## The use of bioflavonoid diquertin in the complex therapy of type 2 diabetes mellitus

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The progression of the prevalence of type 2 diabetes mellitus (DM) has acquired the character of a "non-communicable epidemic" and, according to WHO experts, the number of patients with type 2 diabetes should double from 143 to 380 million people from 1997 to 2025 [1]. The course of DM is complicated by the development of specific vascular complications, the so-called microangiopathies, and the rapid progression of atherosclerosis, leading to cardiovascular mortality in patients with DM 4–5 times more often than in the general population. The severity of type 2 diabetes is aggravated over time not only by the progression of micro- and macroangiopathies, but also by the increase in insulin deficiency, leading to the need for insulin replacement therapy. According to statistics, annually 5-10% of patients with type 2 diabetes need to be transferred to insulin therapy. Thus, after 10–20 years from the onset of the disease, each patient with type 2 diabetes needs insulin.



At present, the role of chronic hyperglycemia in the development of diabetic vascular complications is generally recognized, and more and more data appears confirming the damaging effect of "glucose toxicity" on the secretory capabilities of the insular apparatus. The mechanisms of the damaging effect of chronic hyperglycemia remain largely unclear, but it is assumed that free radicals formed during glucose autoxidation play an important role in the development of these disorders. The powerful cytotoxic effect of free radicals, used by nature to destroy pathogenic microorganisms and its own defective mutant cells, is fraught with potential danger, since the uncontrolled leakage of free radicals can lead to irreversible damage to lipid molecules, proteins and nucleic acids. That is why in a living organism there are regulatory mechanisms that limit the accumulation of these highly toxic products: these are natural antioxidants, such as vitamins C, E, glutathione and antioxidant enzymes (catalase, superoxide dismutase - SOD and glutathione peroxidase - GP). Excessive accumulation of free radicals, leading to the development of oxidative stress, in DM may be due, on the one hand, to glucose autoxidation under conditions of hyperglycemia and, on the other hand, to a decrease in the activity of antioxidant defense.

The pathogenesis of type 2 diabetes, according to modern concepts, is due to two key disorders: the development of insulin resistance in peripheral target tissues and inadequate secretion of insulin necessary to overcome the barrier of insulin resistance. Both of these defects mutually reinforce each other: due to compensatory hyperinsulinemia, insulin resistance is aggravated, and due to a decrease in insulin sensitivity, the need for insulin secretion increases [2]. The resulting hyperglycemia, which causes oxidative stress due to glucose autoxidation, leads to damage to the phospholipid layer of the plasma membranes of target tissues and b cells, contributing to the progression of insulin resistance and a decrease in the secretory capabilities of the insular apparatus due to apoptosis of b cells. By reducing the severity of oxidative stress with the help of antioxidant therapy, it is theoretically possible not only to slow down the progression of diabetic vascular complications and insulin deficiency, but also to reduce the resistance of cells to insulin, thereby contributing to a better compensation of carbohydrate metabolism.



Numerous studies are devoted to the study of the angioprotective and antioxidant properties of natural flavonoids, including those in diabetic micro- and macroangiopathy [3, 4]. The studies of Robak and Gryglewsky (1988) showed that natural flavonoids, in contrast to non-flavonoid antioxidants, have a more pronounced effect due to the fact that they not only “trap” oxygen free radicals, its so-called active forms (AR), but also favorably affect both the vascular wall and the hemostasis system [4]. This explains the great interest that is manifested today in natural flavonoids, in particular in the study of their properties.

Angioprotective Flavonoids
are polyphenols of plant origin. The features of the antioxidant action of these substances are that they can inactivate not only hydroperoxide (LO2•) and alkoxyl (LO•) lipid radicals, but also the superoxide anion radical (O2•) [5]. The presence of antiradical properties in extracts of some plants is based on the fact that the chemical structure of flavonoids contains an aromatic ring and OH groups attached to it, which are able to inhibit the processes of lipid peroxidation (LPO) at the stage of oxygen initiation and active [6].



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A possible mechanism for the utilization of oxygen radicals is the ability of the hydroxyl compound of flavonoids to donate a hydrogen atom and bind more toxic compounds, thus neutralizing them [7].



Phenolic antioxidants are commonly referred to as any Ar(OH)n compounds in which one or more hydroxyl groups (OH) are connected to an aromatic nucleus (Ar), while the molecules may contain several Ar(OH)n fragments. An analysis of the comparative activity of flavonoids showed that the presence of two hydroxyl groups in the B-ring is very important.

ortho positions in and hydroxyl reaction In groups in positions C-3 [6].
the reaction ÿrOH + RO2ÿ•ArO2• + ROOH, no disappearance of the free valence is observed, and only the replacement of the hydroperoxide radical RO2• by phenoxy ArO2• takes place, however, in this case, the effect of inhibition of free radical oxidation is achieved, due to the greater stability of ArO2•, which practically does not participate in continuation of oxidation chains. Flavonoids can restore the activity of L-tocopherol by donating a hydrogen atom L to the tocopherol radical [7], the latter is formed when it donates its own hydrogen atom from the hydroxyl group to the peroxyl radical, thus interrupting the LPO chain. Also a possible mechanism of action of flavonoids Cu.



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Dihydroquercetin (DKV; diquertin is a domestic drug produced by JSC Plant of Ecological Equipment and Ecological Nutrition "DIOD", Moscow) is a 3, 3, 4, 5, 7-pentahydroxyflavone, which is obtained from the crushed wood of Siberian larch (Larix cibirica L). According to the chemical structure, DHQ is a compound related to quercetin and is its analog hydrogenated at the heterocyclic fragment. In addition, in terms of its chemical properties, DHQ is an active antioxidant [8]- free radical scavenger. In the work of V.K. Kolkhir et al. (1995) revealed capillaroprotective and antioxidant properties of DHQ (in some cases exceeding the effect of quercetin), combined with anti-inflammatory, gastro- and hepatoprotective, hypolipidemic and diuretic effects [9]. It probably has direct antiradical activity, mainly due to interaction with lipid radicals. At the same time, DHQ, like quercetin, is a scavenger of superoxide anions [8]. As a substance with a high degree of biological activity, DHQ has a number of positive effects on metabolic reactions and the dynamics of pathological processes. Its ability to reduce the content of low-density lipoproteins (LDL) in the blood [2] makes it possible to consider DHQ derivatives as prophylactic and therapeutic agents against atherosclerosis. The ability of the drug to inhibit the oxidation of the liposomal membrane from egg phospholipids induced by ferrous sulfate or the Fe2+-ascorbate system was noted. Moreover, the antioxidant activity of DHQ is comparable to that of a-tocopherol. It was also found that taxifolin, an analogue of DHQ, inhibited AR in [3]. In the lens of erythrocytes, we have previously demonstrated the ability of the natural bioflavonoid diquertin to suppress the activity of lipid peroxidation processes in the membranes of erythrocytes and platelets of patients with type 2 diabetes, which manifested itself in a decrease in the content of malondialdehyde (MDA) in the cell membrane, an increase in the activity of the key antioxidant enzymes SOD, catalase and



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GP in erythrocytes, a decrease in platelet aggregation activity associated with a decrease in the calcium content in platelets and thromboxane production [10]. The use of diquertin in the complex therapy of patients with type 2 DM contributed to a decrease in the activity of the Na+/H+ exchanger in the erythrocyte membrane and an increase in NO production, determined by the level of nitrites and nitrates in the patients' plasma [11, 12]. All this convincingly proves the positive effect of DHQ on the functional activity of formed elements, blood rheology, and endothelial dysfunction in DM, which helps slow down the progression of diabetic vascular complications, as we have shown in the example of diabetic preproliferative retinopathy in patients with type 2 DM [12]. However, the most effective result of using diquertin in comparison with other antioxidants was a significant decrease in HbA1c levels by 7% from baseline ($p<0.05$), without changing the dose of concomitant hypoglycemic therapy [10].

All of the above prompted us to investigate the possible effects of diquertin on insulin sensitivity and the secretory capabilities of the insular apparatus, comparing them with the antioxidant activity of the drug.



The purpose of this study is to study the effect of dihydroquercetin (dikvertin) on the oxidative status, insulin sensitivity and secretory capacity in patients with type 2 diabetes.

Materials Methods

The study included 40 pre-compensated (HbA1c 6.69±0.2%) patients (16 men and 24 women) aged 56.2±8.5 years with DM duration 0.4±0.12 years. Body mass index (BMI) was 33.3±6.3 kg/m2. Study participants were randomly randomized to receive diquertin 120 mg daily for 12 weeks in addition to oral hypoglycemic therapy (metformin at a daily dose of 2000–2500 mg), or to a comparison group (no antioxidant therapy was performed). As a control, we examined 20 healthy volunteers, comparable in age, who had no indications of impaired carbohydrate tolerance and the presence of DM in relatives.



Before and at the end of the study, HbA1c was monitored using a DCA 2000 Analyzer (Bayer) using latex inhibition of immunoagglutination using the Hemoglobin A1c Reagent Kit. Blood serum lipids were determined by the enzymatic method using Boehringer-Mannheim kits. The content of the secondary product of free radical lipid oxidation, MDA, in LDL was determined by the reaction with 2-thiobarbituric acid at 532 nm on a Hitachi 557 instrument [13]. To assess insulin sensitivity before and after a course of antioxidant therapy, we used the calculated mathematical models HOMA-IR (Homeostasis Model Assessment) [14] and ISI (Insulin Sensitivity Index) [15], which, according to most researchers, most clearly correlate with " gold standard" in assessing insulin [16]. sensitivity To assess the secretory capabilities of the insular apparatus, the index of basal insulin secretion was used -

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ISecrHOMA [14] and insulin release index - InsulinoGenic Index (IGI), determined by the ratio of the area under the curve of insulin response to the area under the curve of glycemic fluctuations during the oral glucose tolerance test (OGTT) [15]. The IRI level was determined by radioimmunoassay using Immunotec kits (Hungary).



Statistical processing was carried out on a computer using a special statistical package "SPSS 9.0" (SPSS Inc., USA). To determine the significance of differences between the compared groups, Student's t-test was used. The reliability of dynamic changes in the studied parameters before and after treatment was determined using non-parametric methods of analysis of variations (Wilcoxon test). Differences were considered significant at $p<0.05$. tables ÿ±m.

All averages are presented values. Results discussion in in form



As mentioned above, patients were included in the study only if satisfactory compensation of carbohydrate and lipid metabolism was consistently achieved in accordance with the criteria of the European Diabetes Policy Group (1998). At the same time, despite satisfactory indicators of carbohydrate and lipid metabolism in patients with type 2 diabetes, dyslipidemia persists, manifested in hypercholesterolemia and hypertriglyceridemia, along with an increase in LDL and a decrease in high-density lipoprotein (HDL) levels compared with donors ($p<0.001$; table . one)). The appointment of natural flavonoids led to a significant decrease in MDA in plasma lipoproteins, a decrease in cholesterol (CH) and 1).

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normalization of the blood lipid spectrum revealed by us, which manifested itself in a significant decrease in cholesterol, triglycerides and an increase in HDL against the background of a decrease in LDL ($p<0.05$), indicates a hypolipidemic effect of the drug, which confirms the data of K.Igarachi et al. [5], who revealed a decrease in LDL in the blood plasma and liver of rats under the influence of DHQ. It is known that at

Under oxidative stress, free radical oxidation of lipids, leading to the accumulation of lipid peroxides, inhibits the key enzyme of cholesterol catabolism in the liver, microsomal 7a-hydroxylase [17], which disrupts the enzymatic regulation of cholesterol catabolism and should lead to the maintenance of its stable high level in the blood. Under these conditions, hepatocytes can secrete into the bloodstream very low density lipoproteins (VLDL), including oxidized LDL, which undergo oxidative degradation to form MDA. It is possible that blocking free radical lipid oxidation with the help of flavonoids, which is manifested by a significant decrease in the formation of MDA, removes the toxic effect of lipoperoxides on hepatic 7a-hydroxylase and thereby contributes to an increase in cholesterol catabolism and an increase in HDL, as well as a decrease in hepatic production of VLDL.



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In the treatment of patients suffering from type 2 diabetes with diquertin, a significant decrease in HbA1c from 6.69±0.15 to 6.124±0.096% ($p<0.01$) was obtained, accompanied by an improvement in glycemic control, according to the data of basal and postprandial glycemia, which may be due to a decrease in the production of reactive oxygen species when carbohydrate metabolism is compensated and, as a result, a decrease in the formation of AGEs, which include HbA1c. However, the reduction of basal glycemia without changing the dose of concomitant hypoglycemic therapy, as shown in Table. 2, suggests an increase in the sensitivity of peripheral tissues and, first of all, the liver to circulating insulin, which determines. Based on this assumption, we calculated insulin sensitivity by two methods - HOMA-IR and ISI [14, 15]. In order to exclude the variability of changes, insulin sensitivity was assessed simultaneously with OGTT, during therapy.



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As can be seen from the table. 2 data, a significant decrease in the insulin resistance index (IR) according to the HOMA model was obtained from 3.05±0.39 to 1.61±0.25 ($p<0.005$) and an increase in the insulin sensitivity index ISI from 87.3±14.1 up to 128.2±24.1 observed 1).

($p<0.05$). group comparisons such changes not (rice.

In order to confirm the relationship between the severity of oxidative stress and insulin resistance, we conducted a correlation analysis between the level of the secondary product of lipid peroxidation - MDA in LDL and the HOMA IR insulin resistance index. We obtained a direct correlation between the level of MDA in LDL and the HOMA-IR index with a correlation coefficient of $r=0.755$ ($p<0.005$). Thus, by reducing the manifestations of oxidative stress with the help of the flavonoid antioxidant diquertin (DHQ), we simultaneously obtained a decrease in insulin resistance, significantly correlated with stress.



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In the group of patients treated with diquertin in combination with metformin, we noted a significant decrease in HbA1c by 7% from baseline ($p<0.05$) without changing the dose of concomitant hypoglycemic therapy. The mechanism of such a positive effect of diquertin on carbohydrate metabolism may be associated with a decrease in insulin resistance of peripheral tissues, which we identified when calculating the HOMA insulin resistance index, which is indirectly confirmed by a decrease in the index of basal insulin secretion calculated by this method. It seemed interesting to us to reveal the effect of the antioxidant flavonoid diquertin on the secretory capabilities of the insular apparatus. To this end, in patients who achieved compensation of carbohydrate metabolism while taking metformin, we performed OGTT with the determination of the initial (fasting) concentration



insulin and through cm and 2 h after reception glucose.

After daily OGTT After 3 months of taking patients Diquertin, the OGTT was repeated. The levels of glycemia and insulinemia were determined at the same time intervals. As can be seen from the data presented in Table. 3 and in fig. 2, after a 3-month course of diquertin, a significant increase ($p<0.05$) in stimulated insulin secretion, expressed as a percentage compared to the basal level of IRI, was achieved. At the same time, the level of basal insulinemia decreased compared to the initial level ($p<0.05$), which indicates a decrease in insulin resistance of peripheral tissues. When calculating the insulin release index, defined as the ratio of the area under the insulin response curve (AUCins) to the area under the glycemic change curve (AUCgluc) during OGTT, we obtained a significant increase in the insulin release index (IGI) ($p<0.01$).

To elucidate the possible relationship between oxidative stress and the functional state of the b-cell, we assessed the correlation between the improvement in the secretory capabilities of the insular apparatus by the percentage increase in the level of IRI at the peak of glucose absorption (after 1 hour) with a decrease in the severity of oxidative stress, determined by the level of MDA in LDL plasma of our patients. patients identified on the background of the use of diquertin. The inverse correlation we obtained $r=-0.411$ ($p<0.05$) between the percentage increase in IRI and the decrease in MDA, in our opinion, indicates a positive effect of diquertin on the secretory capabilities of the insular apparatus due to the removal of reactive oxygen species and a decrease in the manifestations of b-cells.

A possible mechanism for the utilization of oxygen radicals is the ability of the hydroxyl compound of flavonoids to increase [7]. donate hydrogen compounds. As mentioned above, the presence of two hydroxyl groups in the ortho positions in the B-ring and a hydroxyl group in the C-3 position is very important for the activity of flavonoids [6]. The new flavonoid diquertin contains hydroxyl groups. Our data coincided with the data of a number of authors [18] and may indicate that polyhydroxylated aglycone flavonoids are powerful LPO inhibitors, emphasizing once again the importance of the hydroxyl group in the flavone core. The hydroxyl group in position 7 dissociates first and is the main site of attack by the peroxyl radical [7, Diquertin contains a hydroxyl group in position 7.

Thus, summarizing the data obtained, we can conclude that the domestic bioflavonoid diquertin has undoubted antioxidant properties, which reduces the risk of progression of diabetic angiopathy, improves glycemic control and insulin sensitivity. At the same time, the inclusion of the antioxidant diquertin in the complex therapy contributed to an increase in the secretory capabilities of the insular apparatus, which allows us to hope for the preservation of residual insulin secretion during long-term use of antioxidant therapy.

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