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SENSITIZING CAPACITY OF USNIC ACID DERIVED FROM LICHENIZED FUNGI

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Seven forest workers were observed to have allergic contact dermatitis caused by exposure to d-usnic acid derived from lichenized fungi (6); patch test responses using l-usnic acid were negative (5). Three of these patients were tested with Usno®[1] applied in concentration 1 % in white petroleum jelly U.S.P., with positive results. Usno® is a water soluble compound of usnic acid which has been marketed in Europe since 1954 as a broad-spectrum antibiotic for topical therapy (8). A review of reports of the systemic toxicity of usnic acid indicated that such toxicity was low (9).

It was decided to investigate the sensitizing capacity of d-usnic acid for guinea pig and human skin.

Methods and Materials

a. *Guinea pig*

Groups of 10 Hartley strain albino guinea pigs (300–400 gm) were utilized, housed in wire mesh cages and fed with University of British Columbia Prescription guinea pig pellets No. 51609 supplemented with a twice weekly ration of lettuce. Horticultural grade vermiculite was used as litter.

Open patch tests

The posterior surface of the ear and adjacent retroauricular skin was shaved. d-Usnic acid dissolved in equal parts of acetone and olive oil B.P. was applied to the skin by pipette, dose 0.5 ml. per pig.

Closed patch tests

A gauze pad (7/8 inch×1 inch) wetted with 0.5 ml. of the test solution applied to the shaved back of the guinea pig was occluded with a standard size (1 1/2 inch× 2 inch) Elastoplast® covering. The animal was then placed in a restrainer described by Buehler (1) and restrained, but not immobilized by a rubber belt. The patches were left on the animals for six hours daily for 3 days.

Intradermal tests

d-Usnic acid, dissolved in dimethylsulphoxide, was injected intradermally into a fore-foot pad, dose 0.1 ml. per pig.

Injection with Freund's adjuvant

Usnic acid with complete Freund's adjuvant was injected into the skin of the back at four sites in each of a group of six pigs, (total dose per pig—usnic acid 4 mg. and adjuvant 0.25 ml.).

Eliciting dose

14 days later the groups of pigs were challenged by application of usnic acid, dis-

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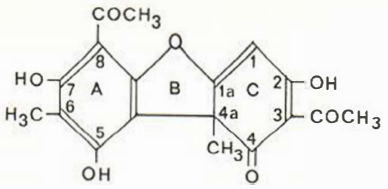

Fig. 1.

solved in equal parts of acetone and olive oil B.P., to the depilated abdominal skin in doses 0.25 ml. and in estimated concentrations 0.77 %, 0.38 %, 0.19 % and 0.09 % spread by a glass rod. In some cases, usnic acid dissolved in dimethylsulphoxide was injected intradermally.

b. *Human*

Closed 48 hour patch tests were carried out on the skin of the outer upper arm. A modified Draize technique was first employed (4) and then a maximization technique described by Kligman (3) was employed to assess the sensitizing capacity of d-usnic acid for human skin. The sample of d-usnic acid, obtained from Koch-Light Laboratories, Colnbrook, Bucks, England, was 95 % pure (by titration) and the melting point was 206°C–208°C. Usnic acid is a partially hydrogenated type of dibenzofurane having an angular methyl group at position 4a (which confers stereoisomeric properties) and is classified as a phloroglucinol derivative (1). Fig. 1.

Results

a. *Guinea pig*

Usnic acid was without primary irritant effect in concentrations up to 50 % in white petroleum jelly, U.S.P.

No evidence of induced dermatitis from the eliciting doses was observed.

b. *Human*

Twenty white adult male patients, aged 60–85 years, receiving institutional care at Shaughnessy Hospital, Vancouver, for a variety of diseases were patch tested with usnic acid in concentrations of 10 % and 50 % in while petroleum jelly, U.S.P. Patients with lymphomatous disease were excluded. No primary irritant effect was observed. Two weeks later, the patients were patch tested with usnic acid 1 % in white petroleum jelly, U.S.P. No positive responses were observed.

At a patch test unit at Shaughnessy Hospital, usnic acid 1 % in white petroleum jelly, U.S.P. was added to a chemical screening patch test set. No positive responses were observed in over 100 patients. This figure does not include cases in which allergy to lichens was suspected clinically and confirmed by patch testing. For the past two years, one of us (J.C.M.) has applied 10 %–20 % d-usnic acid to his left inner upper arm under occlusive patches for varying periods, total exposure probably 3/4 of the two year period, and intermittently preceded by Freoderm® inflammation of the skin (about 30 applications). No positive reactions were observed.

The following study was performed with prisoner-volunteers who were not forest-workers. Usnic acid 10 % in white vaseline was applied to the outer upper arm of 12 white adult male individuals and reapplied at 48 hour intervals for 12 exposures. After a rest period of seven days, eliciting patch tests were carried out. No positive responses were observed. Under the conditions of this modified Draize test, it appeared that usnic acid was not a potent sensitizer for human skin.

After completing the above Draize test, we attempted to sensitize a group of volunteers with an even more rigorous schedule. We followed the procedure of Kligman (3), consisting of five separate 48 hour exposures to usnic acid in concentration 10 % in petrolatum, preceded in each instance by a 24 hour exposure (at the same site) to 10 % sodium lauryl sulphate. This method yields maximum sensitization rates for some low grade sensitizers.

A panel of 24 men was so treated with no evidence of sensitization noted in any.

Discussion

Usno® is prepared from *Cladonia alpestris* which, according to the makers, contains

l-usnic acid. Observations that forest workers with allergic contact dermatitis reacted to d-usnic acid and Usno® but not to l-usnic acid suggest that the sample of Usno® which was used for patch testing here contained the d-form also. Possibly lichens containing d-usnic acid were harvested with C. *alpestris* or racemization occurred during the process of manufacture. Regardless of these possibilities, usnic acid in pharmaceutical preparations is likely to have a low sensitizing capacity for human skin.

In view of the problem of sensitivity to topically applied antibiotics, such preparations appear to merit further attention. Also, *in vitro* studies of Usno® showed a marked antibiotic activity against yeasts (2).

Our experience with clinical sensitization of forest workers with usnic acid must be placed in perspective. We suspect from the data presented here that the sensitization potential of this compound is low and we happened on such an instance. Physiologic and biochemical explanation of such unusual occurrences remains a tantalizing target for future study.

SUMMARY

The sensitizing capacity of d-usnic acid, which has been reported to cause allergic contact dermatitis, was investigated for guinea pig and human skin. Attempts to sensitize guinea pigs were unsuccessful. Using a modified Draize technique and then a maximisation technique which yields maximum sensitization rates for some low grade sensitizers in the cases of 12 and 24 volunteers respectively, no evidence of sensitization was noted in any. d-Usnic acid, used as a topical antibiotic in pharmaceutical preparations, is likely to have a low sensitizing capacity for human skin.

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