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Comparative Study on the Effects of Ointments of Tinidazole, Hydrocortisone and Clobetasol on Animal Models for Inflammatory Dermatitis in Mice

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Abstract To understand further the possible clinical effects of tinidazole ointment at relatively high concentration (2%) for atopic dermatitis (AD), we performed a comparative study with readily available topical corticosteroids, clobetasol propionate (0.005 or 0.05%) and hydrocorotisone butyrate (0.1%) (hereafter referred as clobetasol and hydrocortisone, respectively), on inflammatory dermatitis in mice. We also observed the effects of combined application of tinidazole with clobetasol (0.005%, one tenth of the clinical use) in comparison with tinidazole itself, clobetasol (0.05%) or hydrocortisone (0.1%) on the animal model. All ointments suppressed inflammatory dermatitis induced by trinitrochlorobenzen (TNCB) or oxazolone. The rank order of the potency to suppress the ear edema was clobetasol (0.05%), tinidazole (2%) with clobetasol (0.005%) > clobetasol (0.005%) > tinidazole (2%) in TNCB-induced dermatitis, and hydrocortisone (0.1%), clobetasol (0.05%) > tinidazole (2%), tinidazole with clobetasol (0.005%) > clobetasol (0.005%) in case of oxazolone-induced dermatitis. We confirmed that tinidazole (2%) suppresses immediate and late phase reactions in mice passively sensitized with anti-DNP IgE Mab. In addition, tinidazole (2%) was much more potent than hydrocortisone (0.1%) in suppressing the amount of scratching, presumably due to itching, in passively sensitized mice. These results indicate that the advantage of using ointments of tinidazole would be that it has stronger anti-itching effects than corticosteroids.

Key words: tinidazole, hydrocortisone, clobastasol, ointment, inflammatory dermatitis, mouse

Introduction

Adult atopic dermatitis (AD) is a chronic inflammatory skin disease with significant morbidity[1], and it seems that topical corticosteroids remain one of the most efficient treatments available. However, it is important to develop new treatments for AD, because of concerns about resistance to steroid therapy or potential adverse actions including skin atrophy.

Topical cream or gel containing metronidazole has been used for the treatment of rosacea[2)3)4)] and seborrheic dermatitis[5]. We have recently reported that ointments of metronidazole and tinidazole at relatively high concentrations (1-4%) suppress the immediate and late phase reaction (IPR and LPR) of biphasic ear edema of mice sensitized with ovalbumin or passively sensitized by monoclonal anti-dinitrophenol (DNP) IgE-antibody, trinitrochlorobenzene (TNCB)-induced inflammatory dermatitis with enhanced vascular permeability[6]. In

addition, the effects of tinidazole ointment to suppress IPR, LPR and number of scratching reaction was equi- or more potent than those of a readily available topical hydrocortisone butyrate (LOCOID®)[6]. These observations indicate that ointments of metronidazole or tinidazole possesses anti-inflammatory, immunosuppressive and anti-itching effects, and could possibly be applied clinically to human inflammatory skin disease including atopic dermatitis in addition to rosacea and acne vulgaris[2)3)4)5)].

In an attempt to obtain further understanding of the possible clinical effects of tinidazole on AD, we performed comparative study with clobetasol propionate (DERMOVATE®)[7] and hydrocortisone butyrate (LOCOID®) (classified as group I and II steroid respectively) on the chronic contact hypersensitivity response (CHR) in mice with inflammatory dermatitis.

Materials and Methods

Animals

Male mice (NC / Nga Tnd Crj), 5 weeks old weighting 14.0 - 17.6g, were obtained from Charles River, Japan Inc. After taming and quarantining for 2 weeks with abnormality, all the healthy mice (body weight at the beginning of the sensitization treatment was 19.0 - 24.7g) were used for the experiments. The temperature and humidity were kept at 22 ± 3 °C and 50 ± 15 %, and ambient lighting was automatically regulated on a 12 hour light / dark cycle (lighting : 7 : 00 - 19 : 00). Five mice were maintained in each of polycarbonate cages (W215 x D320 x H130 mm). The animals had free access to solid feed MF for mouse (Oriental Yeast Co., Ltd.) and drinking water (city tap water).

Prior to the sensitization hair was removed by hair clippers from sites of sensitization, and hair removal cream was applied for several minutes, then it was washed off. The animals were divided into several groups according to the experiments and each group consisted of 5 - 10 mice.

Application of Drug

Ointments of tinidazole (2%) and ointment base (FIN-001 ; Yoshitomi Pharmaceutical Co., Ltd) were applied to the sites of the mice. Briefly, ointment base (FIN-001) was a mixture of stearyl alchol, RHEODOL® MS 165 and RHEODOL® SUPER TW-L 120 (Kao Corporation), white petrolatum, liquid paraffin, glycerin, methylparaben, propylparaben and purified water. Tinidazole was mixed with the ointment base to give the final concentration (2%) used in the present experiments. We also used commercially available LOCOID® ointment (hydrocortisone butyrate 0.1%, Torii Pharmaceutical Co., Ltd, Tokyo Japan) and DERMOVATE® ointment (clobetasol propionate 0.05%, Glaxo Smith Kline K. K.) as readily applicable topical corticosteroids.

Animal models

1. Trinitrochlorobenzene (TNCB)-induced dermatitis in mice

We used TNCB to sensitize the mice as reported previously[8]. Twenty-five μL of 2% TNCB solution was applied to the inside and outside of the left pinna (50 μL / ear) of the mice by micropipette. The same amount of acetone was applied to the unsensitized control group. As a challenge, 0.5% solution of TNCB was applied from day 0 to day 24 or 36, once every two days (50 μL / ear) and the thickness of pinnae was measured. Each ointment was applied once a day throughout the experiments 2 hours before the sensitization (day 0 - day 24 or day 0 - day 36, respectively). 10 μL

ointments was put on the pinna with a glass syringe (for 250 μL). To evaluate the effects of ointments, area under the curve ($AUC_{0-19 \text{ or } 24 \text{ day}}$) was also calculated as reported previously[6].

2. Ear edema induced by oxazolone in mice

The sensitization was performed by applying 50 μL of oxazolone solution (7% (w / v) in acetone) to the abdomen with a micropipette, and acetone was applied to the non-sensitized control group. The challenge was done 6 days after the sensitization by applying 5 μL of oxazolone solution to inside and outside the left pinna (10 μL / ear). The right pinna received no treatment. The test ointments were applied 4, 5 and 6 days after the sensitization, and 2 hr before the challenge. Twenty four hours after the challenge, mice were killed by the dislocation of cervical vertebrae, and a part of the pinna (a circle area of 6 mm diameter) was obtained from both pinna with a puncher. The difference in the weight between the punched parts of left and right pinnae was used as an indicator for dermatitis.

3. IgE dependent ear swelling and scratch model in mice

Ten mice of each group were passively sensitized by intravenous injection of 1 ml physiological saline containing 10 μg of monoclonal anti dinitrophenol IgE antibody (anti-DNP-Mab) according to Katayama et al[9]. One mL of physiological saline was injected into the unsensitized control group. Twenty four hours after he passive sensitization, the challenge (5 μL of 0.75% DNFB solution) was applied to the inside and outside of the right ear (10 μL / mouse). Scratching reaction and the increase in ear thickness (ear edema) were then observed as reported previously[9]. After the challenge, each mouse was isolated and then the number of scratching reactions was counted every 30 minutes up to 90 minutes. Scratching of the pinna at least twice by the hindpad was counted as one scratching reaction, and was distinguished from grooming actions by forepad.

Each ointment was applied to the inside of the right ear 2 hours before DNFB application. Three mg of ointment base was applied to the mice in the unsensitized and test groups.

Statistics: The mean value ($\pm$SEM) of various parameters was obtained in various experiments. The differences between the control and the unsensitized group were analyzed by Student's t-test, and the difference between the control and test group was analyzed by Tukey method.

The following drugs were used: monoclonal anti dinitrophenyl (DNP) antibody (mouse IgE isotype), 2,4-dinitro-1-fluorobenzene (DNFB), (Sigma Chemical Co., St Louis, MO, USA), Trinitrochlorobenzene (TNCB), oxazolone, acetone, ethanol (Wako Pure Chemical Industries, Ltd), LOCOID® ointment (hydrocorotisone butyrate 0.1 %, Torii Pharmaceutical Co., Ltd, Tokyo), tinidazole (Yoshitomi Pharmaceutical Co., Ltd) and DERMOVATE® ointment (clobetasol propionate) (Glaxo Smith Kline K. K.).

Results

Effects of tinidazole and hydrocortisone on TNCB-induced dermatitis in mice.

As reported previously[6], during repeated application of TNCB, the ear thickness gradually increased and the peak value was obtained at 16 - 20 days after the start of experiments.

As shown in Fig. 1, ointment base significantly suppressed the increase in the ear thickness in the mice compared to the control group treated with no ointment. Tinidazole (2%) significantly suppressed the ear thickness 1 or 24 hours after the challenge, after 8th or 12th day, respectively. In addition, 48 hours after the challenge, tinidazole (2%) suppressed the ear thickness, except 2nd and 32th days (data not shown).

Hydrocortisone (0.1 %) significantly suppressed the increase in ear thickness compared to the ointment base and was more potent than tinidazole, except 1 and 24hr after the challenge at day 4th and 12th, respectively.

We used area under the curve ($AUC_{0-24\ day}$) to compare the effects of tinidazole and hydrocortisone at 24 hr after the daily challenge with TNCB, since the maximum increase in ear thickness was observed at this timing. The rank order of the potency was hydrocortisone > tinidazole > ointment base.

Effects of tinidazole and clobetasol on TNCB-induced dermatitis in mice.

Further to evaluate the effects of tinidazole on inflammatory dermatitis, we used clobetasol with or without tinidazole. As shown in Fig. 2, ointment base, tinidazole (2%) with or without clobetasol (0.005%), and clobetasol (0.05 or 0.005%) significantly suppressed the increase in ear thickness, 24 hours after the challenge with TNCB. We used $AUC_{0-19\ day}$ to compare the effects of tinidazole with those of clobetasol, and the rank order of the potency was clobetasol (0.05%) > tinidazole (2%) with clobetasol (0.005%) > clobetasol (0.005%) > tinid-

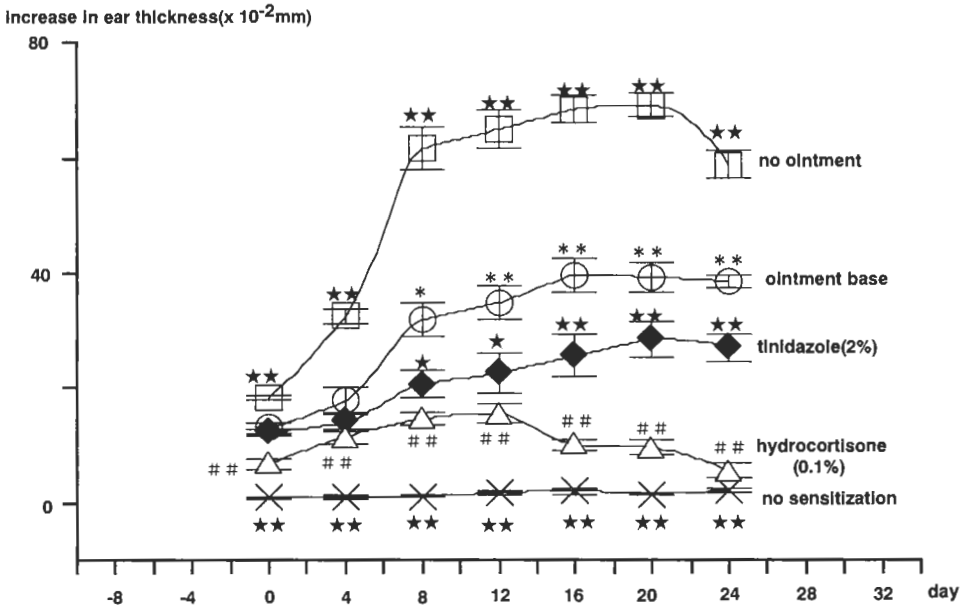

Fig. 1 Effects of ointment base, tinidazole (2%) and hydrocortisone (0.1%) ointments on TNCB-induced ear swelling in mice. Ear thickness was measured at 1, 24 hr after TNCB application. Each point is the mean ± SEM of the difference between left and right pinnae from 8 animals.
$P^{*} < 0.05$, $P^{**} < 0.01$; significantly different from ointment base (t-test). $P^{\#\#} < 0.01$; significantly different from no ointment (t-test), $P^{*} < 0.05$, $P^{**} < 0.01$; significantly different from tinidazole ointment.

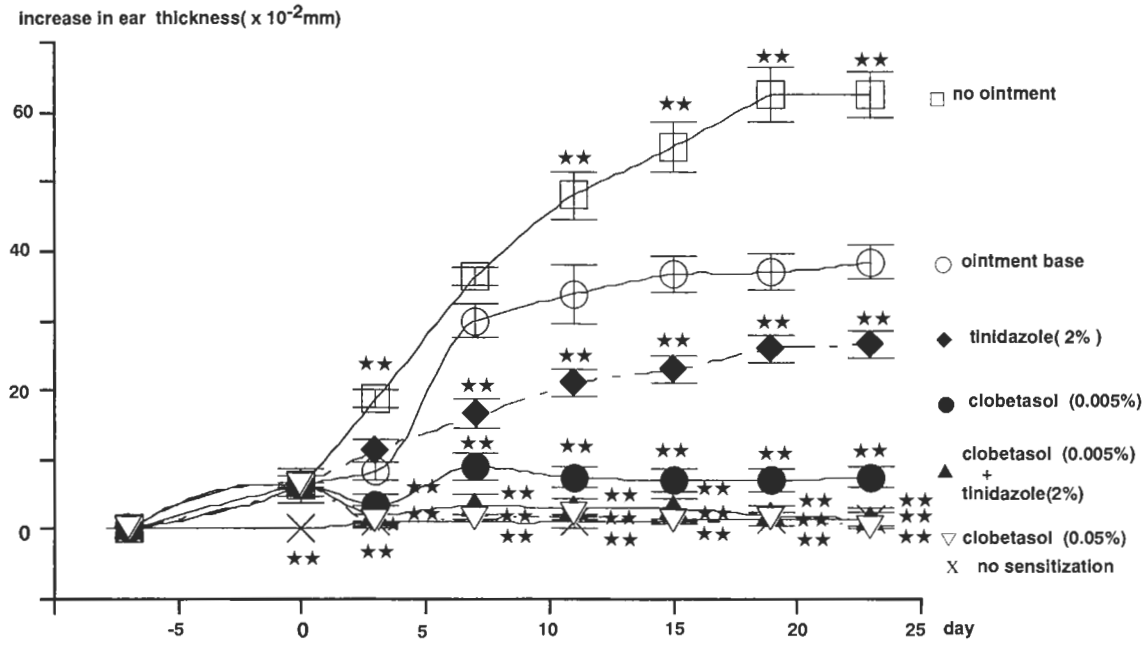

Fig. 2 Effects of ointment base, tinidazole (2%), clobetasol (0.005%) with or without tinidazole (2%) ointments on TNCB-induced ear swelling in mice. Ear thickness was measured at 24 hr after each TNCB application and each point represents the mean ± SEM of 10 animals.
P* < 0.05, P** <0.01 ; significantly different from ointment base (t- test).

azole (2%) > ointment base (Fig. 3). These observations indicate that clobetasol at relatively low concentration (0.005%, one-tenth of the clinical use) suppresses the increase in ear thickness, and that tinidazole (2%) with clobetasol (0.005%) together produces a greater effect comparable to that produced by 0.05% clobetasol (Fig. 3).

Effects of tinidazole, hydrocortisone and clobetasol on oxazolone-induced ear edema in mice.

To study further and compare the effects of tinidazole with hydrocortisone and clobetasol on the animal model for inflammatory dermatitis, we used oxazolone to sensitize the mice. At present, it is considered that type IV allergic reaction through Th1 cell is also involved in addition to Th2 cell mediated allergic reaction in atopic dermatitis[10]. Thus, it is intriguing to observe the effects of tinidazole ointment on oxazolone-induced ear edema model, since it is thought that the dermatitis is due to the type IV allergic reaction[11]. In sensitized mice, the weight of the ear significantly increased upon giving the challenge compared with non-sensitized mice, thereby indicating that sensitization was induced by oxazolone (Fig. 4).

Fig. 4 shows the effects of ointment base with or without clobetasol (0.005%), tinidazole, clobetasol (0.05%) and hydrocortisone (0.1%) on sensitized mice. Ointments of tinidazole (2%) with or without clobetasol (0.005%) significantly suppressed the ear edema compared to the respective controls, namely ointment base with or without clobetasol (0.005%).

Concerning the clobetasol and hydrocor-

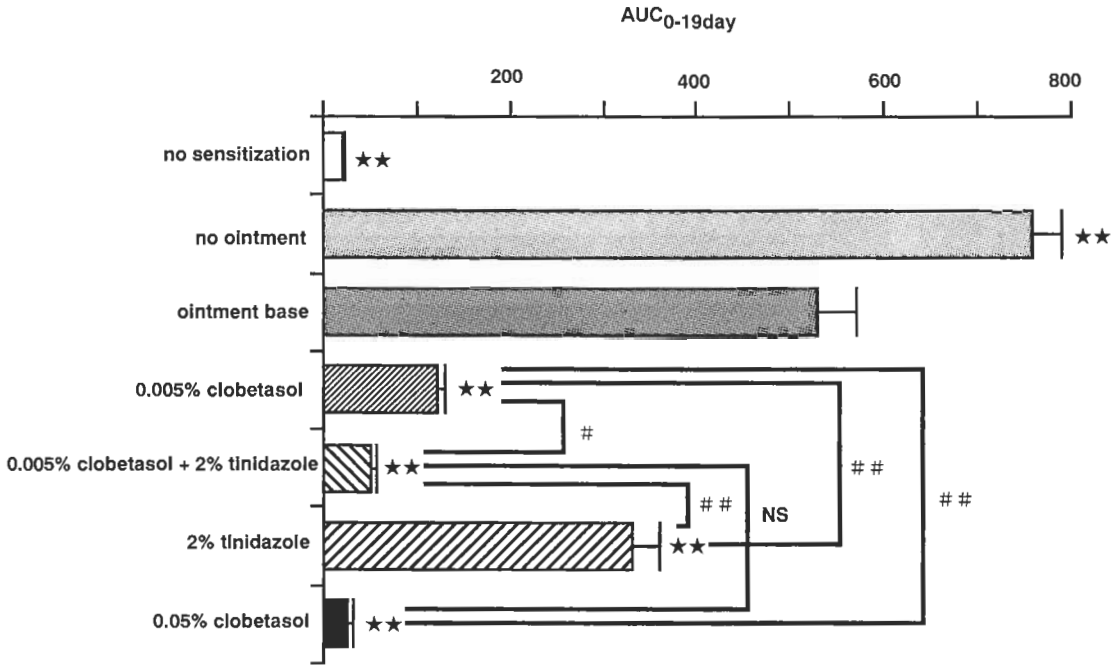

Fig. 3 Effects of ointment base, tinidazole (2%), clobetasol (0.05%) with or without tinidazole (2%) ointments on TNCB-induced ear swelling in mice. Ear thickness was measured at 24 hr after each TNCB application, and area under the curve ($AUC_{0-19\ day}$) of increase in ear thickness (Fig. 2) were calculated.
$P^{**} < 0.01$; significantly different from ointment base (t-test).
$^{*}P < 0.05$, $^{\#\#}P < 0.01$; significantly different (Tukey method).

tisone, corresponding ointment base were not available in the present experiments, and therefore we used mice treated with no ointment as control. Clobetasol (0.05%) and hydrocortisone (0.1%) significantly suppressed the increase in ear thickness compared to control (Fig. 4).

There was no significant difference in the potency of clobetasol (0.05%) and hydrocortisone (0.1%) to suppress the edema, although both agents were more potent than tinidazole with or without clobetasol (0.005%). In addition, these was no additive effect when tinidazole was applied with clobetasol (0.005%) in this animal model.

Effects of ointments of tinidazole with or without clobetasol, and hydrocortisone on allergic ear edema and scratching.

To evaluate further the effects of tinidazole, we used passively sensitized mice with anti-DNP-Mab. When the passively sensitized mice were challenged with DNFB, a biphasic ear edema was observed. Namely, significant immediate (IPR) and late phase reactions (LPR) were provoked in the sensitized but not the unsensitized mice. Tinidazole with or without clobetasol (0.005%), and hydrocortisone suppressed both IPR and LPR (Fig. 5).

We also observed the effects of ointments of tinidazole, clobetasol and hydrocortisone on scratching reaction of ears, since itching is one of the characteristics of inflammatory dermatitis and scratching results from the itching. Fig. 6 shows the number of scratching reactions during periods of 0-30, 0-60 and 0-90 minutes after the challenge with DNFB. Ointments of tinidazole without clobetasol and of hydrocortisone, but not

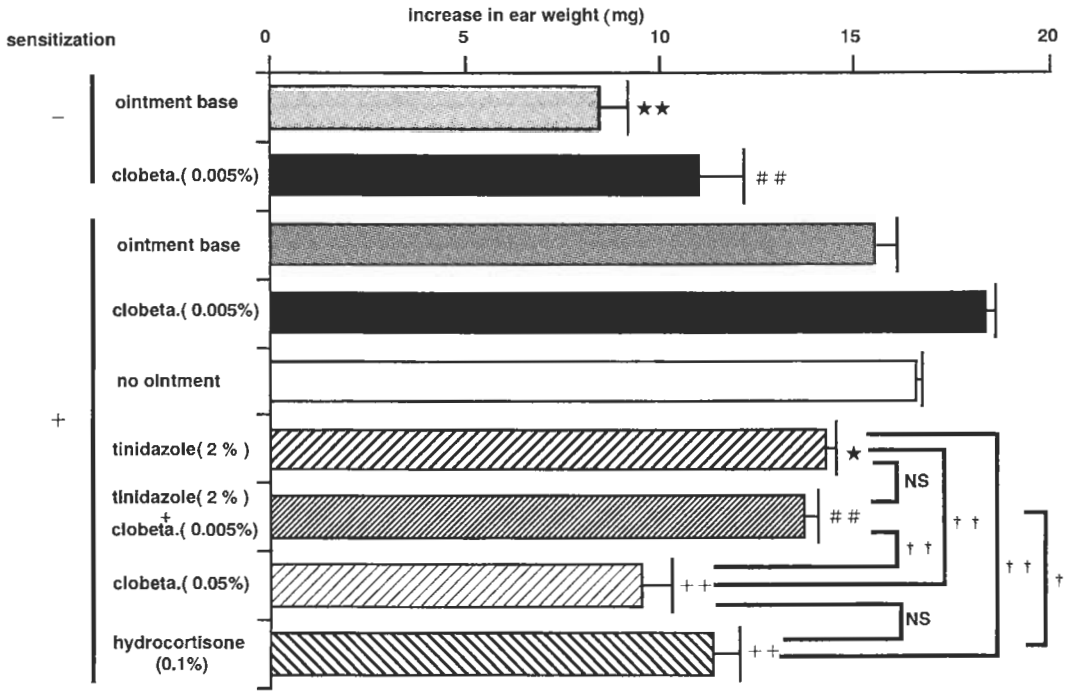

Fig. 4 Effects of tinidazole (2%), clobetasol (0.005 or 0.05%) with or without tinidazole and hydrocortisone (0.1%) on oxazolone -induced ear edema. Each column represents the mean ± SEM increase in weight of ear punches from 8 animals. Ointment volume used was 10 μL / site.
$P^{*} < 0.05$, $P^{**} < 0.01$; vs. sensitization (+) / placebo-ointment (t- test). $P^{\#\#} < 0.01$ vs. sensitization (+) / clobetasol (0.005%) (t-test). NS ; not significant, $P^{++} < 0.01$ vs. sensitization (+) / no ointment (t-test). NS ; not significant $P^{+} < 0.05$, $P^{\dagger} < 0.01$; significantly different (Tukey method).

the tinidazole with clobetasol (0.005%), significantly suppressed the number of scratching reactions during 0-30 and 0-60 minutes after the sensitization. Clobetasol (0.005%) alone had no significant effect. During 0-90 min, tinidazole with clobetasol (0.005%) also suppressed the number of scratching reactions, although the effects were much less than for tinidazole without clobetasol. Interestingly, tinidazole was more potent than hydrocortisone (0.1%) in suppressing the number of scratching.

Discussion

We used chronic contact hypersensitivity response (CHR) model to evaluate the potency of anti-inflammatory, immunosuppressive and anti-itching effects of tinidazole in comparison with those of readily available corticosteroid ointments. The acute CHR provoked by a single epicutaneous administration of a contact sensitizing agent in a pre-sensitized animal has been widely used as an animal model to evaluate topical or cystemic anti-inflammatory compounds, because of simplicity of the method[12)13)]. However, the experimental results obtained with the acute CHR model are not strictly applicable for the allergic inflammation in AD, since AD is generally considered to be due to repeated epicutaneous exposure to various antigens and environmental allergens[14)]. Accumulating evidence indicate that immedi-

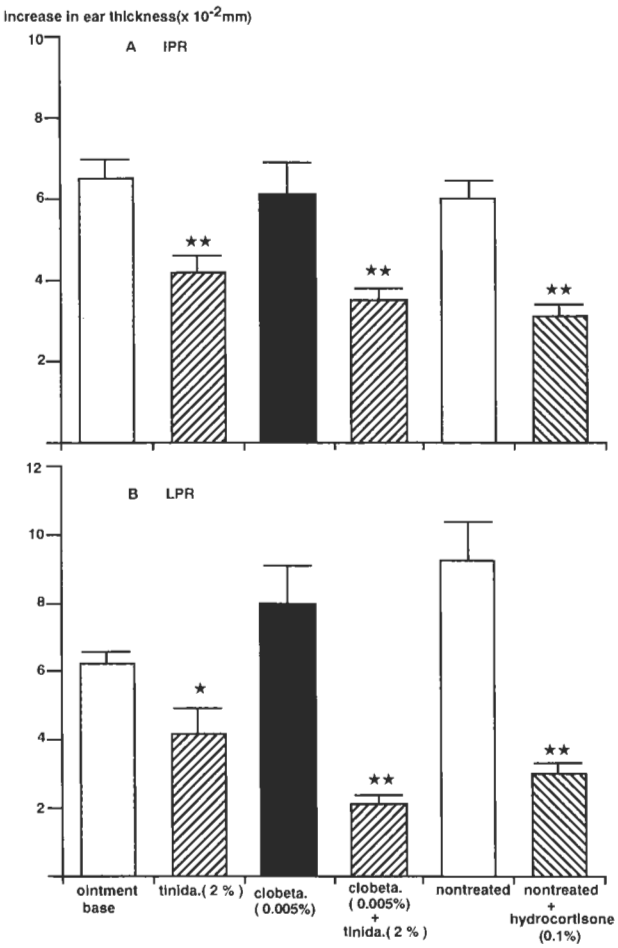

Fig. 5 Effects of tinidazole (2%), clobetasol (0.005%) with or without tinidazole (2%) and hydrocortisone (0.1%) ointments on IgE-mediated biphasic cutaneous reactions in mice. The IPR and LPR were measured 1.5 and 24 hr after DNFB application, respectively. Ointments were applied 2 hr before DNFB application. Each column represents the mean ± SEM of differences in pinna thickness from 10 animals. P* < 0.05, P** < 0.01 significantly different from ointment base (t-test).

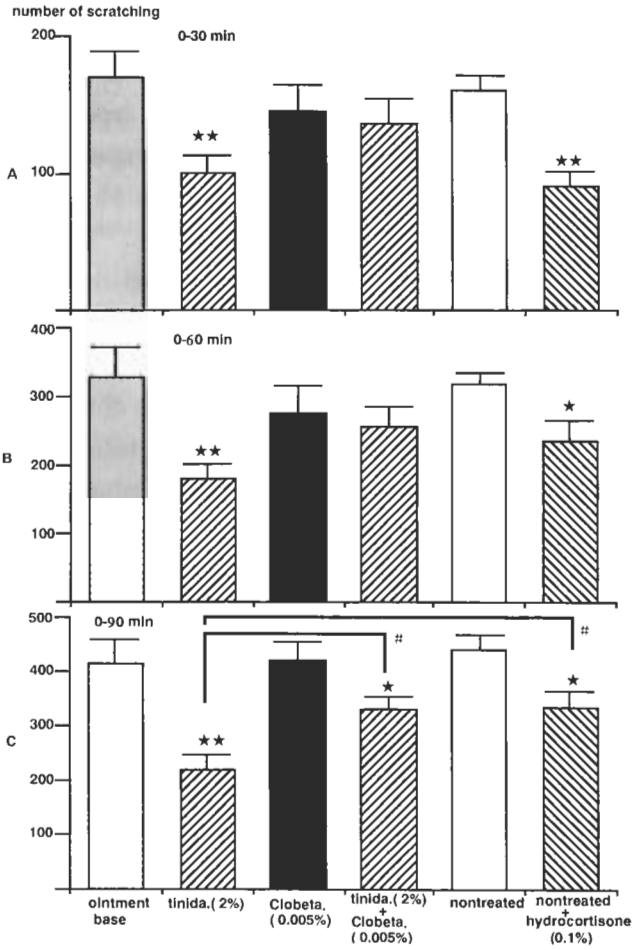

Fig. 6 Effects of tinidazole (2%), clobetasol (0.005%) with or without tinidazole and hydrocortisone (0.1%) on IgE-mediated scratching reaction in mice. To observe the effects of ointments on the scratching reaction, we used mice passively sensitized with anti-DNP-Mab and challenged with DNFB. Ointments were painted 2 hr before DNFB application. The number of scratching was counted for 0 - 30 min (A), 0 - 60 min (B) and 0 - 90 min (C) after DNFB application. Each column represents the mean ± SEM of 10 animals. P* <0.05, P** < 0.01 significantly different from the control (t-test). #P < 0.05 (Tukey method).

ate (IgE-mediated mast cell type), late (IgE-mediated Th2 type) and delayed (IgE-independent Th1 type) allergic reactions are involved in AD[15)16)17)18)], although the precise mechanisms involved in AD are still unclear. In this respect, it is known that repeated administration of antigen often evokes responses which are similar to those observed in AD. Namely, repeated application of a contact sensitizing agent results in

a shift in the hypersensitivity from a typical delayed phase response to an immediate phase response (IPR) followed by a late-phase response (LPR)[19)20)]. These types of responses (IPR, LPR) were antigen specific, and associated with local cytokine pattern due to a Th1- or Th2-type profile. Thus, the chronic CHR model appears to mimic many, if not all, events occurring in the skin of patients with AD.

The present results obtained with chronic CHR mice could be summarized as follows: Tinidazole, hydrocortisone and clobetasol suppressed, (i) TNCB or oxazolone-induced inflammatory dermatitis and (ii) IgE dependent IPR and LPR and scratching reaction. The rank of the potency to suppress the ear edema induced by TNCB was clobetasol (0.05%), tinidazole (2%) with clobetasol (0.005%) > clobetasol (0.005%) > tinidazole (2%), and in case of oxazolone-induced ear edema, hydrocortisone (0.1%), clobetasol (0.05%) > tinidazole (2%), tinidazole with clobetasol (0.005%) > clobetasol (0.005%). In addition, ointment base alone significantly suppressed the ear edema induced by TNCB or oxazolone. This was not observed in the previous experiments[6)].

In the treatment of AD, corticosteroids and emollients are widely used[21)22)]. However, in some patients, these treatment are not very effective, especially in those with atopic dermatitis continuing from childhood into adult life. Some of these patients show persistently severe atopic dermatitis or recurring episodes of severe dermatitis. In addition, it is well known that steroids have various adverse actions, including skin atrophy, and there are restrictions concerning the long term clinical applications especially to the face. An additional complication of use of corticosteroids in children is growth retardation. Azathioprine has been used as a steroid-sparing agent but may be associated with bone marrow suppression and abnormal liver function[21)22)]. Therefore, there is a need for safe and effective alternative therapies in severe AD.

Several reports have suggested that immunosuppressants including cyclosporin (CyA) or tacrolimus are effective in the treatment of atopic dermatitis. Topical formulation of tacrolimus have been used successfully in a series of open trials performed in Japan with patients with atopic dermatitis[23)24)]. A large placebo-controlled double-blind, multicenter clinical trial involving over 200 adult patients with AD proved the safety and efficacy of topical tacrolimus[25)]. It was also reported that oral administration of CyA in children and adults with AD is effective in improving the sympton and signs of disease and the quality of life[26)27)28)]. However, most patients relapsed within a few weeks after withdrawal of treatment, although in some patients long remission was seen[26)].

In the present experiments, we confirmed that tinidazole in relatively high concentration (2%) has anti-inflammatory and immunosuppressive effects, although it was less potent than those of clobetasol (0.05%) or hydrocortisone (0.1%). In addition, we found that combined application of tinidazole with clobetasol (0.005%, one-tenth of the clinical use) shows additive effects to suppresses the ear edema in TNCB -induced but not in the oxazolone-induced dermatitis in the mice. The reason for the differential effects on the animal models for dermatitis of the combined application of tinidazole and clobetasol is unknown. However, these observations indicate that tinidazole is useful as a potential corticosteroid sparing agent in the treatment of patients with AD.

The present and previous experiments[6] indicate that tinidazole is more potent than hydrocortisone or clobetasol concerning anti-itching action. The mechanisms involved in the itching are very complex. Early investigators recognized that many types of mildly damaging stimuli (e.g., mechanical, electrical, thermal) caused pruritus whereas more intense injury evoked pain. These mildly damaging stimuli might directly excite itch-signalling sensory neurons, or indirectly produce pruritus through the release of pruritogens from injured tissue, or evoke an axon reflex[29]. A number of chemicals are known to evoke pruritus. In addition to histamine and mast cell degranulators including substance P and compound 48 / 80, substances such as kallikrein, bradykinin, papain or trypsin have been shown to produce experimental pruritus. Furthermore, recent studies revealed that vanilloid (capsaicin)-sensitive neurons transmit noxious information (usually perceived as itching or pain) to the central nervous system[30]. In addition, it was demonstrated that a combination of at least four inflammatory mediators, namely, bradykinin, histamine, serotonine and prostaglandin E_2, act together to activate the vanilloid receptor 1 (VR1)[31][32]. The precise mechanisms involved in the anti-itching effects of tinidazole are unknown. However, tinidazole but not clobetasol suppresses the current evoked by the activation of VR1 (unpublished observations by R. Inoue & Y. Ito). The combined application of tinidazole (2%) with clobetasol (0.005%) was less effective than tinidazole alone to suppress the scratching reactions in the present experiments (Fig. 6), and this might be because clobetasol has no anti-itching action. Therefore the advantages of using tinidazole ointments would be that it have stronger anti-itching effects than corticosteroid, and can be applicable to the face with fewer adverse effects. It should be stressed that metroimidazoles including tinidazole have few side effects, if any, on the face for long period of treatment[2][3][4][5][33].

Taking into account that tinidazole has anti-inflammatory, immunosuppressive, anti-itching and bactericidal effects, the present and previous studies strongly suggest the possible clinical application of tinidazole ointments to the inflammatory skin diseases including AD.

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(和文抄録)

チニダゾール，ハイドロコルチゾン及びクロベタゾール軟膏の皮膚炎モデルマウスに及ぼす効果の比較研究

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我々はニトロイミダゾール誘導体であるメトロニダゾール及びチニダゾール軟膏（1-4%）が抗原，ハプテン及びmonoclonal anti-dinitrophenol (DNP) IgE-antibody (anti-DNP IgE Mab) で誘発した皮膚炎モデルマウスの皮膚炎，血管透過性亢進ならびに掻痒に起因すると考えられるスクラッチ反応を抑制することを発見した．そこで比較的高濃度（2%）のチニダゾール軟膏のハプテン及びanti-DNP IgE Mabによる皮膚炎モデルマウスに及ぼす効果を，汎用されているステロイド剤であるクロベタゾール(0.005%及び0.05%）及びハイドロコルチゾン（0.1%）の効果と比較研究した．またチニタゾールと臨床使用濃度の1/10の濃度のクロベタゾールの合剤の効果を，チニダゾール，クロベタゾール（0.05%）及びハイドロコルチゾン（0.1%）の単剤のそれと比較検討した．

チニダゾール（2%），ハイドロコルチゾン（0.1%）およびクロベタゾール（0.005%および0.05%）軟膏はTNCB及びオキサゾロンにより誘発したマウスの耳浮腫を抑制し，その効力の順序はTNCB皮膚炎の場合，クロベタゾール（0.005%）またはチニダゾール（2%）とクロベタゾール(0.005%)の合剤＞チニダゾール(2%)であり，オキサゾロン皮膚炎の場合，ハイドロコルチゾン（0.1%）またはクロベタゾール（0.05%)＞チニダゾール（2%）またはチニダゾールとクロベタゾール(0.005%)の合剤＞クロベタゾール(0.005%)の順であった．またチニダゾール軟膏（2%）がanti-DNP IgE Mabにより受動感作したマウス耳浮腫の即時相及び遅発相を抑制することを確認した．さらに特筆すべきことは，チニダゾール軟膏（2%）がanti-DNP IgE Mabにより受動感作したマウスの痒みによると考えられるスクラッチ反応をハイドロコルチゾン（0.1%）よりはるかに有効に抑制したことである．これらの実験結果は臨床応用に際してのチニダゾール軟膏の利点はその抗掻痒効果がステロイド剤より強いことにあると考えられた．