Study of Antidiabetic Activity of a New Ultralow-Dose Antibody Preparation on the Model of Streptozotocin Diabetes in Rats

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Antidiabetic activity of a new ultralow-dose preparation of antibodies to insulin receptor β-subunit was revealed on the model of streptozotocin diabetes in rats: treatment with this preparation in a daily dose of 2.5 ml/kg (for 50 days through a gastric tube) normalized blood glucose level, restored glucose tolerance in the oral glucose test and body weight gain, and improved animal survival. By its hypoglycemic activity and effect on glucose tolerance the preparation was not inferior to classical drugs for the treatment of types 1 and 2 diabetes mellitus insulin (12 U/kg) and glybenclamide (8 mg/kg).

Key Words: *diabetes mellitus; streptozotocin; insulin; glybenclamide*

According to estimations of the World Health Organization, more than 180 million people all over the world suffer from diabetes mellitus (DM). By the year 2030 their number can increase more than 2-fold [4]. The main criteria of DM are elevated blood glucose level after overnight fasting and impaired glucose tolerance [9].

The main form of drug therapy for type 1 DM is insulin replacement therapy; type 2 DM is treated by sugar-reducing drugs (sulfonylurea derivatives, biguanides, meglitinides, *etc.*). Classical drug therapy of DM, despite its efficiency, cannot completely compensate for disorders developing in DM and moreover, can cause side effects [1,2,7]. For this reason, the search for new hypoglycemic drugs remains a pressing problem [3].

Streptozotocin diabetes is a widely used experimental model of DM [11]. The search for antibiotic producers with antitumor activity among *Streptomyces achromogenes strains* revealed a nitroderivative component called streptozotocin in one of the tested strains. This agent induced multiple disorders in coiled DNA strands in laboratory rodents after enteral or parenteral treatment [8]. In parallel, indirect changes in glucokinase activity were detected in the same experimental animals [12].

Further studies showed that intragastric treatment with streptozotocin (total dose of 40 mg/kg) for 5 days was associated with the development of insulinopenic diabetes-like state (IPDLS), with autoimmune disorders (AID) in the pancreas recorded with the same incidence as in patients with type 1 DM. On the other hand, it was found that the kallikrein-kinin system, but not T lymphocytes, played the leading pathogenetic role in this model of AID [10]. Single intravenous injection of streptozotocin in a dose of 30-40 mg/kg to rats resulted in transitory IPDLS. Increasing intravenous dose of streptozotocin to 60-70 mg/kg led to the development of severe functional disorders paralleled by multiple DNA breaks [6].

We evaluated the antidiabetic potential of a new drug for oral treatment belonging to the ultralow-dose antibodies [5] and containing antibodies

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to insulin receptor β-subunit (ULD AB-IRb) in ultralow doses.

MATERIALS AND METHODS

Experiments were carried out on 130 outbred male albino rats (250-300 g). Diabetes mellitus was induced by single intravenous injection of streptozotocin (50 mg/kg). Blood glucose was measured after 72 h. Rats with DM (blood glucose concentration of at least 15 mmol/liter) were divided into groups. Controls (n=60) daily received distilled water (2.5 ml/kg intragastrically) for 50 days. Animals of experimental group 1 (n=20) were subcutaneously injected with insulin (Actrapid) in a daily dose of 12 U/kg (2 injections daily; 50 days). Group 2 animals (n=20) received glybenclamide (Maninil, Berlin-Chemie) in a daily dose of 8 mg/kg (twice daily intragastrically). Group 3 animals (n=20) received ULD AB IRb (Materia Medica Holding) in a single daily dose of 2.5 ml/kg (intragastrically). Intact group consisted of 10 rats.

Body weight, blood glucose level (after overnight fasting by the glucose oxidase method using Glucose FKD kits), and water consumption were evaluated in all experimental groups on days 3, 7, 14, 21, 28, 35, 42, and 50 of treatment; 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.

The significance of differences between the groups was evaluated using Student's t test.

RESULTS

Streptozotocin injection to rats induced the development of pronounced hyperglycemia: blood glucose concentration increased 4-6.5 times in controls in comparison with intact animals and reached 17.90±0.06 mmol/liter by the end of the experiment (Fig. 1). In addition, body weight loss was observed in control animals by the end of the experiment: these rats lost 47% body weight after 50 days, while intact rats gained 20% during the same period. Water consumption increased 2.7 times after streptozotocin intoxication in comparison with controls (Table 1).

Insulin treatment for 50 days led to reduction of blood glucose levels in rats with experimental DM to 8.96±0.05 mmol/liter (p<0.001 *vs.* the control; Fig. 1), but the severity of polydipsia virtually did not change (Table 1). Body weight in rats treated with insulin did not change throughout the experiment.

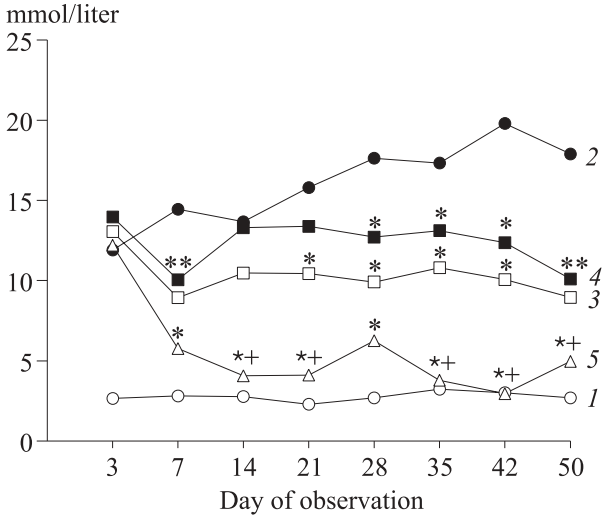

Fig. 1. Dynamics of blood glucose level in rats with experimental DM. *1*) intact; *2*) control; *3*) experimental group 1 (insulin); *4*) experimental group 2 (glybenclamide); *5*) experimental group 3 (ULD AB IRb). p<0.05 compared to: *control, +experimental groups 1 and 2.

Glybenclamide treatment also was associated with a reduction of hyperglycemia (to 10.10±0.03 mmol/liter on day 50 in comparison with the control; p<0.001; Fig. 1), which however did not prevent body weight loss and polydipsia (Table 1).

Treatment with ULD AB IRb led to normalization of blood glucose level in rats with DM as soon as after 7 days (Fig. 1). From this day to the end of the experiment this parameter in animals of this group did not differ from that in intact rats and was significantly (p<0.05) lower in comparison with blood glucose levels in animals treated with insulin and glybenclamide (Fig. 1). The appearance, behavior, and body weight gain of rats treated with ULD AB IRb did not differ from the parameters in intact animals. In addition, this treatment reduced water consumption (by 22.5% in comparison with the control group; p<0.05).

TABLE 1. Body Weight Gain and Water Consumption by Animals of Experimental Groups; Day 50 of Treatment

Group	Body weight gain, %	Water consumption, ml/rat
Intact	20	57.32±4.63
Control	-47	151.78±10.50
Experimental		
1 (insulin)	0*	189.23±21.92
2 (glybenclamide)	-30.5	164.05±15.76
3 (ULD AB IRb)	12.5*	117.57±8.38*

Note. *p<0.05 compared to the control.

TABLE 2. Survival of Experimental Rats

Group	Number of animals by the start of experiment	Survival (in %) on experimental day							
		3	7	14	21	28	35	42	50
Control	60	100	50	40	35	35	26,6	16,7	15
Experimental 1 (insulin)	20	100	70	50	50	50	40	35	20
Experimental 2 (glybenclamide)	20	100	70	65	50	50	40	35	20
Experimental 3 (ULD AB IRb)	20	100	80	60	50	50	45	40	30

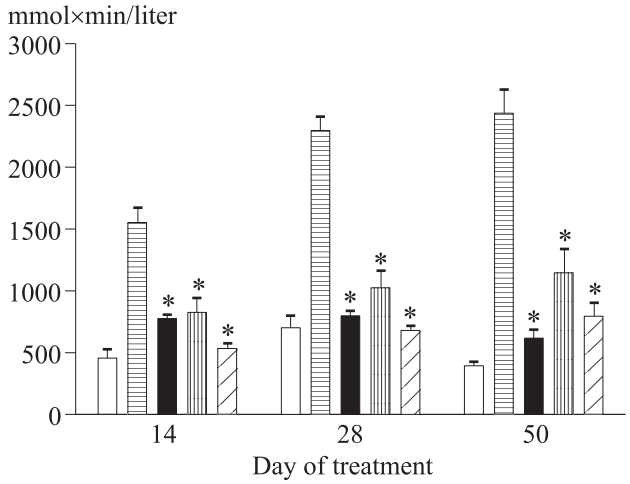

Fig. 2. Dynamics of AUC for oral glucose tolerance test in rats with DM during experimental treatment. Light bars: intact; horizontally-hatched bars: control; dark bars: experimental group 1 (insulin); vertically-hatched bars: experimental group 2 (glybenclamide); cross-hatched bars: experimental group 3 (ULD AB IRb). $p<0.05$ compared to the control.

Delayed death of experimental animals after streptozotocin injection was observed during the entire period of observation (Table 2), but no significant differences between the control and experimental groups were found by this parameter.

Glucose tolerance decreased 3-6-fold after streptozotocin injection (Fig. 2) and increased significantly ($p<0.05$) after intragastric insulin and glybenclamide treatment in comparison with the control group (2.5-3 and 2-2.5 times, respectively; Fig. 2). Treatment with ULD AB IRb also improved glucose tolerance in diabetic rats (2.4-3.7 times in comparison with the control, $p<0.05$) and was no less effective that glybenclamide and insulin treatment.

Hence, antidiabetic activity of ULD AB IRb preparation was demonstrated on the model of experimental streptozotocin-induced DM in rats: the treatment promoted normalization of blood glucose level, improvement of glucose tolerance (according to oral glucose tolerance test), body weight gain, though this treatment did not appreciably improved survival of experimental animals. By its hypoglycemic effect and effect on glucose tolerance ULD AB IRb preparation was no less effective than classical drugs for the treatment of types 1 and 2 DM (insulin and glybenclamide).

The results prompt further studies of antidiabetic effect of ULD AB IRb preparation.

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