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VEGETABLE-BASED TRITERPENOID MILIACIN LIMITS INSULIN RESISTANCE IN RATS IN EXPERIMENT

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Abstract
Vistar rats received the vegetable-based triterpenoid 3-b-methoxy-D18-oleanene in the amount of 0.2 mg per kg of body weight simultaneously with a high-calorie diet. At the end of the 4-week experiment, in the group of rats receiving miliacin in combination with a high-calorie diet, serum glucose and insulin levels did not differ significantly from those of the control group and the group of rats receiving miliacin. At the same time, the concentration of glucose and insulin in the animals of this group was, on average, 30% lower than in the group of rats fed a high-calorie diet. Comparisons of HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) for the groups "high-calorie diet" and "high-calorie diet and miliacin" showed that the consumption of miliacin in combination with a high-calorie diet led to a significant decrease in the insulin resistance index by 2 times. The use of miliacin for the prevention of IR seems promising from the point of view of its effectiveness shown by the absence of a toxic effect in an experiment on animals. The results of the study broaden the understanding of biological effects range of miliacin as a promising agent for insulin resistance decrement.

Keywords. Miliacin, high-calorie diet, insulin resistance.

INTRODUCTION

Insulin resistance (IR), according to modern concepts, is one of the leading risk factors for type 1 and type 2 diabetes mellitus, metabolic syndrome and associated arterial hypertension, cardiovascular diseases, ischemic heart disease and other socially significant diseases. The most important component of decrement of the described diseases development risk is the identification and timely prevention of IR.

It was shown that IR and insulin deficiency in diabetes mellitus increase the rate of lipid peroxidation, which is accompanied by a relative or absolute decrease in the activity of the antioxidant support network. For this reason, it seems relevant to use compounds of plant origin for the prevention of IR [13], which are characterized, along with a pronounced antioxidative activity and hypoglycemic effect, low toxicity. In particular, the use of the triterpenoid compound 3-b, 30-dihydroxy-20 (29) -en-2-one, exhibiting hypoglycemic and antidiabetic activity, as the treatment diabetes mellitus of 1 type and/or 2 type has been described [21].

Among the triterpenoids practically used in medicine is miliacin, which is part of millet oil [6,7,10], used to treat trophic ulcers, infected wounds and some other diseases [5,7]. Miliacin stimulates nonspecific defense factors and prevents their apparent depression under conditions of toxic damage to the body with tetrachloromethane [11], induces hypercellularity of lymphoid organs [4], stimulates the immune response [8], has antioxidative, membrane-protective and antiapoptotic [11] activity, demonstrates the ability to regulate gene expression that controls the redox balance of the cell [9], prevents structural and functional disorders of the organs of immunogenesis and the liver under the action of xenobiotics [1,4,12,15], reduces systemic pathological endotoxinemia during bacterial infection, increasing the survival rate of the infected animals and decreasing the evidence of hypoplasia of central organs of immunogenesis: thymus and red bone marrow.

It has been experimentally shown that miliacin has a wide spectrum of biological activity, including those associated with its ability to prevent oxidative stress [4], while the triterpenoid itself does not change the redox balance, but protects it under conditions of oxidative stress induction, which is especially important, based on the concept of the reactive oxygen intermediates and lipid peroxidation products' role in the mechanisms of IR development [1]. At the same time, the issue of the ability of miliacin for IR decrement has not been studied.

The goal of the research was to study the effect of the vegetable-based triterpenoid miliacin on the insulin resistance decrement in rats in the experiment.

MATERIALS AND RESEARCH METHODS

Experimental animals, male Vistar rats (branch of the «Stolbovaya» nursery, Moscow region, Stolbovaya settlement), in the amount of 180 individuals with an initial weight of 170 g, were kept in standard plastic cages at room temperature in compliance with day / night cycles in a ratio of 12 /12 hours. The rats were on a standard diet (balanced pelleted feed "ProCorm" by Biopro, Novosibirsk) with free access to water.

The animals were divided into 4 groups. The first group was the control group. Animals of the second group, in order to create insulin resistance in them, got margarine containing saturated fatty acids (hereinafter referred to as a high-calorie diet, full diet). The amount of margarine was selected in such

a way that it increased the daily caloric content by about 9-10%, compared with the control, and was about 1.0 gram at the beginning of the experiment. As the weight of the animals increased due to their natural growth, the amount of the added product increased proportionally, and in such a way that the proportion of additional fat intake provided 10% of the caloric content of the total diet. Animals of the third group were fed with the vegetable-based triterpenoid 3-b-methoxy-D 18 -oleanene (miliacin) at a dose of 2 mg / kg body weight in combination with 25 µl of a solution of neutral fats (TAG) containing polyunsaturated higher fatty acids of sunflower oil, on an empty stomach. Animals of the fourth group got margarine simultaneously with miliacin at a dose of 2 mg / kg of body weight in combination with 25 µl of the neutral fats solution (TAG). The total duration of the experiment was 4 weeks.

A week before the end of the experiment, the animals were subjected to a glucose tolerance test (GTT) and the concentration of glucose in the blood was determined. For this, the animals received per os carbohydrate in the form of a 20% glucose solution at the rate of 2 g / kg of body weight. Glucose concentration was determined in whole blood using an Accu Chek Performa glucometer. Along the tail vein, a puncture was made with lancets, the device was brought up with an inserted test strip, the glucometer automatically took 1.5 µl of blood and printed the glucose concentration on the

display. At the same time, the initial glucose level was assessed (0 min.), And then after 30, 60, 90 and 120 minutes [2].

The experiment was carried out in accordance with ethical standards and recommendations for the humanization of work with laboratory animals, which are reflected in the "European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes " (Strasbourg, 1985). The animals were euthanized by decapitation, after which blood was collected in VenoSafe vacuum tubes (Belgium) with a coagulation activator (silicon coating). To obtain serum, a tube with coagulated blood was placed in an "EVA 200" centrifuge and centrifuged for 15 minutes at 2400 rpm. Then, using a dispenser, the serum was taken and poured into «Eppendorf» tubes for further biochemical study. Biochemical parameters including measurements of glucose concentration in mixed blood in the fasting state, insulin level in blood serum in the fasting state with subsequent calculation of the HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) index [19] was calculated by the formula: [glucose, in the fasting state, mmol / L] x [insulin, in the fasting state, µU / ml] /22.5, as well as the construction of a curve reflecting the glucose tolerance test, for quantitative interpretation of the ROC curve (area under the error curve), the AUC indicator was taken (area under ROC curve), which is based on the measurement of the area under the glycemic curve relative to the baseline.

The results of the studies were processed by the methods of variation statistics using the software package for PC Microsoft Excel 7.0, STATISTICA 13.0, including the methods of parametric analysis (Student's test). To isolate significant correlation coefficients, the level of significance adopted for biomedical research was selected ($p < 0.05$).

RESULTS AND ITS DISCUSSION

Table 1 shows significant differences in the concentration of blood glucose and insulin when consuming miliacin.

Table 1. Effect of miliacin on glucose and insulin concentrations in serum in a standard and high-calorie diet

Set of experiments	Glucose, mmol/L	Insulin, Uu/l	HOMA-IR
I-Control n=45	5,34±0,25	11,16±0,71	2,65±0,26
II-Miliacin n=45	4,87±0,29	7,39±0,41	1,58±0,12
III- High calory diet n=45	8,54±0,42	17,38±1,92	6,60±0,43
IV- High calory diet and Miliacin n=45	5,9±0,31	12,15±1,61	3,19±0,22
Significance of differences	D1-2>0,05 D1-3<0,001 D1-4>0,1 D3-4<0,001	D1-2<0,01 D1-3<0,02 D1-4>0,05 D3-4<0,05	D1-2<0,001 D1-3<0,001 D1-4>0,1 D3-4<0,001

It was found that intact rats were characterized by physiological levels of glucose and insulin. Oral administration of miliacin led to a significant decrease in glucose and insulin levels by 10 and 51%, respectively. The nutritional intervention of margarine into the diet of animals led to hyperglycemia and hyperinsulinemia, which was reflected in an increase in the glucose and insulin concentration in the blood serum in the group of rats kept on a high-calorie diet. The increase in the studied parameters was 60 and 55%, in comparison with the control. Comparison of glucose and insulin concentrations in the groups of rats kept on a high-calorie diet and in the group of animals receiving miliacin showed a significant increase in the levels of the studied parameters in the group of rats getting a high-calorie diet. The increase was 75% and 135%, respectively, for glucose and insulin. In the group of rats receiving miliacin in combination with a high-calorie diet, serum glucose and insulin levels did not differ significantly from those of the control group and the group of rats getting miliacin. At the same time, the concentration of glucose and insulin in the animals of this group was on average 30% lower than in the group of rats fed a high-calorie diet.

As a result, the insulin resistance index HOMA-IR in the group of animals receiving miliacin was 1.7 lower than in the control; in the group of rats kept on a high-calorie diet, it was 2.5 higher than in the control; for animals, combined with a high-calorie diet getting miliacin, was 1.2 times higher than in the control (for this group, the differences were not significant). HOMA-IR comparisons for the "high-calorie diet" and "high-calorie diet and miliacin" groups showed that the consumption of miliacin in combination with a high-calorie diet led to a significant decrement in the insulin resistance index by 2 times.

The figure shows the indicators of the glucose tolerance test in the compared groups.

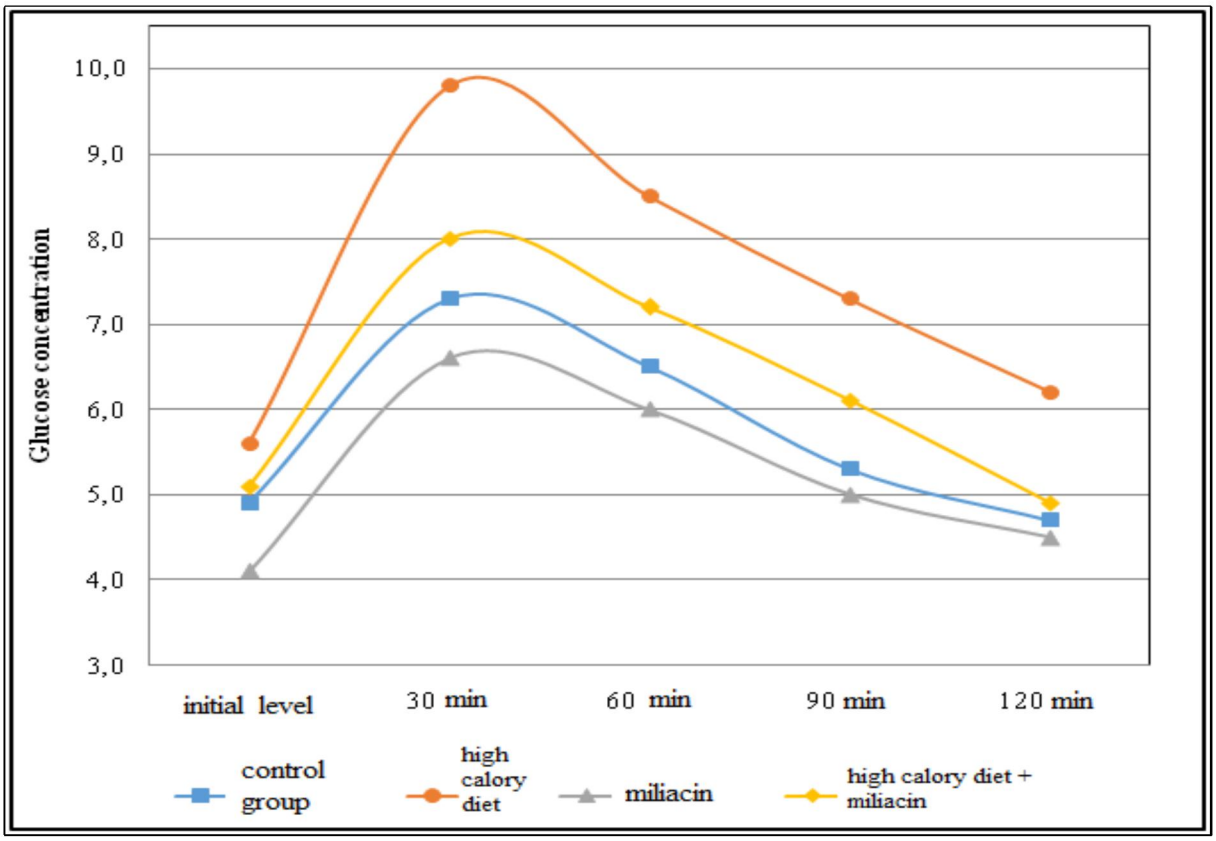

Figure. Glycemic curves reflecting the glucose tolerance test of the compared groups of animals

The glycemic curve shows that long-term consumption of saturated fats by animals affects glucose tolerance. This is due to both higher values of glucose concentration in fasting state (114%) and by 30th minute (134%) relative to control group, as well as a slower return to the initial level at 60th (138%) and 90th (132%) minutes of the experiment.

A lower glucose level was noted throughout the test in animals that regularly consumed miliacin, relative to the control group, but at the same time being within the normal range.

The glucose tolerance test showed lower blood glucose levels in animals fed a high-calorie diet in combination with a daily dose of miliacin. Thus, in rats of this group, the glucose concentration significantly decreased in fasting state by 37%, at 30th minute by 48%, 60th - 42%, 90th - 46%, 120th - 38% compared to animals fed a high-calorie diet.

Table 2 shows the significant differences in the AUC area (under the curve) in the compared groups.

Table 2. AUC area (under the curve) (conditional units)

Set of experiments	AUC area (under the curve) (conditional units)
I-Control n=45	96±12
II-Miliacin n=45	70.9±9
III- High calory diet n=45	210±27
IV- High calory diet + Miliacin n=45	123±15
Significance of differences	D1-2>0,1 D1-3<0,001 D1-4>0,1 D3-4≤0,01

Thus, animals that got the vegetable-based triterpenoid 3-b-methoxy-D [8-oleanen in an amount of 0.2 mg per kg of body weight simultaneously with a high-calorie diet did not have significant differences in glucose and insulin levels in the blood serum at the end of the 4-week experiment, in comparison with the indicators of the control group and the group of rats administered miliacin and kept on a standard diet. At the same time, the glucose and insulin concentration for animals of this group was, on average, 30% lower than in the group of rats fed a high-calorie diet. HOMA-IR comparisons for the "high-calorie diet" and "high-calorie diet and miliacin" groups showed that the consumption of miliacin in combination with a high-calorie diet led to a significant decrement in the insulin resistance index by 2 times.

RESULTS AND DISCUSSION

The antihyperglycemic effects of vegetable-based triterpenoids described in the literature are described by various mechanisms, including modulation of insulin secretion [16], increase in glucose metabolism [20], activation of enzyme systems that generate cyclic AMP [18], and an increase in peripheral glucose disposal [17].

The mechanisms by which the antiglycemic effect of miliacin occurs are most likely associated with its antioxidant and membrane-stabilizing action [3]. In particular, the participation of miliacin in the regulation of cell redox balance by canceling MT-induced expression of the

prooxidant factor CYP-2E1 gene and restoring the suppressed MT expression of the gene of one of the leading factors of «Glu red» antioxidant defense was described. [4,7]. The understanding of the realization of the membrane-protective action of the triterpenoid is most likely associated with its ability to integrate into membranes, due to its hydrophobicity, to strengthen the packing strength of phospholipids and to shield the latter from attack by radical particles [14]. By exerting a modifying effect on lipids, miliacin acts as a structural membrane protector, capable of maintaining the membranes viscosity at the level that ensures the necessary activity of membrane-dependent processes under conditions of generation of reactive oxygen intermediates.

Conclusions: Thus, the advantages of using miliacin for the prevention of IR appears to be promising both from the point of view of its effectiveness, low toxicity shown in an experiment on animals, and from the point of view of the availability of raw materials for the extraction of triterpenoid. The results of the study broaden the understanding of biological effects range of miliacin as a promising agent for insulin resistance decrement.

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