Some Pharmacological Properties of Immunotropic Preparation from Porcine Skin

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We studied pharmacological properties of immunotropic preparation from porcine skin (skin resorption effect and acute and chronic toxicity). It was demonstrated that the preparation did not damage the skin during evaluation of skin-resorption effect; evaluation of acute and chronic toxicity revealed no changes in the general state of experimental animals and histological structure of visceral organs and skin; evaluation of chronic toxicity showed that the preparation induced no changes in blood biochemical parameters compared to the control.

Key Words: *immunotropic preparation; skin; acute toxicity; chronic toxicity; skin resorption effect.*

The development of new immunotropic preparation is an important problem of modern immunopharmacology. Immunotropic preparations are now used in the treatment of many pathologies, including skin diseases. Psoriasis is a prevalent skin disease. The etiology and pathogenesis of this disease are not quite clear, but recent investigations showed that hyperproliferation and incomplete differentiation of keratinocytes in psoriasis are preceded by immunological shifts. Previous studies demonstrated a shift in the regulation of T cells towards T helper type 1 cells and T cytotoxic type 1 cells, which manifested in increased production of cytokines secreted by Th1 and Tc1 lymphocytes (IFN-γ, IL-2, TNF, *etc*.) and in decreased production of cytokines secreted by Th2- and Tc2 cells (IL-10, IL-4, IL-5, *etc*.) [4]. The development of new immunotropic preparations correcting this shift is an actual problem.

We proposed an acetone method for isolation of immunotropic preparation from porcine skin [3]. Using this method we isolated 3 immunotropic preparation, one of them (working name K-activin) inhibited proliferation and stimulated differentiation of human keratinocytes in a primary culture [1,2]. Since hyperproliferation of keratinocytes and their incomplete differentiation are typical of psoriasis, we hypothesized that this preparation can produce a therapeutic effect in this condition.

The aim of the present study was to evaluate the skin resorption effect and acute and chronic toxicity of K-activin.

MATERIALS AND METHODS

Pharmacological properties of K-activin were studied on outbred albino mice, outbred albino rats, guinea pigs, and Chinchilla rabbits obtained from Kryukovo nursery.

The preparation was administered via a noninvasive route (in the form of ointment). Cream Unny containing lanolin, olive or sunflower oil, and water (1:1:1) was used as the basis for the ointment. K-activin was dissolved in distilled water to a concentration of 900 μg/ml and mixed with sunflower (or olive) oil and lanolin. The concentration of T-activin in the ointment was 0.03%.

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The skin resorption effect was studied on 5 outbred albino mice and 5 outbred albino rats by placing their tails into 0.03% K-activin ointment for 4 h. Signs of local irritation (disturbed integrity of the skin on the tail) were evaluated visually.

Acute toxicity was studied on 3 animal species: outbred albino mice, outbred albino rats, and guinea pigs. We used 20 mice weighing 15-20 g (5 mice per group differing by the dose and administration route), 12 guinea pigs weighing 301-443 g, and 12 outbred albino rats weighing 217-328 g (3 animal per group). Aqueous solution of K-activin was injected intraperitoneally (therapeutic and 100-fold therapeutic doses) or applied to the skin (therapeutic dose). Control animals received intraperitoneal injection of physiological saline.

The animals were continuously observed during the first day. The general state of the animals, their behavior, and intensity and pattern of locomotor activity were recorded. The state of the skin and hair, the color of visible mucosae and the position of the tail were evaluated. The irritating effect was scored (by the degree of erythema and edema). Fodder and water consumption and the weigh of feces were determined. The animals were weighed before and after the experiment. The effect of the preparation was evaluated by macro- and microscopic examination. The animals were decapitated after 72 h, samples of the heart, lung, liver, kidney, spleen, intestine, colon, and skin were collected for histological examination. Sections were stained with hematoxylin (after Rego) and eosin and by the method of Van Gieson.

Chronic toxicity of 0.03% K-activin ointment was studied for evaluation of possible damaging effect of the preparation during its long-term use. The study was performed on 15 Chinchilla rabbits for 60 days. The animals were divided into 3 groups (5 per group). In groups 1 and 2 rabbits, 0.03% K-activin ointment was daily applied on a cleaned hair-free skin (36 cm^2) and hair-free damaged skin (4-cm^2 scratch). In group 3 rabbits (controls), the hair was pulled out (36 cm^2; 2 rabbits) and a 4-cm^2 scratch was inflicted (3 rabbits), but no ointment was applied.

The animals were daily observed throughout the experiment. Their behavior, food consumption, and the state of the skin, hair, and mucosae were recorded. The irritating effect was scored (visually, by the degree of erythema and edema). Biochemical parameters of the blood were determined every 10 days. The blood from control and experimental rabbits was collected from the ear vein into a centrifuge tube. The samples were allowed to stay at room temperature for 30 min for clot formation and centrifuged at 1500 rpm for 10 min; the serum was collected and total protein, albumin, triglycerides (TG), HDL, cholesterol (CH), potassium, calcium, iron, glucose, total bilirubin, urea, creatinine, alkaline phosphatase, AST, ALT, amylase, and lactate dehydrogenase (LDH) were assayed.

AST, ALT, and amylase were measured on a Reflotron biochemical analyzer (Boehringer Mannheim), other parameters were assayed on a Humalyzer 2000 biochemical analyzer (Human).

The animals were decapitated after 60 days, samples of the heart, lung, liver, kidney, spleen, intestine, colon, and skin were collected for histological examination. Sections were stained with hematoxylin (after Rego) and eosin and by the method of van Gieson.

RESULTS

Evaluation of the skin-resorption effect of 0.03% K-activin ointment showed that the integrity of the skin on the tails was not impaired.

Experiments on acute toxicity revealed no changes in animal state. None animals died. Body weight, skin, and hair were unchanged. Neither intraperitoneal injection of K-activin, nor application of the aqueous solution on the skin surface affected animal behavior and food consumption. Histological examination of organs and tissues revealed no significant differences from the control groups after both application on the skin and intraperitoneal injection in therapeutic and 100-fold therapeutic doses in all animal species.

In experiments on chronic toxicity of K-activin, the rabbits were active, had good appetite, and gained body weight throughout the experiments. In group 2 rabbits, the scratch was covered with a crust; no inflammatory edema and tenderness were observed. The crust detached on days 4-6 and minor pigmentation, peeling, and hyperemia disappeared on day 10. In experimental rabbits, no reliable difference from the control group by histological picture of visceral organs and tissues were noted after application of K-activin ointment. Histological picture of scratched skin treated with K-activin ointment demonstrated successful reparation of the epidermis and connective tissue components and practically did not differ from the state of the skin in the damaged area in control animals. Application of K-activin ointment on depilated or damaged skin did not disturb the histological picture of visceral organs and skin. Biochemical parameters in both experimental groups did not significantly differ from the corresponding parameters in the control group. The levels of TG, HDL, and CH

considerably differed between decades, but within one decade no significant differences between the experimental and control groups were noted.

Thus, K-activin did not damage the skin during evaluation of skin-resorption effect; evaluation of acute and chronic toxicity revealed no changes in the general state of experimental animals and histological structure of visceral organs and skin; evaluation of chronic toxicity showed that the preparation induced no changes in blood biochemical parameters compared to the control.

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