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Effect of Topical Preparation of Mycophenolic Acid on Experimental Allergic Contact Dermatitis of Guinea-pigs Induced by Dinitrofluorobenzene

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Abstract—The effects of a topical preparation of mycophenolic acid on the experimental allergic contact dermatitis induced by dinitrofluorobenzene was investigated. Visual assessment of skin reactions showed significant efficacy of a topical preparation of mycophenolic acid. This efficacy appeared from the early stage and endured up to 3 days. Morphological changes in the epidermis and dermis layers of animals treated with a mycophenolic acid cream were moderate compared with that in animals treated with vehicle only. In particular, hyperkeratosis was strongly suppressed. Since suppression of inflammatory cell infiltration was also observed, this efficacy might reach to the epidermis and dermis layer.

Mycophenolic acid (6-4-hydroxy-6-methoxy-7-methyl-3-oxo-5-phthalanyl)-4-methyl-4-hexenoic acid) as purified from the penicillium species by Gosio (1896) possesses biological properties including antibiotic (Florey et al 1946), antiviral (Cline et al 1969), and anti-cancer activities (Carter et al 1969). It has an immunosuppressive property (Mitsui & Suzuki 1969; Allison et al 1991) and a potent pharmacological action in the treatment of psoriasis (Jones et al 1975; Lynch & Roenigk 1977). Long-term study in the treatment of psoriasis supports its efficacy (Marinari et al 1977).

Most of the mycophenolic acid administered either orally or parenterally is rapidly metabolized in the liver to the glucuronide and more than 90% of the glucuronide is excreted in the urine (Lintrup et al 1972). Therefore, a large dose of mycophenolic acid is required for the full pharmacological function. The parenteral administration of mycophenolic acid at a high dose may increase adverse effects and the chance of opportunistic infectious diseases (Lynch & Roenigk 1977). Because of these metabolic problems, topical application has been suggested. However, a successful transdermal delivery of mycophenolic acid has not been reported.

In this study, we prepared topical formulations of mycophenolic acid to avoid the first-pass effect and to exert the pharmacological action locally. To elucidate the efficacy of each preparation, visual assessment on the experimental allergic contact dermatitis of guinea-pigs induced by 2,4-dinitrofluorobenzene (DNFB) was carried out. Furthermore, morphological changes over the period of treatment were observed.

Materials and Methods

Animals

Outbred female Hartley strain guinea-pigs, 200–300 g, were purchased from Japan Laboratories Animals Co. Ltd, Japan. They were maintained under conventional conditions and used in groups of 8–9.

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Chemicals

Mycophenolic acid (Fig. 1) was kindly supplied by Professor G. Tamura and Dr K. Ando. Isopropyl myristate (IPM-EX) and polyoxyethylene(5)glyceryl monostearate (TMGS-5) were purchased from Nikko Chemicals Co. (Tokyo, Japan). Isotridecylmyristate (MITD) and isoprene glycol were purchased from Kurare Co. (Tokyo, Japan). Diethyl sebacate, di-iso-propanolamine, stearic acid, and carboxyl vinyl polymer were purchased from Wako Pure Chemicals Co. (Tokyo, Japan). Cetyl alcohol and cetyl palmitate were purchased from Tokyo Chemicals Co. and Hayashi Co. (Tokyo, Japan), respectively.

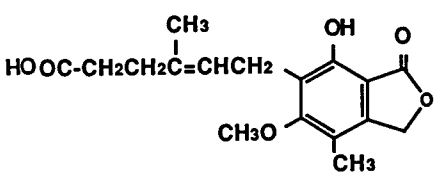

FIG. 1. Structure of mycophenolic acid.

Preparation of mycophenolic acid cream

Mycophenolic acid cream was prepared according to the recipe in Table 1. More than 70 different various solvents were tested to dissolve mycophenolic acid; only TMGS-5 could dissolve mycophenolic acid and therefore mycophenolic acid dissolved in TMGS-5 was mixed with a lipophilic base, adjusting to 5% mycophenolic acid at the final concentration. Then aqueous phase including isoprene glycol, di-iso-propanolamine, and distilled water (or ethanol) was added to the lipophilic base. The mixture was emulsified with heating at 80°C. At the final step, carboxyl vinyl alcohol was added and the mixture was well emulsified on ice. Cream-1 contained 5% ethanol; cream-2 did not contain

Table 1. Composition of mycophenolic acid cream in distilled water or 5% ethanol.

Constituent	%
Mycophenolic acid	5·0
Isoprene glycol	7·0
Diethyl sebacate	8·0
Cetyl alcohol	2·0
Cetyl palmitate	8·0
Stearic acid	3·0
Polyoxyethylene(5) glyceryl monostearate (TMGS-5)	5·0
Isopropyl myristate (IPM-EX)	3·0
Isotridecylmyristate (MITD)	2·0
Di-iso-propanolamine or ethanol	2·0
Carboxyl vinyl polymer	0·2

ethanol. As a reference betamethasone-gentamicin cream, a steroidal drug with moderate efficacy, was used. Betamethasone-gentamicin contained 0·06% betamethasone (Shionogi Pharmaceutical Co., Osaka, Japan).

Experimental allergic contact dermatitis

Experimental allergic contact dermatitis of guinea-pigs was induced by DNFB (Nakarai, Tokyo, Japan), according to the method of Aldridge et al (1985). Briefly, cyclophosphamide (Sigma) was dissolved in distilled water and injected intraperitoneally at a dose of 300 mg kg^{-1}. Three days after the injection of cyclophosphamide, 50 μL 10% (w/v) DNFB dissolved in acetone:olive oil (1:1) was epicutaneously applied to the dorsum of one ear for sensitization. On day 8, animals were challenged with 20 μL DNFB solution (0·1% or 0·5%) dissolved in acetone:olive oil (4:1) and applied to the shaved flank. Drug (20 μL) was applied topically after DNFB, to the flank 9 h later and at similar times on ensuing days. The initial application occurred immediately after drying of the test solution. The corresponding vehicles were topically applied to the opposite side of the flank. The topical applications of drug regimen were assessed twice daily and assessment of skin reaction was carried out 24, 48 and 72 h after the applications. The scores of skin reaction were defined as follows: 0 = no change; 1 = pink spot; 2 = pink; 3 = red but not elevated; 4 = red and elevated.

Statistical analysis

The results were described as the mean ± s.d. and compared using Student's *t*-test. $P < 0·05$ was considered significant.

Histological observation

Animals were killed at 24, 48 or 72 h after treatment. Skin specimens of each flank were removed and fixed by 10% neutralized formalin solution. Specimens were embedded in paraffin wax. Sections were stained with haematoxylin-eosin according to the original method. Morphological changes were observed for hyperkeratosis, parakeratosis, acanthosis, intracellular oedema, basal membrane expansion, spongiosis, and inflammatory cell infiltration, under the light microscope.

Results

Effects of topical preparations of mycophenolic acid on DNFB-induced allergic dermatitis in guinea-pig

Allergic contact dermatitis was induced by either 0·1% or 0·5% DNFB. Since 0·5% DNFB caused severe tissue damage and hampered the evaluation of skin reactions, 0·1% DNFB was used in all subsequent experiments. Skin-reaction scores during cream treatment are shown in Table 2. Cream-1 and cream-2, significantly ($P < 0·05$) suppressed skin reactions induced by DNFB at 24 h after treatment. This efficacy of cream-2 endured up to 72 h after treatment. While betamethasone-gentamicin had no effect on days 1 or 2, the efficacy of mycophenolic acid cream was evident from the early stage of experimental dermatitis. On the third day of treatment, the efficacy of betamethasone-gentamicin on skin reactions was similar to that of mycophenolic acid creams.

Morphological changes of epidermis and dermis of experimental allergic contact dermatitis

Normal epidermis and dermis are shown in Fig. 2. There was no hyperkeratosis, parakeratosis, acanthosis, intracellular oedema or expansion of basement membrane. Morphological changes of the epidermis and dermis layers of the group treated with vehicle only are shown in Fig. 3. Twenty-four hours after the application of vehicle only, inflammatory cell infiltration was observed in epidermis, with some formation of spongiotic vesicles. In the upper layer of the dermis, inflammatory cell infiltration was also found. Frank hyperkeratosis, parakeratosis, acanthosis, and intracellular oedema were observed 48 h later. While hyperkeratosis and parakeratosis were recognized, little intracellular oedema was observed 72 h after treatment. As well as acanthosis, elongated acanthotic rete ridges were observed after both 48 and 72 h. Histomorphological observation showed serial changes of acute dermatitis.

Morphological changes of the epidermis and dermis layers treated with mycophenolic acid

Overall, the degree of efficacy in groups treated with cream-1 and cream-2, were similar regarding morphological changes. Fig. 4 shows typical morphological changes of skin treated with cream-2. Twenty-four hours after treatment, slight expansion of basal membrane and intracellular oedema were observed. A relatively smaller amount of spongiosis and inflammatory cell infiltration was recognized compared with the group treated with vehicle alone. Morphological changes of skin 48 h after treatment were observed (Fig. 4). Although slight hyperkeratosis, intracellular oedema, and expansion of basal membrane were observed, the change was smaller than that in the group treated with vehicle. Seventy-two hours after treatment, only slight acanthosis and spongiosis were observed. While betamethasone-gentamicin could not suppress hyperkeratosis, even 72 h after treatment (data not shown), cream-1 and cream-2 strongly suppressed hyperkeratosis.

Discussion

There has been no successful topical regimen of mycophenolic acid reported so far. In this study, we demonstrated that a topical preparation of mycophenolic acid was effective in improving the experimental allergic contact dermatitis induced by DNFB. This effect endured up to at least three days. The degree of efficacy was better than that of betamethasone-gentamicin, especially in the early stage of treatment. DNFB-induced delayed hypersensitivities are frequently applied as a model of allergic dermatitis (Rullan et

Table 2. Evaluation of efficacy of mycophenolic acid cream on DNFB-induced allergic dermatitis of guinea-pigs.

Drug	24 h	48 h	72 h
Vehicle-1	1·4±0·8	1·4±0·7	1·1±0·8
Cream-1	0·4±0·8*	0·6±0·6*	0·6±0·5
Vehicle-2	1·7±0·5	1·4±0·5	1·3±0·5
Cream-2	0·6±0·4**	0·8±0·5*	0·8±0·5*
Control (no drug)	1·6±0·5	1·4±0·5	1·6±0·5
Betamethasone-gentamicin	1·6±0·7	1·3±0·7	0·8±0·4†

* $P<0·05$ (vs vehicle-1: cream-1 not containing mycophenolic acid), ** $P<0·001$ (vs vehicle-2: cream-2 not containing mycophenolic acid), † $P<0·05$ (vs control), mean±s.d. (n=8–9).

al 1984). This model reflects the allergic contact skin reaction. The evaluation of immunosuppressants on this model is well correlated with clinical efficacies (Rullan et al 1984; Aldridge et al 1985; Cole et al 1988). The results in this study might suggest a clinical efficacy of mycophenolic acid at least equal to that of betamethasone.

We expected the ethyl ester to exert better pharmacological activity than that of mycophenolic acid because of enhancement of penetration. However, we failed to demonstrate this. From the morphological observations, the improvement of skin reactions seemed to reach to the epidermis and dermis layer. In particular, the suppressive effect of mycophenolic acid cream on hyperkeratosis was marked. Since mycophenolic acid creams inhibited inflammatory cell infiltration, we can assume that mycophenolic acid reaches the dermis layer.

Mycophenolic acid has interesting pharmacological activities including an immunosuppressive function equivalent to that of cyclosporin A, without any serious side-effects. Although the compound is well-established, its evaluation as a therapeutic agent has not been exploited. Mycophenolic acid is a potent inhibitor of inosine monophosphate dehydrogenase and guanosine monophosphate synthetase (Carter et al 1969). The compound and related analogues, therefore, inhibit the biosynthesis of guanosine nucleotides. Sircar & Schwender (1983) have demonstrated that mycophenolic acid strongly inhibits synthesis of soybean lipoxygenase. They suggested that this inhibition may intervene in the production of leukotriene in the epidermal system, and may contribute to the efficacy in psoriasis.

Jones et al (1975) evaluated therapeutic efficacy of mycophenolic acid in 29 patients with severe psoriasis. Preliminary clinical trials showed non-toxicity of mycophenolic acid, at doses of up to 6400 mg daily (Jones et al 1975). Long-term toxicity studies of mycophenolic acid in rabbits also showed no apparent signs of toxicity (Adams et al 1975). Most of mycophenolic acid administered by the parenteral route is rapidly metabolized to the glucuronide. Several adverse reactions associated with high dose of mycophenolic acid have been reported (Lynch & Roenigk 1977). In general, the

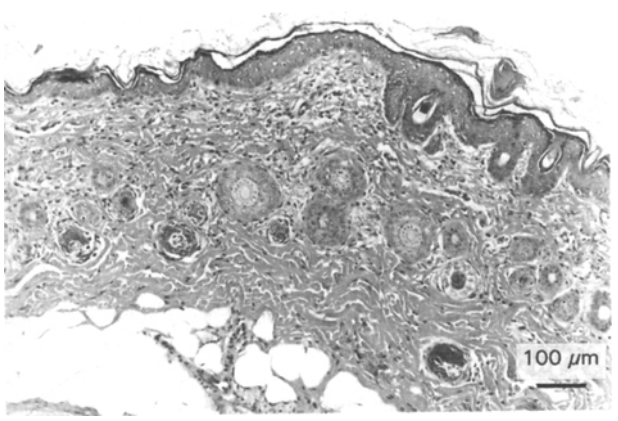

FIG. 2. Morphology of normal epidermis and dermis layer of guinea-pig under the light microscope.

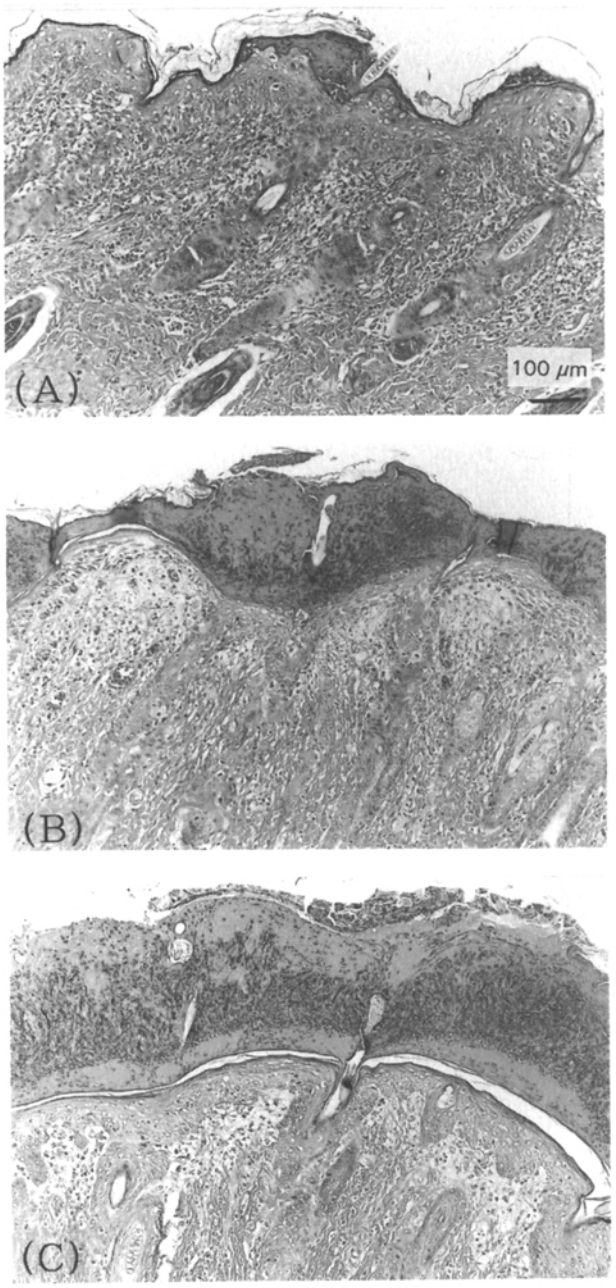

FIG. 3. Morphological changes of epidermis and dermis treated with vehicle only. Allergic contact dermatitis induced by DNFB and treated with vehicle only, 24 (A), 48 (B), 72 (C) h after the treatment. Tissue sections were observed under the light microscope.

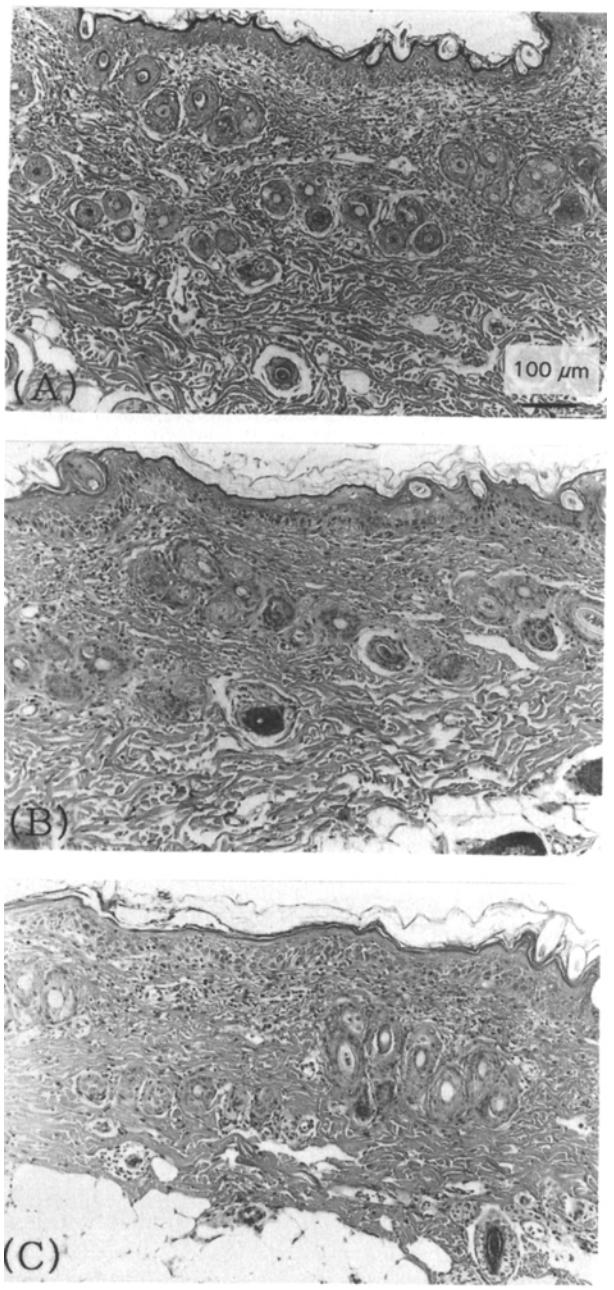

FIG. 4. Morphological changes of epidermis and dermis treated with mycophenolic acid cream. Allergic contact dermatitis induced by DNFB and treated with mycophenolic acid cream 24 (A), 48 (B), 72 (C) h after the treatment. Tissue sections were observed under the light microscope.

side-effects appeared to be dose-related and were particularly common above a daily dosage of 4800 mg.

Since mycophenolic acid is a relatively small molecule, it would be expected to penetrate more efficiently than cyclosporin A. By circumventing the metabolic problems and because of its interesting immunosuppressive activities, topical preparation of mycophenolic acid could be useful in the treatment of allergic dermatitis and psoriasis. Since our study demonstrated efficacy on experimental allergic contact dermatitis, a topical preparation of mycophenolic acid would be promising in the treatment of this condition.

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