Contact Dermatitis, 2000, 43, 79–81
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CONTACT DERMATITIS
ISSN 0105-1873

Topical application of docosanol- or stearic acid-containing creams reduces severity of phenol burn wounds in mice

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Because of their reported antiviral and anti-inflammatory activities, cream formulations containing *n*-docosanol (docosanol) or stearic acid were tested for effects on chemically-induced burns in mice. In this model, injury was induced by painting the abdomens of mice with a chloroform solution of phenol. This was followed by the topical application of test substances 0.5, 3, and 6 h later. Progression of the wounds was assessed by a single evaluator after 8 h, using a numerical score of gross morphology. Docosanol- and stearic acid-containing creams substantially and reproducibly lessened the severity and progression of skin lesions compared to untreated sites with a 76% and 57% reduction in mean lesion scores, respectively. Untreated wounds appeared red and ulcerated; docosanol cream-treated wounds showed only slight erythema.

Key words: docosanol; stearic acid; burns; phenol; irritant contact dermatitis; chemical burns; prevention; treatment; mouse. © Munksgaard, 2000.

Accepted for publication 14 March 2000

Phenol, also known as carbolic acid or monohydroxybenzene, is extremely caustic in high concentrations and can cause life-threatening burns in industrial or household settings (1–3). Phenol matricectomy is one of the most common procedures performed in the podiatrist's office and correct care of the chemically-induced burn is required (4). Although copious lavage with water is the most effective immediate treatment for most types of chemical burns, polyethylene glycol solutions or isopropyl alcohol have proven to be better solvents for washing phenol burns than water (5, 6).

In chemical burns, the skin is denatured through chemical disruption and alteration of physical properties. In this regard, chemical burns differ from thermal, electrical or radiation burns, which result from energy directly absorbed into the skin (7). However, burn injuries all have common features. Inflammatory responses resulting from a burn include predictable alterations in the dermal vasculature (8). In the dermis directly affected by the injury, acute thrombosis and occlusion of vessels occurs. A vascular response also occurs in the uninjured dermis bordering the site of injury, resulting in diminished blood flow. If blood flow is maintained or restored to the area, the tissue survives (9,10). If blood flow is interrupted for sufficient time, progressive ischemia enlarges both the area and depth of the burn site.

The compound *n*-docosanol (docosanol), a 22-carbon, straight-chain, saturated alcohol, while under development as an anti-herpes agent (11), was also found to have anti-inflammatory activity in various model systems, including murine models of collagen-induced arthritis (12) and Langerhans cell migration in assays described by Cowing (13). Stearic acid, an 18-carbon saturated fatty acid, appears to exert similar activity (14). Anti-inflammatory activity of triacontanol, the homologous 30-carbon alcohol, has also been reported (15). The current paper describes experiments conducted to evaluate the effect of topical administration of creams containing 10% docosanol or 10% stearic acid on the chemically-induced burn, or contact dermatitis, caused by phenol.

Materials and Methods

Docosanol (>98% pure) was obtained from M. Michel and Company, Inc., New York, NY. Stearic acid (NF grade) was from Henkel Corporation. Creams containing docosanol or stearic acid were

Table 1. Effect of docosanol- or stearic acid-containing creams on the severity of phenol-induced burns in mice

Treatment	No. mice[a)]	Mean score SD[b)]	*p*-value[c)]
untreated	16	4.04 (0.11)	
docosanol 10% cream	18	0.98 (0.20)	<0.001
stearic acid 10% cream	12	1.74 (0.41)	<0.001
cream vehicle	7	4.14 (0.26)	not significant

[a)] All mice received a different treatment on each of 2 burn sites.
[b)] The following scale was used for evaluation: 0=normal color; 1=almost normal color; 2=red; 3=very red and having a red margin and 4=ulceration.
[c)] Compared to untreated. Student *t*-test, unpaired, two-tailed. *p*-value docosanol 10% cream versus stearic acid 10% cream <0.01.

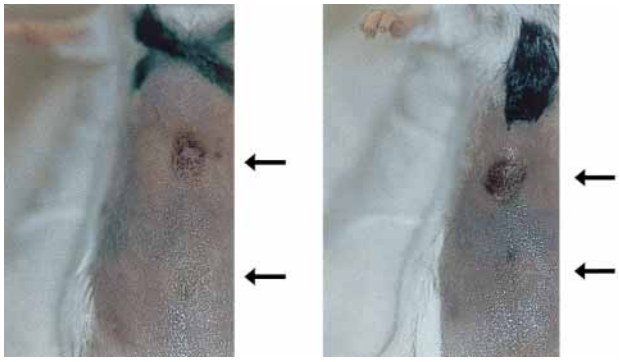

Fig. 1. Photographs of untreated (upper sites) compared to docosanol-treated (lower sites) phenol burns. The photograph shows 2 different mice untreated or treated with docosanol 10% cream 0.5, 3, and 6 h after phenol application. The approximate area of application is indicated by arrows.

provided by Avanir Pharmaceuticals. Vehicle creams were prepared with the same method used to prepare the docosanol (or stearic acid) creams except that docosanol (or stearic acid) was omitted. Other chemicals were reagent grade or the equivalent.

Recovery from chemically-induced burns was measured using female CAF-1 mice greater than 12 weeks of age. The hair on the abdomen of the mice was removed using a hair clipper and then by shaving with a razor. A preparation of phenol:chloroform (1:1; v:v) saturated with Tris buffer, pH=8.0 was used to induce the burn. Phenol:chloroform (5 μl) was applied to the denuded skin in a circular pattern covering an area 5–8 mm in diameter and dried by fanning for 30 s, with 2 burn sites per animal. Test substances were applied 0.5, 3, and 6 h later; with each mouse receiving 2 different treatments. The creams were rubbed in gently but completely.

At 8 h after application of phenol:chloroform, the wounds were assessed by a single evaluator, blinded as to the treatment pattern, and scored with the following scale: 0=normal color; 1=almost normal color; 2=red; 3=very red and having a red margin and 4=ulceration. Intermediate ratings 0.33 above or below integer values were used for more and less severity, respectively, compared to the integer rating standard. A score of 0.5 was used for wounds just noticeably reddened, but not yet equivalent to a typical "1" rating.

Results

Phenol in concentrated solutions causes a severe chemical burn. With the volume and concentration of phenol (50% solution in chloroform) applied in this study, untreated burns develop into circular wounds approximately 1.5 cm in diameter that appear red and ulcerated after 8 h. The therapeutic efficacy of creams containing docosanol or stearic acid was examined in this system. Typical results are tabulated in Table 1 and illustrated in the photographs of Fig. 1. Treatment of the phenol burns with creams containing docosanol or stearic acid 0.5, 3, and 6 h later clearly reduced lesion severity. Whereas untreated or vehicle-treated control animals showed severe erythema and ulceration (mean scores of 4.0–4.1), a statistically significant therapeutic benefit was observed in lesions treated with 10% stearic acid creams (mean score=1.74; $p<0.001$) and a larger effect was observed following lesion treatment with 10% w/w docosanol-containing creams (mean score 0.98; $p<0.001$). Only minimal erythema developed in the docosanol cream-treated sites. Efficacy in this murine phenol burn model has been used routinely as part of quality control to evaluate lots of docosanol creams after preparation for use in non-clinical studies; therefore, numerous lots of docosanol-containing creams have been tested. Creams containing 12% or 20% docosanol or 5%, 12%, or 20% stearic acid, when examined in this model, exhibit responses similar to those observed with 10% docosanol cream or 10% stearic acid cream, respectively (not shown). The effects of treatment are highly reproducible with little variation from animal to animal.

Discussion

These results demonstrate that creams containing docosanol and stearic acid consistently exhibit burn-healing properties with up to 76% (docosanol) and 57% (stearic acid) reductions in mean lesion scores from phenol-induced wounds.

In this mouse phenol burn model, docosanol was first applied 30 min after the initial chemical

burn. The effect of further-delayed treatments was not investigated. Preliminary experiments conducted with thermally induced burns in rats and mice (unpublished data) indicate that heat-induced burns may also be benefited by treatment with docosanol, but that early treatment may be important for optimal effect.

The mechanism for how docosanol or stearic acid mediates effects on burns is unknown. One possibility is that they may act as vasodilators to maintain vascular flow and protect against burn progression. Alternatively, increased migration of Langerhans cells from the skin to the regional lymph nodes is a major feature of irritant contact dermatitis (16). Several known topical immune response modifiers suppress – or enhance – Langerhans cell migration (17). Lipid molecules applied to the skin may help to restore the epithelial permeability barrier and help regulate this aspect of the cutaneous immune response (18).

In its current formulation, docosanol cream may be useful as a first-aid type cream for the treatment of minor chemical or thermal burns to ameliorate pain and to limit burn progression into a more serious injury. Docosanol properly formulated may prove to be therapeutically useful in the treatment of more severe burns including those resulting from phenol exposure. Further study seems warranted.

Acknowledgements

The authors gratefully acknowledge Ms. Regina R. McFadden and Ms. Joceyln Mahittipongse for expert technical assistance and Dr. Jagadish C. Sircar for advice concerning the study and manuscript. The study was funded by Avanir Pharmaceuticals.

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