EFFECT OF ORAL ADMINISTRATION OF INSULIN WITH A SYNTHETIC POLYMER ON ALLOXAN DIABETES

V. G. Baranov, L. L. Shchukovskaya,
M. F. Belovintseva, V. A. Kropachev,
and I. A. Donetskii

UDC 615.272.3.541.64

KEY WORDS: combination of insulin with synthetic polymer; alloxan diabetes; oral administration; blood glucose level; absorption.

The production of insulin in a form suitable for enteral administration still remains an urgent problem. There have been several reports of the use of insulin in experiments on diabetic animals [3, 4, 6, 10, 12, 13] whose authors have used different factors to protect the hormone against the action of proteolytic enzymes and to improve its absorption through the intestinal epithelium: fat emulsions, liposomes, and surfactants. The authors cited have stated that on enteral administration of insulin the blood glucose level of the animals falls and the insulin concentration in the plasma rises, but as a rule the effect was short-lived — not more than 3 h.

In previous investigations [1, 2] on intact rabbits the present writers showed that oral administration of insulin with various types of synthetic polymers had a marked hypoglycemic action during 6 h of observation. Addition of low-molecular-weight surfactants potentiated the hypoglycemic effect. The blood glucose level was found to be lowered by a greater degree if the combination was introduced into the mouth than into the stomach through a tube, and it was accordingly postulated that absorption of insulin with the polymer may begin actually within the oral cavity. Ishida et al. [7] also showed that insulin in the presence of a surfactant is absorbed through the oral mucosa in dogs and they suggested a new therapeutic form.

The aim of the present investigation was to study the action of a combination of insulin and a synthetic polymer administered by the oral route to rabbits with alloxan diabetes.

EXPERIMENTAL METHOD

Chinchilla rabbits weighing 2.5-3 kg were given alloxan by intravenous injection in a dose of 130-140 mg/kg as a 10% solution. Blood was taken from the animals 10 days later for testing. Observations lasted 3 months. The animals with alloxan diabetes were divided into two groups: 1) 14 rabbits with a fasting blood glucose level of 250-430 mg/100 ml, 2) 17 rabbits with a blood glucose level of 160-250 mg/100 ml. The polymer with which insulin was combined consisted of a strong polyelectrolyte with viscosity-average molecular weight of 3800 daltons, conventionally called "polythenate" (this polymer was previously called "polythene" [2]). The dose of insulin was 32 Units/kg. The combination of insulin with the polymer in 3 ml of physiological saline (pH 5.0) was introduced into the mouth. The blood glucose was determined by the method in [9] in the fasting state, after starvation for 18 h, and in blood samples taken every 2 h during the 6-10 h after administration of the insulin preparation. The numerical results were subjected to statistical analysis by the Student—Fisher test.

EXPERIMENTAL RESULTS

Oral administration of the insulin—polymer complex in a dose of 32 Units/kg caused a marked fall in the blood glucose level of all rabbits with alloxan diabetes during 10 h of observation (Table 1). The maximal decrease was observed after 4 and 6 h. In the animals of group 1, with a high initial blood glucose level, oral administration of the insulin—polymer combination lowered the blood glucose level by a maximum of 71 and 70%, compared with 44 and 43% after 4 and 6 h, respectively, in the animals of group 2. The action of insulin alone

Laboratory No. 15, Institute of Macromolecular Compounds, Academy of Sciences of the USSR. Department of Endocrinology, S. M. Kirov Postgraduate Medical Institute, Leningrad.
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 3, pp. 86-87, March, 1983. Original article submitted June 28, 1982.

0007-4888/83/9503-0357$07.50

TABLE 1. Changes in Blood Glucose Level (in mg/100 ml) in Rabbits with Alloxan Diabetes after Oral Administration of Insulin Alone and of a Combination of Insulin (32 Units/kg) with Polymer, and Also Subcutaneous Injection of Insulin—Protamine Suspension in a Dose of 1 Units/kg (M ± m)

Preparation	Number of observations	Time after administration of preparation, h					
		0	2	4	6	8	10
Control (diurnal fluctuations)	10	336±20	343±23	337±33	330±37	382±48	377±47
Insulin alone	10	305±19	301±23	270±22 (11%)	264±26 (14%)	303±30	309±27
Combination of insulin with polymer:							
animals of group 1	14	338±14	170±25* (50%)	99±18* (71%)	101±18* (70%)	107±31* (68%)	133±26* (61%)
animals of group 2	17	197±14	154±13* (22%)	111±12* (44%)	113±9* (43%)	—	—
Insulin—protamine suspension	6	330±5	167±22 (49%)	119±17 (64%)	130±23 (61%)	142±22 (57%)	159±23 (52%)

Legend. *$P \leqslant 0.002$ compared with insulin alone. Percentage decrease of blood glucose level compared with initial concentration, taken as 100%, given in parentheses.

(Table 1) was much weaker and the fall in the glucose level was only 11 and 14%. Differences between the hypoglycemic action of the combined preparation and of insulin alone are highly significant ($P \leqslant 0.002$).

The combination of insulin and polymer thus had a stronger hypoglycemic action in animals with a high initial blood glucose level. Sensitivity to insulin is known to be increased in animals with experimental diabetes, permeability of the cell membranes is changed, and the insulin-inactivated activity of the liver is depressed [11]. These factors were evidently less marked in the animals of group 2.

The hypoglycemic action of the insulin—polymer combination administered orally in a dose of 32 Units/kg was compared with the action of an insulin—protamine suspension, injected subcutaneously in a dose of 1 Unit/kg; the results were found to be comparable and not to differ significantly (Table 1). Data in the literature show that investigators giving insulin by the enteral route usually use large doses. The reason is that only 0.5-8% of the total quantity of the preparation exerts its physiological action compared with that obtained by intramuscular injection. Ishida et al. [7], for instance, gave dogs 27 Units/kg orally, whereas Shichiri et al. [13] gave 25-50 Units/100 g body weight into the jejunum of rats with diabetes three times a day. When polymer implants were used [5, 8], large doses of insulin also were given and it was found that about 3% of the administered dose was absorbed.

It is a particularly important fact that the combination of insulin and polymer, when tested by oral administration, proved to be long-acting and even 10 h after administration the blood glucose level was still reduced by more than 60% compared with the initial level. Observations on a rabbit with a high blood glucose showed that if the insulin—polymer combination was administered orally once a day for 6 days the fasting blood glucose level was reduced from 357 to 228 mg/100 ml and the decrease expressed as a percentage increased from 41 and 48 to 85 and 81 after 4 and 6 h, respectively.

The results indicate that diabetes can be controlled in animals by oral administration of a combination of insulin with a synthetic polymer. The polymer protects the hormone from destruction by proteolytic enzymes and, acting as a surfactant, it also perhaps facilitates its absorption.

LITERATURE CITED

1. V. G. Baranov, M. F. Belovintseva, L. L. Shchukovskaya, et al., Probl. Éndokrinol., No. 6, 88 (1975).
2. V. G. Baranov, L. L. Shchukovskaya, V. A. Kropachev, et al., Probl. Éndokrinol., No. 6, 41 (1979).
3. V. P. Komissarenko, Probl. Éndokrinol., No. 1, 40 (1938).
4. I. N. Kononenko, A. V. Stefanov, and V. K. Lishkov, in: Abstracts of Proceedings of the 2nd All-Union Congress of Endocrinologists [in Russian], Leningrad (1980), p. 127.

5. H. M. Creque, R. Langer, and J. Folkman, Diabetes, 29, 37 (1980).
6. G. L. Haberland, J. Pütter, and W. Puls, Med. Pharmacol. Exp., 14, 297 (1966).
7. M. Ishida, Y. Machida, N. Nambu, et al., Chem. Pharm. Bull., 29, 810 (1981).
8. R. Langer and J. Folkman, Am. Chem. Soc. Polymer Preprints, 18, 379 (1977).
9. N. Nelson, J. Biol. Chem., 153, 375 (1944).
10. H. M. Patel and B. E. Ryman, FEBS Lett., 62, 60 (1976).
11. H. M. Patel and B. E. Ryman, Biochem. Soc. Trans., 5, 1054 (1977).
12. M. Shichiri, R. Kawamori, Y. Goriya, et al., Acta Diabet. Lat., 15, 175 (1978).
13. M. Shichiri, R. Kawamori, Y. Goriya, et al., Endocrinol. Jpn., 23, 493 (1976).

MITOTIC INDEX IN THE CORNEAL EPITHELIUM FOLLOWING TRAUMA TO TISSUES DIFFERING IN PROLIFERATIVE ACTIVITY

A. B. Denisov

UDC 612.841.1.014.2:612.6]-063

KEY WORDS: proliferation; trauma; corneal epithelium; skin; muscle; salivary gland.

Maintenance of a certain number of cells in a tissue is an important component of homeostasis. According to many workers [8] products formed as a result of trauma are regulators of cellular homeostasis. However, despite long searches for specific growth stimulators, all substances isolated have been shown to possess relative tissue specificity [8]. Moreover, growth stimulators have been studied after trauma to strongly proliferating tissues. It is still not clear whether inactively proliferating tissues have the ability to secrete growth stimulators.

The object of this investigation was to compare the nonspecific action of trauma to tissues differing in their ability to undergo post-traumatic hyperplasia on proliferation.

EXPERIMENTAL METHOD

Experiments were carried out on 169 mature noninbred rats of both sexes. In the experiments of series I and II the rats were immobilized and the skin shaved in the dorsal region, after which the epithelium of the skin was scarified until multiple pinpoint hemorrhages appeared (area of injury about 20% of body surface). In the experiments of series III 50% of the right quadriceps femoris muscle was resected in the rats. A mock operation, with simple incision of the skin and fascia, was performed on the control rats. In the next four series of experiments about 20% by weight of the right submandibular salivary gland was removed, and in the control rats only the capsule of the gland was divided. All traumatic manipulations were carried out under pentobarbital anesthesia (50 mg/kg). Mitoses were blocked 4 h before the rats were killed by intraperitoneal injection of colchicine (3 mg/kg). The corneal epithelium (CE), which is one of the tissues with the highest level of proliferation, was chosen as test object. Furthermore, proliferation in CE is easily changed by the action of various modifying agents [1, 2, 4]. The mitotic index (MI_c) was calculated as the sum of prophases and c-mitoses (in promille) by the method described in [4]. The dose of colchicine used did not induce preprophase inhibition of mitosis and it completely blocked metaphase (the number of ana- and telophases was under 1%). In the experiments of series I and II the animals were killed on the 2nd, 3rd, 5th, and 7th days, in the remaining experiments on the 3rd day (the length of the mitotic cycle in CE cells). The results were subjected to statistical analysis [7]. Significance was assessed by the U test [3].

EXPERIMENTAL RESULTS

In both experiments trauma gave rise to reproducible and repeated changes in proliferation in CE which occurred despite the developing stress, which was reflected in atrophy of

Department of Pathological Physiology, N. A. Semashko Moscow Medical Stomatologic Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 95, No. 3, pp. 88-89, March, 1983. Original article submitted May 25, 1982.

0007-4888/83/9503-0359$07.50