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COMPARATIVE STUDY OF THE EFFECT OF VARIOUS FORMS OF QUERCETIN ON EXPERIMENTAL DIABETES

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Abstract

Background and aims: *Diabetes mellitus (DM) is a multifactorial metabolic disorder characterized by hyperglycaemia caused by insulin deficiency or insulin resistance. It is a global public health problem. This study aimed to determine specific pharmacological effect of quercetin in water soluble and liposomal preparations in experimental diabetes mellitus.* ***Material and methods:*** *We examined the effect of Corvitin and Lipoflavone (at the dose of 10 mg / kg body weight) in a comparative study in white rats with type 1 diabetes and type 2 diabetes coupled with obesity. To simulate the forms of diabetes mellitus most analogous to those in humans we used Streptozotocin at the doses of 30 mg / kg and 50 mg / kg. We tested the levels of glucose, glycosylated hemoglobin, C-reactive protein, and interleukins 6 and 4 in the blood.* ***Results:*** *In animals with type 1 and type 2 diabetes Lipoflavone significantly reduces glucose and glycosylated hemoglobin levels compared to the rats treated with Corvitin. When administered to animals with diabetes, the effect of quercetin in liposomal form on the concentrations of IL-6, IL-4 and C-reactive protein is also larger compared to the water-soluble form.* ***Conclusions****: Water soluble quercetin preparation Corvitin and to a larger extent liposomal preparation of this flavonoid, Lipoflavone, show anti-inflammatory effect and restore key parameters of carbohydrate metabolism in experimental type 1 diabetes mellitus and type 2 diabetes coupled with obesity, reducing blood glucose and glycosylated hemoglobin levels.*

key words: *diabetes; quercetin; antioxidants; cytokines.*

Background and aims

Diabetes mellitus (DM) is a multifactorial metabolic disorder characterized by hyperglycemia caused by the lack of insulin or insulin resistance [1]. Today, DM is a problem affecting health systems worldwide. The number of DM patients in the developed countries of the world reached 2 to 6% of the total population [2,3]. According to the latest estimates of the

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International Diabetes Federation, 415 million (1 in 11 people) suffer from diabetes; by 2040 this number will increase to 642 million or nearly 10% of the population [4,5].

In patients with diabetes, chronic hyperglycemia is accompanied by significant physiological, biochemical and histological changes [6]. These changes inevitably lead to the development of endothelial dysfunction and are the cause of diabetes-specific micro- and macro-angiopathies and associated complications. Antioxidants, in particular flavonoid quercetin, are known to alleviate pathological processes linked to the progression diabetes, including limiting the incidence of oxidative stress damage [7]. However, there are no studies comparing the effect of water-soluble and liposomal preparations of quercetin in type 1 and 2 diabetes, including their ability to restore key parameters of carbohydrate metabolism and produce anti-inflammatory effect.

The objective of our study, therefore, was to determine specific pharmacological effect of quercetin in water-soluble and liposomal forms in experimental diabetes mellitus.

Materials and methods

Quercetin preparations: water soluble *Corvitin* (BHFZ, Ukraine) and liposomal *Lipoflavon* (Biolik, PAT, Ukraine) were used for a comparative study of their effect on type 1 diabetes mellitus and type 2 diabetes mellitus coupled with obesity. White Wistar rats were assigned into eight groups: 1, 2 - control (intact animals); 3, 4 - animals with diabetes mellitus (DM); 5, 6 - animals with diabetes receiving *Corvitin*; and 7, 8 - animals with diabetes receiving Lipoflavon. Both preparations were administered intraperitoneally at 10 mg of quercetin / kg body weight for 2 weeks.

To model type 1 diabetes, streptozotocin (STZ) was used at a dose of 50 mg / kg. Once administered at such a dose, it causes acute necrosis of β cells of the Langerhans islets, sharply inhibiting their insulin-producing capacity and causing expressive hyperglycemia. To model type 2 DM with obesity, STZ was administered at a dose of 30 mg / kg body weight to the animals with obesity produced by maintaining them for 4 weeks on a high-energy diet [8]. Obesity caused by the diet is accompanied by the emergence of moderate resistance of peripheral tissues to insulin, thus the subsequent low-dose STZ injection instigates the development of β-cell dysfunction with suppressed insulin secretion [9]. These changes correspond to the stages of human type 2 diabetes pathogenesis.

To confirm disease progression, blood glucose levels were monitored using a standard kit (Filisit Diagnostics Company, Ukraine) and glycosylated haemoglobin erythrocytes (HbA1c) using a standard reagent kit (Reagent Company, Ukraine). Only the animals with 10.0 mmol / l glucose level were used in subsequent tests. Blood concentrations of C-reactive protein (CRP), pro-inflammatory interleukin-6 (IL-6) and anti-inflammatory interleukin-4 (IL-4) were determined using a set of reagents from Vector-Best Company (Russia).

The rats were euthanized using thiopental sodium narcosis (50 mg/kg), after which biochemical studies were performed. The animals with type 1 diabetes were euthanized on the 31st day of the experiment, and with type 2 diabetes on the 113th day. All manipulations with experimental animals were carried out in compliance with the rules of the *European Convention for the Protection of Vertebrates Used for Research and Other Scientific Purposes* and the Ukrainian *Scientific and Practical Recommendations for the Keeping of Laboratory Animals and Working with Them* [10].

Student's t-test and descriptive statistics analysis were carried out using Excel software (Microsoft, USA) and STATISTICA 6.0 (Statsoft, USA). In in the instances of not normal sampling distribution, the nonparametric Mann–Whitney U test was performed. The difference between the studied parameters was considered statistically significant at $p<0.05$.

Results

We demonstrate that at the end of the experiment, glucose and HbA1c levels in animals with type 1 diabetes increased 2.5-fold and by 98%, respectively. In the animals with type 2 diabetes, glucose levels increased 2.3-fold compared to the control group, while HbA1c increased by 84%.

In our experiments, in animals with type 1 and 2 diabetes, the level of the proinflammatory cytokine IL-6 increased (by 336% for DM 1 group and 396% for DM 2 group) while at the same time anti-inflammatory cytokine IL-4 decreased (by 43% for DM 1 group and 62% for DM 2 group, Table 1). This is consistent with the increase of serum C-reactive protein (CRP) concentrations by 122% in our experimental animals with type 2 DM and obesity.

In animals with type 1 DM, *Lipoflavon* significantly reduced the level of glucose and HbA1C (by 23% and 17%, respectively), compared to the group of animals that received *Corvitin*. At the same time, *Lipoflavon* treatment was associated with normalization of HbA_1C level. Compared to the water-soluble preparation of quercetin, *Lipoflavon* also significantly (by 25%) reduced blood glucose level in the animals with type 2 diabetes and obesity.

Table 1. The effect of *Corvitin* and *Lipoflavon* on the levels of IL-6 and IL-4 in type 1 diabetes mellitus and type 2 diabetes mellitus with obesity (M±m)

Index	*Experiment series*			
	Control (n=7)	*DM 1 (n=6)*	*DM 1+ Corvitin (n=9)*	*DM 1+ Lipoflavon (n=10)*
IL-6, ng/L	14.89±0.79	64.97±2.29 $p<0.01$	40.01±0.98 $p<0.001$ $p_1<0.001$	26.48±1.87 $p<0.001$ $p_1<0.001$ $p_2<0.001$
IL-4, ng/L	25.28±0.27	14.45±0.77 $p<0.01$	20.49±0.98 $p<0.01$ $p_1<0.001$	23.05±0.72 $p>0.05$ $p_1<0.001$ $p_2<0.01$
Index	*Control (n=7)*	*DM 2 (n=8)*	*DM 2 + Corvitin (n=10)*	*DM 2 + Lipoflavon (n=11)*
IL-6, ng/L	12.60±0.28	62.51±0.94 $p<0.001$	32.88±0.76 $p<0.001$ $p_1<0.001$	27.48±0.50 $p<0.001$ $p_1<0.001$ $p_2<0.001$
IL-4, ng/L	24.42±0,50	9.23±0.37 $p<0.001$	13.75±0.37 $p<0.001$ $p_1<0.001$	17.15±0.32 $p<0.001$ $p_1<0.001$ $p_2<0.001$
Notes: 1. p is significant in comparison with the markers of control animals; 2. p_1 is significant between the DM 1 and DM 2 groups; 3. p_2 is significant between the kuytgroups DM 1 + *Corvitin* and DM 2 + *Corvitin*.				

As the data presented in Table 1 demonstrates, administration of water-soluble form of quercetin to the animals with type 1 diabetes leads to a decrease in concentration of

IL-6 by 38%, while the concentration of IL-4 increases by 42%. Liposomal form of quercetin contributed to a decrease in IL-6 levels by 59% and increase of IL-4 by 60%. Thus, *Lipoflavon* exceeded the effect of *Corvitin* on concentration of IL-6 by 34%, and IL-4 by 13%. The level of IL-4 in the *Lipoflavon* treatment returned to the normal values.

Treating the animals with type 2 diabetes with water-soluble form of quercetin caused the decrease in concentration of IL-6 by 47%, while concentration of IL-4 increased by 49%. In this series of experiments *Corvitin* reduced the level of C-reactive protein by 33%. The liposomal form quercetin reduced the concentration of IL-6 by 56%; increase the concentration of IL-4 by 86%; and reduced the level of CRP by 50%. *Lipoflavon* activity exceeded that of *Corvitin* in terms of its effect on the levels of IL-6 by 16 %, IL-4 by 25 %, and CRP by 26 % (Table 1).

Discussion

Hyperglycemia in diabetes is believed to trigger activation of various processes that lead to oxidative stress, endothelial dysfunction, the development of atherosclerotic changes; it is also an important risk factor for macro- and microvascular complications [11]. HbA1C is not only an indicator of the extent of hyperglycemia, but also having an increased affinity for oxygen, it slows down the flow of oxygen to the tissues, which leads to the development of generalized hypoxia and metabolic disorder in peripheral tissues. HbA1C increase in the patients with diabetes correlates with the incidence and severity of DM complications [12].

Proinflammatory cytokines promote the expression of adhesive molecules, recruit general inflammatory cells to the locus of atherosclerotic inflammation increasing their functional activity, and stimulate the endothelial procoagulant activity [8]. This is consistent with the increase of serum CRP concentrations in animals with type 2 DM and obesity. CRP is believed to play a leading role in developing negative effects of vascular inflammation, causing destabilization of atheroma in addition to its initiation and progression [13]. In addition, obesity increases the production of IL-6 adipose tissue, which contributes to the development of insulin resistance, while the level of this cytokine directly correlates with the level of C-reactive protein in the blood. These facts point to a connection between obesity, inflammation and insulin resistance.

The positive effect of quercetin on blood glucose level in diabetes is attained through the inhibition of glucose absorption in the intestine and increase of its absorption by peripheral tissues [14]. Quercetin also accelerates the use of glucose in liver and skeletal muscle cells by activating key glycolysis enzymes, hexokinase and pyruvate kinase, reducing the activity of glycogen phosphorylase and stimulating glycogen synthesis in the liver and skeletal muscle [15]. In vitro, quercetin was shown to inhibit α-glucosidase activity [16]. The decrease in HbA_1C observed in our studies of this flavonoid may be associated with inhibition of oxidative stress.

The anti-inflammatory activity of quercetin is corroborated the fact that it blocks key enzymes of the arachidonic acid cascade, 5-lipoxygenase and cyclooxygenase. This property contributes to the stabilization of lipid peroxidation processes in biomembranes. Thus, quercetin preparations can be appropriate for use to alleviate disorders characterized by inflammation processes, cytolysis and free radical oxidation [17].

Inhibitory activity of quercetin molecule against IL-6 is governed by the presence of OH group at the positions 3 and/or 4 on the B ring.

The presence of unsaturated (double) bond in the C2-C3 position, the nature of hydroxylation / methoxylation of A and B rings, and the presence of 3 OH-rings C contribute to its lipoxygenase / cyclooxygenase inhibitory activity [18].

The quercetin-mediated reduction of the products of the arachidonic acid cascade leads, among other things, to a decrease in apoptosis level [2]. As our study demonstrates, *Lipoflavon* exceeded *Corvitin* in its impact on glucose level, HbA_1C, C-reactive protein, IL-6 and IL-4 in experimental diabetes, which is probably due to the presence of phosphatidylcholine liposomes in this formula, increasing antioxidant activity, membrane stabilization and membrane-reparative properties of *Lipoflavon* [2].

This study was carried out on a sample of small size. It is therefore essential to validate our findings with a larger sample size to determine the features of nitroxydergic processes more completely.

Conclusions

Water-soluble preparation quercetin *Corvitin* and to a greater extent liposomal preparation of this flavonoid *Lipoflavone* show anti-inflammatory activity in experimental type 1 DM and type 2 DM with obesity, as is confirmed by their positive effect on the balance of proinflammatory-anti-inflammatory cytokines and C-reactive level protein.

Corvitin and, to a greater extent, *Lipoflavon* in experimental type 1 DM and type 2 DM with obesity restore key indicators of carbohydrate metabolism, reducing the concentration of serum glucose and glycosylated hemoglobin of erythrocytes.

Conflict of interest. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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