

Original Article

Changes in Mass Measurement Indices, Cardiointervalogram Parameters and Duration of Swimming in Animals with Experimental Type 2 Diabetes Mellitus Treated with Drugs Exerting Antioxidant Properties

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Abstract

Introduction: Diabetic cardiomyopathy is common in patients with labile type I diabetes, with a tendency to ketoacidosis, reduced body weight, and affection of small blood vessels. This research aimed to determine the nature of the reaction of the autonomic nervous system and changes of biometrical indices in experimental diabetes type 1 and under the influence of different forms of quercetin. **Material and Methods:** White outbred mature male rats were used in the experiments. For diabetes type 1 modeling, a single intraperitoneal streptozotocin injection (50 mg/kg) was used. In 14 days after the injection, animals were divided into three groups: animals with diabetes without treatment; rats that were injected with a water-soluble form of quercetin; rats that were injected with the liposomal form of the bioflavonoid. Bodyweight, heart weight, heart mass ratio, tolerance of animals to physical activity (swimming test), and glucose level in the blood were defined. The state of the autonomic nervous system was estimated according to the indices of cardiointervalography. **Results:** Quercetin preparations at experimental diabetes type I contributed to the improvement of the mass measurement parameters and functional state of the autonomic regulation of heart activity, causing normalization of the majority of its indices and, as a result, increased the tolerance of animals to physical activity (duration of swimming). Under the influence of the preparations, vagal influence on the heart has been progressively reduced, and the restoration of balance between the tonus of sympathetic and parasympathetic divisions of the autonomic nervous system and centralization of control of heart rhythm were observed. It is important to mention that for most biometrical indices and indices of heart rhythm variability, the activity of the liposomal form of quercetin was more pronounced than its water-soluble form. It is possible that this effect was due to liposomes. **Conclusion:** The liposomal form of quercetin exhibited higher activity against most biometric and heartbeat rate indicators.

Keywords: diabetes, heart, autonomous nervous system, quercetin.

Introduction

The severity of diabetes mellitus (DM) is determined not only by its widespread but also by the rapid development of the complications that cause disability and mortality [1]. Both types of DM worsen the condition of both the coronary arteries and myocardium due to the development of diabetes-specific microangiopathy, macroangiopathy, metabolic disorders, and diabetic autonomic neuropathy [2]. Type 1 diabetes occu-

pies only 10-15% in the morbidity structure; its danger and social importance, above all, lies in the affliction of young people [3]. Among the severe complications of type 1 diabetes, an important place is the impaired functioning of the heart and blood vessels, which underlies the development of diabetic cardiomyopathy, the pathogenesis of which also includes an imbalance in the neurohumoral regulation [2, 4, 5]. Among such patients, myocardial infarction and stroke are diagnosed 3-5 times and 2-3 times more often, respectively,



compared to the population of the same age [1]. According to the literature, at the initial stages of the pathology, there is an increase in sympathetic influence on the background of lesions of the vagus nerve. In conditions of severe diabetes, both cholinergic and adrenergic regulatory processes are attenuated, leading to heart failure [3, 4, 5]. These pathophysiological disorders of cardiac activity progress, and contribute to the development of autonomic neuropathy of the heart, and later, myocardial infarction [1].

Recently, an active search for new substances and the improvement of bioavailability of existing ones in the fight against this disease is underway [6]. Given the role of free radicals in the development of various pathological processes in diabetes, antioxidants such as plant phenols, capable of counteracting toxic effects on cells, are relevant and occupy a significant niche.

Recently, taking into account the role of free radicals in the development of various pathological processes, there is an active search for new substances among plant phenols that can counteract their toxic effects on cells. Because of this, the use of agents having polytropic pharmacological properties is considered promising.

Such substances include polyphenols, including quercetin, which is highly effective in treating cardiovascular pathologies, particularly acute disorders of the coronary circulation and myocardial infarction, and the complex therapy of chronic heart failure [7, 8]. On this basis, it was logical to investigate the role of quercetin in changes in the parameters of autonomic regulation of cardiac activity in experimental type 1 DM.

Our study aimed to determine the nature of the response of the autonomic nervous system and the changes in biometric indicators of the cardiac activity in experimental type 1 diabetes, using the water-soluble and liposomal forms of quercetin.

Material and Methods

The experiments were performed on 64 white Wistar male rats weighing 120-150 g, which were divided into four groups: I – control (intact); II, III, IV – rats with type 1 diabetes, which was reproduced by a single intraperitoneal injection of 50 mg/kg streptozotocin (STZ), (“Sigma-Aldrich”, USA) on a 0.1 molar citrate buffer (pH 4,5) [9]. Rats of groups III and IV were administered intraperitoneally a water-soluble prepara-

tion of quercetin (WQ), (Corvitin) and the liposomal form of quercetin (LQ), (Lipoflavon), respectively, at a dose of 10 mg/kg in terms of the active substance. The drugs’ administration began two weeks after the start of type 1 DM modeling and was carried out within 14 days [10]. Animal euthanasia involved performing a state of deep anesthesia (thiopental sodium, 50 mg/kg). The studies were carried out following the national and international recommendations for the protection of animals used for experimental and other scientific purposes (Strasbourg, 1986; Law of Ukraine, No. 3447-IV, 2006) and in accordance with the requirements of the Bioethics Commission of I. Horbachevsky Ternopil National Medical University (Protocol No. 29, May 20, 2015).

Modeling type 1 DM was confirmed by determining the concentration of glucose in the blood using a standard set, LLC “Filisit diagnostika”, Ukraine. Subsequently, the animals in which glucose levels were not lower than 10.8 mmol/L 14 days after the STZ injection were used [10]. HbA1C levels in red blood cells were determined using a standard reagent kit (JSC “Reagent”).

The role of the autonomic nervous system in cardiac activity was assessed by cardiointervalography (CIG). Mathematical analysis of heart rate variability allows determining the functional state and correlation of the influence of the adrenergic and cholinergic units of the autonomic nervous system on the work of the sinoatrial node [11, 12]. An electrocardiogram was recorded in the II standard lead and analyzed with the help of the Cardiolab-CE computer complex, the duration of 1000 sequentially located R-R cardio intervals with precision to 0.001 seconds. The following parameters were evaluated:

- heartbeat rate (HBR, min⁻¹);
- mode (Mo, sec) – the duration of the R-R interval, which is most commonly found on the electrocardiogram segment under study;
- the amplitude of mode duration (AMo, %) – the ratio of the number of cardio intervals that correspond to the total number of cardio intervals analyzed (1000);
- the variation range of cardio intervals (ΔX , sec.) – the difference between the highest and lowest R-R duration in a sample;
- index of tension (IT), reflects the degree of centralization of heart rate management and is determined by the formula: $IT = AMo / (2 \cdot \Delta X \cdot Mo)$;
- vegetative equilibrium index (VEI = $AMo / \Delta X$, conv. un)
- characterizes the relationship between the activity

of the sympathetic and parasympathetic units of the autonomic nervous system;

- regulatory processes adequacy indicator (RPAI = AMo/Mo, conv. un) – reflects the correspondence between the activity of the sympathetic nervous system and the level of functioning of the sinoatrial node;
- vegetative rhythm indicator (VRI, 103) – assesses the activity of the autonomous circuit of regulation, namely the participation of parasympathetic influences in changes of the heart rhythm.

The weight of the animals was determined by weighing in accordance with the standard operating procedure (“Weighing Animals”), before morning feeding, always at the same time.

The animals’ heart weight was determined by weighing on torsion scales and calculating the mass ratio of the heart (MRH) by the formula:

$$\text{MRH (\%)} = (\text{Mheart}/\text{Manimal}) \cdot 100$$

The level of physical endurance of rats was determined using a swimming test [13].

Statistical processing of the results was performed using Student’s t-test. In cases where the sample distribution was different from normal, the non-parametric Mann – Whitney test was additionally used. The difference between the studied parameters was considered statistically significant at $p \leq 0.05$.

Results and Discussion

As the results show, at the end of the observation period, the mean serum glucose level in animals with type 1 diabetes increased from (5.09 ± 0.21) to (17.96 ± 0.79) mmol/L ($p < 0.001$) and was higher than the corresponding control group by 253%. At the same time, the content of glycosylated hemoglobin (HbA1C) increased from (5.27 ± 0.63) to $(10.43 \pm 0.34)\%$, by 98% ($p < 0.001$) (Figure 1).

Hyperglycemia in diabetes is the trigger mechanism for the activation of various processes that lead to oxidative stress, endothelial dysfunction, the development of atherosclerotic changes, and is a significant risk factor for macro- and microvascular complications [4]. In addition, there is a linear relationship between hyperglycemia and manifestations of vascular inflammation [14]. With a high affinity for oxygen, HbA1C causes a slowdown in oxygen uptake, resulting in peripheral tissue hypoxia and metabolic disorders [4].

During the experiment, it was found that animals with DM had slowed weight gain by 16% relative

to the control group. Such changes are characteristic of type 1 diabetes and are considered a response to the insulin deficiency condition [4, 15] due to the activation of lipolysis with reduced insulin levels.

In parallel, the cardiac mass increased by 26% and MRH by 56%, which may indicate the presence of myocardial hypertrophy in diabetes [6] and the progression of the hypoxic state [4]. In this series, 20% of animals have died during the experiment, which is also characteristic of this experimental model [15]. Exercise tolerance of type 1 DM was reduced by 79% compared to the control group.

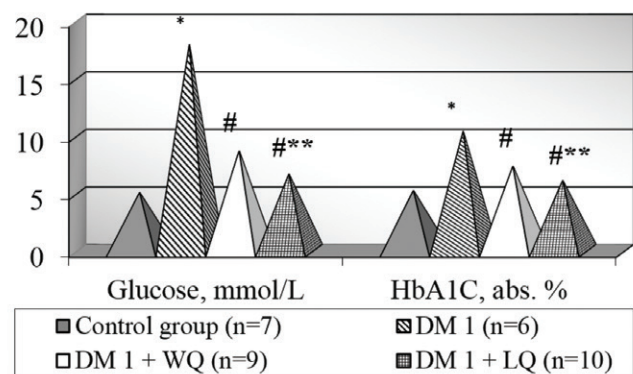


Figure 1: Changes in glucose and glycosylated hemoglobin levels in type 1 diabetes mellitus and after Corvitin and Lipo-flavone administration.

Notes: * is significant in comparison with the markers of control animals;

is significant between type 1 DM;

** is significant between the 1 DM and WQ groups.

The pathology development was accompanied by a violation of the functional state of autonomic regulation of cardiac activity, as indicated by significant changes in CIG indicators (Table 2). In animals with type 1 DM, there was a shift in the vegetative balance. Thirty days after the onset of type 1 diabetes, the HBR in animals decreased by 22% against a background of aMo growth of 42%, a decrease in AMo by 25%, an increase in their ΔX index by 48% and, as a consequence, a decrease in IT by 36%. These changes testify the manifestation of the stress syndrome of regulatory systems, which are confirmed by significant changes in the autonomic balance, namely a decrease in control of the heart rhythm by the sympathetic and an increase by the parasympathetic level of the autonomic nervous system. The data obtained do not contradict the known facts of autonomic dysregulation detected in type 1 experimental diabetes mellitus [11, 12].

The decline in IT and bradycardia refer to a depletion of reserves and a failure of adaptation, which is a

Table 1: Influence of WQ and LQ on the indices of mathematical analysis of the heart rhythm in experimental type 1 DM (M±m).

| Index | Experimental group | | | |
|-------------------------------|------------------------|------------------------|-------------------------------------|---|
| | Control group (n=7) | Type 1 DM (n=6) | Type 1 DM + WQ (n=9) | Type 1 DM + LQ (n=10) |
| Weight of the animal, g | 187.00±4.47 | 156.33±1.54 p<0.001 | 164.56±3.07 p<0.01 p1<0.001 | 177.90±5.83 p>0.05 p1<0.001 p2<0.05 |
| Weight of the heart, g | 0.77±0.08 | 0.97±0.04 p<0.05 | 0.96±0.04 p<0.05 p1>0.05 | 0.94±0.08 p<0.05 p1>0.05 p2>0.05 |
| MRH | 0.41±0.05 | 0.64±0.03 p<0.01 | 0.57±0.02 p<0.05 p1>0.05 | 0.50±0.04 p>0.05 p1<0.05 p2<0.05 |
| Duration of swimming, seconds | 210.09 ±14.40 | 44.63±6.42 p<0.01 | 119.98±18.02 p<0.001 p1<0.001 | 128.40±12.10 p<0.01 p1<0.001 p2>0.05 |

Note: p is significant in comparison with the markers of control animals, p1 – is significant in comparison with the markers of type 1 DM, p2 – is significant in comparison with the markers of type 1 DM + WQ

poor prognostic factor [12]. A decrease in HBR in STZ-induced diabetes was also reported in other studies [16].

A significant decrease in indicators such as VRI by 26%, VEI by 15%, IT by 36% and RPAI by 36%, compared with control, indicates a decrease in the activity of the humoral adrenergic link and increase the activity of the autonomous loop of regulation. The adequacy of regulatory processes in the conditions of type 1 diabetes has decreased significantly, reflecting the reduced degree of compensatory adaptive regulation processes.

The cause of impaired autonomic regulation of cardiac activity may be apoptosis of neurocytes. Literature has shown that many products of radical-dependent reactions can induce apoptosis of neurons [4]. Cytomorphological methods revealed apoptotic destruction of cultured in vitro neurocytes because of hypoxic effects, glutamate-induced ischemia, oxidative stress, or donor nitric oxide (NO) [4].

In the groups of animals with diabetes, where a correction with Corvutin and Lipoflavon was performed, marked improvements in glucose and glycosylated hemoglobin blood levels were noted (reliability of differences with the group of animals with diabetes accounted for all cases < 0.05). In particular, under the influence of Corvutin, the glucose level in the blood se-

rum decreased by 51% (from (17.96 ± 0.79) to (8.72 ± 0.82) mmol/L), and glycosylated hemoglobin by 29% (from (10.43 ± 0.34) to (7.39 ± 0.12 %)). When using Lipoflavon, glucose levels decreased by 63% (from (17.96 ± 0.79) to (6.70 ± 0.59) mmol/L) and HbA1C by 41% (from (10.43 ± 0.34) to (6.14 ± 0.23) %), compared with the group of animals with diabetes that did not receive correction drugs. Lipoflavon significantly reduced glucose by 23% and glycosylated hemoglobin by 17% compared to Corvutin (Figure 1).

On the other hand, numerous studies suggest that the use of antioxidants, in particular quercetin, limits the incidence of cardiovascular diseases and their constant satellite - atherosclerosis [17]. It is known that the maximum antioxidant effect of quercetin manifests itself after 14 days [18]. In the WQ correction group, an increase of 5% in the body weight of experimental animals with DM was observed. The liposomal form of quercetin contributed to a 14% increase in body weight over the same study period.

At the same time, the water-soluble form of quercetin had no effect on the cardiac mass and MRH but increased the animal's exercise tolerance (swimming duration) by 169%. In the group of animals with type 1 diabetes, in which the LQ correction was per-

formed, the average swimming duration increased by 188% against the decrease in MRH by 22% with unchanged heart weight.

After comparing the activity of water-soluble and liposomal forms of quercetin, it was found that LQ significantly changed body weight (by 8%) and MRH (by 12%) compared to WQ. Moreover, under the influence of LQ, these two indicators have normalized. Indicators of heart weight and mean swimming time of animals did not differ significantly when using both drugs (Table 1). It is known that Quercetin has the ability to

increase adaptation to hypoxia, which is necessary for ischemic heart damage; probably, due to this property, quercetin preparations increased the tolerance of animals to exercise. In the group of animals with type 1 DM, which underwent WQ correction, the following changes in CIG were observed: reduction of Mo by 24% and ΔX by 27%, and growth of AMo by 23%, HBR by 18%, IT by 31% and RPAI by 36% at unchanged VEI and VRI levels. Moreover, under the influence of the water-soluble form of quercetin, the normalization of Mo, AMo, ΔX, IT and RPAI indicators occurred (Table 2).

Table 2: Influence of WQ and LQ on the mass measurement indices and tolerance to physical activity in experimental type I DM (M±m).

| Index | Control group (n=7) | Experimental group | | |
|-------------------------|------------------------|----------------------------|--|---|
| | | Type1 DM (n=6) | Type 1 DM + WQ (n=9) | Type 1 DM + LQ (n=10) |
| Mo, sec | 0.12±0.003 | 0.17±0.02 p<0.01 | 0.13±0.003 p>0.05 p ₁ <0.01 | 0.12±0.001 p>0.05 p ₁ <0.001 p ₂ >0.05 |
| AMo, % | 51.86±3.67 | 39.00±2.67 p<0.05 | 47.78±2.05 p>0.05 p ₁ <0.05 | 47.50±2.01 p>0.05 p ₁ <0.05 p ₂ >0.05 |
| ΔX, 10 ³ sec | 3.71±0.42 | 5.50±0.34 p<0.05 | 4.00±0.50 p>0.05 p ₁ <0.05 | 3.80±0.20 p>0.05 p ₁ <0.001 p ₂ >0.05 |
| HBR, min ⁻¹ | 514.71±11.97 | 402.67±29.19 p<0.01 | 474.56±9.48 p<0.05 p ₁ <0.01 | 486.40±4.98 p<0.05 p ₁ <0.001 p ₂ >0.05 |
| IT | 62569.57±5885.64 | 39960.33±3762.74 p<0.05 | 52526.78± 3083.59 p>0.05 p ₁ <0.05 | 63603.00± 1898.07 p>0.05 p ₁ <0.001 p ₂ <0.05 |
| VEI, conv. un. | 14.62±0.56 | 12.43±0.99 p<0.05 | 13.41±1.80 p<0.05 p ₁ >0.05 | 14.70±0.57 p>0.05 p ₁ <0.05 p ₂ <0.05 |
| VRI, 10 ³ | 2.44±0.22 | 1.81±0.19 p<0.05 | 2.15±0.19 p<0.05 p ₁ >0.05 | 2.43±0.10 p>0.05 p ₁ <0.05 p ₂ <0.05 |
| RPAI, conv. un. | 0.44±0.03 | 0.28±0.04 p<0.01 | 0.38±0.03 p>0.05 p<0.05 | 0.38±0.02 p>0.05 p ₁ <0.05 p ₂ >0.05 |

Note: p is significant in comparison with the markers of control animals, p₁ – is significant in comparison with the markers of type 1 DM, p₂ – is significant in comparison with the markers of type 1 DM + WQ.

In the group of animals with type 1 diabetes, in which the LQ correction was performed, the following changes in the cardiogram were observed: a decrease in Mo by 29% and ΔX by 31%, an increase in AMo by 22%, HBR by 21%, IT by 59%, VEI 18%, VRI 34%, RPAI by 36%. Moreover, when using the liposomal form of quercetin, indices such as Mo, AMo, ΔX , IT, VEI, VRI, and RPAI did not differ much from similar values found in the control group.

When comparing the indicators in both groups receiving correction drugs, we noted that the activity of LQ outperformed WQ in terms of IT (21%), VEI (10%), and VRI (13%).

The increase of the ΔX indicator in both cases of correction testifies the strengthening of self-regulation mechanisms and a good flow of compensation [11]. In addition to quercetin, lyophilized LQ powder also includes lecithin, the presence of which further gives membrane-protective, anti-inflammatory, and antioxidant properties [10, 14]. Due to the presence of liposomes as carriers of the active substance of drugs, LQ retains a more stable and long-lasting effect compared to WQ, which is explained by the property of the liposomes themselves to act as a depot of the drug and to protect the active substances from destruction. In liposomal forms, the active substance is released gradually over a long time due to the hydrophilic surface of the liposomes and can be stored in the bloodstream for about two days. In our view, these pharmacokinetic and pharmacodynamics features of the liposomal form of quercetin contributed to its higher cardioprotective activity in type 1 diabetes mellitus compared to the water-soluble form of this flavonoid.

Conclusions

Preparations of quercetin in experimental type 1 diabetes contributed to the improvement of the mass measurement parameters and the functional state of autonomic regulation of cardiac activity, causing normalization of most indicators of the latter, and, consequently, increasing the tolerance of animals to exercise. Under the influence of drugs, the vagal influence on the heart progressively decreased and the correlation between the tone of the sympathetic and parasympathetic parts of the autonomic nervous system and the heart rhythm management centralization occurred. The liposomal form of quercetin exhibited

higher activity against most biometric and HBR indicators.

Conflict of Interest

The authors declare that there is no conflict of interest.

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