Antioxidant effects of the bioflavonoid diquertin in the complex therapy of type 2 diabetes mellitus

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Type 2 diabetes mellitus (DM) is one of the most common diseases affecting the population of both economically developed and developing countries. According to JMReaven (17), type 2 DM is a “chronic, incurable, progressive disease”, the course of which

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 type 2 diabetes mellitus 4-5 times more often than in the general population. Severity of type 2 diabetes over time, it is aggravated not only by the progression of micro- and macroangiopathies, but also by the increase in insulin deficiency, leading to the need for replacement insulin therapy. According to statistics annually 5-10% of patients with type 2 diabetes need a transfer for insulin therapy, i.e. already after 10-20 years from the onset of the disease, each patient with type 2 diabetes needs insulin.

The pathogenesis of type 2 diabetes, according to modern concepts, is due to two key disorders: the development insulin resistance in peripheral target tissues and inadequate insulin secretion required to overcome the barrier of insulin resistance. Both of these defects mutually reinforce each other: due to the compensatory hyperinsulinemia exacerbates insulin resistance, by reducing insulin sensitivity, the need for insulin secretion increases (10). The resulting hyperglycemia, which causes oxidative stress for due to glucose autooxidation, leads to damage to the phospholipid layer of the plasma membranes of target tissues and ÿ-cells, contributing to the progression of insulin resistance and a decrease in the secretory capabilities of the insular apparatus due to apoptosis of ÿ-cells. By reducing the severity of oxidative stress with the help of antioxidant therapy, it is theoretically possible not to

only to slow down the progression of diabetic vascular complications and insulin deficiency, but also to reduce insulin resistance, thereby contributing to a better compensation of carbohydrate metabolism.

Numerous studies are devoted to the study of angioprotective and antioxidant properties of natural flavonoids, including in diabetic micro- and macroangiopathy (11,18). Research by J.Robak and RJ Gryglewsky (1988) showed that natural flavonoids have a more pronounced effect than non-flavonoid antioxidants, since not only

"capture" oxygen free radicals, its so-called "active forms", but also favorably affect both on the vascular wall and on the hemostasis system (18) This explains the great interest that is manifested today in natural flavonoids, in particular, in the study of their angioprotective properties. Flavonoids are polyphenols of plant origin. Features of their antioxidant action

are that they can inactivate not only hydroperoxide (LO2•) and alkoxyl (LO•) lipid radicals, but also the superoxide anion radical (O2•) (12). 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 is attached OH-groups attached to it, which are capable of inhibiting LPO processes at the stage of oxygen initiation and transmission electrons from one active form to another (16).

Dihydroquercetin (dikvertin) - DKV - a new domestic patented drug, which is 3, 3, 4, 5, 7-pentahydroxyflavone, which is obtained from crushed wood of Dahurian larch (Larix dahurica T) and Siberian larch (Larix cibirica L). Chemical structure, DHQ is a compound related to quercetin. It is its hydrogenated analog at the heterocyclic fragment. In addition, DKV, in its own way, chemical properties is an active antioxidant (8), i.e., a substance that binds free radicals. IN The work of V.K.Kolkhir et al.(3) revealed capillary-protective and antioxidant properties of DHQ (exceeding in some cases, the effect of quercetin), combined with anti-inflammatory, gastro- and hepatoprotective, hypolipidemic and diuretic effects (3). It probably has direct antiradical activity mainly due to interaction with lipid radicals. IN

same time, DHQ, like quercetin, is a scavenger superoxide anions (8). As a substance with a high degree of biological activity, DHQ has a whole range of positive effects on metabolic reactions and dynamics of various pathological processes. Its ability to reduce the content of low lipoproteins in the blood density (LDL) (11), allows us to consider DHQ derivatives as a means of preventing and treating atherosclerosis. The ability of the drug to inhibit the oxidation of the liposomal membrane from egg phospholipids was noted, induced by ferrous sulfate or Fe2 + - system ascorb, and the antioxidant activity of DHQ is comparable to that of ÿ-tocopherol. It has also been established that taxifollin, an analog of DHQ, inhibited reactive radicals in the rat lens, as well as the accumulation of sorbitol in human erythrocytes (11). Previously, we demonstrated the ability of natural

bioflavonoid DHQ inhibit the activity of lipid peroxidation processes in erythrocyte membranes 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 superoxide dismutase, catalase and glutathione peroxidase in erythrocytes, a decrease in aggregation platelet activity associated with a decrease in platelet calcium and thromboxane production (7). The use of DHQ in the complex therapy of patients with type 2 diabetes contributed to a decrease in the activity of Na+/H+-exchanger in the erythrocyte membrane and an increase in NO production, determined by the level of nitrites and nitrates in blood plasma (1, 2). 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 to slow down the progression of diabetic vascular complications, as it was shown by us on the example of diabetic preproliferative retinopathy in patients with type 2 diabetes (2).

TABLE 1. Dynamics of metabolic parameters during DHQ therapy (M±m)

Indicator	Donors	Comparison group		Group 1 (DKV)	
		after 6.69±0.15	6.69±0.15	5.82±0.54±0.48	1.38±0.28
Hb A1c%	5.5±0.1	1.22±0.23	4.01±0.9	3.68±0.65	0.42±0.29
Cholesterol	4.79±0.45	statistically significant		5.68±0.9#	4.86±0.50 **
(mmol/l) THC	0.94±0.18			1.16±0.27#	0.98±0.22 **
(mmol/l) HDL	1.58±0.26			1.2±0.18#	1.41±0.19**
(mmol/l) LDL	2.78±0.38			3.96±0.75#	3.01±0.55**
(mmol/l) MDA in LDL (nmol/	1.18±0.15			1.613±0.278#	1.211±0.198*

*- mg protein) p< 0.05 compared with baseline,

However, the greatest effect of using DHQ in comparison with other antioxidants showed a significant decrease in HbA1c levels by 7% from baseline (p<0.05), without changing the dose of concomitant sugar-lowering therapy (7).

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

The aim of this study was to study the effect of DHQ on the oxidative status and course of diabetic
retinopathy in patients with type 2 diabetes.

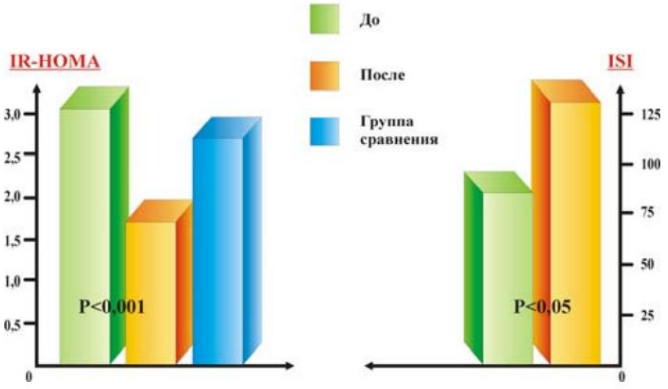
The study included 40 precompensated (Hb A1c - 6.69±0.2%) patients (16 men and 24 women) aged 56.2±8.5 years with DM duration 0.4±0, 12 years old, body mass index 33.3±6.3 kg/m2. Patients were randomly assigned to either

a group receiving in addition to oral sugar-lowering therapy (metformin at a daily dose of 2000-
2500 mg) DHQ at a daily dose of 120 mg for 12 weeks, or in a comparison group that did not receive an antioxidant therapy. As a control, 20 healthy
volunteers comparable in age, without indication of
intolerance to carbohydrates and the presence of sugar
relatives with diabetes.

Before and at the end of the study, Hb A1c was monitored using a DCA 2000 Analyzer (Bayer) by latex inhibition of immunoagglutination using
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(4).

To assess insulin sensitivity before and after the course of antioxidant therapy, we used the calculated mathematical models HOMA-IR (Homeostasis
Model Assessment) (15) and ISI (Insulin Sensitivity Index) (14), which, according to most researchers, most clearly correlate with the "gold standard" in assessing
insulin sensitivity – “euglycemic clamp
technical"(9).

Рисунок 1. Динамика индекса инсулинорезистентности HOMA и чувствительности к инсулину ISI на фоне терапии диквертином



To assess the secretory capabilities of the insular apparatus, we used the basal insulin secretion index, ISecrHOMA (15), and the insulin release index, InsulinoGenicIndex (IGI), determined by the area ratio
under the insulin response curve to the area under the glycemic fluctuation curve during an oral glucose tolerance test (14). The level of immunoreactive insulin (IRI) was determined by radioimmunoassay.
analysis using Immunotek kits (Hungary).

Statistical processing of the results was carried out on computer using the special statistical package SPSS version 9.0 (SPSS inc. USA). To determine the reliability of differences between the compared
groups used Student's t-test. Reliability of dynamic changes in the studied parameters
before and after treatment were determined using nonparametric methods of analysis of variance (Wilcoxon test). Differences were considered significant at p<0.05. Everything
the average values in the tables are presented as M±m.

As mentioned above, patients were included in the study only if they consistently achieved satisfactory compensation for carbohydrate and lipid metabolism in accordance with the criteria of the European Diabetes Policy.
Group (1998). However, as can be seen from Table. 1 despite
on satisfactory indicators of carbohydrate and lipid
metabolism in patients with type 2 diabetes, dyslipidemia persists, manifested by hypercholesterolemia and hypertriglyceridemia, along with an increase in the content of LDL and a decrease in the level of high density lipoproteins (HDL) according to
compared with those of donors (p<0.001). Purpose
natural flavonoids led to a significant decrease in MDA in plasma lipoproteins and a decrease in the content
cholesterol (CH) and triglycerides (TG) almost to the control level (see Table 1).

The normalization of the blood lipid spectrum revealed by us, which manifested itself in a significant decrease in cholesterol, TG and an increase in HDL levels against the background of a decrease in LDL levels (p<0.05), indicates a hypolipidemic effect of the drug, which confirms the data (12), which revealed a decrease in the content LDL in blood plasma and liver of rats under action of the DKV. It is known that oxidative stress
free radical lipid oxidation leading to accumulation of lipid peroxides inhibits a key enzyme
catabolism of cholesterol in the liver - microsomal 7ÿ hydroxylase (5), which disrupts the enzymatic regulation
catabolism of cholesterol and should lead to the maintenance of its consistently high blood levels. Under these conditions, hepatocytes can secrete lipoproteins into the bloodstream.
very low density (VLDL), including oxidized LDL, which undergo oxidative degradation with the formation of MDA. It is possible that blocking
free radical oxidation of lipids with the help of flavonoids, which is manifested by a significant decrease in the formation of MDA, removes the toxic effect of lipid peroxides
on hepatic 7ÿ-hydroxylase and thus promotes
an increase in cholesterol catabolism and an increase in HDL levels, and also reduce hepatic production of VLDL.

Table 2. Changes in carbohydrate metabolism and insulin resistance criteria against the background of the use of DHQ in patients with type 2 diabetes (M±m)

Indicator	Glycemia basal naya, (mmol/l)	Glycemia post prandial, (mol/l)	Hb A1c, %	IR-HOMA	ISI
Before taking DKV (n=40)	6.31±0.15	11.11±0.69	6.69±0.15	3.05±0.39	87.3±14.1
After taking DKV (n=20)	5.28±0.12, p<0.001	9.73±0.64, p<0.05	6.124±0.096, p<0.01	1.61±0.25, p<0.001	128.2±24.1, p<0.05
Control group (n=20)	6.48±0.16, p>0.1	-	6.648±0.21, p>0.1	2.67±0.73, p>0.1	-

In the treatment of patients suffering from type 2 diabetes with DKV, a significant decrease in HbA1c was obtained from 6.69±0.15% to 6.124±0.096% (p<0.01), accompanied by improvement 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 during compensation carbohydrate metabolism and, as a result, a decrease in the formation of end products of nonenzymatic glycation, which include HbA1c. However, the reduction in 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, in our opinion, causes a decrease in

gluconeogenesis and basal glycemia.

Based on this assumption, we calculated insulin sensitivity using 2 methods, HOMA-IR and ISI(14,15). In order to exclude variability in changes, insulin sensitivity was assessed simultaneously with an oral glucose tolerance test, against which the patients continued to take the basic sugar-lowering therapy.

FDI.

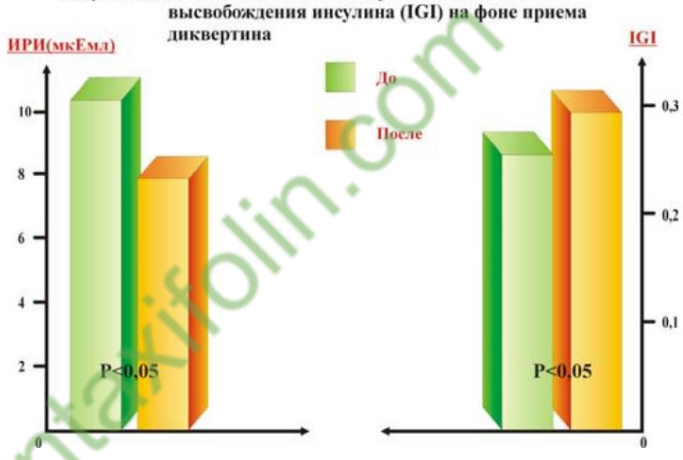
As can be seen from the table. 2 data, a significant decrease in the insulin resistance index (IR) according to the HOMA model was obtained from 3.05±0.39 to 1.61±0.25 (p<0.005) and increase in insulin sensitivity index ISI from 87.3±14.1 to 128.2±24.1 (p<0.05). In parallel evaluated comparison group, we do not have such changes found (Figure 1.)

In order to confirm the relationship of severity oxidative stress and insulin resistance, we conducted a correlation analysis between the level of secondary POL–MDA product in LDL and HOMA-IR insulin resistance index. A direct correlation was found between them (r=0.755, p<0.005). Thus, reducing the manifestations oxidative stress with the help of the flavonoid antioxidant DHQ, we simultaneously obtained a decrease in insulin resistance, significantly correlating with a decrease in oxidative stress.

In the group of patients treated with DHQ in combination with sulfonylurea drugs, there was a significant decrease in Hb A1c by 7% from baseline (p<0.05) without changing the dose of concomitant sugar-lowering therapy. Mechanism of the positive effect of DHQ on carbohydrate metabolism may be associated with reduced insulin resistance 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 using this method. However, we

it seemed interesting to determine the effect of the antioxidant of the flavonoid series DHQ on the secretory capabilities insular apparatus. To this end, patients who have compensation of carbohydrate metabolism while taking metformin, we conducted an oral glucose-tolerant a test to determine the concentration of insulin initially (on an empty stomach) and 1 and 2 hours after taking glucose.

Рисунок 2. Динамика базальной секреции ИРИ и индекса высвобождения инсулина (IGI) на фоне приема диквертина



After the test, patients were prescribed DKV in daily dose of 120 mg. After 3 months of taking DHQ, testing was repeated with the determination of the level of glycemia and insulinemia in the same time intervals. As can be seen from Table. 3 and fig. 2, after a 3-month course of DKV was achieved a significant increase (p<0.05) in stimulated insulin secretion, expressed as a relative percentage of compared with baseline IRI. At the same time, the level basal insulinemia decreased (p<0.05) compared with initial, which indicates a decrease in insulin resistance of peripheral tissues. When calculating the index release of insulin, determined by the ratio area under the insulin response curve (AUCins) to the area under the glycemic change curve (AUCgluc) during oral glucose tolerance test, we obtained a significant increase in the insulin release index (IGI) (p< 0.01), see table. 3 and in fig. 2.

In order to elucidate the possible relationship between oxidative stress and the functional state of the ÿ-cell, the correlation of improvement in secretory capabilities was assessed. insular apparatus according to the relative percentage of the 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 the secondary product LPO - MDA in plasma LDL against the background of the use of DHQ). received by us inverse correlation (r= -0.411, p<0.05) between percentage increase in IRI and a decrease in MDA testifies to our view on the positive effect of the use of DHQ on the secretory capabilities of the insular apparatus due to removal of reactive oxygen species and thus reducing the occurrence of oxidative stress leading to apoptosis of ÿ-cells. A possible mechanism for the utilization of oxygen radicals is the ability of a hydroxyl compound

flavonoids donate a hydrogen atom and bind more toxic compounds, thus neutralizing them (6).

Phenolic antioxidants are commonly referred to as any Ar(OH)n compounds in which one or more hydroxyl groups (OH) are attached to an aromatic nucleus (Ar); in this case, the molecules can contain several Ar(OH)n fragments. Analysis of the comparative activity of flavonoids showed (16) the importance of the presence of 2 hydroxyl groups in ortho positions in the B-ring and hydroxyl group in position C-3. The new flavonoid DHQ has hydroxyl groups at these positions.

Essentially, in the reaction ÿrOH + RO2ÿÿAr O2ÿ+ ROOH free valency disappears, and only hydroperoxide the RO2ÿ radical is replaced by the phenoxy ArO2ÿ, however, at this achieves the effect of inhibition of free radical oxidation, due to the greater stability of ArO2ÿ, which practically does not participate in the continuation of oxidation chains. Flavonoids can restore

activity of L-tocopherol, donating a hydrogen atom L to the tocopherol radical (6), the latter is formed when it donates its own hydrogen atom from the hydroxyl group to the peroxyl radical, interrupting such chain POL at once. Possible mechanism of action flavonoids can be chelation of metal ions Fe and Cu. Our data coincided with the data of a number of authors (13) and may indicate that polyhydroxylated aglycone flavonoids are potent inhibitors

LPO, which once again emphasizes the importance of hydroxyl groups in the flavone core. Hydroxyl group in the 7th position dissociates first and is the main site of attack by the peroxyl radical (6,13). As part of the DKV there is a hydroxyl group in the 7th position.

Summarizing the obtained data, it can be concluded that undoubted antioxidant properties of domestic bioflavonoid DHQ, which reduces risk of progression of diabetic angiopathy, improved glycemic control and insulin sensitivity. Inclusion in the complex therapy of an antioxidant DKV, contributed to an increase in the secretory capabilities of the insular apparatus, which allows us to hope for the preservation of residual secretion of insulin during prolonged use of antioxidant therapy.

Table 3. Dynamics of insulin response in response to a carbohydrate load before and after taking diquertin in patients with type 2 diabetes (M±m)

Parameters	IRI0, µU /ml	IRI1 hour, µU /ml	IRI 2 hour, µU/ml ÿIRI = (IRI1-IRI0), % AUC ins/ AUC glitch 59.6±12.9		
Before DKV	11.29±1.8	53.9±8.8	427.2±48.8 0.251±0.043 46.3±10.3 763.6± 168.8*		0.295±0.048**
In 3 months.	7.99±1.3*	73.1±21.6			

*- p<0.05, **- 0<0.01

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