

Preliminary Pharmacological Study of Purified Snake Enzymatic Cream Isolated from Agkistrodon Halys Venom

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(Received March 4, 2003; Accepted June 24, 2003)

To investigate the pharmacological and toxicological actions of purified snake enzymatic cream (PSE) isolated from Agkistrodon Halys Venom, erythematous dermatitis was induced by applying dinitrofluorobenzene (DNFB) on the back skin of guinea pigs. Consequent local alterations at different doses of PSE were observed and compared using negative control methods. In skin irritation and acute toxicological experiments, 500 times the effective doses were used in artificially lesioned skin. The marking criteria in the former were based on local manifestations, and in the latter, on changes of some indicators including body weight, breath, and heart rate before and after the experiments. PSE significantly alleviated erythematous dermatitis ($p < 0.01$, t test) without systemic or local adverse drug reactions within 7 days. PSE as a new drug candidate poses a bright prospect in the prevention and treatment of dermatitis.

Key words—Snake; venom; enzyme; dermatitis; guinea pig

INTRODUCTION

At present, eczema, dermatitis and other intractable dermatoses are still frequently encountered diseases harmful to people's health. But the medicines used to treat them are mainly hormonal. Though these provide a therapeutic effect to a certain degree, they are not advisable to be used for a long period because they have many side-effects and the diseases easily recur.

From the 1980's, professor Hao Wenxue began to treat antithrombotic diseases with snake venom antithrombotic enzyme (Svate), which, recently, was found to be effective in the treatment of many intractable skin diseases such as dermatomyositis, eczema, scleroderma, and psoriasis.¹⁾ In fact, the medicinal use of snakes was already recorded in Shen Nong's "Canon of Materia Medica" compiled more than 2,000 years ago, and in Li Shi-zhen's "Compendium of Materia Medica",²⁾ however no such reports seem to be available in other countries.

Usually, the dosage form of Svate is mainly by injection, which may inconvenience patients. For this reason, the authors have long been searching for an externally used new medicine for treating various kinds of dermatoses, which should be simple and convenient to use, with high therapeutic effect and low

toxicity. Finally, on the basis of Svate-3 (Snake Venom Antithrombotic Enzymes), with Agkistrodon Halys from Jiangsu Province and Jejiang Province as materials and through the processes of isolation and purification, we produced a cream of pure snake venom enzyme (PSE).³⁾ Here, we have carried out a series of experiments on PSE in order to evaluate its safety in clinical application.

MATERIALS AND METHODS

The venom from Agkistrodon Halys was made into a dry powder through the processes of vacuum freezing and drying by the Research Center for the Prevention and Treatment of Senile Diseases.³⁾ The dry powder, containing mainly arginine esterase and a small quantity of nerve growth factors, was made into PSE with an oil-in-water emulsion which consisted of vegetable oil, glycerin, stearic acid, glyceryl monostearate and azon.³⁾

The specific method of preparation: (1) Oil-phase: stearic (111 g), monoglyceride (23 g), liquid paraffin (124 g), castor oil (124 g), and vaseline (10 g) were dissolved in a water bath at 70~80°C. (2) Water-phase: Nipagin ethyl ester (1 g) was dissolved in boiled water. Shortly after the solution became cool, glycerin (62 g), triethanolamine (10 g) and ketone nitride in proper quantity were added to it. The oil phase was added slowly into the water phase, or vice versa, then mixed thoroughly. When the solution

cooled down to room temperature, pure snake venom enzyme was added and stirred thoroughly.

Although it was not a simple component, we called it purified snake enzymatic cream (PSE) for convenience.

1) Experiment on the Therapeutic Effect of PSE on Dermatitis⁴⁾ 30 white, healthy adult guinea pigs (Hartely) of both sexes, weighing 418 ± 73 g provided by the Department of Experimental Animals of China Medical University (CMU), were depilated on the back skin of both sides, and the hairless area was 20 cm^2 . The left side skin was sensitized by 1% freshly-prepared DNFB (from the Department of Immunology of CMU), 0.5 ml of which was smeared twice in 14 days, then 7 days after smearing the second time, the right was provoked by 0.5 ml of 0.1% DNFB. Another 7 days later, erythematous dermatitis occurred.

According to body weights (BW) and sexes, all animals were randomly divided into 3 groups (10 animals each), including a high-dose of PSE (2.49 USP/kg, 1 USP equivalent to enzymatic activity (Arginine esterase hydrolyzes the benzoyl-L-arginine ethyl ester) leading to 0.003 variation in optical density (253 nm) per minute, American Pharmacopoeia), low-dose (0.62 USP/kg) and a vehicle group. PSE or vehicle was administered on the whole area of dermatitis as soon as it formed, then covered with sterilized gauze and adhesive plaster covering the smearing area. All animals were fed by special staff assigned in the department. Constant room temperature was maintained at 18°C and ventilation was done twice a day. After being smeared with the medicine, whether or not the erythema, edema, exudation and eschar appeared, the area was observed once a day.

2) Experiment on Skin Irritation of PSE⁵⁾ 20 guinea pigs (198 ± 6 g) were depilated by 40 cm^2 on both sides of the back, then divided into 2 groups, an intact skin and damaged skin group. For the latter, the skin of animals was cut artificially (both sides: $2 \times 2 \text{ cm}^2$, of subcutaneous depth) with surgical scissors under ether anesthesia. The groupings and ad-

ministration were as shown in Table 1.

PSE at a dose of 52.5 USP/kg (equivalent to 500 times the effective clinical dose) were smeared only once, evenly, on the right side of the intact group, while the same dose of PSE was spread on the right side of the damaged group soon after the skin was cut. At the same time, vehicle was administered on the left side of both groups. All area covered with drugs was covered by suitable methods, and 24 hrs later the residues of the drug were washed out with warm water, then, the occurrence of erythema and edema in the site smeared by drugs was observed at 1, 24, 48, and 72 hrs, respectively.

The marking criteria of skin irritation reaction and estimation of irritation intensity of tested drugs should be conducted according to the related regulations of the SDA (State Drug Administration) of China in order to calculate the mean marks and evaluate the irritation intensity of PSE.⁶⁾ The marking criteria were not shown here.

3) Experiment on Acute Skin Toxicity of PSE Preliminary experiments showed that no death occurred at a dose of 500 times greater than the estimated clinical dose. Animals were grouped, depilated and administered as in experiment 2 (see Table 1). Before the drug was administered, respiration and ECG (lead I) were recorded under wakened states.

The observation time should be 7 days continuously after administration of the drugs. Different from the skin irritation experiment in which the marking criteria were based on local manifestations, in the acute skin toxicological experiment, the indicators were based on changes in BW, breath, heart rate, central nervous system, and activities of the limbs before and after the experiments. Once death occurred, postmortem and pathological examination should be done.

RESULTS

1) The Therapeutic Effect of PSE on Dermatitis All animals suffered from moderate or severe erythematous dermatitis (without exudation) within

Table 1. Animal Groupings in Experiments of Skin Irritation and Acute Skin Toxicity

	Left side	Right side
Intact group (10 animals)	vehicle	52.5 USP/kg PSE
Damaged group (10 animals)	vehicle	52.5 USP/kg PSE

72 hr after they passed through DNFB provocative contact. The time of natural regression was about 5 to 6 d, and the area of dermatitis healed under scattered crusts. The time of dermatitis recovery was reduced from 4.9 ± 1.2 d (mean \pm SD) in the control group to 2.8 ± 1.0 d (mean \pm SD, $p < 0.01$, t test) in the high dose group, and to 3.7 ± 1.2 d (mean \pm SD, $p < 0.05$, t test) in the low dose group, which is shown in Table 2. When comparing the recovery time between the administered groups (high dose and low dose) and control group, we found that PSE significantly reduced the time of the natural regression of dermatitis ($p < 0.05$, t test).

2) Skin Irritation Experiment on PSE Administration of PSE did not lead to erythema or edema within 72 hrs in any group, indicating that smearing PSE 52.5 USP/kg on either intact or damaged skin one time had no stimulatory action. The total marking of irritation was 0 to 1, indicating no erythema was seen, or was seen with difficulty. The skin irritation intensity of PSE ranged from 0 to 0.49, indicating no irritation.

3) Acute Skin Toxicity Experiment on PSE No obvious abnormalities were found in the skin, hair, eyes or mucous membrane with naked eyes, compared with the controls, nor were any found in respiration, circulation, central nervous system, activities of limbs, while BW increased from 206 ± 18 g

to 236 ± 25 g in both the intact and damaged skin groups (Table 3). No death occurred. The results suggest that no obvious toxic reaction occurred within 7 days after PSE 52.5 USP/kg was administered.

DISCUSSION

PSE has a therapeutic effect on dermatitis caused by DNFB without irritation or acute toxic action on the skin of guinea pigs, even if a dose 500 times the clinical dose is administered.

In 1990, Liu Zi-hua reported that 1173 cases of 10 kinds of dermatoses, such as simple pityriasis, pompholyx, etc., were treated with PSE cream and particularly good therapeutic results were gained.⁶⁾ In 1998, Chen Shu-zhen et al. applied the purified snake enzymatic cream to treat 300 cases of chronic eczema with an effective rate of 95%.⁷⁾

PSE may improve local microcirculation and the permeation of hair follicles and sebaceous glands by dissolving cutin, and can further alleviate the dystrophy of cells and maintain the normal processes of cornification.⁸⁾ Besides, PSE contains some essential trace elements, such as Cu, Fe, Zn and so on, which may combine with organic molecules to form metal complexes inhibiting the formation of inflammatory substances and effectively eliminating the chain-reactions of oxygen free radicals.⁸⁾ The small quantity of nerve growth factors in PSE can accelerate the heal-

Table 2. The Effect of PSE Cream on Dermatitis Caused by DNFB in Guinea Pigs

Group	Dose (USP/kg)	Number of animals	Subsiding period of dermatitis (day, Mean \pm SD)*
High dose	2.49	10	2.8 ± 1.0
Low dose	0.62	10	3.7 ± 1.2
Vehicle	—	10	4.9 ± 1.2

Table 3. The Results of Acute Toxicity Experiment of PSE Cream (52.5 usp/kg, $n = 20$, mean \pm SD)

Time of applying medicine	Body weight (g)	Respiration Times/min	Heart rate Times/min
Before administration	206 ± 18	127 ± 8	353 ± 20
After administration instantly	—	—	—
1 h	—	128 ± 15	353 ± 20
24 h	—	115 ± 20	336 ± 31
7 d	236 ± 25	120 ± 19	342 ± 29

Note: The respiration or heart rate from 2 to 6 d were not obviously different from those on 7 d, and are not shown here.

ing processes of damaged skin.⁹⁾ However, the definite mechanisms of the medicine still need further study.

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