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EFFECT OF VITAMIN E AND OTHER AMELIORATORY AGENTS ON THE FENVALERATE-MEDIATED SKIN SENSATION

(Fenvalerate; skin sensory sensations; vitamin E; piperonyl butoxide; benzocaine; pyrethroid)

L.A. MALLEY, S.Z. CAGEN, C.M. PARKER, T.H. GARDINER, G.A. VAN GELDER and G.P. ROSE

Toxicology Research, Shell Development Company, P.O. Box 822, Houston, TX 77001 (U.S.A.)

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SUMMARY

Previous investigations have demonstrated that dermal exposure to fenvalerate or other synthetic pyrethroid insecticides can produce a skin sensory response characterized by an itching/tingling sensation in humans and animals. The objective of this investigation performed in guinea pigs was to establish treatments which would be effective against pyrethroid-mediated skin sensation. Two classes of agents were tested. Barrier agents, which block penetration of substances through the skin, did not significantly reduce the fenvalerate-mediated skin sensations. Post-treatments with steroidal Dermolate® , anti-histamine Delamine® or anti-inflammatory aspirin did not significantly reduce the pyrethroid-mediated skin sensation. However, Bicozene® (a local anesthetic cream) and Tashan® (a vitamin A, D, and E-containing cream) were effective in reducing the pyrethroid-mediated skin sensations. Prior (30 min and 5h) dermal application of vitamin E was found to be effective in significantly reducing the fenvalerate-mediated skin sensation; even when applied 29 h prior to fenvalerate exposure, there appeared to be a reduced skin response. Piperonyl butoxide (PBO), a pesticide synergist, reduced the fenvalerate skin sensations when applied either directly to the skin or in conjunction with the pyrethroid.

INTRODUCTION

Synthetic pyrethroids have become popular insecticides as a result of their efficacy and low mammalian toxicity. Some individuals exposed to pyrethroids, either in the laboratory, in manufacturing, or in the field, have reported transient sensory

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Abbreviation: PBO, piperonyl butoxide

responses in the skin following pyrethroid exposures. These effects have been described as transient tingling/itching sensations, most frequently experienced on facial skin [1–4]. The design of the animal model is based on monitoring the animals response (licking, scratching, biting and rubbing) to the pyrethroid-treated exposed skin. Investigations in our laboratory using this approach have led to the development of an animal model to evaluate the cutaneous sensation caused by various pyrethroids [5]. Using the animal model, the skin sensations occurred in the absence of any overt skin irritation. The peak response of guinea pigs treated on shaved skin test-sites with various synthetic pyrethroid preparations occured 1 h after application and lasted approx. 4 h. It was noted that all pyrethroids tested (cypermethrin, permethrin, deltamethrin, fenvalerate and flucythrinate) produced this skin sensation response, although the level of response varied with the dose and the pyrethroid [5,6].

The aim of this investigation was to establish treatments that might be effective against the skin sensation experienced by incidentally or accidentally exposed individuals. To accomplish the objective, two types of agents were examined for their effects on pyrethroid-induced skin sensations: (a) agents which could block penetration through the skin and (b) agents which have pharmacologic actions to reduce skin sensation. Fenvalerate was used in these studies as a typical representative of the alpha cyano-containing class of pyrethroids.

MATERIALS AND METHODS

Chemicals

Fenvalerate (cyano(3-phenoxyphenyl)methyl-4-chloro-*o*-(1-methyl)benzene acetate) was used as either a emulsifiable concentrate formulation (Pydrin® 2.4 EC, Shell Chemical Co.) diluted in water, or as the technical material diluted in either ethanol or corn oil. The agents tested for their amelioratory action on the fenvalerate-induced skin sensations were as follows: aspirin (acetylsalicylic acid, Sigma Chemical Co.); Vaseline® (Chesebrough-Ponds); Kerodex® (Ayerst Labs.); PBO (Pfaltz and Bauer); hydrocortisone (Dermolate®, Schering Corp.); double anti-histamine gel (Di-Delamine® Commerce Drug Co.); Bicozene® cream (Creighton Products Corp.); Tashan® (Block Drug Co.); vitamin E acetate (Jack Eckerd Corp.) and vitamin E (Calbiochem Behring Corp.).

Animals

Male Duncan Hartley guinea pigs obtained from Camm Research Institute (Wayne, NJ) were used in this study. Standard laboratory chow (Purina guinea pig chow) and tap water were available ad lib.

Animal preparation

Skin sites were prepared approx. 24 h prior to treatment by clipping the back and

trunk area down to the lateral midline on both sides of the animal, using an electric clipper with a surgical gauge head. The guinea pigs were further treated with a depilatory lotion applied for 5 min and then rinsed off. 3–8 Animals were used per dose group.

Testing procedures

(1) Pyrethroid treatment. On the day of testing, two testing procedures were used: (a) 0.1 ml fenvalerate (2.4% pyrethroid, w/v) was applied to one flank, and 0.1 ml of a vehicle control substance (e.g., ethanol or the blank emulsifiable concentrate formulation) was applied to the opposite flank such that there was a clear 344/A3 distinction between treatment areas; or (b) 0.1 ml fenvalerate (2.4% pyrethroid w/v) was applied to both flanks either prior to or following the amelioratory test agent. The compounds were applied by 'painting' over the test area using a syringe fitted with a ball-tipped dosing needle.

(2) Amelioratory treatments. Pretreatments were applied at various times prior to application of the pyrethroid either to the skin sites receiving the pyrethroid or to both the pyrethroid skin sites and the vehicle skin sites. Post-treatments were applied within 15 min of pyrethroid application in exactly the same manner as with the pretreatments. After dosing, the animals were placed in individual transparent cages for observation.

Scoring of the pyrethroid-mediated skin sensation

The response was quantified by counting the number of times the animal turned to lick, scratch, bite or rub the control or test treatment skin sites during pre-defined time intervals. Beginning 10 min after test or control exposure, observations were made every 15 min for a 5-min period during the first hour following exposure (i.e., 4 observations). Further 5-min observations were made hourly for the next 3 or 4 h. A more detailed description of the rationale behind the scoring procedure has previously been reported [5].

Statistics

Studies involving the comparison of fenvalerate-treated animals alone with animals treated with fenvalarate plus an amelioratory agent (pyrethroid treatment) were analyzed by analysis of variance, with individual means compared by the least significant difference test [7]. Studies involving comparison of fenvalerate to fenvalerate plus amelioratory agent (ameliatory treatment) applied on the same animal were made using Student's paired *t*-test. Statistical evaluations were made for the cumulative scores from the observations made over the 4-h post-treatment period. The level of statistical significance was taken as $P<0.05$.

RESULTS

Application of fenvalerate to guinea pig skin either diluted in ethanol or as an emulsifiable concentrate (EC) formulation resulted in cumulative (seven 5-min observations over a 4-h period) mean score of 73 ± 12 turns to the pyrethroid-exposed treatment site and 2.3 ± 2 turns to the vehicle control-exposed treatment site. These figures are derived from the mean results from the pyrethroid-treated and control-treated animals tested as part of the undermentioned two studies. This result has subsequently been used in the following studies to express the animal response data after amelioratory treatment in terms of a percentage of the fenvalerate-alone response.

Pretreatment with Vaseline® did not produce any protection (81 ± 21%) compared to the fenvalerate-alone response. The barrier creams Silicote® and Kerodex® produced a small reduction in the cumulative response to fenvalerate (64 ± 14, and 71 ± 21%, respectively). However, these results were not significantly different from the pyrethroid-alone response (Table I).

Post-treatment (15 min after fenvalerate exposure) with a hydrocorticoid cream (Dermolate®) resulted in a reduction (63 ± 8%) in the pyrethroid-mediated response, but again this was not statistically significant. Post-pyrethroid exposure to anti-histaminergic gel (Di-Delamine®) and to aspirin resulted in no appreciable changes from pyrethroid-alone treated animals (Table II).

Post-treatment (15 min after fenvalerate exposure) with a local anesthetic cream (Bicozene®) resulted in a significantly reduced response compared to the pyrethroid-alone cumulative response ($P<0.05$). A similar degree of protection was seen when a vitamin A, D, E containing cream (Tashan®) was applied after fenvalerate application (29 ± 15% of the pyrethroid alone).

Based on the above findings, the effect of pre-treatment (30 min prior to pyrethroid exposure) with vitamin E acetate was examined. Application of 0.1 ml

TABLE I

THE EFFECT OF BARRIER TREATMENTS ON THE FENVALERATE-MEDIATED SKIN SENSATIONS IN GUINEA PIGS

Agent[a]	% Mean control response[b]	Level of protection[c]
Vaseline®	81 ± 21	no
Silicote®	64 ± 14	no
Kerodex®	71 ± 22	no

[a] Agents were applied to shaved skin sites immediately prior to application of fenvalerate.

[b] Data based on the cumulative mean score over a 4-h period for the fevalerate-mediated response, which in these studies was 75 ± 12.

[c] Protection was based on the test response being significantly less than the fenvalerate-alone skin response at $P<0.05$, $N=6$–8.

TABLE II

THE EFFECT OF VARIOUS PHARMACOLOGICAL AGENTS ON THE FENVALERATE-MEDIATED SKIN SENSATIONS IN GUINEA PIGS

Agent[a]	Pharmaceutical action	% Mean control response[b]	Level of protection
Dermolate®	Glucocorticoid	63 ± 8	no
Di-Delamine®	Antihistamine	90 ± 26	no
Aspirin®	Anti-inflammatory	92 ± 38	no
Bicozene®	Local anesthetic	32 ± 9	yes
Tashan®	Vitamin A,D,E, cream	29 ± 15	yes

[a] Agents were applied 15 min after application of fenvalerate, with the exception of Aspirin, which was applied to the skin 30 min prior to fenvalerate treatment.

[b] The data are based on the cumulative mean score over a 4 h period to the fenvalerate-mediate response, which in these studies was 75 ± 12.

[c] Protection was based on the response being significantly less than the fenvalerate-alone skin response at $P<0.05$, $N=6$–8.

of either a 5% or a 50% (w/v) solution of vitamin E acetate in corn oil resulted in significant reduction ($P<0.05$) in the response (41% and 14%, respectively) compared to the fenvalerate-alone response. Furthermore, this study showed that corn oil vehicle controls also showed a significantly reduced response over the pyrethroid-alone treated animals, but this reduction was smaller than that produced by vitamin E (Table III).

A time-course study was used to establish the extent of the protective effect of vitamin E on the pyrethroid-mediated response. The results showed that there was a significantly reduced response to fenvalerate following a 5-h pre-exposure period with vitamin E (50% w/v in corn oil), whereas there was no such effect with corn oil after a 5-h pre-exposure period (Table IV). The response to fenvalerate was still reduced after a 29-h pre-exposure period to vitamin E, but not significantly.

TABLE III

THE EFFECT OF PRIOR ADMINISTRATION OF VITAMIN E ON THE FENVALERATE-MEDIATED SKIN SENSATION RESPONSE IN GUINEA PIGS

Treatment[a]	Cumulative mean response[b]	% Control
Fenvalerate	85 ± 5	100
5% Vitamin E (in corn oil)	35 ± 5	41*
50% Vitamin E (in corn oil)	12 ± 15	14*
Corn oil alone	46 ± 15	57*

[a] Vitamin E acetate (5% and 50% in corn oil) and corn oil alone were applied as pretreatments 30 min prior to fenvalerate.

[b] The 4-h cumulative mean score is based on 4 animals per group.

* Significantly different from the fenvalerate-alone skin response at $P<0.05$.

TABLE IV

THE EFFECT OF VITAMIN E AT VARIOUS PRETREATMENT TIMES ON THE FENVALERATE-MEDIATED SKIN SENSATION RESPONSE IN GUINEA PIGS

Pretreatment time	% of 4-h cumulative mean fenvalerate response[a]	
	Vitamin E	Corn oil
0.5 h	14 ± 18*	57 ± 18*
5.0 h	44 ± 13*	99 ± 17
29.0 h	63 ± 17	92 ± 14

[a] Data based on the cumulative mean score over a 4-h period for the fenvalerate-alone mediated response, which in this study was 85 ± 5, $N=4$–6.
*Significantly different from the fenvalerate-alone skin response at $P<0.05$.

A further study was conducted using piperonyl butoxide (a chemical often used in conjunction with pyrethroids). PBO was used either in conjuction with the EC formulation of fenvalerate, or applied directly (0.1 ml) to the test site prior to fenvalerate exposure. In both cases PBO resulted in a significant ($P<0.05$) reduction in the response, compared to the fenvalerate-alone skin response (Table V).

DISCUSSION

Synthetic pyrethroids produce sensations characterized by itching/tingling sensations of the skin in humans [4]. What is supposed to be a similar sensation in laboratory animals has previously been quantified [5]. The studies reported here confirm the previous data that the pyrethroid fenvalerate does induce skin sensations in guinea-pigs, which can be measured in terms of the animals, response to the treated test site. Using this approach, a series of experiments was carried out to investigate whether two classes of amelioratory agents could modify the fenvalerate-mediated skin sensations.

TABLE V

THE EFFECT OF PIPERONYL BUTOXIDE (PBO) ON THE FENVALERATE-MEDIATED SKIN SENSATION RESPONSES IN GUINEA PIGS

Treatment	% Cumulative mean fenvalerate-alone response[a]
Fenvalerate + PBO in the EC formulation	30 ± 5*
0.1 ml PBO applied directly to the skin prior to fenvalerate	17 ± 11*

[a] Data based on the cumulative mean score over a 4-h period to the fenvalerate-mediated response, which in this study was 61 ± 9, $N=4$–6.
*Significantly different from the fenvalerate-alone skin response at $P<0.05$.

Barrier creams, traditionally used to minimize dermal exposure to chemicals, were ineffective in preventing or significantly reducing the fenvalerate-mediated skin sensations. The reason for their lack of effect is difficult to understand, but may be due either due an incomplete barrier being created or to the lipophilic nature of fenvalerate, which allows it to diffuse through the barrier. Either explanation is possible, as only minute quantities of the pyrethroid are required to elicit the skin response.

Several agents with pharmacological actions were tested to prevent or alleviate fenvalerate-mediated skin sensations. Bicozone® (local anesthetic cream) and Tashan® (vitamin cream) were the only effective agents against the fenvalerate-mediated skin effects. The effectiveness of Bicozone® and the lack of effect of the anti-histamine and steroidal creams confirms the belief that the skin sensations are due to the effect of the pyrethroid on the sensory nerve terminals in the skin, rather than a classical skin irritation or sensitization effect [5]. Since some individuals become sensitized to local anesthetics with long-term use, no further investigations were undertaken using this approach.

Prior administration (30 min) of vitamin E, one of the components of Tashan®, was found to be effective in reducing fenvalerate-mediated skin sensations. Prior administration (30 min) of corn oil containing minute amounts of vitamin E was also effective in reducing the response to fenvalerate. A time-course showed that application of vitamin E, up to at least 5 h prior to fenvalerate exposure, significantly reduced the response compared to fenvalerate alone. In addition, vitamin E was effective, even if applied 29 h prior to fenvalerate exposure; corn oil was not effective, however, applied at 29 h or 5 h prior to fenvalerate treatment. The mechanism by which vitamin E exerts its effect on pyrethroid-mediated response is not known. However, vitamin E might possibly exert a pharmacological action, such as stabilizing critical membranes [8] or affecting nerve conduction by enhancing nerve energy metabolism [9]. Although the mechanism by which vitamin E exerts beneficial effects on nerves is not completely understood, it has been shown to provide protection against other neurotoxic compounds such as methyl mercury and misonidazole [10,11]. Further work will, however, be required to elucidate the mechanism by which vitamin E provides an amelioratory action. Since completion of these studies, vitamin E has been shown to alleviate the skin sensation response reported in in humans following ear-lobe application [4].

PBO is often used as a pesticide synergist, acts as an inhibitor of the oxidative metabolism of xenobiotics and has been noted to enhance the acute toxicity of pyrethroids [12,13]. Based on in vitro studies, fenvalerate itself, rather than a metabolite, can be considered reponsible for the observed skin sensation response. As PBO prevents the breakdown of fenvalerate, it was expected that PBO would enhance the skin sensation response, as more fenvalerate would be locally available. However, application of PBO, either in conjunction with fenvalerate (i.e., incorporated into the pyrethroid formulation) or applied directly to the skin test site im-

mediately prior to fenvalerate treatment, reduced the skin sensation response to fenvalerate. Cagan et al. [5] noted that high concentrations of fenvalerate applied to the skin resulted in apparent suppression of nerve activity, expressed as a reduction in skin sensation responses. It is possible that the observed reduction in skin sensation with PBO is a result of nerve suppression, due to more fenvalerate being present to elicit this effect. However, PBO might be directly affecting nerve energy metabolism or be competing for receptor sites at the nerve terminals. Further studies are necessary to establish the mechanism by which PBO exerts this effect.

In conclusion, the skin-sensation response is mediated by the pharmacological action of fenvalerate on the sensory nerve terminals in the skin, as evidenced by the inhibitory effects of the local anesthetic cream containing benzocaine. The skin sensation does not appear to be a classical irritation response, as antihistamine or steroidal treatment did not alleviate the skin responses. Traditional barrier creams were also ineffective in preventing fenvalerate-mediated skin sensations. Vitamin E application (both pre- and post-application) afforded the 'best protection', the greatest reduction in the fenvalerate-mediated skin sensation response. The effectiveness of vitamin E is thought to be associated with its membrane-stabilizing properties, although its exact mode of action is not known.

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