Pharmacology of Somatrotropin Pegylated by Electron-Beam Synthesis Nanotechnology

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Pharmacological characteristics of somatotropin pegylated using electron-beam synthesis nanotechnology (PEG-STH) were studied. Oral PEG-STH stimulated the intensity of protein and lipid metabolism and endochondral bone growth without modifying the processes of periosteal and endosteal bone formation. Specific activity of this substance administered orally significantly surpassed that of parenteral non-modified growth hormone.

Key Words: *somatotropic hormone; pegylated somatotropin; electron-beam nanotechnology; preparations for regenerative medicine*

Low production of pituitary somatotropin (somatotropic hormone, growth hormone, STH) is a prevalent cause of growth delay in children and pituitary dwarfism (nanism). In clinical practice these conditions are most often corrected by relpacement therapy. STH preparations are administered exclusively parenterally and by long courses in the majority of cases [2,10,14]. The development of side effects due to immunogenic activity of hormone protein largely limits the use of these drugs [9,14]. On the other hand, protein toxicity can be reduced by their immobilization on low-molecular-weight polyethylene glycol (PEG) using electron-beam synthesis nanotechnology. The resultant drugs are protected from proteolytic enzymes and hence and can be administered *per os* [5,11-13].

We studied specific activity of oral STH pegylated (immobilized) by electron-beam synthesis nanotechnology (PEG-STH).

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MATERIALS AND METHODS

Experiments were carried out on 23-25-day-old male Wistar rats (intense growth phase; 50-60 g). Certified animals were bred at Breeding Center of Experimental Biological Clinic of Laboratory Animal, Institute of Pharmacology.

Pegylated STH was developed at Institute of Pharmacology in collaboration with Scientific Future Management. The drug was administered intragastrically in a dose of 0.9 mg/kg 5 days a week for 3.5 weeks (17 doses over 25 days). Immobilization of STH molecules on low-molecular-weight PEG (molecular weight 1500 Da) was performed using electron-beam synthesis nanotechnology using directed flow of fast electrons [4]. Non-conjugated recombinant somatotropin (Scientific Future Management) served as the reference drug. It was injected subcutaneously in a dose equivalent by STH content in pegylated preparation (0.05 mg/kg) according to a similar protocol. This dose was determined in preliminary experiments as the threshold dose for this STH preparation. Controls

intragastrically received PEG solution in an equivalent volume according to the same protocol.

Body weights, body weight increment, tail length (from fur line to the tip), and tail length increment were recorded on days 7, 14, 21, and 27. Blood levels of protein, triglycerides, cholesterol, urea, and glucose were measured on day 27. Biochemical measurements were carried out on a semi-automated biochemical analyzer (Cormay). In addition, the length of the femoral bone from the *trochanter major* top to the *fossa intercondylaris* was measured, its endochondral growth was evaluated by thickness of the cartilaginous epiphyseal plate in the proximal part of the bone, and periosteal and endosteal bone formation were evaluated by the width of the bone marrow channel and thickness of the cortical plate in the middle part of the diaphysis. The tibial bone for histological studies was fixed in 10% formalin, decalcinated in formic acid, and dissected transversely in the central part of the diaphysis. The proximal fragment was then embedded in paraffin and transverse sections 10 μ thick were sliced through the middle of the bone marrow channel. The preparations were stained with hematoxylin and eosin [1]. The measurements were carried out using computer processing of graphic data. The results were processed by methods of variation statistical using the Student *t* test and nonparametric Mann–Whitney *U* test.

RESULTS

Treatment with STH preparations caused no changes in body weights of experimental animals: the parameters in experimental rats virtually did not differ from the control. The tail length increased: its increment was recorded in animals subcutaneously injected with non-conjugated STH and in those injected with PEG-STH. However, changes in these parameters reached the level of statistical significance only after conjugated hormone: 105.1 and 109.2% of control, respectively. The absence of appreciable differences from the reference group seemed to be due to insufficient dose of the drug for manifestation of morphometric changes during this period [8].

On the other hand, both drugs induced significant shifts in the blood biochemistry. After administration of non-modified and pegylated hormone, we observed an increase in serum levels of triacylglycerides (to 129.7 and 150.0% of the control, respectively) and cholesterol (to 106.7 and 138.9% of the control, respectively). This reflects expected intensification of lipid metabolism under the effect of anabolics [2,10]. At the same time, a significant increase in serum protein and urea concentrations (to 107.6 and 129.3% of control, respectively) was observed in rats receiving PEG-STH, but not non-conjugated hormone, which reflects more rapid protein metabolism [10]. Interestingly, treatment with STH-based drugs (possessing contrinsular activity, according to published data [2,10]) caused no changes in blood glucose level. This seemed to be due to high plasticity of the endocrine system in young experimental animals and realization of the compensatory mechanisms (primarily pancreatic) [2].

The state of the bones confirmed stimulation of anabolic processes. Treatment with STH drugs led to an increase in femoral bone length (Table 1). In addition, the cartilaginous epiphyseal plate of the proximal part of the bone thickened significantly (Fig. 1), this indicating its more rapid endochondral growth.

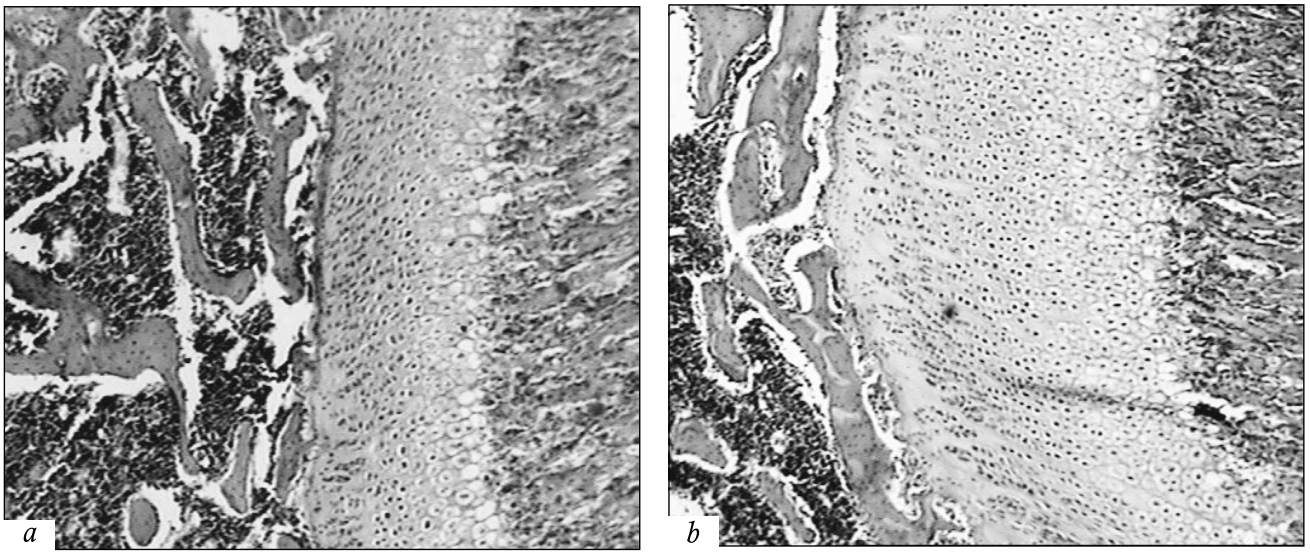

Fig. 1. Cartilaginous epiphyseal growth plate of the proximal tibia in control animals receiving PEG solution (*a*) and PEG-STH (*b*). Hematoxylin and eosin staining, ×60.

TABLE 1. Effects of STH Drugs on the Femoral Bone Parameters of Male Wistar Rats ($X \pm m$)

Group	Length of femoral bone, mm	Thickness of epiphyseal growth plate in proximal part, mm	Width of bone marrow channel, mm	Thickness of cortical plate, mm
Control	25.1±0.2	0.44±0.01	2.11±0.12	0.25±0.02
STH	25.6±0.1*	0.52±0.03*	2.02±0.09	0.24±0.01
PEG-STH	25.97±0.10*+	0.53±0.04*	2.20±0.07	0.25±0.01

Note. $p<0.05$ in comparison with: *control, +STH.

Similarly as in the above cases, these changes were more pronounced in animals receiving PEG-STH. On the other hand, the test STH preparations caused no changes in the width of the bone marrow channel and thickness of the cortical plate (Table 1), hence that they did not stimulate ossification processes, which is important for possible PEG-STH use as a drug for growth stimulation in children [2].

Hence, STH pegylated by electron-beam synthesis nanotechnology exhibited a pronounced anabolic effect associated with significant stimulation of growth. Its advantages in comparison with available STH preparation [2,10,14] is significantly higher specific activity and most convenient oral route of administration. In addition, the maximum conjugation of metabolism intensity and stimulation of *de novo* tissue formation in response to PEG-STH indicated good prospects of this preparation for regenerative medicine [3,4,15].

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