第３２号

出典：チニダゾールの抗炎症作用

ORIJINAL PAPER

Effects of metronidazole and tinidazole ointments on models for inflammatory dermatitis in mice.

Arch Dermatol Res (2003) 294 : 544–551
DOI 10.1007/s00403-002-0381-4

ORIGINAL PAPER

K. Nishimuta · Y. Ito

Effects of metronidazole and tinidazole ointments on models for inflammatory dermatitis in mice

Received: 11 August 2002 / Revised: 24 October 2002 / Accepted: 11 December 2002 / Published online: 29 January 2003

Abstract We investigated the effects of 1–4% ointments of metronidazole and tinidazole (derivatives of nitroimidazole) on models of inflammatory dermatitis evoked by antigen, hapten and monoclonal anti-dinitrophenol (DNP) IgE antibody in mice. Metronidazole and tinidazole ointments (1) suppressed the late-phase reaction (LPR) of biphasic ear edema in mice sensitized with ovalbumin (OA), (2) suppressed trinitrochlorobenzene-induced inflammatory dermatitis, (3) suppressed the immediate phase reactions and LPR in mice passively sensitized with anti-DNP IgE mAb, and (4) enhanced vascular permeability and the number of scratching reactions, presumably due to itching, in passively sensitized mice. These results strongly indicate that metronidazole and tinidazole 1–4% ointments possess antiinflammatory, immunosuppressive and antiitching effects, and have the potential for clinical use in the treatment of human inflammatory skin diseases including atopic dermatitis in addition to rosacea and acne vulgaris.

Keywords Metronidazole · Tinidazole · Ointment · Inflammatory · Dermatitis · Mouse

Introduction

Topical cream or gel containing metronidazole has been used for the treatment of rosacea [1], and topical 1% metronidazole gel has also been shown in a recent study to be effective in the treatment of seborrheic dermatitis [2]. Although the precise mechanisms involved in its beneficial effects in rosacea and seborrheic dermatitis are unknown, metronidazole is classified therapeutically as an antiprotozoal and antibacterial agent [3]. Recent studies, however, have confirmed that metronidazole may possess antiinflammatory actions, and the beneficial effects of metronidazole in rosacea and acne vulgaris are attributable to its antiinflammatory activity rather than to its antibacterial activity [1, 4]. There are also reports that nitroimidazole derivatives may suppress immune responses, since intraperitoneal administration of metronidazole inhibits swelling of the ears induced by 2,4-dinitro-1-fluorobenzene (DNFB) in mice after sensitization [5], and significantly inhibits increases in anti-TAB (anti-*Salmonella typhi*, *S. paratyphi A* and *S. paratyphi B*) antibody titer to TAB vaccine in rabbits [6]. It is also known that intraperitoneal administration of metronidazole inhibits the delayed immune reaction to intravenous injection of ovine erythrocytes, and leukocyte migration in two animal models of cellular immune responses [7].

It is generally accepted that human atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by chronic relapsing inflammation [8]. Evidence for cell-mediated immune hyperactivity, the presence of mediators derived from eosinophils and elevated serum IgE levels in AD patients support this view. To treat the pruritic and eczematous plaques in patients with AD, various external preparations with or without glucocorticoids are widely used. However, there are some clinical restrictions on the application of steroids to the face, and it seems important to develop new drug preparations for AD.

Thus, in the present study, we investigated the effects of ointments of metronidazole and tinidazole, a nitroimidazole derivative that has been shown to be more potent than metronidazole as an antiprotozoal and antibacterial agent [9], in models of inflammatory dermatitis in mice. In some experiments the effects were compared with those of FK506 ointment and a readily available topical corticosteroid, Locoid. The present results provide the first experimental evidence of the clinical potential of tinidazole and metronidazole ointments at quite high concentrations (1–4%) in the treatment of inflammatory skin diseases including AD.

K. Nishimuta · Y. Ito (✉)
Department of Pharmacology,
Graduate School of Medical Sciences,
Kyushu University, 812-8582 Fukuoka, Japan
Tel.: +81-92-6426075, Fax: +81-92-6426079,
e-mail: yushi@pharmaco.med.kyushu-u.ac.jp

Materials and methods

Animals

Male mice (NC/Nga Tnd Crj) at 5 weeks of age and weighing 14.3–17.3 g were obtained from Charles River, Japan. After taming and quarantining for 2 weeks to reveal any abnormality, all the healthy mice (body weight at the beginning of sensitization 19.0–24.7 g) 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-h light/dark cycle (lighting 7 A.M. to 7 P.M.). Mice were housed (five mice per cage) in polycarbonate cages (width 215, depth 320, height 130 mm). The animals had free access to solid feed (MF for mouse; Oriental Yeast Company) and drinking water (city tap-water).

Prior to sensitization hair was removed from the sensitization sites using clippers followed by hair removal cream which was left for several minutes before washing out. The animals were divided into several groups according to the experiments, each group consisting of five to ten mice.

Drugs

Metronidazole and tinidazole ointments (final concentrations 0.5–5%; Development Headquarters, Yoshitomi Pharmaceutical Company), FK506 ointment (Protopic ointment; Fujisawa Pharmaceutical Company), and placebo ointment (ointment base W50083; Yoshitomi Pharmaceutical Company) were applied to the prepared sites of the mice. The ointment base W50083 is a mixture of stearyl alcohol, Rheodol MS-50 (Kao Corporation), white petrolatum, liquid paraffin, Rheodol Super TW-L 120 (Kao Corporation), glycerin, methylparaben, propylparaben and purified water. Metronidazole and tinidazole were mixed with the ointment base to the final concentrations used in the experiments. We also used commercially available Locoid ointment (hydrocortisone butyrate 0.1%; Torii Pharmaceutical Company, Tokyo, Japan) as a readily available topical corticosteroid.

Other drugs used were: chicken egg albumin (ovalbumin, OA), monoclonal anti-dinitrophenyl (DNP) antibody, mouse IgE isotype (Sigma Chemical Company, St Louis, Mo.), acetone, ethanol (Wako Pure Chemical Industries), and trinitrochlorobenzene (2,4,6-trinitrochlorobenzene, TNCB; Tokyo Kasei Kogyo Company).

Animal models

Antigen-induced biphasic ear edema in mice

Mice were sensitized by a single injection of 0.5 ml OA in physiological saline solution containing aluminum hydroxide gel (OA 20 μg/ml, aluminum hydroxide gel 2 mg/ml) into the abdomen. On day 15 or 16 after sensitization, 10 μl OA in physiological saline solution (OA 0.5 mg/ml) was injected intradermally into the inside of both pinnae (challenge with antigen) using a microliter syringe and a 3LG needle. These mice were the ear-swelling model. The thickness of the pinnae was measured at 1 h and 24 h after challenge using a thickness gauge. The difference in the thickness measured before and after challenge was determined as an indicator of ear edema. Test ointments (3 mg) were applied 2 days before challenge to the inside of both pinnae once a day for 3 days, and on the day of challenge the ointments ere applied 2 h before challenge. Ointment base was applied to the mice of the unsensitized control group.

TNCB-induced dermatitis in mice

TNCB was used to sensitize the mice as reported previously [10]. TNCB 2% solution was applied to the left pinna of the mice using a micropipette, 25 μl to the inner and 25 μl to the outer side (50 μl/ear). The same amount of acetone was applied to the unsensitized control group. After sensitization, a 0.5% solution of TNCB was applied from day 7 (day 0) to day 28 once every 2 days (50 μl/ear) and the thicknesses of the pinnae were measured. Each ointment was applied repeatedly once a day throughout the experiments (day 0 to day 27) or from day 16 to day 28. Ointment (10 μl) was applied 2 h before sensitization to the pinna using a 250-μl glass syringe.

We also used TNCB to sensitize the mice by applying 150 μl of sensitization solution to the thorax, abdomen and footpad using a micropipette. The challenging solution (10% TNCB in basco olive oil) was spread on the hair-depleted skin surface from the neck to the back. Sensitization was judged to be present if the clinical manifestations showed a score of more than six points [11]. Sensitization of all mice was complete after four treatments with the challenge solution. The clinical manifestations were evaluated as they are for human atopic dermatitis in terms of pruritus/itch, erythema/hemorrhage, excoriation/erosion and scaling/dryness. These four items were classified into the four categories subclinical, mild, moderate and severe scored as 0, 1, 2 and 3, respectively. The scoring was carried out independently by two examiners and the mean value was used. The scoring was carried out just before treatment with the test substances and 3 and 7 days after the consecutive applications. The ointments were applied twice a day, from 9:00 A.M. to 10:00 A.M. and from 4:00 P.M. to 5:00 P.M. for seven consecutive days.

The mean value and standard error (mean±SE) of the score for each group was calculated, and the effects of the test substances were evaluated using the Wilcoxon test on the 3rd and 7th days. The level of significance was taken as 5% ($P<0.05$).

IgE-dependent ear swelling and scratch model in mice

Ten mice of each group were passively sensitized by intravenous injection of physiological saline (1 ml) containing 10 μg of anti-DNP mAb according to the method of Katayama et al. [12]. The unsensitized control group received 1 ml physiological saline. After 24 h, 5 μl of a 0.75% DNFB solution was applied to the inside and outside of the right ear (10 μl/mouse). When DNFB was applied, scratching activity and increases in ear thickness (ear edema) were evaluated as described previously [12].

After challenge, each mouse was isolated and the scratching reactions counted during 30-min periods up to 90 min. Scratching the pinna at least twice with the hindpad was counted as one scratching reaction, and grooming actions with the forepad were distinguished from scratching reactions.

Each ointment was applied to the inside of the right ear 2 h before DNFB application. Ointment base (3 mg) was applied to the mice of the unsensitized and test groups.

Accelerated vascular permeability induced by IgE in mice

We also used the mice passively sensitized by anti-DNP-mAb to examine the vascular permeability, and the Evans blue dye method described previously [13]. The increase in the ear thickness (ear edema) was determined 1.5 and 24 h after challenge with DNFB, since it has been found that accelerated vascular permeability is involved in the initial edema (during the initial 1.5 h). We compared the effects of tinidazole and FK506 ointments on the vascular permeability in the pinnae after passive sensitization. The tinidazole and FK506 ointments (10 μl of each) were applied to the inside of the right ear 2 h before DNFB application.

Statistics

The mean values (±SEM) of various parameters were obtained in the various experiments. The differences between the control and the unsensitized group were analyzed using the *t*-test, and the differences between the control and test group were analyzed using Dunnett's method.

Results

Effects of tinidazole and metronidazole ointments on ear edema

On challenge with OA solution, immediate and late phase reactions (IPR and LPR, respectively) of ear edema were provoked in the control (sensitized mice), but not in the unsensitized group.

Tinidazole ointment (1% and 3%) and metronidazole ointment (3%) showed no effect on the IPR (Fig. 1A). However, both ointments at 3% significantly suppressed the LPR (Fig. 1B). Ointments with various concentrations of tinidazole (0.1, 0.5, 1 and 2%) were used to determine the minimal effective dose, which was found to be 2% (data not shown). In contrast, FK506 ointment (0.1%) dose-dependently suppressed both the IPR and LPR in the sensitized mice (Fig. 1C, D).

Tinidazole and metronidazole ointments suppressed the LPR of the biphasic ear edema provoked by OA challenge in the sensitized mice. We therefore compared the effects of tinidazole with those of FK506 during longer time periods using TNCB-induced dermatitis in mice [11].

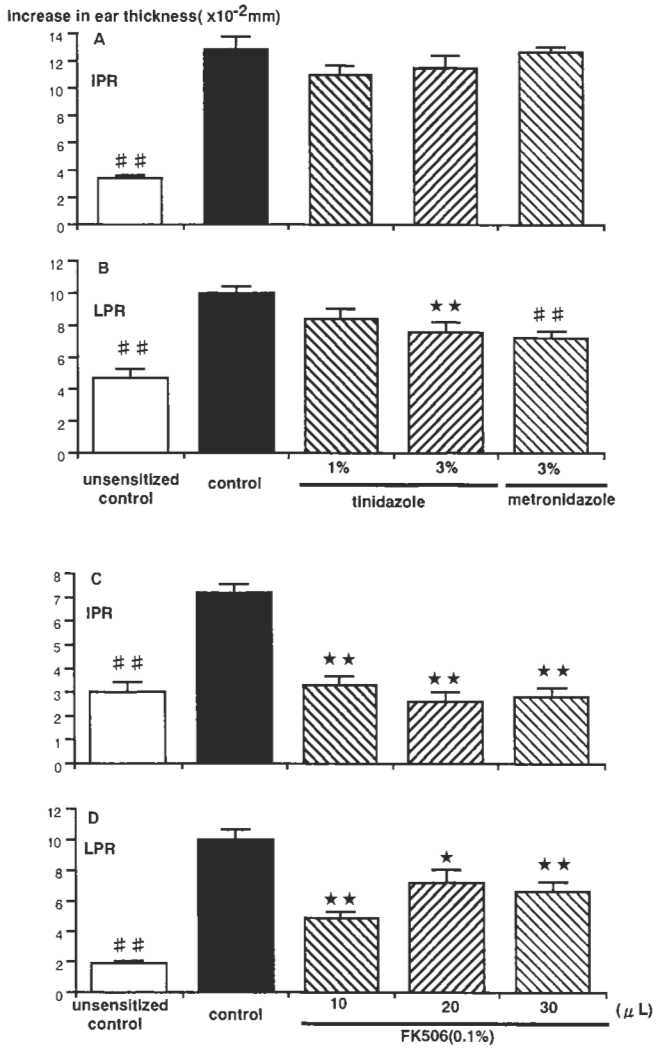

Fig. 1A–D Effects of tinidazole and metronidazole ointments (**A**, **B**) and FK506 ointment (**C**, **D**) on the IPR and LPR in OA-challenged mice. Ear thickness was measured 1 h (IPR) and 24 h (LPR) after OA challenge. Mice were challenged intradermally with OA solution. Each ear was treated with each ointment once a day for 3 days. Each column represents the mean±SE from eight animals. $^{*}P<0.05$, $^{**}P<0.01$, vs control (Dunnett's method); $^{\#\#}P<0.01$, vs control (t-test)

Effects of tinidazole on TNCB-induced dermatitis

The thickness of the pinnae gradually increased after challenge with TNCB, and the maximum value was observed about 24 h after challenge. During repeated applications of TNCB, the maximum value gradually increased and the peak occurred on day 16. The thickness of the pinnae of the sensitized control group (0.730±0.04 mm) on day 16 was about three times that of the unsensitized control group

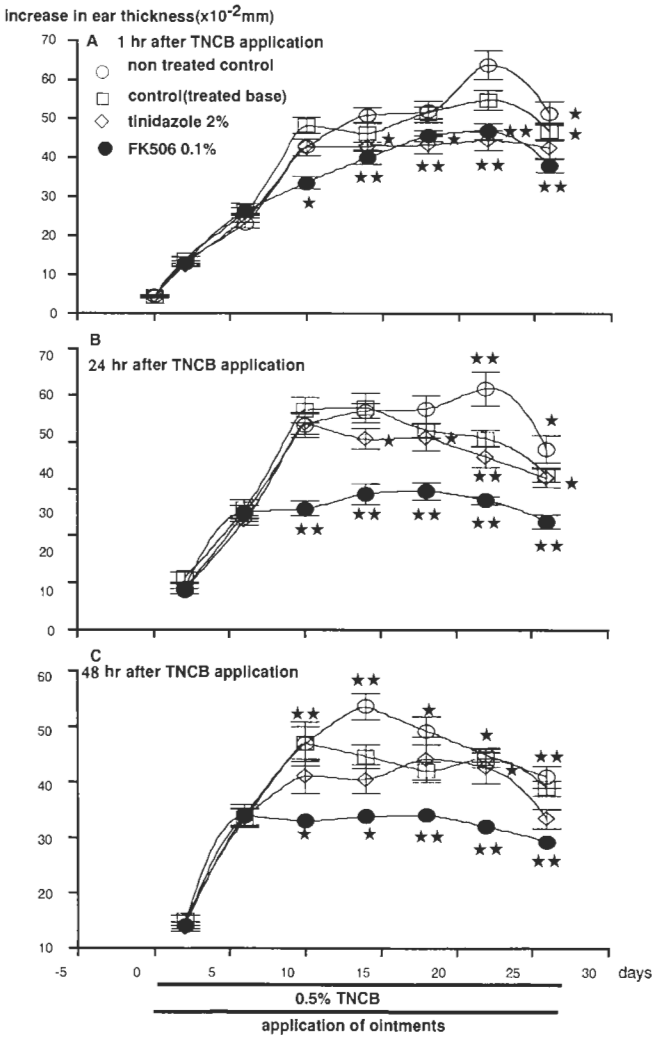

Fig. 2A–C Effects of tinidazole and FK506 ointments on TNCB-induced ear swelling in mice. Ear thickness was measured 1 h (**A**), 24 h (**B**) and 48 h (**C**) after TNCB application. $^{*}P<0.05$, $^{**}P<0.01$, vs control (t-test, means±SEM, n=8)

(0.233±0.002 mm). Tinidazole and FK506 ointments were applied during the period day 16 to day 20 after sensitization. Following treatment with tinidazole (2%) and FK506 (0.1%) ointments, significant suppression of TNCB-induced ear edema was seen on day 24 (to 0.363±0.021 and 0.379± 0.021 mm, respectively) and day 28 (to 0.308±0.025 and 0/273±0.011 mm, respectively).

Tinidazole and FK506 ointments were then applied throughout the experiments from day 0 to day 27, and the thicknesses of the pinnae were measured 1, 24 and 48 h after challenge with TNCB. As shown in Fig. 2, the maximum thickness of the pinnae gradually increased and reached a plateau at about 10–15 days after the start of the repeated applications of TNCB (0.5%) as measured 1, 24 and 48 h after challenge with TNCB. After 6–10 days treatment, tinidazole (2%) suppressed the increase in ear thickness as measured 1 and 24 h after challenge with TNCB, but the suppression was less clear at 48 h after challenge (Fig. 2). FK506 ointment (0.1%) showed stronger inhibitory effects on the increase in the thickness of the pinnae than tinidazole ointment when the area under the curve (AUC) was employed to evaluate the effects of the ointments. The AUC values in relation to the control for tinidazole (2%) and FK506 (0.1%) were 0.88 and 0.24, 0.97 and 0.77, and 0.92 and 0.73 after 1, 24 and 48 h, respectively. The decrease in the thickness of the pinnae was greater in groups treated from day 0 than in those treated from day 8 (Fig. 2).

To study the effects of tinidazole on inflammatory dermatitis, the mice were sensitized with TNCB by applying it to the thorax, abdomen and footpad, and the effects of various concentrations of tinidazole ointment (1, 1.5, 2, 3 and 5%) were investigated.

Concerning the general condition of the mice, there was no conspicuous abnormality throughout the experiments. However, in some mice, injury to the ears due to the inflammatory dermatitis and scratching was marked. These observations indicate that in this model of inflammatory dermatitis, scratching of the ears was greatly enhanced, and this was probably due to the intense itching.

Table 1 shows the changes in the mean score in each group during the course of the experiments. There was no difference in the mean score between the control (group 1) and the treated groups (groups 2, 3, 4, 5 and 6) before the application of tinidazole ointment (1, 1.5, 2, 3 and 5%, respectively). After treatment with tinidazole ointment, the scores were significantly reduced, and the effects were dose-dependent. With regard to the symptoms of dermatitis (pruritus, flare and hemorrhage, excoriation, crust formation and dryness), the improvements were prominent in groups 5 and 6 treated with 3% and 5% tinidazole ointments, respectively.

Effects of tinidazole, metronidazole, FK506 and Locoid ointments on allergic ear edema and scratching

When mice passively sensitized with anti-DNP-mAb were challenged with DNFB, a biphasic ear edema was observed as in the case of mice actively sensitized with OA. That is, a significant IPR and LPR of the ear edema occurred in the control but not in the unsensitized group. Tinidazole, metronidazole and FK506 ointments suppressed both the IPR and LPR of the ear edema (Fig. 3).

The effects of tinidazole were compared with those of Locoid ointment as an example readily available topical corticosteroid. As shown in Fig. 4, tinidazole and Locoid were equipotent in suppressing the IPR and LPR.

We also investigated the effects of tinidazole and metronidazole ointments on scratching of the ears, since itching is one of the characteristics of inflammatory dermatitis and the scratching reaction is due to the itching. After challenge with DNFB, each passively sensitized mouse was isolated and the scratching reactions counted. Table 2 shows the number of scratching reactions during the periods 0–30, 0–60 and 0–90 min after challenge with DNFB. There were significantly more scratching reactions in the control group than in the unsensitized group. Tinidazole ointment (1% and 2%) suppressed the number of scratching reactions during the periods 0–30 and 0–90 min after challenge. However, the 4% tinidazole ointment showed no statistically significant inhibitory effect on the scratching reaction. The minimum concentration of tinidazole causing suppression of scratching was 1%.

Similar experiments were conducted with metronidazole ointment (1%, 2% and 4%). During the periods 0–60 and 0–90 min after DNFB challenge, the number of scratch-

Table 1 Effects of tinidazole ointment (1–5%) on inflammatory dermatitis in the mice induced by TNCB. Six groups (group 1, control; groups 2–6, treated with 1, 1.5, 2, 3, and 5% tinidazole, respectively) of five mice each were used. The mice were sensitized with TNCB and challenged one to four times with the antigen. Sensitization was considered to be present if the clinical manifestation score was more than 6 (see Methods), and sensitization was complete after four challenges in all mice. After sensitization, the ointments were applied once a day for 7 days. The values shown are the mean scores (±SE)

Group	Treatment	Control	3 days after treatment		7 days after treatment	
				P value		*P* value
1	Control	7.2±0.49	5.8±0.37		5.2±0.37	
2	Tinidazole 1%	7.0±0.32	4.8±0.20	0.034	3.2±0.20	0.041
3	Tinidazole 1.5%	7.2±0.58	5.0±0.45	0.041	3.0±0.55	0.041
4	Tinidazole 2%	7.0±0.55	3.4±0.51	0.042	2.0±0.55	0.043
5	Tinidazole 3%	7.4±0.40	3.2±0.37	0.041	1.8±0.37	0.038
6	Tinidazole 5%	7.2±0.49	1.6±0.24	0.038	0.2±0.20	0.041

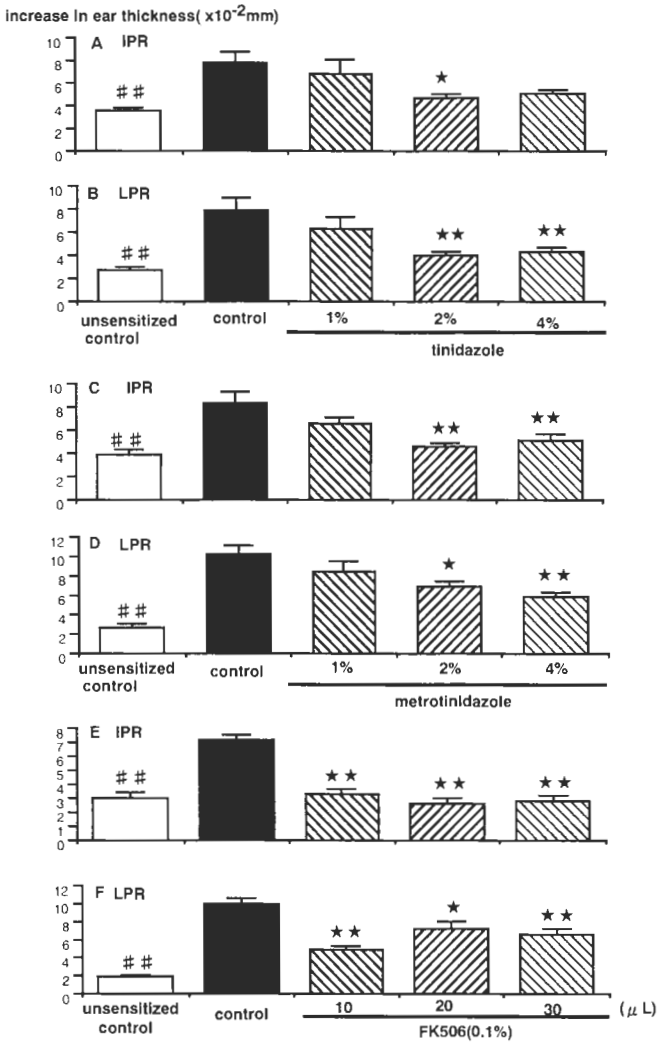

Fig. 3A–F Effects of tinidazole, metronidazole and FK506 ointments on IgE-mediated biphasic cutaneous reactions in mice. The IPR and LPR were measured 1.5 h and 24 h after DNFB application, respectively. Tinidazole, metronidazole and FK 506 ointments were applied 2 h before DNFB application. Each column represents the mean±SEM from ten animals. $^{*}P<0.05$, $^{**}P<0.01$, vs control (Dunnett's method); $^{\#\#}P<0.01$, vs control (*t*-test)

ing reactions increased significantly in the control group, and 4% metronidazole ointment significantly reduced the number of scratching reactions (data not shown). The effects of FK506 ointment (0.1%) on the scratching reaction were also investigated. The application of 30 μl FK506 (0.1%) significantly suppressed the number of scratching

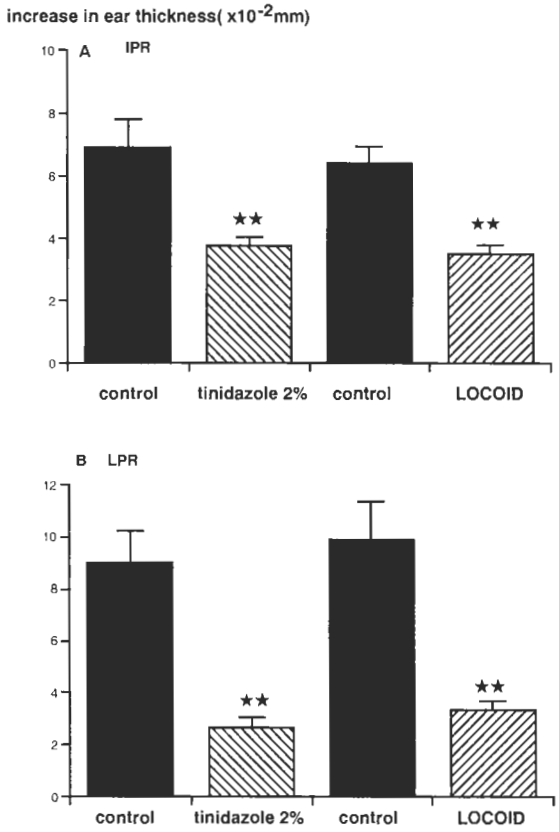

Fig. 4A, B Effects of tinidazole ointment (2%) and Locoid on IgE-mediated biphasic cutaneous reactions in mice. The IPR (**A**) and LPR (**B**) were measured 1.5 h and 24 h after DNFB application, respectively. Each ointment (ointment base, tinidazole and Locoid) was applied 2 h before DNFB application. Each column represents the mean±SE from 12 animals. $^{**}P<0.01$ (*t*-test)

Table 2 Effects of tinidazole ointment (0.1, 0.5, 1, 2 and 4%) on IgE-mediated scratching reaction. To determine the effects of tinidazole ointment on the scratching reaction, mice were passively sensitized by injection of anti-DNP-mAb and were challenged with DNFB. Tinidazole ointment was applied 2 h before DNFB application. Scratchings were counted during the periods 0–30, 0–60 and 0–90 min after DNFB application. The values shown are the mean number of scratchings (±SE) (ten animals per group)

Group	Period after DNFB application (min)					
	0–30		0–60		0–90	
		P value		*P* value		*P* value
Unsensitized control	106.7±20.50		176.6±32.90		255.9±46.4	
Control	191.8±24.70	<0.05[a]	350.1±45.20	<0.01[a]	477.1±24.50)	<0.01[a]
Tinidazole 1%	115.8±11.40	0.0389[b]	221.0±30.50		315.8±37.90	0.0089[b]
Tinidazole 2%	112.0±17.30	0.0287[b]	220.7±35.40		277.2±39.80	0.0011[b]
Tinidazole 4%	139.5±26.80		280.6±39.20		397.4±40.0	

[a]Versus unsensitized control (*t*-test)
[b]Versus control (Dunnett's method)

Table 3 Effects of tinidazole (2%) and Locoid on the IgE-mediated scratch reaction in mice. Each ointment was applied 2 h before DNFB application. In the control mice, the ointment base (W50083) was applied. Scratchings were counted during the periods 0–30, 0–60 and 0–90 min after DNFB application. The values shown are the mean number of scratchings (±SE)

Group	Period after DNFB application (min)					
	0–30		0–60		0–90	
		P value[a]		*P* value[a]		*P* value[a]
Control	179.0±20.0		329.9±45.0		423.3±46.6	
Tinidazole 2%	103.5±13.7	0.0052	181.9±24.5	0.0085	229.0±29.4	0.0019
Control	169.0±15.4		321.7±19.1		434.5±29.8	
Locoid	95.1±14.9	0.0023	231.0±31.2	0.0214	337.6±32.1	0.0374

[a]Versus control (*t*-test)

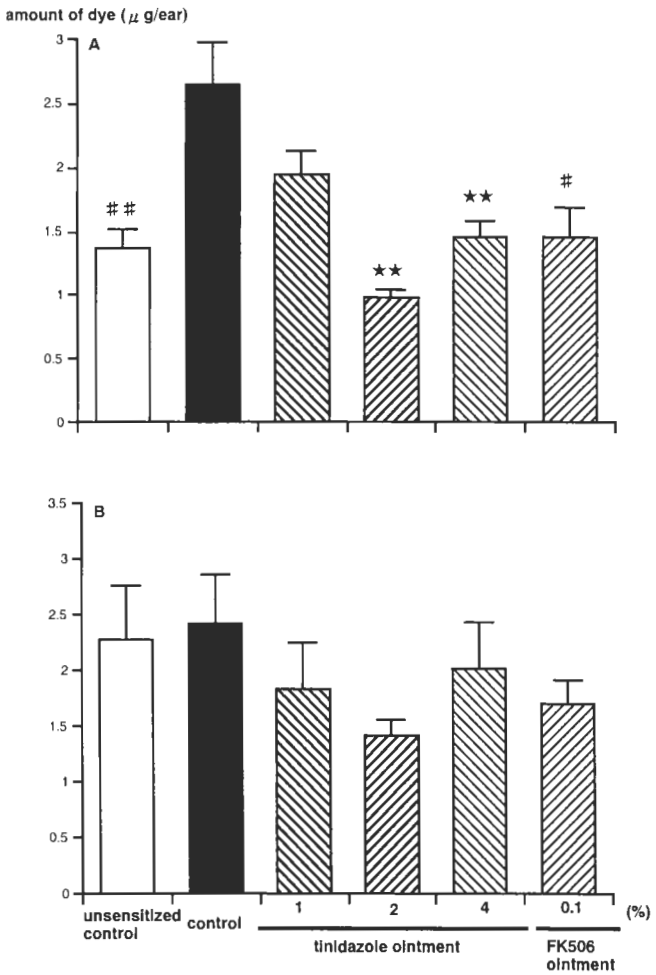

Fig. 5A, B Effects of tinidazole and FK506 ointments on IgE-mediated increase in vascular permeability of the ear. Mice were injected intravenously with 0.5 ml 0.5% Evans blue saline solution 0.5 h before being killed 1 h (**A**) and 1.5 h (**B**) after DNFB application. Ears were removed to measure the amount of extravasated dye. Each column represents the mean±SE of seven or eight animals. $^{**}P<0.01$, vs control (Dunnett's method); $^{\#}P<0.05$, $^{\#\#}P<0.01$, vs control (*t*-test). At 1.5 h after DNFB application (**B**), no significant difference was observed between the control and unsensitized control (*t*-test). Tinidazole ointment (3%) was applied 2 h before challenge with DNFB

reactions in a similar manner to metronidazole and tinidazole during the periods 0–60 and 0–90 min (data not shown).

The potency of tinidazole in suppressing the number of scratching reactions was compared with that of Locoid. As shown in Table 3, tinidazole and Locoid suppressed the number of scratching reactions to the same extent during the 0–30 min period after challenge. However, tinidazole was more potent than Locoid during the 0–60 and 0–90 min periods after challenge.

Effects of tinidazole and FK506 ointments on IgE-dependent vascular permeability

Mice passively sensitized with anti-DNP-mAb were also used to study the effects of tinidazole and FK506 ointments on vascular permeability as measured by the Evans blue dye method. During the period 30–60 min after DNFB application, extravasated dye from the skin of the pinna increased significantly in the control group (Fig. 5A). Treatment of the pinnae with tinidazole (2% and 4%) and FK506 (0.1%) ointments suppressed the increase in vascular permeability significantly (Fig. 5A).

However, during the period 60–90 min after DNFB application (Fig. 5B), there was no difference in the amount of extravasated dye between the control and the unsensitized control groups. During this period, tinidazole (1%, 2% and 4%) and FK506 (0.1%) ointments showed no effects on the amount of extravasated dye (Fig. 5B).

Discussion

Tinidazole and metronidazole were used as antiprotozoal agents when first introduced, but it was soon shown that the two compounds have strong actions against obligate anaerobes, and they have been widely used clinically to prevent infections by *Trichomonas* and anaerobes after operations [3, 9]. The main mechanism involved in their action is thought to be related to the reduction of the nitro group of nitroimidazoles by the microorganism, which in turn causes functional impairment of the cleavage of double-stranded DNA, thereby inhibiting mitosis and proliferation of the microorganisms [3]. Recent studies have

also indicated that metronidazole decreases neutrophil-generated free radicals at sites of inflammation with the aid of palmitoleic acid in the skin and inhibits oxidative tissue injury under in vivo conditions [14].

It has also been shown that oral administration of metronidazole with tetracycline is very effective in the treatment of patients with rosacea [15]. The first clinical trial in which an external preparation of metronidazole was used for the treatment of rosacea was carried out in 1960, although positive effects were not observed [16]. Then it was proved in double-blind trial that an ointment with new base containing 1% metronidazole was effective for rosacea [17], with no adverse reactions [18, 19]. These studies indicate that the development of a base for the ointment is also important in the topical use of metronidazole, and that topical metronidazole is equipotent to orally administered metronidazole. In the treatment of rosacea, the combination regimen of systemic tetracycline and topical metronidazole has been recommended, and it has been reported that topically applied metronidazole maintains remission at significantly higher levels than in untreated patients after weaning from systemic tetracycline [1]. Metronidazole is effective in the treatment of rosacea at relatively low concentrations at which it has no antibacterial action. Thus, it was of interest to study the mechanisms involved, and it turned out that metronidazole suppresses the production of free radicals by neutrophils, but not those produced by the xanthine/xanthine oxidase (X/XO) system. The authors suggested that the antiinflammatory actions of metronidazole in suppressing the generation of free radicals by neutrophils might be involved in its effects on rosacea [14, 20].

On the other hand, AD is regarded as a disease mediated by type 2 helper T cells (Th2), since T cell clones obtained from lesions of AD mainly release IL-4, a Th2-related cytokine [21]. However, in recent studies using RT-PCR, the dominant expression of IFN-α mRNA, a Th1-related cytokine, rather than IL-4 mRNA, has been demonstrated in some lesions of AD [22, 23]. Similarly, in studies using in situ hybridization and immunohistochemistry, mRNA expression of both Th2 and Th1 cytokines has been shown, and the expression of INF-γ mRNA is dominant rather than IL-4 in lesions of AD [24]. These observations indicate that Th1 may also be closely related to skin lesions of AD.

Repeated elicitation of contact hypersensitivity by TNCB resulted in a shift in the time course of antigen-specific contact hypersensitivity from a delayed-type to an early-type response, a reflection of a shift in cutaneous cytokine expression from a Th1 to a Th2 profile. The repeated elicitation of contact hypersensitivity in this animal model indicates that it is a reproducible model of chronic skin inflammation [10, 25].

In summary, treatment of NC mice with tinidazole and metronidazole 1–4% ointments (1) suppressed the LPR of biphasic ear edema in mice sensitized with OA; (2) suppressed TNCB-induced inflammatory dermatitis; (3) suppressed IgE-dependent IPR and LPR; (4) suppressed the scratching reaction; and (5) enhanced vascular permeability. Furthermore, tinidazole (2%) was found to be equipotent or more potent than Locoid in suppressing the IPR and LPR and the number of scratching reactions. Some of those results were also observed in other mouse strains including BALB/C.

In animals sensitized with specific antigens, exposure to the relevant antigen causes an immediate reaction (IPR) including measles or flare in the skin and bronchoconstriction of the airways. In addition, reactions such as edema and erythema are also triggered by stimulation with higher concentrations of antigens, and these reactions last for 6–24 h (LPR). It is considered that such biphasic reactions correspond to the clinical manifestations in human chronic allergic diseases [25].

Katayama et al. [12] have reported that in mice passively sensitized with murine monoclonal anti-DNP IgE antibody, challenge with DNFB evokes a biphasic dermatitis which has peak responses at 1 and 24 h after challenge. The IPR is characterized by a rapid increase in capillary permeability and the LPR by skin thickening with significant infiltration of eosinophils and other inflammatory cells. This model might serve as an animal model for human AD. Several antiallergic agents have been investigated in this animal model of a biphasic skin reaction. It was found that histamine H_1 receptor antagonists, including diphenhydramine and homochlorcyclizine, and the allergic histamine release inhibitors, tranilast and amlexanox, clearly inhibit the IPR but not the LPR [26]. Concerning the LPR, of the antiallergic agents investigated, only glucocorticoids including prednisolone and dexamethasone suppressed the reaction [26]. These observations indicate that IgE antibody-dependent biphasic skin reactions consist of a mast cell- and a histamine-dependent IPR, and a mast cell-independent but IgE-dependent LPR.

In the present experiments, metronidazole and tinidazole ointments were effective in suppressing the IPR and LPR in this animal model. It should be stressed that both ointments suppressed the LPR of the biphasic skin reaction and the scratching reaction due to the itching, and that tinidazole (2%) were equipotent or more potent than Locoid. The precise mechanisms involved in the inhibitory action of the two ointments on the LPR and scratching are unknown at present. However, at least, the inhibitory effects on the LPR might correlate with the immunosuppressive effects of tinidazole and metronidazole which selectively inhibit certain aspects of the cell-mediated immune response [27, 28]. The beneficial effects of metronidazole in acne vulgaris and rosacea have recently been shown to be attributable to its antiinflammatory activity rather than its antibacterial activity [1, 4].

At present, topical steroids are widely used as the most effective antiinflammatory agent for the treatment of patients with AD. However, it is well known that steroids have various adverse effects, especially on the face, including acne and hypertrichosis. Indeed, there are restrictions concerning the long-term clinical application of steroids to the face (see for example the guidelines for the therapy of AD of the Ministry of Health and Welfare in Japan). In this respect, it should be stressed that tinidazole

ointment was found to be equipotent or more potent than Locoid in suppressing the IPR, the LPR and the number of scratching reactions. At present, at least, it is generally considered that metronidazole has no adverse effects on the face [1, 18, 19]. Thus, the advantages of using metronidazole or tinidazole ointments are that the ointments have stronger anti-itching effects than corticosteroid, and can be applied to the face with far fewer side effects, if any, than corticosteroids.

Following a toxicological evaluation of nitroimidazoles with particular reference to their carcinogenic, mutagenic and teratogenic potential, it has been reported that nitroimidazoles are essentially free of cancer risk or other serious toxic side effects. Teratogenicity tests of metronidazole, ornidazole and tinidazole in animals have shown negative results. A follow-up for 10 or more years of 771 women first treated with metronidazole between 1960 and 1969 for trichomoniasis has revealed no excess of any form of cancer attributable to the treatment, and no excess cancer risk has so far come to light in the Kaiser-Permanente follow-up of nearly 2500 patients given at least one prescription of metronidazole between 1969 and 1973 [29].

In consideration of the anti-inflammatory, immunosuppressive, anti-itching and bactericidal effects of tinidazole and metronidazole, the present results strongly suggest the possible clinical use of the ointments of both agents in relatively high concentrations (1–4%) in inflammatory skin diseases including AD, in addition to rosacea and acne vulgaris.

References

1. Wilkin J (1999) Use of topical products for maintaining remission in rosacea. J Arch Dermatol 135:79–80
2. Parsad D, Pandhi R, Negl KS, Kuma B (2001) Topical metronidazole in seborrheic dermatitis. Dermatology 202:35–37
3. Nair MD, Nagarajan K (1983) Nitroimidazole as chemotherapeutic agents. Prog Drug Res 27:163–252
4. Bannatype RM (1999) Metronidazole, its bioactive metabolites and acne. Clin Med Res Opin 15:289–299
5. Rockwell S, Irvin CG, Hederlank MH (1983) Inhibition of delayed hypersensitivity by metronidazole and misonidazole. Int J Radiat Oncol Biol Phys 9:701–706
6. Kohli J, Bhattacharya SK, Gupta VS, Sen P (1987) Effect of metronidazole on immune mechanism in experimental animals. Indian J Exp Biol 25:177–180
7. Sen P, Chakaravarty AK, Kohli J (1991) Effects of some imidazoles on cellular immune responses. An experimental study. Indian J Exp Biol 29:867–869
8. Grewe M, Bruijnzeal-Koomen CAFM, Schöpf E, Thepen T, Langeveld-Wildschut AG, Ruzicka T, Krutmann J (1998) A role for Th1 and Th2 cells in the immunopathogenesis of atopic dermatitis. Immunol Today 19:359–361
9. Carmine AA, Brogden RN, Heel RC, Speight TM, Avery GS (1982) Tinidazole in anaerobic infections. A review of its antibacterial activity, pharmacological properties and therapeutic efficacy. Drugs 24:85–117
10. Kitagaki H, Fujisawa S, Watanabe K, Hayakawa K, Shiohara T (1995) Immediate-type hypersensitivity response followed by a late reaction is induced by repeated epicutaneous application of contact sensitizing agents in mice. J Invest Dermatol 105:749–755
11. Leung DY, Hirsch RL, Schneider L, Moody C, Takaoka R, Li SH, Meyerson LA, Mariam SG, Goldstein G, Hanifin JM (1990) Thymopentin therapy reduces the clinical severity of atopic dermatitis. J Allergy Clin Immunol 85:927–933
12. Katayama I, Tanei R, Yokozeki H, Nishioka K, Doi Y (1990) Induction of eczematous skin reaction in experimentally induced hyperplastic skin of Balb/C mice by monoclonal anti-DNP IgE antibody: possible implications for skin lesion formation in atopic dermatitis. Int Arch Allergy Appl Immunol 93:148
13. Rogers DF, Boschetto P, Barnes PJ (1989) Plasma exudation: correlation between Evans blue dye and radiolabeled albumin in guinea-pig airways in vivo. J Pharmacol Methods 21:309–315
14. Akamatsu H, Oguchi M, Nishijima S, Asada Y, Takahashi M, Ushijima T, Niwa Y (1990) The inhibition of free radical generation by human neutrophils through the synergistic effects of metronidazole with palmitoleic acid: a possible mechanism of action of metronidazole in rosacea and acne. Arch Dermatol Res 282:449–454
15. Saihan EM, Burton JL (1980) A double-blind trial of metronidazole versus oxytetracycline therapy for rosacea. Br J Dermatol 102:443–444
16. von Prinz L (1960) Die Behandlung der Rosacea papulopustulosa mit metronidazole (Vagimid). Dsch Gesundheits-Wesen 35:1804–1805
17. Nielsen PG (1983) Treatment of rosacea with 1% metronidazole cream. A double-blind study. Br J Dermatol 108:327–332
18. Breneman D, Stewart D, Hevia O, Hino PD, Drake LA (1998) A double-blind, multicenter clinical trial comparing efficacy of one-daily metronidazole 1 percent cream to vehicle in patients with rosacea. Cutis 61:44–47
19. Jorizzo JL, Lebwohl M, Tobey RE (1998) The efficacy of metronidazole 1% cream once daily compared with metronidazole 1% cream twice daily and their vehicles in rosacea; a double-blind clinical trial. J Am Acad Dermatol 39:502–504
20. Miyachi Y, Simamura S, Niwa Y (1986) Anti-oxidant action of metronidazole: a possible mechanism of action in rosacea. Br J Dermatol 114:231–234
21. Hamid Q, Boguniewicz MZ, Leung DY (1994) Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. J Clin Invest 94:870–876
22. Grewe M, Gyufko K, Schopf E, Krutmann J (1994) Lesional expression of interferon-γ in atopic eczema. Lancet 343:25–26
23. Ohmen JD, Hanifin JM, Nickoloff BJ, Rea TH, Wyzykowski R, Kim J, Jullien D, McHugh T, Nassif AS, Chan SC, et al (1995) Overexpression of IL-10 in atopic dermatitis. Contrasting cytokine patterns with delayed type hypersensitivity reactions. J Immunol 154:1956–1963
24. Tanaka Y, Anan S, Yoshida H (1990) Immunohistochemical studies in mite antigen-induced patch test sites in atopic dermatitis. J Dermatol Sci 1:361–368
25. Kitagaki H, Ono N, Hayakawa K, Kitazawa T, Watanabe K, Shiohara T (1997) Repeated elicitation of contact hypersensitivity induces a shift in cutaneous cytokine milieu from a T helper cell type1 to a T helper cell type2 profile. J Immunol 159: 2484–2491
26. Nagai H, Sakura T, Inagaki N, Mori H (1995) An immunopharmacological study of the biphasic allergic reaction in mice. Biol Pharm Bull 18:229–245
27. Miller JJ (1980) The imidazoles as immunosuppressive agents. Transplant Proc 12:300–303
28. Rockwell S, Kapp DS (1982) Immunosuppression by hypoxic cell radiosensitizers. A phenomenon of potential clinical importance. Int J Radiat Oncol Biol Phys 8:1071–1074
29. Roe FJ (1985) Safety of nitroimidazoles. Scand J Infect Dis Suppl 46:72–81