Study of Hypoglycemic Activity of Subetta and Rosiglitazone on the Model of Streptozotocin-Induced Diabetes Mellitus in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 1, pp. 62-64, January, 2012
Original article submitted November 16, 2010

Antidiabetic activity of Subetta was revealed on the model of streptozotocin-induced diabetes mellitus in rats. Intragastric administration of this preparation in a dose of 5 ml/kg for 50 days reduced blood glucose levels, urine levels of glucose and ketone bodies, restored glucose tolerance in the oral glucose test, improved general condition and increased the survival rate of animals. The effectiveness of the drug was not inferior to that of rosiglitazone (8 mg/kg).

Key Words: *Subetta; rosiglitazone; diabetes mellitus; streptozotocin*

Diabetes mellitus (DM) is one of the most common endocrine diseases. According WHO data, more than 180 million people all over the world suffer from this pathology [1]. The main criteria for DM are fasting blood glucose levels above 6.7 mmol/liter and impaired glucose tolerance [5]. Streptozotocin-induced diabetes is a widely used experimental model of DM [6], because administration of streptozotocin simulates gradual dysfunction of pancreatic β-cells [4] as well as impaired glucose tolerance and development of related disorders.

Combined preparation Subetta contains ultra-low doses of antibodies to β-subunit of the insulin receptor and ultra-low doses of antibodies to endothelial NO-synthase. Previous studies on the model of streptozotocin-induced diabetes mellitus in rats showed that hypoglycemic activity of ultra-low doses of antibodies to insulin receptor β-subunit administered orally was not inferior to that of insulin and glybenclamide [2]. It is also known that ultra-low doses of antibodies to endothelial NO-synthase protect endothelial cells [3], which is important for the prevention and treatment of vascular complications of DM (neuropathy, nephropathy, *etc*.).

Here we compared hypoglycemic activity and effectiveness of Subetta and rosiglitazone on the model of streptozotocin-induced diabetes in rats.

MATERIALS AND METHODS

Experiments were carried out on 70 outbred male albino rats (162-408 g). DM was induced by single intravenous injection of streptozotocin (50 mg/kg). Blood glucose was measured after 72 h. Rats with developed DM (blood glucose concentration of at least 12 mmol/liter) were divided into groups. Controls (n=20) daily received distilled water (intragastrically, 5 ml/kg once a day) for 50 days. Animals of experimental group 1 (n=20) intragastrically received rosiglitazone (Avandia, GLAXO WELLCOM C.A.) in a dose of 8 mg/kg/day, twice a day. Group 2 animals (n=20) daily received Subetta (Materia Medica Holding) intragastrically in a single dose of 5 ml/kg. Intact group comprised 10 rats.

Body weight, water consumption, and blood and urine glucose levels were evaluated after overnight fasting by the glucose oxidase method using Glucose FKD kits; fasting level of ketone bodies in the urine

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0007-4888/12/1531-0054

was measured in all experimental groups on days 3, 7, 14, 21, 28, 35, 42, and 50 of treatment using PHAN® diagnostic strips (Lachema). Glucose tolerance (1 g/kg glucose orally) was evaluated on days 14, 28, and 50, and the areas under the time/concentration curves (AUC) were calculated by the trapezium method.

Significance of differences between the groups was determined using Student's *t* test.

RESULTS

Single streptozotocin injection to rats induced significant increase in blood glucose concentration (up to 15.94±2.99 mmol/liter *vs.* 3-5 mmol/liter in intact rats; Fig. 1) and as a consequence, urine glucose concentration (up to 3.56±0.94 mmol/liter *vs.* 0-0.1 mmol/liter in intact rats) and ketone bodies (up to 2.50±0.65 *vs.* 0 in intact rats). Oral glucose tolerance test showed that glucose utilization rate in peripheral blood on days 14 and 50 after streptozotocin administration was lower that in intact animals by 4.6 and 4.1 times, respectively ($p<0.001$; Fig. 2). These changes indicate the development of DM; the severity of DM remained unchanged throughout the experiment (Fig. 1, 2). In control rats, DM was accompanied by pronounced polydipsia and polyuria and decreased food consumption. Body weight loss attained 32.2% of initial values by the end of the experiment (Table 1). The rats had gray and dull fur; multiple hemorrhages and necrotic areas were seen on the skin, limbs, and tail. Lesions of the eyelids and conjunctiva, blurred pupil and sclera with numerous hemorrhages of various sizes were noted. Healing of small wounds was slow and was accompanied by infection and purulent inflammation leading to gangrene of the tail and limbs. Mortality in this group was 80% by the end of the experiment.

Rosiglitazone, the reference drug, reduced blood glucose level in rats as soon as on day 14 of treatment. Glucose level in this group did not return to intact values (from 8 to 10 mmol/liter), but was lower than in the control group (>15 mmol/liter throughout the experiment; Fig. 1). A decrease in the level of glucose and ketone bodies in the urine was observed starting from day 21 of treatment. By day 50, fasting levels of glucose and ketone bodies in the urine were 1.45±0.68 and 0.75±0.43 mmol/liter, respectively (vs. 3.37±0.90 and 1.13±0.38 mmol/liter in control group). In oral glucose tolerance test, AUC decreased by 35.6 and 46.3% on days 14 and 50 of rosiglitazone treatment (Fig. 2). In this group, the severity of polydipsia and poliuria was significantly lower, and food consumption was slightly higher than in control. The exterior appearance and general condition of rats also changed, body weight decreased by 12.3% on day 14 of obser-

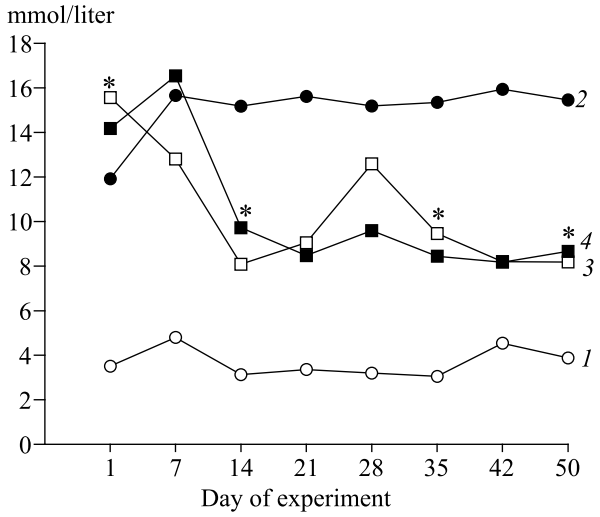

Fig. 1. Effect of test drugs on plasma glucose levels in rats with streptozotocin-induced DM. *1*) intact; *2*) control; *3*) Subetta; *4*) rosiglitazone. *$p<0.05$ in comparison with the control group.

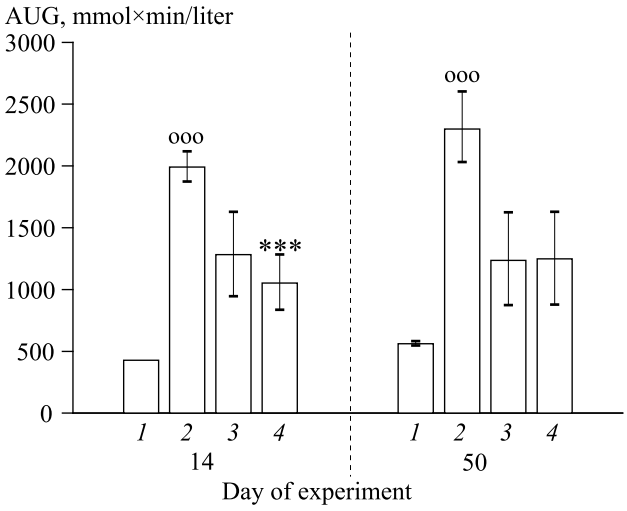

Fig. 2. Effect of test drugs on glucose tolerance in oral glucose tolerance test in rats with streptozotocin-induced DM. *1*) intact; *2*) control; *3*) rosiglitazone; *4*) Subetta. $p<0.001$ in comparison with: ***control group, °°°intact group.

TABLE 1. Body Weight Dynamics and Survival of Experimental Rats

Group	Changes in body weight by experimental day 50, % of initial value (before streptozotocin injection)	Survival, %
Intact	12.9	100
Control	-32.2*	20
Rosiglitazone	-15.0	20
Subetta	1.6	30

Note. *$p<0.05$ in comparison with intact group.

vation, but fully recovered to the initial level by day 50 (Table 1). Improvement of general state was not associated with changes in survival of experimental animals: only 20% rats survived to the end of the observation period.

Subetta reduced blood glucose level as soon as after 7 days. On days 14-50, glucose concentration ranged from 8 to 10 mmol/liter (except for day 28; Fig. 1). Urine levels of glucose and ketone bodies decreased starting from day 14 of Subetta treatment and on day 50 were 1.41±0.57 and 0.75±0.34 mmol/liter, respectively (*vs*. 3.37±0.90 and 1.13±0.38 mmol/liter in control group, $p<0.05$). Subetta treatment increased glucose utilization in oral glucose tolerance test by 47.2% ($p<0.05$) and 45.7% on days 14 and 50, respectively (Fig. 2). Reduced DM severity influenced appearance and behavior of rats; body weight gain was even lower than in the intact group, but higher than in controls (Table 1). Survival in this group was higher than in controls by 10% (Table 1).

Thus, hypoglycemic activity of Subetta was not inferior to that of the reference drug rosiglitazone. In rats, Subetta reduced the severity of DM (decrease in glucose level in the blood plasma and urine and concentration of ketone bodies in urine and normalization of glucose tolerance), improved general condition of animals (lower body weight loss and better skin condition), and somewhat reduced animal mortality.

The results suggest that further clinical trials of Subetta in treating patients with DM are promising.

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