p = 0,065$), a hypertriglyceridemia (>1,7 mmol/l) 82,6 % and 57,1 % cases respectively ($\chi^2 = 8,003$, $p = 0,005$). The LPNP high level (>3,0 mmol/l) at patients of 1 group was defined at

72,5 %, in 2nd group at 51,0 % of patients ($\chi^2 = 4{,}800$, $p = 0{,}028$). Low concentration of LPVP (>1,2 mmol/l) was observed at 68,1 % patients with NIDDM with IBS and at 38,7 % of patients without IBS ($\chi^2 = 11{,}584$, $p = 0{,}001$). The Atherogenic Coefficient (AC) was higher at patients with NIDDM and IHD (82,6 %) in comparisons with patients without IBS (63,3 %) ($\chi^2 = 4{,}680$, $p = 0{,}031$) (Fig. 2).



1 — hyperholesterinemia; 2 — hypertriglyceridemia; 3 — high level of LDL;
4— low level of HDL; 5 — high level of CA; $p < 0{,}05$ on all pairs of indicators

Figure 2. The frequency of lipid metabolism disorders in patients NIDDM with IHD and without IHD

Apparently (Table 2), patients with IHD had on average values of OHS, TG, LPVP authentically higher than at patients without IHD. Statistically significant decrease concentration of LPVP showed noted at patients with NIDDM and IHD in comparison with patients without IBS ($p < 0{,}001$). Authentically the KA high levels are revealed in patients with NIDDM.

Table 2

Lipid metabolism in patients NIDDM with IHD and without IHD

Parameters	NIDDM with IHD, $n = 69$	IDDM without IHD, $n = 49$
TCH, mmol/l	6,4±1,3	5,4±1,0*
Triglycerides mmol/l	2,8±1,2	1,9±0,9*
HDL, mmol/l	1,11±0,22	1,28±0,14*
LDL, mmol/l	4,0±1,3	3,3±0,9*
CA, U	5,4±1,3	4,4±0,9*

Note. * — p < 0,05.

Miroalbuminuria is revealed in 56,5 % number of patients with NIDDM and IHD and in 40,8 % patients without IBS ($\chi^2 = 2{,}234$, $p = 0{,}135$). The proteinuria was found in 28,9 % of patients of 1 group and in 24,5 % of ($\chi^2 = 0{,}110$, $p = 0{,}741$).

The content of uric acid in both groups without changes. However, in patients with NIDDM and IHD had an average value of uric acid authentically above than at patients without IBS (303,5±71,5 and 264,8±51,3 mmol/l respectively, $p < 0{,}05$).

By correlation analysis of patients with NIDDM and IHD it was revealed significant interrelations between morning glycemia and duration of NIDDM ($r = 0{,}36$, $p < 0{,}05$), the TG level ($r = 0{,}47$, $p < 0{,}05$), LPVP ($r = -0{,}41$, $p < 0{,}05$). Similar data are obtained for postprandial glycemia: interrelation with values of LPNP ($r = 0{,}21$, $p < 0{,}05$), uric acid ($r = 0{,}31$, $p < 0{,}05$) and diastolic arterial pressure ($r = 0{,}20$, $p < 0{,}05$) also was revealed. The HbAc1 level didn't correlate with NIDDM duration and with level of TG and LPNP, LPVP, uric acid, meanwhile was however connected with the OHS level of serum of blood ($r = 0{,}24$, $p < 0{,}05$).

42 patients with NIDDM (60,9 %) patients have earlier a myocardial infarction. patients with NIDDM with IHD and IM in the anamnesis and without IM only reliable distinction on NIDDM duration is noted, but not revealed statistically significant difference on BWI, AG duration, to the GARDEN and DAD levels, indicators of a carbohydrate and lipid changes, values of uric acid, a daily proteinuria.

Discussion

Frequency of combination of NIDDM and IHD can reach 45–55 %, and IHD developed early [5, 9–12]. Our results confirmed a high frequency of IHD in patients with NIDDM as 58,5 %; more often among persons till 50 years (63,8 %) testify to the high frequency of IHD.

According to some authors at sick SD 2 types Existence of an autonomous neiropathy with alteration of afferent nerves of heart can be one of cause developing of painless ishemia of a myocardium and atypical symptoms of IHD which increase risk development of sudden death [2, 7, 10, 13]. It is necessary treat of all symptoms of ischemia of a myocardium not only of typical attacks of stenocardia.

NIDDM consider as one of major factors of risk of heart function insufficiency. At patients with NIDDM types with IHD in 60,9 % cases there are a chronic heart insufficiency which depended of NIDDM duration. These data are coordinated with Framingemky's results, results of the Russian epidemiological research EPOHA-HHI as with American national register NHANES.

High frequency of cardiovascular diseases in patients with NIDDM is caused by various risk factors. In diabetes the risk of development of IHD may be determinaed by obesity, smoking, AG, a dislipidemiya, and also with a hyperglycemia, an insulin resistance and by hyperinsulinemia. Our results showed that in patients with NIDDM+IHD duration of diabetes and AG of the GARDEN and DAD level authentically more high and fact of smoking is established more often comparatively with patients without IBS. The Body Weight Index as a giperinsulinemia indicator, was increased as high values of index a waist circle to a circle of hips (OT/OB) as in patients with NIDDM+IHD as in NIDDM without IHD. It is a not direct confirmation of importance role of smoking and AG as of abdominal obesity in development of macrovascular complications in NIDDM. More high morning and postprandial glycemia and HbA were revealed in patients with NIDDM+IHD as more high frequency of hyper triglycerinemia more high level of LPLD and KA level, the low concentration of LPHD omparatively with patients with NIDDM only.

Researches UKPDS (1998), Honolulu Heart Study (1999), DECODE (The Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe) (1999) established reliable decrease of the frequency of macrovascular complications among patients with lower glycemia level and also adverse predictive value of a postprandial hyperglycemia in development of cardiovascular complications [14].

Thus, results of this research confirm important role of disorders of the glucose and lipid metabolism as risk factors of atherogenesis.

Today more often the great significance is attached to influence of factors as microalbuminuria, proteinuria and of uric acid as risk factors for developing of cardiovascular diseases.

Among patients with NIDDM+IHD frequency of a microalbuminuria made 56,5 % that will be coordinated with results of D.R.Meeking et al. (1999) [15]. Patients had authentically more high aised level of uric acid comparatively with patients with NIDDM only. Microalbuminuria is a marker developing of atherosclerosis [9] and the raised content of uric acid can promote damage endothelium of arteries and create predisposition to fixation of cholesterol, to activate adhesion and aggregation of platelets [16].

Conclusions

1. In patients with NIDDM are revealed the high frequency of painless form of ischemia of a myocardium (17,4 %), atypical symptoms (59,4 %) of IHD, chronic heart function insufficiency (60,9 %) and in 63,8 % early development of IHD (till 50 years).

2. In patients with NIDDM+IHD is revealed longer current of NIDDM and of AG, decompensation glucose metabolism and marked disorders of a lipid metabolism as high frequency of the hypertriglyceridemiya, high LPNP level, and low level of LPVP, authentically high level systolic and diastolic blood pressure as of uric acid concentration comparatively with patients without IHD.

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2 типтегі қант диабетімен ауыратындарда жүректің ишемиялық ауыруының пайда болу қауіпі мен қарқын алуының факторлары және аурудың өтуінің клиникалық ерекшеліктері

Қант диабетімен ауыратындардың 2 типінде төмендегі аурулардың жоғары жиілігі байқалған: ауырсынусыз болатын миокард ишемиясы (17,4 %), жүректің ишемиялық ауыруы (59,4 %), созылмалы жүрек қысымы жетімсіздігі (60,9 %) және жүректің ишемиялық ауруының ерте дамуы (63,8 %). Қант диабетімен ауыратындардың 2 типінде жүректің ишемиялық ауруының даму қауіпінің себебі қант диабеті ауруының, артериалды гипертонияның ұзақ уақыт өтуі, көмірсу алмасуының ауыр декомпенсациясы, липидтер алмасуының бұзылуы, зәр қышқылының көбеюі болып табылады.

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Клинические особенности течения, факторы риска развития и прогрессирования ишемической болезни сердца у больных сахарным диабетом 2 типа

У пациентов, больных сахарным диабетом 2 типа, отмечена высокая частота безболевой ишемии миокарда (17,4 %), атипичных симптомов ишемической болезни сердца (59,4 %), хронической сердечной недостаточности (60,9 %) и раннее развитие ишемической болезни сердца (63,8 %). Факторами риска развития ишемической болезни сердца у больных сахарным диабетом 2 типа являются: длительное течение сахарного диабета, артериальная гипертония, тяжелая декомпенсация углеводного обмена, нарушения липидного обмена, повышенный уровень мочевой кислоты.

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UDC 616.831–005.1/.379–008.64–07

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Methods predicting the probability of stroke in patients with type 2 diabetes

Risk developing of a stroke in patients with diabetes 2.8 times is higher comparatively with health persons. Authors proposed the mathematical model «stroke-risk factors» for definition probability of developing of stroke at patients with diabetes 2 types based on standard methods carried out in medical service. The analysis of mathematical model allowed to establish the speed of increase of a stroke for urgent hospitalization of patients.

Key words: diabetes, stroke, macrovascular complications, mathematical model of stroke-risk factor, macroangiopathy, cerebral disorders, predicting of stroke.

Introduction

There is a rapid increase in the incidence of diabetes mellitus (DM) now, dominant share of which is type 2 diabetes. Type 2 diabetes is up to 95 % of all cases of diabetes [1]. The significance of this trend is dramatically, not only within manifestation of disease by itself, but also the close pathogenetic association with type 2 diabetes with the development of micro-and macrovascular complications.

Diabetic macroangiopathy is a frequent complication of diabetes with a primary lesion of the coronary, cerebral and peripheral blood vessels. One of the significant pathology that develops on the background of macroangiopathy — an acute cerebrovascular accident (stroke), and encephalopathy as its predecessor. Stroke is one of lea ding causes of death in the world and is the major reason of permanent disability. [2] According to WHO, more than 30 million cases of stroke registered annually [3]. Disability as a result of a stroke varies from 40 to 80 % [4].

According to the MRFIT research the risk of stroke among patients with diabetes was 2.8 times higher than in patients without diabetes. Stroke occurs in 2.08 times mo re frequently in patients with diabetes according to our study [5].

The main theories to explain the development of macroangiopathy in type 2 diabetes include hyperglycemia, dislipidemia, oxidative stress, insulin resistance, hyperinsulinemia, endothelial dysfunction. Difficult to say which mechanism is primary, but it is absolutely clear that they are part of each other and aggravate the disease, accelerating the formation of macrovascular complications [6, 7].

Due to the high risk of stroke in patients with type 2 diabetes it is necessary to carry out preventive measures. It is necessary to develop a method that forecasts the risk of stroke, taking into account possible risk factors to implement an effective prevention of stroke in patients with diabetes.

On the base of Centre of primary health care in Karaganda standard laboratory and hardware methods of investigation were performed. This methods are conducted regularly in patients with type 2 diabetes. A mathematical model predicting the likelihood of stroke in patients with type 2 diabetes was developed according to this data.

The purpose of the study was to develop a mathematical model of «stroke-risk factors» to determine the likelihood of stroke in patients with type 2 diabetes.

Materials and methods

In the laboratory study included 94 participants aged 40 to 83 years, with an equal inclusion of women and men of different national and ethnic origin. There were 60 patients with diabetes mellitus in the compensatory stage with cerebral disorders in the group, 9 of them had a stroke for the current year. The group of comparison consisted of 34 participants with diabetes without stroke matched for age and sex. The criteria for inclusion in the control group were age 40–80 years, normal blood pressure, BMI within 18.5–2.5.

Surveys conducted with all of the participants in the study. The questionnaire presented questions to identify risk factors (gender, age, height, weight, family history of diabetes and stroke, the presence of hypertension and its duration, the presence of diabetes and its duration, smoking, alcohol abuse, lack of exercise, diabetes, unbalanced diet, obesity, emotional stress, use of oral contraceptives), as well as questions that re-

veal the presence of symptoms of ischemic attacks (sudden headache, nausea, ringing in the ears, dizziness, weakness, loss of consciousness, motor and speech impairment). After conducting the survey all participants underwent the following survey methods.

Measurement of BMI. Body mass index was calculated by Adolph Quetelet formula: I = m/h^2. Measurement of systolic and diastolic blood pressure. The patient was asked to relax and calm down for 15 minutes before measurement. SBP and DBP were measured by the method of Korotkov with mechanical tonometer. Measurement of respiratory rate. Respiratory rate was measured by the auscultatory at rest. Measurement of heart rate. Heart rate was measured by tactile method for a minute at rest. Testing blood glucose blood sampling was performed in the morning on an empty stomach in the standard terms. For blood collection were used tubes Vacutainer, the level of glucose in the blood was determined by the method of Somogyi-Nelson.

Determination of glycated hemoglobin. Blood sampling was performed in the morning on an empty stomach in the standard terms. For blood collection were used tubes Vacutainer. Determination of glycated hemoglobin produced by using immunological reagents Vital and DR 2800 spectrophotometer with a wavelength 443 nm.

Studies of blood coagulation (PTI, amount of fibrinogen, platelet aggregation, APTT). Blood sampling was performed in the morning fasting in the standard terms. For blood collections were used tubes Vacutainer. The analysis was performed on the singlechannel analyzer parameters of hemostasis Clot-1. Determination of blood biochemical parameters (cholesterol, triglycerides, ALT, AST, total bilirubin, direct bilirubin, urea, creatinine, total protein). Blood sampling was performed in the morning fasting in the standard terms. For blood collections were used tubes Vacutainer. For analysis reagents used with the company Vital biochemical analyzer BioSystemA-15.

Electrocardiogram. ECG study was conducted in 12-lead electrocardiograph for BTL-088D, United Kingdom, 2011. CDSM of brachiocephalic trunk. CDSM of b/c the barrel held scanner MEDISON SONOACE X8, 5–12 MHz linear probe.

According to the questionnaires and surveys conducted by expert assessments by specialists (neurologist) was diagnosed with the presence of cerebrovascular accidents. Statistical processing of the measurement was carried out according to conventional methods in the program Statistica. Square correlation matrix was set to determine the correlation coefficient. The distributions of the parameters subordinated to the normal distribution law [8–10].

Results

There is a significant correlation between the event «stroke» and SBP, duration of hypertension, the percentage of stenosis of the carotid artery in the group of patients with diabetes and cerebrovascular disorders. It is interesting to note that there is a significant correlation coefficient between diabetes disease duration and duration of hypertension. Also, there is a significant correlation coefficient between HbA1 and duration of hypertension (Table).

T a b l e

Significant correlation coefficients

№ X	Parameter	Correlation coefficients between event «stroke» and parameter	Intervals diapason	Intervals with codes	Regression coefficients
1	*2*	*3*	*4*	*5*	*6*
X1	Age	0,19	Less than 60–90	1–4	–0,240133
X2	BMI	0,05	16–31 and higher	1–4	–1,93289
X3	Duration of hypertension	0,23	0–21 and higher	1–6	–4,16929
X4	Stroke	1		0/1	
X5	SBP	0,34	110–191 and higher	1–9	2
X6	DBP	0,08	60–110 and higher	1–5	–1,24113
X7	HR	0,14	Less than 60–81 and higher	1–4	3,9
X8	RR	–0,01	16–21 and higher	1–3	5,31
X9	Blood glucose	0,02	3,3–12 and higher	1–4	–3,24604

Table continues

1	2	3	4	5	6
X10	Cholesterol	0,04	Less than 5.2–8 and higher	1–4	–4,43646
X11	Triglycerides	0,13	0,14–4,5 and higher	1–3	7
X12	PTI	0,11	77–101 and higher	1–4	–4,45344
X13	Fibrinogen	0,02	2,3–7,5	1–3	–4,57874
X14	Platelet agregation	0,04	13–19 and higher	1–3	18
X15	aPTT	–0,03	23–41 and higher	1–3	–4,08824
X16	ALT	0,14	0–61 and higher	1–3	–4,01144
X17	AST	0,07	0–61 and higher	1–3	–2,47517
X18	Total bilirubin	0,14	8,6–20,6 and higher	1–3	–1,29847
X19	Direct bilirubin	0,15	3,4–12 and higher	1–3	–0,666565
X20	Urea	0,07	25–51 and higher	1–4	9,620053
X21	Creatinine	0,07	40–116 and higher	1–5	–7,49193
X22	Total protein	–0,10	Less than 65–86 and higher	1–4	1,42290
X23	Tachycardia	–	Yes/No	1–2	0,229491
X24	Conduction disturbances	–	Yes/No	1–2	1,776381
X25	Violation of repolarization	–	Yes/No	1–2	–0,340166
X26	Arrhythmia	–	Yes/No	1–2	–24,3798
X27	Extrasystole	–	Yes/No	1–2	–27,0057
X28	Normal ECG	–	Yes/No	1–2	1,142954
X29	Percentage of carotid stenosis	0,29 0,58 0,87	0–99 %	1–3	6,095 7,01 7,33
X30	Increase of peripheral resistance	–	Yes/No	1–2	–5,64649
X31	Deformation of VA	–	Yes/No	1–2	–19,4154
X32	Compression of VA	–	Yes/No	1–2	16
X33	Increase acceleration of blood flow	–	Yes/No	1–2	16
X34	Normal DCS	–	Yes/No	1–2	2,914050
X35	Glycosylated hemoglobin	0,02	4–12 and higher	1–9	0,917
X36	Diabetes disease duration	0,18	0–21 and higher	1–6	–1,38249

It was necessary to create a matrix with coded values to develop mathematical model because these studies included both qualitative and quantitative characteristics in different units of measurement.

The next step is: to determine the regression coefficients by method of logistic regression. These factors were the basis for the development of a mathematical model to predict the risk of stroke [9]. The mathematical model allows: to determine the risk of stroke in a patient or a tendency of stroke rise in social groups, to explore the character of change in the probability of occurrence of stroke according to operating factors, to assess the degree of influence of the studied factors on the probability, to predict the occurrence of stroke for given levels of the factors, to determine the optimal levels of factors to indicate required values of parameters [8].

This model is:

$$y = \exp(b0 + b1 * x1 + ... + bi * xi) / \{1 + \exp(b0 + b1 * x1 + ... + bi * xi)\}; \quad (1)$$

$0 < y < 1$, where: y — its occurrence; b0 — free term; b1.... bi — regression coefficients of factors x1... xi; x1... xi — studied factors.

The processing of the regression coefficients were tabulated.

The following is a regression equation that indicates the likelihood of stroke in patients with type 2 diabetes.

Y=EXP(8.155–0.240133*X1–1.93289*X2–4.16929*X3+2*X5-.24113*X6+3.9* X7+ 5.31*X8–
–3.24604*X9–4.43646*X10+7*X11–4.45344*X12–4.57874*X13+18*X14–4. 08824*X15–4.01144*X16–
–2.47517*X17–1.29847*X18–0.666565*X19+9.620053E+00*X20–
–7.49193*X21+1.42290*X22+0.229491*X23+1.776381*X24–0.340166* X25–24.3798*X26–
–27.0057*X27+1.142954*X28+6.0955*X29–5.64649*X30–19.
4154*X31+16*X32+16*X33+2.914050E+01*X34+0.917*X35–1.38249*X3)/1+ EXP(8.155–
–0.240133*X1–1.93289*X2–4.16929*X3+2*X5–1.24113*X6+3.9*X7+ 5.31*X8–3.24604*X9–
–4.43646*X10+7*X11–4.45344*X12–4.57874*X13+18*X14–4.08824*X15–4.01144*X16–2.47517*X17–

–1.29847*X18–0.666565*X19+9.620053 E+00*X20–
–7.49193*X21+1.42290*X22+0.229491*X23+1.776381*X24–0.340166* X25–24.3798*X26–
–27.0057*X27+1.142954*X28+6.0955*X29–5.64649*X30–19.
4154*X31+16*X32+16*X33+2.914050E+01*X34+0.917*X35–1.38249* X3); (2)

It is noteworthy that tests of significance were p = 0.02038, with hi2 = 54.174 for the second group (patients with diabetes), and for the first group of p = 0.03683, with hi2 = 51.352. These significance tests confirm performance of the model.

Regression equation is close to functional. The derivative of the regression equation allows to determine the development trend of the probability of stroke:

$$\frac{dy}{dxi} = \frac{az}{(1+z)^2}, \quad (3)$$

Where: a= b0+b1+…+bi-1; z = exp b0+b1+…bi *x.
Increasing xi

$$\frac{dy}{dxi} \to 0. \quad (4)$$

Function of the probability of each risk factor is hyperbole, asymptotically approaching 1, and the function of the probability of change of speed — hyperbole that tends to 0.

The resulting dependence of the rate of increase of stroke can be used to deter- mine the terms of hospitalization of the patient.

Discussion

There are significant correlation coefficients between the event «stroke» and elevated SBP, duration of hypertension and a high percentage of carotid stenosis, the presence of these factors already predetermines the development of cerebrovascular accidents. It takes such a low number of parameters in patients with diabetes for the development of stroke, due to pathologically altered state of the vas- cular wall, their changed architectonic and endothelial dysfunction.

The glycation end products (GEP) are formed in diabetes. GEP circulating in the bloodstream bind the proteins of the extracellular matrix of blood vessels. In addition, GEP have the following effects: increase permeability between endothelial cells, violate the bioavailability of NO to the smooth muscle cells of arterioles and initiate the secretion of cytokines by macrophages -primary mediators of inflammation [11]. These processes lead to endothelial dysfunction, thickening of the basement membrane, decreased elasticity, increased vascular tone, the violation of their architectonic. According to McDermott, glycosylation end products independently contribute to the development and maintenance of high blood pressure in patients with diabetes mellitus, disrupting normal sensitivity to the action of the vessel walls of vasodilator substances. The irreversibility of the molecules glycosylation end products explains the continued progression of micro- and macrovascular complications even with very good compensation of diabetes [12].

Violation of NO-producing endothelial function is primarily due to the development of angiopathy and atherogenesis. Inadequate NO production not only leads to reduced vascular relaxation and spasm, but also to an increased vascular permeability of proteins and lipoproteins, to the rapid proliferation of smooth muscle cells to unhindered expression of adhesion molecules on endothelial cells to increased thrombosis. All of these processes lead to an imbalance between vasodilator and vasoconstrictor, prothrombotic and antithrombotic, anti-inflammatory and pro-inflammatory, anti-sclerotic, proliferative factors in side of prevalence the latter [6].

As pointed Mkrtumian [6] et al. alteration of endothelium expresses adhesion molecules, including ICAM-1 to facilitate the penetration of monocytes crowded lipids in the subendothelium. These abnormalities are probably promote lipid accumulation in the vascular wall, proliferation and migration into the intima of smooth muscle cells of arteries and increase their production of collagen and elastin, developing platelet microaggregates. Recent shape the microembolisms vasa-vasorum large arterial vessels with local changes of the vascular wall, which ultimately leads to the development of atherosclerosis and thrombosis.

There are three components of the underlying etiology and pathogenesis of stroke: an inadequate cardiovascular activity, altered state of brain vessels and disturbances in coagulation [13, 14]. The above pathological processes lead to abnormalities in both the cardiovascular system and the inadequacy of blood supply of the brain, atherosclerotic vascular changes and activation of coagulation processes [15–17].

As a result, the long duration of hypertension, high SBP and the high percentage of carotid stenosis in patients with type 2 diabetes are sufficient risk factors for the appearance of stroke.

Conclusions

Obtained results led to the following conclusions about sufficient quantity of three risk factors (elevated SBP, prolonged hypertension, and a high percentage of stenosis of the carotid artery) for the occurrence of acute ischemic attack in patients with type 2 diabetes, due to a significant correlation coefficient between the event «stroke» and examined factors. A methodology was developed to predict the occurrence of stroke in patients with type 2 diabetes. It was based on standard methods regularly conducted in hospitals. The analysis of the mathematical model allowed us to establish the rate of increase of stroke in order to monitor its trend and provide timely hospitalization to the patient.

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2 типтегі қант диабетімен ауыратындарда инсульттің пайда болуының ықтималдығын болжау әдістемесі

Мақалада 2 типті қант диабетімен ауыратын пациенттердің инсультке шалдығу мүмкіндігін математикалық үлгілеу бойынша ақпарат ұсынылған. Қарағанды қаласында № 2 АМСК негізінде үлкен көлемде зертханалық және аппараттық зерттеулер жүргізілді. Содан кейін инсульт оқиғасы мен зерттелген факторлардың арасындағы корреляция коэффициенттері анықталды. Келесі кезең логистикалық регрессия әдісімен «тәуекелдің инсульт-факторлары» регрессивті моделін құрастыру болып табылады. Алынған теңдеулер инсульттің болу қауіпін анықтауға мүмкіндік береді. Регрессия теңдеуі өз мәні бойынша детерминделгенге жақын болғандықтан, оны дифференциялауға болады. Әр тәуекел факторы бойынша алғашқы туынды ықтималдықтың өсу жылдамдығы анықталды береді. Бұл қаулы жаңа болып табылады.

Ф.А.Миндубаева, И.А.Кадырова

Методика прогнозирования вероятности возникновения инсульта у больных сахарным диабетом 2 типа

Риск возникновения инсульта среди пациентов с сахарным диабетом в 2,8 раза выше по сравнению с лицами, не имеющими его. Авторами предложена методика математической модели «инсульт-факторы риска» для определения вероятности возникновения инсульта у больных диабетом 2 типа. Методика основана на стандартных методах исследования, регулярно проводимых в лечебных учреждениях. Анализ математической модели позволил установить скорость нарастания инсульта с целью своевременной госпитализации больных.

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UDC 613.2:612.359:616–012

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High specific fluorescent method staining of zinc-insulin complex in pancreatic B-cells

Authors demonstrated results using of high specific fluorescent method histochemical staining of Zn^{+2}-ions in pancreatic B-cells. It was showed in diabetic animals and in animals past mobilization of insulin from B-cells a simultaneous decreasing as of amount of insulin as of Zn^{+2}-ions in cytoplasm of B-cells. Meanwhile formation in B-cells of chemical complexes of derivatives of Diethyldithiocarbamic acid with Zn^{+2}-ions result negative fluorescent reaction for Zn^{+2}-ions but positive reaction for insulin using of insulin staining methods.

Key words: insulin contents, immunohistochemical, immunofluorescent, pseudoisocyanine and aldehyde fuchsine methods, pancreatic B-cells.

It is known that pancreatic B-cells contained a large amount of ions of Zinc [1–3] as salivary glands and prostate. In B-cells zinc ions take part in processes of biosynthesis of insulin as in of storage of insulin by forming of zinc-insulin complex [4, 5]. Pancreas of rat, rabbit, dog, cat, some fish, human, birds, mice, hamster, porcine, hoerst, contained a large amount of zinc. Using of electron microscopy histochemical method it was showed that that zinc concentrated in B-cells in B-granules only contained deposited form of insulin [6] and destruction of B-cells caused by Dithizon which formed in B-cells toxic complexes with zinc-ions, started by destruction of B-granules [7].

Widely known methods staining of insulin as immunohistochemical, immunofluorescent, diethylpseudoisocyanine and some other methods are specific for insulin only but not for staining of zinc ions. Very often in diabetes and intact B-cells there are is a quantity correlation between insulin and zinc ions content: decreasing of insulin content accompanied by decreasing of amount of zinc ions and in opposite, in intact B-cells a large amount of insulin accompanied by a large amount of zinc-ions. Meanwhile for estimate ability of B-cells for storage of insulin in cells it is necessary to use method of staining of zinc-ions whereas staining of insulin is indirect method for to estimate concentration of zinc ins in B-cells.

Aim of work: to study result using of high specific fluorescent methods revealing of zinc ions by using of 8-para(toluenesulphonylamino)quinolin (TSQ), a high specific for Zn^{+2}-ions reagent [8, 9] which formed complex «zinc-TSQ». TSQ is a derivative of 8-oxyquinolin and synthesis was elaborated by Prof. N.N.Voroshzov in 1930 [10]. In UV-light with maximum of absorbance as 360–370 nm, this complex fluoresces brightly green light [11]. Specificity for zinc ions of this method was confirmed in vitro by interaction of pure zinc ions with TSQ that result intensive green fluorescence of solution; using of spectral analysis it was confirmed presence in solution of «zinc-TSQ» complex and correlation of maximum of absorbance of this complex with pure complex synthezed in vitro. Sensitivity of this method is high and concentration of zinc as 10^{-7}–10^{-8} revealed using it [8]. This same time in TSQ is TSQ possess a high diabetogenic activity and injection of 40–50 mg/kg result necrosis, destruction and death of absolute majority of B-cells within 20–30 min past formation of complex «zinc-TSQ» in B-cells [12].

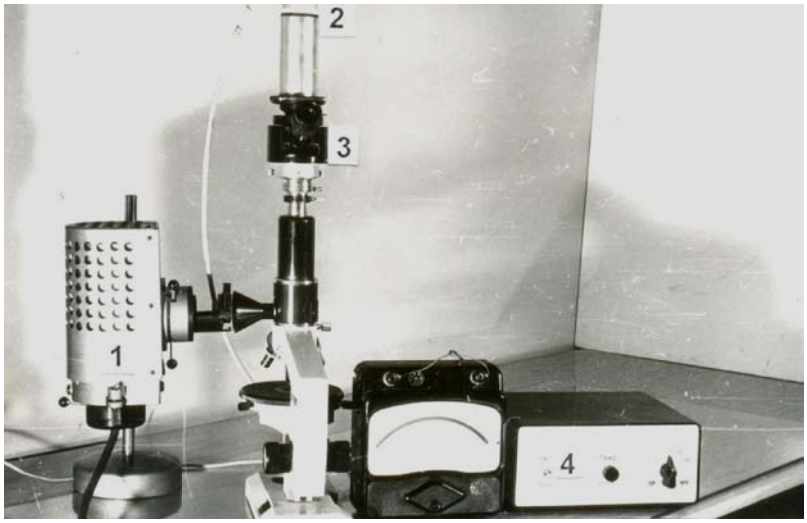
Materials and methods

8 rabbits (2450–2680 g) and 3 Guinea Pigs (320–370 g). Group 1: 3 animals with diabetes caused by Dithizon (48,8–51,6 mg/kg); Group 2: intact rabbits; Group 3: 3 animals past administration of Na salt of diethyldithiocarbamic acid (DCA) (504 and 987 mg/kg) that result temporary non toxic binding of zinc in B-cells; Group 4: 2 Guinea Pigs past administration of DZ (49,5–52,8 mg/kg) and 2 intact Guinea Pig.

Preparing of Dithizon water-ammonia solution: Dithizon (Avocado Chem. Company, USA) — 200 mg.+ 15 ml. of bi-distilled water + 0,25 ml of 25 % ammonia, 10 min mixing on temperature +70º Celsius.

Staining technologies

1. Fluorescent reaction with TSQ. 0,04 % ammonia solution of TSQ was used. Staining procedures: a few drops of TSQ solution place on frozen sections for 10 sec.; 3 times wash by distilled water and investigation on UV-light microscope with measuring of intensity of fluorescence (intensity of fluorescence in control was accepted for 1,00; length of wave of light — 360 nanometers. For quantitative estimation of results of measuring intensity of fluorescence parameter K was calculated as relation: Intensity of fluorescence of B-cells IF1/ Intensity of fluorescence of exocrine tissue cells IF2 (IF1/IF2); intensity of staining of exocrine tissue cells was accepted for 1,00 using of histofluorimetrical complex constructed by G.G.Meyramov and coll. [13] (Fig. 1).



1 — UV-lamp; *2* — photo-electronic multiplier; *3* —diaphragm for a choice of B-cells; *4* — electric device

Figure 1. Histofluorimetrical complex for measurement amount of zinc-insulin complex in pancreatic B-cells

2. For insulin staining the Immunohistochemical (anticorps for insulin from DAKO, Denmark) and Pseudoisocyanine [14] (SERVA, Germany) methods was used. For quantitative estimation of results of measuring intensity of fluorescence parameter K was calculated as relation: Intensity of fluorescence of B-cells IL1/ Intensity of fluorescence of exocrine tissue cells IL2 (IL1/IL2); intensity of staining of exocrine tissue cells was accepted for 1,00. For quantitative estimation of results of measuring density of staining B-cells by Immunohistochemical method parameter K was calculated as relation: Density of staining of B-cells IG1/ Density of staining of exocrine tissue — IG2 (IG1/IG2); intensity of staining of exocrine tissue cells was accepted for 1,00.

For histological analysis Victoria-4 histochemical method (MERCK, Germany) was used [15–18].

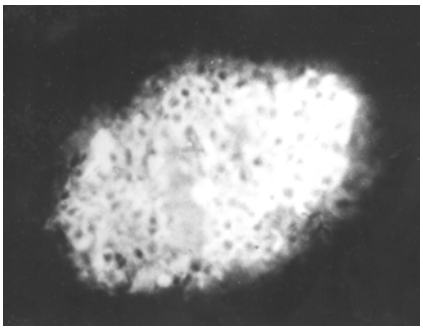
Results

1. Group 1. Immediately past injection of Dithizon (DZ) negative reaction for zinc ions was revealed in B-cells contrary to positive reaction for insulin (fig.2.1, 2.2) that is determined by binding of all amount of zinc in cells by DZ. As result zinc ions not formed with TSQ visible fluorescent complex in cytoplasm of B-cells. Intensity of fluorescence of B-cells: K(IF1/IF2) = 1,04±0,02; control: intact B-cells: K = 2,02±0,07 ($p < 0,001$). Insulin content in B-cells: K(IG1/IG2) = 1,88±0,02 IL1/IL2 = 2,06±0,08 (Table).

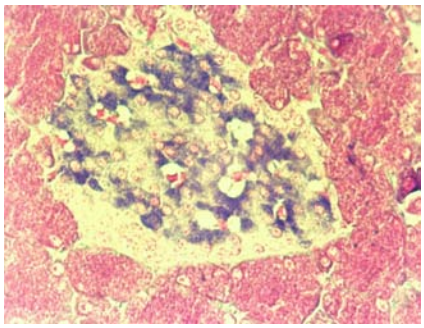
2. Group 2. Animals with diabetes caused by injection of DZ (50,2 and 47,6 mg/kg) 7 days ago. Negative reaction as for zinc-ions with TSQ as for insulin revealed in B-cells on frozen sections of pancreas tissue (fig. 2.3, 2.4) that demonstrated absence in cytoplasm of B-cells as of zinc-ions as of insulin in result of necrosis and destruction of cells: K(IF1/IF2) = 1,08±0,03; control: intact B-cells: K = 2,00±0,08 ($p < 0,001$). Insulin content in B-cells: K(IG1/IG2) = 1,12±0,02; IL1/IL2 = 1,07±0,06 (Table).

3. Group 3. Injection of Na salt of diethyldithiocarbamic acid (DCA) result forming in cytoplasm of B-cells of not toxic complex zinc-DCA for a few hours. A negative reaction for zinc-ions (fig. 2.5) was revealed in B-cells past injection of DCA that is explained by formation of complex zinc-DCA that is why TSQ not formed fluorescent complex zinc-TSQ in cells [7]: K = K(IF1/IF2) = 1,02±0,04; control: intact

B-cells: K = 1,98±0,05 (p < 0,001). Positive reaction for insulin (fig. 2.6). Insulin content in B-cells: K(IG1/IG2) = 1,85±0,04; IL1/ IL2 = 2,02±0,07 (Table).



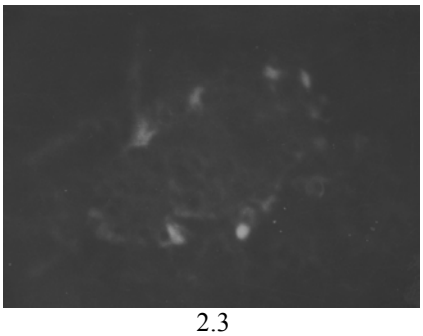
2.1



2.2

2.1 — Pancreatic islet of intact rabbit. TSQ fluorescent reaction for zinc. Intensive fluorescence (a large amount of zinc in B-cells); UV-light microscopy; ×140;

2.2 — Pancreatic islet of intact rat. Victoria-4 staining method. Normal content of deposited insulin in B-cells (blue-violet color); ×280;



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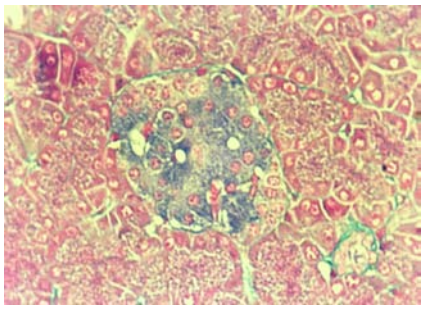
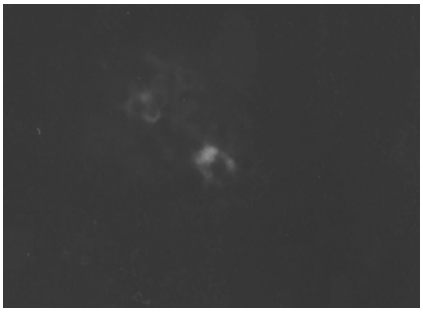


2.4

2.3 — Pancreatic islet of diabetic rats. TSQ fluorescent reaction for zinc. Completely negative reaction for zinc (absence of fluorescence) determined by destruction of B-cells and by absence of zinc-ions in cytoplasm; UV-light microscopy; ×140;

2.4 — Pancreatic islet of diabetic rats. Victoria-4 staining method. Decreasing of insulin content in B-cells and of size and number of islets in sections; ×280;



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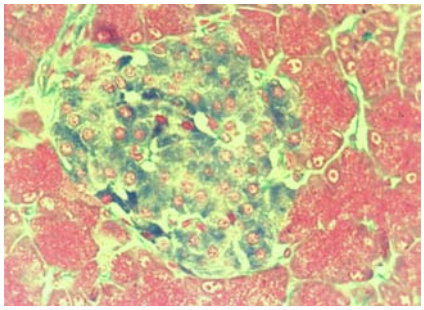

2.6

2.5 — Pancreatic islet of rabbit. DCA 987 mg/kg. TSQ fluorescent reaction for zinc. Completely negative reaction for zinc (absence of fluorescence) determined by not diabetogenic temporary binding of zinc-ions by DCA; UV-light microscopy; ×140;

2.6 — Victoria-4 staining method. Normal content of deposited insulin in B-cells (blue-violet color); ×280

Figure 2. Zinc-ions and insulin content in B-cells of intact and experimental rats

4. Group 4. B-cells of Guinea Pig contrary to many other animals (rabbit, rat, dog, fish, cat, hamster, porcine) not contained zinc ions and biochemical nature of processes of insulin storage in B-cells of Guinea Pig now not cleared yet.

Negative reaction for zinc-ions with TSQ was revealed in B-cells: K = (IF1/IF2) = 0,98±0,04; control: intact B-cells of rat: K = 1,97±0,06 (p < 0,001). Insulin content in B-cells: K(IG1/IG2) = 1,82±0,04; IL1/IL2 = 1,91±0,05 (Table).

T a b l e

Insulin and Zinc content in pancreatic B-cells (parameter K)

No.	Conditions of experience	Insulin (IG, IL) and Zinc content (IF) in B-cells (parameter K)		
		insulin (IG)	insulin (IL)	zinc (IF)
1	5 min. past injection of DZ	1,88±0,02*	2,06±0,08*	1,04±0,02
2	Diabetes caused by DZ (48,8–51,6 mg/kg)	1,12±0,02*	1,07±0,06*	1,08±0,03*
3	DCA (987 mg/kg)	1,85±0,04	2,02±0,07	1,02±0,04
4	Guinea Pig (intact)	1,82±0,04	1,91±0,05	0,98±0,04
5	Rabbit (intact)	1,92±0,04	2,02±0,06	1,98±0,06*

Note. * — p < 0,005.

Discussion

In 1961 E.Boshevolnov and G.Serebrarakova informed about ability of TSQ, a derivative of 8-oxyquinolin, to form in vitro complexes with Zn^{+2}-ions and with ions of Cadmium (Fig. 3, 4).

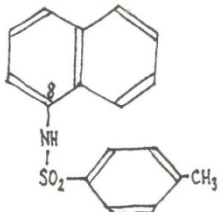

Figure 3. 8-para(toluene-sulphonylamino)quinolin (TSQ)

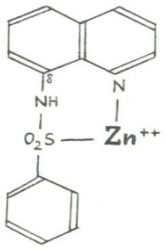

Figure 4. Complex Zn^{+2} – 8-para(toluene-sulphonylamino)quinolin (TSQ)

Zn^{+2}-TSQ complex radiates intensive green fluorescence under UV-light 360–370 nm length of wave and Cd-TSQ — intensive yellow fluorescence that was confirmed by spectral analysis of spectrum of absorbance. Past long time prolonging testing in Institute of High Pure Chemicals (Moscow) TSQ was proposed as fluorescent reagent for identification of very small amounts of zinc in solutions and tissues. Later in laboratory of Lasaris Y.A. and coll., Karaganda, TSQ was tested for revealing in vitro and in intact and diabetic animals of a large amount of zinc-ions. TSQ is high specific reagent for staining of zinc-ions in pancreatic B-cells. Now there are not other methods for revealing of zinc-ions in B-cells. It is known that zinc-ions take part in processes of storage of insulin by formation of complex zinc-insulin in B-cells. Very often there are parallelism between content of zinc and insulin in cytoplasm of B-cells and is possible to stain insulin in B-cells for estimate a content of zinc-ions in cells.

Results of using for many years of this method revealing of zinc-ions showed that in 3 cases method demonstrated a full coincidence with content of insulin in B-cells: 1) in intact animals; 2) in animals with experimental diabetes; 3) in animals after removing of zinc-insulin complex from B-cells by drugs. That is why this method can be used not only for estimate of zinc-ions content in B-cells but for insulin content too.

In one case results of TSQ-reaction can not correspond to quantity contained of zinc-ions in B-cells: some chemicals formed complexes with zinc in B-cells for short period and this time in fluorescent reaction for zinc will be negative despite of presence a large amount of metal in cytoplasm of cells.

This method demands following conditions: for fixing of tissue of a pancreas to use the alcohol sated with hydrogen sulfide (H_2S) or to use sections of frozen pancreas tissue. Filters for UV-microscopy: UV-filter between UV-lamp and microscope and yellow filter for ocular of microscope.

Acknowlegement

Authors are thankful for methodical assistance and financial supporting of investigation to Prof. G.G.Meyramov.

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Панкреаттық В-жасушаларда Zn^{2+} иондарын бояудың жоғары арнаулы гистохимиялық әдістері

Авторлар В-жасушаларда мырыш иондарын анықтайтын жоғары арнаулы люминесцентті әдісті қолданудың нәтижелерін көрсеткен. Бір мезгілде тәжірибелік диабетте В-жасушаларда және осы кешенді В-жасушалардан жұмылдыру кезінде де мырыш ионы мен инсулин мөлшерінің төмендейтіні көрсетілген. Сонымен қатар жасушаларда мырыш иондарының диэтилтиокарбоамин қышқылының туындыларымен байланысы — мырыш иондарына күрт кері әсер, ал инсулинге айқындалған оң әсер етуімен қатар жүреді.

А.Г.Мейрамова, К.-Д.Конерт, А.М.Айткулов, А.Р.Алина, Г.О.Жузбаева,
З.Т.Кыстаубаева, А.П.Андреева, О.Л.Коваленко, А.А.Жузжасарова, С.С.Тыржанова

Высокоспецифичный гистохимический метод окраски ионов Zn^{+2} в панкреатических В-клетках

Авторами показаны результаты использования высокоспецифичного люминесцентного метода выявления ионов цинка в В-клетках. Отмечено, что одновременное параллельное снижение содержания ионов цинка и инсулина наблюдается в В-клетках при экспериментальном диабете и после мобилизации комплекса из В-клеток. Между тем связывание ионов цинка в клетках производными диэтилдитиокарбаминовой кислоты сопровождается резко отрицательной реакцией на цинк с сохранением хорошо выраженной положительной реакцией на инсулин.

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UDC 613.2:612.359

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Long time prolonged elimination of zinc-insulin complex from B-cells not result dysfunction of cells

Authors showed that almost complete elimination of zinc-insulin complex from cytoplasm of B-cells caused by 3 days prolonged administration of Glibenclamide to animals accompanied by complete disappearing of insulin and zinc-ions from B-cells. Next 6–7 days free of using of Glibenclamide result parallel complete recovery of amount of insulin and zinc in B-cells without any changes of histostructure of islets and function of B-cells.

Key words: rats Vistar, histology, insulin, B-cells, dissociation of complex, secretion, pancreatic islet, histostructure.

Pancreatic B-cells of many sorts of animals and of human contained a large amount of zinc-ions which take part in process of forming deposited form of insulin in cells which concentrated in B-granules of cells. In pancreas tissue B-cells contain large amounts of zinc. The major role of zinc is the binding of insulin in hexamers [1]. Zinc ions and insulin create a hexameric, crystalline structure, comprising 2 zinc ions and 6 insulin molecules, which is stored in the secretory granules until secreted in response to metabolic demands [2]. Zinc in B-cell secretory granules is involved in the storage and stabilization of the insulin hexamere in B-cells [2, 3]. Zinc ions appear to play important significance in process of microcrystallization of the precipitated insulin granules. May be it is advantage in condensing the stored hormone [2].

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It is known that prescription of Sulphorea result elimination of complex insulin-zinc from B-cells [4]. It was showed that 1 week past of partial or almost complete elimination of zinc-insulin complex from B-cells, function of cells are restored. It is not investigated question: are eliminated from B-cells zinc and insulin, insulin only or insulin and part of zinc-ions. Previously it was reported that binding of zinc-ions in B-cells by diabetogenic or not diabetogenic chelat active chemicals result a complete binding of zinc-ions and dissociation of complex 1,5–2 h later: chemicals are removed from cells and zinc remains in the cytoplasm of B-cells [5].

Meanwhile now is not cleared what is insulin and zinc content in cytoplasm of B-cells more long time after elimination of insulin from B-cells.

Aim of work: to investigate insulin and zinc ions content as state of histostructure of pancreatic islets more long period later, as 30 days, after almost complete elimination of zinc-insulin complex from B-cells.

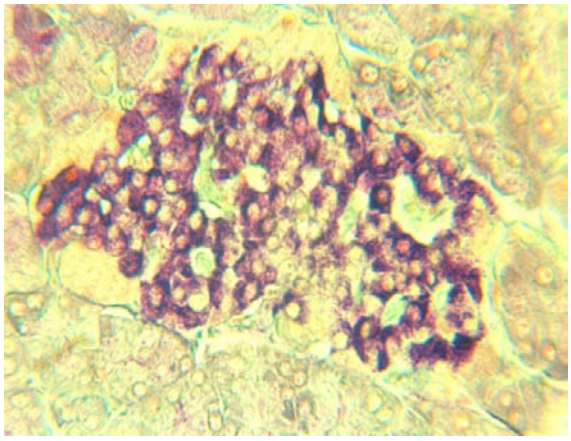
Materials and methods

26 rats Vistar 170–185 g were used. 2 % starch suspension of Glibenclamide (GB) used for peroral administration to animals in doses 10 and 25 mg/kg for 6 days 1 time daily. Blood Glucose control: 3 h, 24 h, 3 days 15 days and 30 days after past administration of GB). Histostructure of pancreas tissue and insulin content in B-cells were studied 30 days after administration of GB.

Histology. Pancreas tissue were fixed in Bouin. Sections 4–5 mcm were stained by hematoxylin and eosine; insulin staining by aldehyde fucshine [6], pseudoisocyanine [7–9] and zinc-ions — by 8-para(toluenesulphonylamino)quinolin (TSQ) [9]. Intensity of fluorescence of insulin and of zinc-ions was measured by fluorescent histofluorimetric complex constructed by G.G.Meyramov [10]. For transmission electron microscopy samples of pancreas tissue were fixed in 2,5 % Gluthar-aldehyde. Ultrafine sections of tissue contrasted by Reynolds [11] and were investigated on electron microscope JEM-7A.

Results

Blood Glucose concentration past administration of GB. Results showed that maximal decreasing of BG concentration was observed 3 h past first administration of 10 mg/kg (-approx. 20 %) and of 25 mg/kg (-28 %). 24 h past administration there are some not authentically prevalence of BG level in comparison before administration of GB. Next period from 3rd days until 30th days BG concentrations was not changed.



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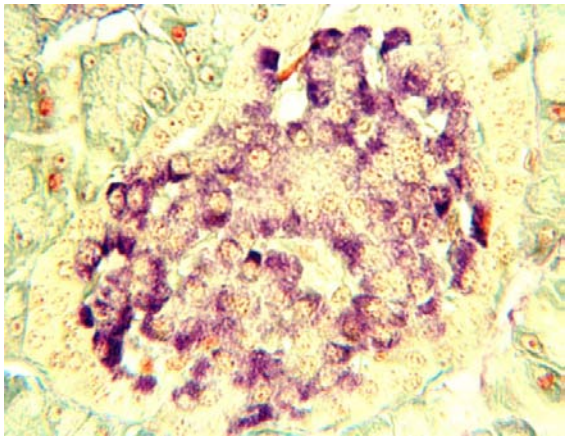

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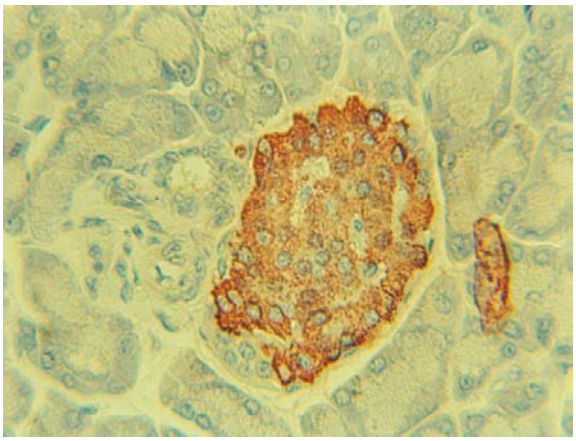

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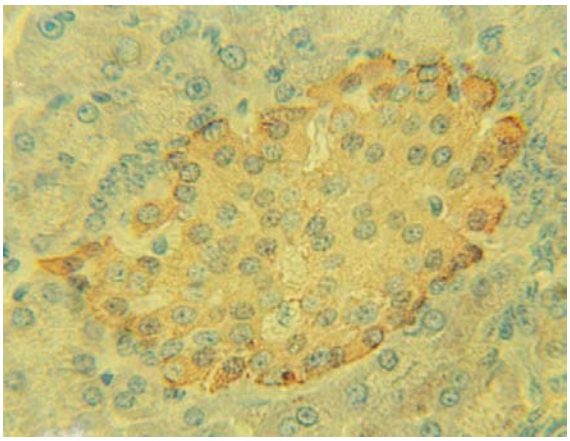

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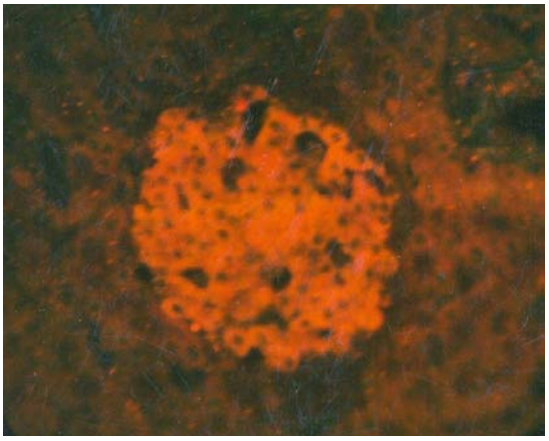

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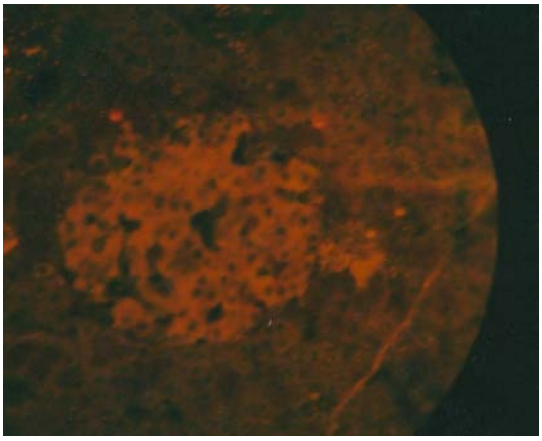


1.6

1.1 — Pancreatic islet of intact rat. Aldehydefucshine; ×280;
1.2 — Pancreatic islet 3h past action of GB 10 mg/ kg. Decreasing of insulin content in B-cells. Aldehydefucshine; ×280;
1.3 — Pancreatic islet of intact rat. Immunohistochemistry; ×280;
1.4 — Pancreatic islet 3h past action of GB 25 mg/kg. Decreasing of insulin content in B-cells. Immunohistochemistry; ×280;
1.5 — Pancreatic islet of intact rat. Pseudoisocyanine; ×200;
1.6 — Pancreatic islet 3h past action of GB 10 mg/kg. Decreasing of insulin content in B-cells. Pseudoisocyanine; ×200

Figure 1

Table 1

Blood Glucose concentration past administration of GB

	Dose	Blood Glucose concentration, mM					
		before	3 h	24 h	3 days	15 days	30 days
1	Control (intact)	5,1±0,31	5,0±0,21	4,9±0,14	5,2±0,28	5,1±0,25	5,1±0,32
2	GB, 10 mg/kg	4,7±0,22	3,8±0,16 $p < 0,05$	4,8±0,15	4,8±0,23	4,9±0,17	4,6±0,23
3	GB, 25 mg/kg	4,6±0,15	3,3±0,14 $p < 0,05$	4,9±0,12	5,1±0,29	4,8±0,15	4,5±0,13

Table 2

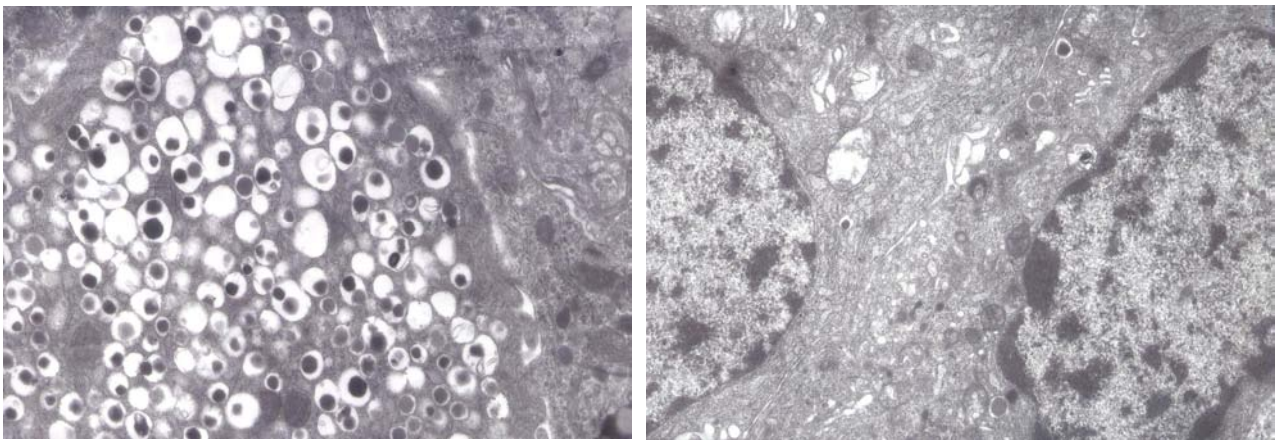
Insulin and Zinc content in pancreatic B-cells past administration of GB (parameter K)

	Condition	Insulin and Zinc content in B-cells (K)					
		before GB		3 h after GB		30 days after GB	
		insulin	zinc	insulin	zinc	insulin	zinc
1	GB, 10 mg/kg	2,04±0,05**	2,00±0,03	•1,62±0,04**	•1,71±0,03	1,94±0,06	1,87±0,05
2	GB, 25 mg/kg	1,95±0,06*	1,97±0,05	1,38±0,04*▪	1,62±0,04▪	2,00±0,07	1,96±0,06
3	Intact	2,02±0,05	1,98±0,06	1,96±0,07	1,94±0,08	1,97±0,04	2,02±0,05

Note. * — $p < 0,01$; ** — $p < 0,05$; ▪ — $p < 0,05$; • — $p < 0,05$.

Results of estimation of insulin and zinc content in B-cells showed evident decreasing amount as of insulin as of zinc-ions 3 hours after GB administration (Table 2; Fig. 1.1–1.6; Fig. 2.1, 2.2). Decreasing is more marked past administration of 25 mg/kg — for almost 30 % comparatively with approximately 20 % past ad- ministration of 10 mg/kg. Results showed that a coincidence of results between the contents in B-cells of insulin and zinc is available only for intact B-cells and after 24 h and 30 days past administration of drug. 3 hours after administration of GB results showed more intensive decreasing of insulin content comparatively with content of zinc-ions (Table 2). Results obtained before action of GB were authentically prevailed in compared with zinc ions past administration of both doses of GB. 30 days past administration of both doses of GB insulin and zinc content in B-cells were restored completely. There are not any histological changes in pancreatic islets 30 days after action of GB (Fig. 1.2, 1.4).

We found discrepancy more marked differences between results measuring of insulin and zinc-ions content 3 h past action of GB 25 mg/kg (Table 2): approximately 30 % of insulin are eliminated from B-cells and 18–20 % of zinc-ions only eliminated contrary to parallelism of results 30 days past action of GB. It is possible to suppose that part of amount of zinc-ions after dissociation of complex is eliminated from B-cells and part remain in cells (Fig. 3).



2.1 2.2

2.1 — Intact pancreatic islet. B-cells. Large number of B-granules contained zinc-insulin complex; ×3450;
2.2 — Islet 3 h past administration to animal of GB 25 mg/kg. Marked decreasing of number of B-granules; ×2920

Figure 2. Transmission electron microscopy

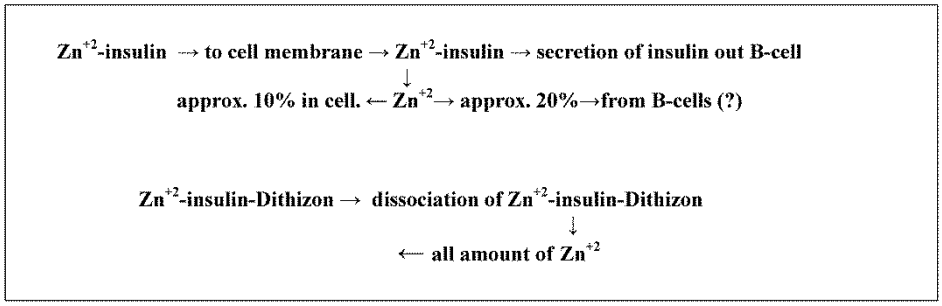


Figure 3. Dissociation of complexes Zn^{+2}-insulin and Zn^{+2}-insulin-Dithizon in B-cells

Conclusions

1. Elimination of insulin from B-cells by GB accompanied by partial decreasing of zinc-ions content 3 h after action and completely is restored 30 days later. Insulin content in B-cells reduced for approximately 30 % and of zinc-ions — for 18–20 % 3 hours after administration of 25 mg/kg of GB.

2. There are not any histological changes of histostructure of pancreatic islets 30 days past elimination of zinc-insulin complex from B-cells. Amount of insulin and zinc-ions in B-cells restored completely 30 days later.

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В-жасушалардан мырыш-инсулин кешенінің ұзақ уақыттағы элиминациясы және олардың функциясы бұзылуына катысының жоқтығы

Авторлар «Глибенкламидті» үш күндік енгізуден кейін В-жасушаларынан мырыш-инсулин кешенінің элиминациясы цитоплазмадан мырыш пен инсулиннің толықтай жоғалуына әкеліп соққандығын анықтады. Препаратты енгізуді тоқтатқаннан кейін жасушалардағы мырыш пен инсулиннің мөлшері

толықтай орнына келді. Панкреатиттік өсінділердің гистоқұрылысының жағдайында ешқандай өзгерістер байқалмады.

Г.Т.Тусупбекова, А.М.Айткулов, Л.Вильямс, В.И.Корчин, Л.Г.Тургунова,
З.Т.Кыстаубаева, Г.О.Жузбаева, О.Л.Коваленко, А.Ж.Шайбек,
А.М.Тулиева, К.Т.Кошебаева

Длительная элиминация цинк-инсулинового комплекса из В-клеток, не сопровождающаяся нарушениями их функций

Авторами установлено, что элиминация из В-клеток цинк-инсулинового комплекса, вызванная трехдневным введением Глибенкламида, сопровождалась полным исчезновением из цитоплазмы инсулина и цинка. После прекращения введения препарата содержание цинка и инсулина в клетках полностью восстанавливалось. Никаких изменений состояния гистоструктуры панкреатических островков не выявлено, как и инсулинпродуцирующей функции В-клеток в последующем.

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2013 жылғы «Қарағанды университетінің хабаршысында» жарияланған мақалалардың көрсеткіші. «Биология. Медицина. География» сериясы

ГЕОГРАФИЯ

МЕДИЦИНА

МЕРЕЙТОЙ ИЕСІ

Указатель статей, опубликованных в «Вестнике Карагандинского университета» в 2011 году. Серия «Биология. Медицина. География»

БИОЛОГИЯ

ГЕОГРАФИЯ

МЕДИЦИНА

К ЮБИЛЕЮ УЧЕНОГО