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ORIGINAL PAPER

Anti-inflammatory and anti-itch activity of sertaconazole nitrate

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Abstract Cutaneous fungal infections are frequently associated with an inflammatory component including irritated skin, itching and stinging/burning. Therapeutic anti-fungal agents that have anti-inflammatory activity have the potential to provide clinical benefit beyond fungus eradication. Recently, certain anti-fungal agents have been shown to have intrinsic anti-inflammatory activity, therefore we sought to determine the extent of the anti-inflammatory activity of these compounds. The anti-inflammatory activities of eight anti-fungal agents (butoconazole, ciclopirox olamine, fluconazole, miconazole nitrate, sertaconazole nitrate, terconazole, tioconazole and ketoconazole) were compared in a number of preclinical models of dermal inflammation and pruritus. While butoconazole, ciclopirox olamine, fluconazole, and miconazole nitrate were all found to have anti-inflammatory activity, only sertaconazole nitrate reduced the release of cytokines from activated lymphocytes and mitigated inflammation in animal models of irritant contact dermatitis and neurogenic inflammation. In addition, sertaconazole nitrate inhibited contact hypersensitivity and scratching responses in a murine model of pruritus. Furthermore, the in vitro and in vivo anti-inflammatory activity of sertaconazole nitrate was found to be greater than other topical anti-fungal agents examined. These studies demonstrate that topical administration of clinically relevant concentrations of sertaconazole nitrate resulted in an efficacious anti-inflammatory activity against a broad spectrum of dermal inflammation models and itch. The anti-inflammatory properties of sertaconazole may contribute to the efficacy of the drug in the treatment of cutaneous fungal conditions and provide greater anti-inflammatory activity compared with other anti-fungal agents.

Keywords Inflammation · Itch · Anti-fungal · Sertaconazole nitrate · Cytokine · Lymphocyte

Abbreviations

PHA	Phytohemagglutinin
PBMC	Peripheral blood mononuclear cells
RTX	Resiniferatoxin
TPA	Tetradecanoyl phorbol acetate
TNFα	Tumor necrosis factor-α
IL-2	Interleukin-2
IFNγ	Interferon γ
IL-4	Interleukin-4
GM-CSF	Granulocyte macrophage colony-stimulating factor

Introduction

Cutaneous fungal infections are estimated to be the second most common skin diseases in the United States [35]. Fungal infections are frequently characterized by irritated skin, itching and stinging/burning symptoms [2, 3, 5, 20]. In addition the presence of itching is related with the severity of topical fungal infections [10]. Furthermore, fungal infections and the inflammatory response induced by dermatophytes can have pronounced effects on skin barrier function

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resulting in scaly and hyperkeratotic skin which can lead to skin maceration and tissue damage [2, 19].

Since cutaneous fungal infections often manifest as irritated skin and have an inflammatory component [13], therapeutic agents that have anti-inflammatory activity have the potential to provide additional clinical benefits over agents that simply act by eradicating the infecting organism. Indeed, an anti-fungal/steroid combination, clotrimazole–betamethasone dipropionate (Lotrisone), has been shown to provide clinical benefits in addition to eradication of the dermatophytes [37]. However, long-term use of a corticosteroid in the anti-fungal/steroid combination can result in skin atrophy [6]. Recently, certain anti-fungal agents have been shown to have intrinsic anti-inflammatory activity, and thus may preclude the need for the addition of a corticosteroid [1].

The current study was designed to evaluate the activity of a panel of eight anti-fungal agents (butoconazole, ciclopirox olamine, fluconazole, miconazole nitrate, sertaconazole nitrate, terconazole, tioconazole and ketoconazole) against in vitro and in vivo models of inflammation. We report that sertaconazole nitrate was the most potent anti-fungal studied in reducing the release of cytokines from activated T-cell enriched human lymphocytes. In vivo, sertaconazole nitrate was found to be effective against dermal irritation models of contact hypersensitivity, irritant contact dermatitis, neurogenic inflammation and pruritus; whereas, none of the other anti-fungal compounds evaluated significantly reduced inflammation in all dermal irritation models. The anti-inflammatory and anti-itch properties of sertaconazole may contribute to the efficacy of the drug in the treatment of cutaneous fungal infections, particularly in those cases associated with pronounced inflammation.

Methods

Materials

Oxazolone, resiniferatoxin, tetradecanoylphorbol acetate, ciclopirox olamine, miconazole nitrate and all routine reagents were obtained from Sigma (Sigma Aldrich, St Louis, MO, USA). Substance P was purchased from Bachem (Torrance, CA, USA). Sertaconazole nitrate was obtained from Ferrer Pharmaceuticals (Barcelona, Spain). Butoconazole, fluconazole, terconazole, tioconazole and ketoconazole were synthesized by Johnson & Johnson Pharmaceutical Research and Development. For in vitro studies, sertaconazole nitrate, ciclopirox olamine, butoconazole, miconazole nitrate, fluconazole, terconazole, tioconazole and ketoconazole were all prepared in DMSO at a concentration of 100 mg ml^{-1} prior to dilution in cell culture media. Female CD-1 mice were obtained from Charles River Laboratories (Wilmington, MA, USA). The Institutional Animal Care and Use Committee at Johnson & Johnson approved all procedures used in these experiments.

In vitro studies

T-cell activation

Upon stimulation with phytohemagglutinin (PHA), mature T cells respond by clonal expansion and the secretion of cytokines [34]. Anti-fungal agents were evaluated in vitro at escalating concentrations (0.1, 1, 10, 100 μg ml^{-1}) to assess their ability to inhibit the activation of PHA-stimulated human peripheral blood mononuclear cells (PBMCs). PBMCs were prepared from three different healthy adult male donors by differential centrifugation on Ficoll-Hypaque (Biological Specialty Corporation, Colmar, PA, USA). PBMCs were plated at 1×10^6 cells ml^{-1} in Serum Free Lymphocyte Growth Media (ExVivo 15; Biowhittaker, Walkersville, MD) and 100 μl was added to a flat-bottomed 96-well plate. Human PBMCs were stimulated with 10 μg ml^{-1} purified PHA (Remel, Lenexa, KS, USA) in the presence or absence of anti-fungal agents. PBMCs were then incubated at 37°C at 5% CO_2 for 48 h at which time supernatants were collected and stored at −20°C until assayed. Cytokines were analyzed using commercially available immunoassay multiplex kits (Upstate Biotechnology, Charlottesville, VA, USA) on a Luminex L100 (Luminex Corporation, Austin, TX, USA) a validated assay with a sensitivity that is comparable to that of conventional enzyme-linked immunosorbent assays [11]. Lactate dehydrogenase (Boehringer Mannheim, Indianapolis, IN, USA) was also assessed on the supernatants to determine cytotoxicity. After the supernatant was removed from part of the 96-well plate, the remaining cells were returned to the incubator and proliferation was determined at 72 h by using alamarBlue® (Alamar Bioscience, Sacramento, CA, USA). Results were calculated using Graphpad Prism (GraphPad Software, San Diego, CA, USA) to determine the concentration of each compound that resulted in a 50% reduction of release (IC_{50}) of each cytokine.

In vivo studies

Oxazolone-induced ear edema (contact hypersensitivity)

To assess the potential for anti-fungals to inhibit contact hypersensitivity responses, CD-1 mice were sensitized

with 3% oxazolone (Sigma) (30 mg ml^{-1}) prepared in corn oil:acetone (4:1 ratio) and administered to a shaved abdomen (50 µl). Five days later they were challenged with oxazolone (2%) in acetone, administered to the dorsal left ear (20 µl), with the right ear remaining untreated. One hour after the challenge, anti-fungal agents in a 70% EtOH/30% propylene glycol vehicle or vehicle alone was applied to the dorsal left ear (20 µl) with the right ear untreated ($N = 7$ per group). Maximum response of this model is seen at 24 h where the animals were sacrificed by CO_2 inhalation and 7-mm ear punches were taken. The percentage of inhibition was calculated by comparing the difference in ear weight between vehicle and anti-fungal-treated mice.

Quantitation of pro-inflammatory cytokines from mouse ear biopsies

Biopsies of 7 mm diameter were taken from mouse ears that had been untreated, challenged with oxazolone only, or treated with anti-fungal agents after oxazolone challenge were homogenized on ice in 800 µl cold PBS containing protease inhibitor cocktail (Sigma Aldrich, St Louis, MO, USA) with a Polytron homogenizer. Samples were centrifuged at 1,000 rpm for 10 min at 4°C and supernatants were removed. Cytokine content of the supernatant was determined using a Mouse Multi-cytokine Detection Kit (Upstate Biotechnology, Charlottesville, VA, USA) with a Luminex 100 Multi-Analyte Detector (Luminex, Austin, TX, USA) analyzer.

TPA-induced ear edema (irritant dermatitis)

TPA-induced ear edema was performed by the method reported by Rao et al. [27]. A 0.005% (w/v) TPA solution was made in acetone and 20 µl was applied to the dorsal left ear of CD-1 mice. Anti-fungal agents, administered as a 1% solution in a 70% ethanol/30% propylene glycol vehicle, or vehicle control, were immediately applied to the left ear (20 µl) after the application of TPA, with the right ear untreated ($N = 7$ per group). Maximum response in this model is 5.5 h after TPA application. The mice were sacrificed by CO_2 inhalation and a 7-mm biopsy was removed from each ear. The percentage of inhibition was calculated by comparing the difference in ear weight between vehicle and anti-fungal-treated mice.

Resiniferatoxin-induced ear edema (neurogenic dermatitis)

Neurogenic inflammation was induced by 0.05% resiniferatoxin (RTX) [29] in acetone applied to the dorsal left ear (20 µl) of CD-1 mice. Immediately following RTX treatment, anti-fungal compounds (1%) were applied (20 µl) in 70% EtOH/30% propylene glycol vehicle or vehicle control, to the dorsal left ear while the right ear was untreated ($N = 10$ per group). Maximum response in this model is seen at 30 min. At that time the animals were sacrificed and 7-mm ear punches were taken. The percentage of inhibition was calculated by comparing the difference in ear weight between vehicle and anti-fungal-treated mice.

Substance P-induced itch response

An itch-associated response was induced by intradermal injection of substance P in male CD-1 mice (7–9 weeks old), and scratching behavior was observed and quantitated according to the methodology of Andoh and Kuraishi [4]. In brief, mice were individually housed in a plastic cage for at least 1 h before the experiment for acclimation. Mice were pretreated for 30 min with topical application of an anti-fungal agent prepared in 100% ethanol or with vehicle control, to an area of the back that had been shaved 1 day prior to the experiment. Substance P was prepared in sterile physiological saline, and 300 µg of Substance P or sterile physiologic saline (control) in a volume of 50 µl was injected into the interscapular part of the back. After injection, mice were returned to the cage, and their scratching behaviors were videotaped. The number of scratches elicited during the 30-min period after injection was determined by playback of the videotape.

Statistics

Data analysis—data are presented as mean ± standard deviation. Cytokine release experiments were individually performed from three separate donors of PBMCs. Analysis of variance (ANOVA) with Newman–Keuls post hoc test was used to compare the effects of anti-fungal on cytokine release from PBMCs and on inflammation in murine models with the significance for all tests set at $P < 0.05$.

Results

In vitro studies

Stimulation of human PBMCs with PHA resulted in a release of 3,766.2 ± 213.0 pg ml^{-1} of TNFα, 2,679.2 ± 78.3 pg ml^{-1} of IL-2, 2,492.8 ± 117.3 pg ml^{-1} of IFNγ, 66.34 ± 12.9 pg ml^{-1} of IL-4 and 619.6 ± 21.1 pg ml^{-1} of granulocyte macrophage colony-stimulating factor

(GM-CSF). Fluconazole, terconazole and tioconazole demonstrated very limited inhibitory effects against tested cytokines with IC_{50} values for TNFα, IL-2, and GM-CSF of over 50 μg ml^{-1}. Sertaconazole nitrate, ciclopirox olamine, butoconazole, ketoconazole and miconazole nitrate all inhibited release of IL-2, TNFα, IFNγ, IL-4, and GM-CSF from activated human lymphocytes (Table 1) without inducing cytotoxicity at any concentration as demonstrated by a lack of LDH release (data not shown). Of the compounds tested, sertaconazole nitrate was significantly more potent against the release of all cytokines tested compared with the other anti-fungal agents (Table 1). Sertaconazole nitrate inhibited cytokine release in a dose-dependent fashion (Fig. 1) with IC_{50} values of 0.37, 1.06, 0.21, 4.97, and 0.34 μg ml^{-1}, respectively. Additional studies were conducted to determine whether sertaconazole nitrate had an effect on T-cell proliferation. Sertaconazole nitrate was found to inhibit the proliferation of stimulated human lymphocytes with an IC_{50} of 4 ± 2.5 μg ml^{-1}.

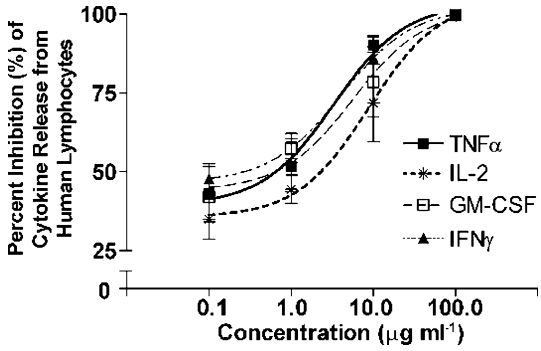

Fig. 1 Sertaconazole nitrate inhibits the release of inflammatory cytokines from activated human lymphocytes. Lymphocyte-enriched human peripheral blood mononuclear cells were stimulated with 10 μg ml^{-1} purified phytohemagglutinin (PHA) in the presence or absence of sertaconazole nitrate. After 48 h, release of cytokines from activated lymphocytes was assayed using ELISA. The results shown are mean ± SD of cytokine release from PBMCs derived from three independent donors. *TNFα* tumor necrosis factor-α, *IL-2* interleukin-2, *GM-CSF* granulocyte macrophage colony-stimulating factor, *IFNγ* interferon γ

In vivo studies

TPA-induced ear edema (irritant dermatitis)

Tetradecanoyl phorbol acetate (TPA) is the main active compound found in croton oil, producing vasodilation, erythema, and edema within 5 h after contact with the skin [39]. Of the anti-fungal agents examined, only butoconazole, ketoconazole and sertaconazole nitrate significantly reduced the TPA-induced edema response. The mean ear weight of TPA-challenged animals treated with sertaconazole nitrate (1%) was 7.23 ± 2.18 mg compared with 14.7 ± 2.68 for controls, indicating a statistically significant reduction (50.7%) in irritant dermatitis ($P < 0.05$; Fig. 2). Butoconazole and ketoconazole also resulted in a significant reduction in irritant dermatitis (37.9% reduction and 33.1% respectively; $P < 0.05$ versus vehicle), however, sertaconazole nitrate was more efficacious in this model. Bethamethasone-17 valerate (0.1%), the positive control in this model, reduced inflammation by 66.9%.

Resiniferatoxin-induced ear edema (neurogenic dermatitis)

Resiniferatoxin (RTX) is a potent analog of capsaicin that activates the neural pathway that mediates neurogenic inflammation and, therefore, can be useful for the assessment of agents that may block neurogenic

Table 1 Inhibitory effect of anti-fungals on phytohemagglutinin (PHA)-stimulated release of cytokines from human peripheral blood lymphocytes

Compound	IC_{50} (mean ± SD μg ml^{-1})				
	TNFα	IL-2	IFNγ	IL-4	GM-CSF
Sertaconazole nitrate	0.37 ± 0.2	1.06 ± 0.1	0.21 ± 0.1	4.97 ± 2.9	0.34 ± 0.03
Ciclopirox olamine	50.51 ± 34.1*	9.18 ± 1.2*	14.55 ± 4.2*	ND	1.51 ± 0.3*
Butoconazole	7.2 ± 0.5*	14.4 ± 5.7*	7.36 ± 2.1*	ND	7.6 ± 3.6*
Miconazole	4.81 ± 0.37*	15.8 ± 1.5*	4.34 ± 1.6*	ND	7.31 ± 2.3*
Fluconazole	> 100	> 100	> 100	ND	> 100
Terconazole	59.1 ± 28.9*	> 100	> 100	ND	65.4 ± 25.4*
Tioconazole	> 100	> 100	> 100	ND	> 100
Ketoconazole	0.65 ± 0.51	41.9 ± 27.9*	0.41 ± 0.62	ND	8.3 ± 4.3*

IC_{50} concentration resulting in 50% reduction in cytokine release, *TNFα* tumor necrosis factor-α, *IL-2* interleukin-2, *IFNγ* interferon γ, *IL-4* interleukin-4, *GM-CSF* granulocyte macrophage colony-stimulating factor, *ND* not determined

*Significant difference compared to the respective sertaconazole nitrate IC_{50} determined using ANOVA with Newman–Keuls post hoc test, the significance for all tests was set at $P < 0.05$

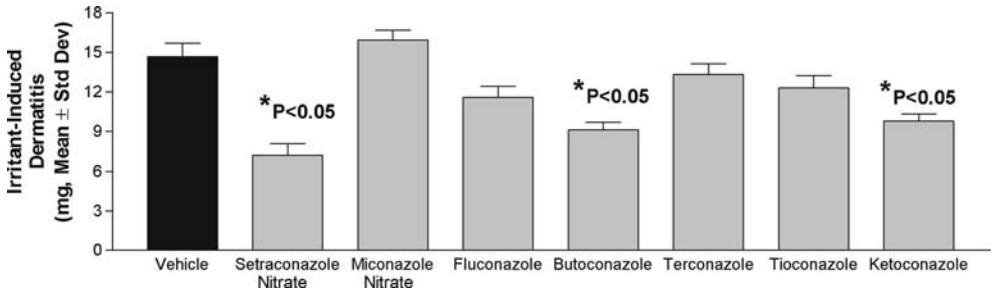

Fig. 2 Reduction of TPA-induced murine dermatitis by topical application of sertaconazole nitrate. CD-1 mice were treated with tetradecanoyl phorbol acetate (TPA) applied to the right ear; the left remained untreated. Immediately after application of TPA (1 μg/ear), anti-fungal agents (1%) were applied to the TPA-treated ear ($N = 7$ per group). The results shown are mean ± SD; an *asterisk* indicates a significant reduction in inflammation compared to the vehicle treated group determined using ANOVA with Newman–Keuls post hoc test. Butoconazole and sertaconazole nitrate significantly ($P < 0.05$) reduced the TPA-induced irritant dermatitis

inflammation [32]. Of the anti-fungal agents examined, only fluconazole and sertaconazole nitrate significantly reduced the RTX-induced edema response. Sertaconazole nitrate 1% elicited a significant reduction in RTX-induced ear edema when compared with controls with mean ear weights of 5.0 ± 1.65 and 8.8 ± 1.52 mg, respectively. This represented a 43.0% reduction in ear edema compared with control ($P < 0.05$; Fig. 3). This effect was greater than that produced by other anti-fungals. Fluconazole also significantly reduced RTX-induced neurogenic dermatitis, reducing ear edema by 29% ($P < 0.05$ versus vehicle); however, sertaconazole nitrate was more efficacious in this model. Indomethacin (1.0%), the positive control in this model, reduced inflammation by 46.5%.

Oxazolone-induced ear edema (contact hypersensitivity) and cytokine detection

Exposure to oxazolone induces an immune-mediated allergic response (contact hypersensitivity) [39, 40]. The activity of sertaconazole nitrate against contact hypersensitivity was ascertained since this was the only anti-fungal agent effective in both irritant and neurogenic dermatitis models. Topical treatment with sertaconazole nitrate 1% significantly inhibited contact hypersensitivity (Fig. 4a) and decreased the content of the pro-inflammatory cytokines TNFα, IL-2, and IFNγ in oxazolone exposed murine skin (Fig. 4b). The mean ear weight in oxazolone-challenged mice treated with sertaconazole nitrate was 6.4 ± 2.4 mg compared with 12.6 ± 1.9 mg for control-treated animals. This represented a 49.7% reduction in edema for the sertaconazole nitrate group ($P < 0.05$). Hydrocortisone (0.01%), the positive control in this model, reduced inflammation by 65.5%. The total cytokine content from biopsies of murine tissue treated with oxazolone increased by 2.5, 5.9 and 1.6-fold for TNFα, IL-2, and IFNγ respectively over control tissue. Topical treatment with sertaconazole nitrate 1% resulted in a significant decrease in the total cytokine content.

Substance P-induced itch response

Since itch is a sensation that promotes the desire to scratch the stimulated area, scratching can be used as

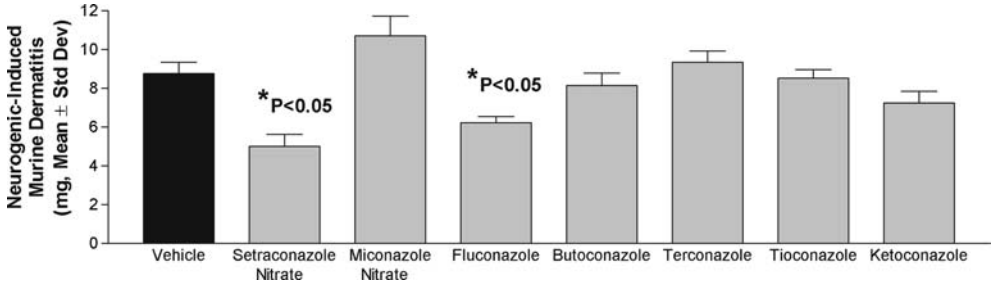

Fig. 3 Reduction of resiniferatoxin-induced neurogenic inflammation by topical application of sertaconazole nitrate. CD-1 mice were treated with resiniferatoxin (RTX) applied to the right ear; the left remained untreated. Immediately after application of RTX (10 μg/ear), sertaconazole nitrate or other anti-fungal agents were applied to the RTX-treated ear ($N = 10$ per group). The results shown are mean ± SD, an *asterisk* indicates a significant reduction in inflammation compared to the vehicle treated group determined using ANOVA with Newman–Keuls post hoc test. Fluconazole and sertaconazole nitrate significantly ($P < 0.05$) reduced the RTX-induced neurogenic inflammation

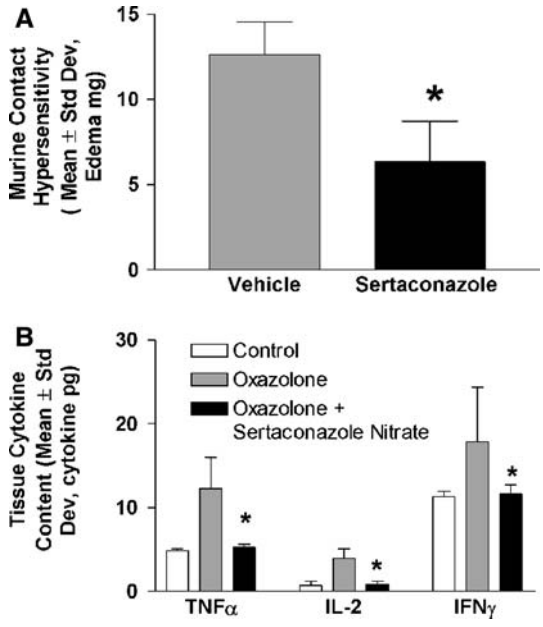

Fig. 4 Reduction of oxazolone-induced murine contact hypersensitivity and cytokine content by topical application of sertaconazole nitrate. CD-1 mice were sensitized to oxazolone and 5 days later challenged with oxazolone applied to the right ear; the left remained untreated ($N = 7$ per group). One hour after application of oxazolone (1 μg/ear), sertaconazole nitrate was applied to the oxazolone-treated ear. **a** Sertaconazole nitrate significantly ($P < 0.05$) reduced the edema resulting from oxazolone-induced contact hypersensitivity and **b** significantly ($P < 0.05$) reduced the oxazolone-induced cytokine release from inflamed tissue. The results shown are mean ± SD, an *asterisk* indicates a significant reduction in inflammation compared to the oxazolone group treated with vehicle alone determined using ANOVA with Newman–Keuls post hoc test

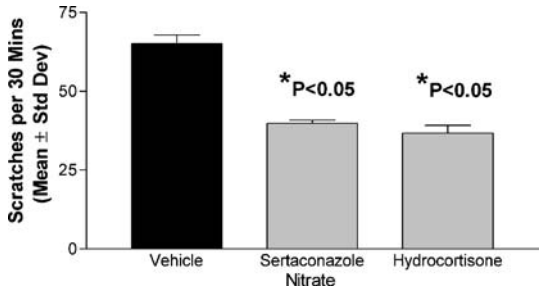

Fig. 5 Reduction of Substance P-induced itch by topical application of sertaconazole nitrate. CD-1 mice ($N = 7$ group) were pre-treated for 30 min with topical application of vehicle, sertaconazole nitrate or hydrocortisone to the shaved back. An itch-associated response is induced by intradermal injection of Substance P (300 μg) in a volume of 50 μl into the interscapular region of the back in mice, and scratching behavior is observed and quantitated. An *asterisk* indicates a significant reduction in scratching compared to the vehicle treated group determined using ANOVA with Newman–Keuls post hoc test. Sertaconazole nitrate significantly ($P < 0.05$) reduced the Substance P-induced itch-associated response

an objective measure of the degree of itching [18]. Substance P is an undecapeptide belonging to the tachykinin family; intradermal injection of substance P has been shown to elicit pruritogenic (itch) responses in humans [4, 18]. In the itch model, vehicle-treated animals scratched a mean of 65.0 ± 8.1 times per 30 min, while animals treated with sertaconazole nitrate 1% scratched a mean of 39.8 ± 2.7 times per 30 min (Fig. 5). This represented a statistically significant reduction (38.7%) in scratching in sertaconazole nitrate-treated animals ($P < 0.05$) comparable to the reduction in scratching in hydrocortisone (1%)-treated animals (43.5%).

Discussion

The findings in the current study indicate that certain anti-fungal agents, independent of fungal eradication, exert anti-inflammatory activity that may provide therapeutic benefits for irritated skin associated with fungal infections. Of the anti-fungal agents examined (butoconazole, ciclopirox olamine, fluconazole, miconazole nitrate, sertaconazole nitrate, terconazole, tioconazole and ketoconazole) only sertaconazole nitrate was found to significantly reduce the release of cytokines from activated lymphocytes and mitigate inflammation in animal models of irritant contact dermatitis and neurogenic inflammation. In addition, sertaconazole nitrate inhibited contact hypersensitivity and scratching responses in a murine model of pruritus. Thus, topical administration of clinically relevant concentrations of sertaconazole nitrate resulted in an efficacious anti-inflammatory activity against a broad spectrum of dermal inflammation models and against itch.

Several lines of evidence indicate that exposure to dermatophytes or isolated dermatophyte proteins can directly mediate the dermal inflammatory responses reported during fungal infection [2]. Treatment of human keratinocytes in culture with the fungus, *Trichophyton*, has been shown to induce release of the pro-inflammatory cytokines TNFα and IL-8 [25]. Peripheral lymphocytes isolated from a patient with a dermatophyte infection and treated in vitro with *Trichophyton* resulted in a significant increase in release of IFNγ, IL-2, and GM-CSF which are involved in the development of a delayed type hypersensitivity responses in skin [16, 17]. This finding is consistent with observations that injection of *Trichophyton* can induce either an immediate or delayed type hypersensitivity response in individuals that have a previous history of fungal infection [30, 36]. Histology of patients with dermatophyte infection reveals an infiltration of skin by

neutrophils and lymphocytes at the site of infection [24]. This infiltration of the skin by polymorphonuclear leukocytes is a primary factor in the development of numerous skin conditions [12, 23]. We demonstrated that sertaconazole nitrate inhibits the release of IL-2, TNFα, IFNγ, IL-4, and GM-CSF from activated human lymphocytes in a dose-dependent fashion (Fig. 1) indicating that the drug is effective in inhibiting the release of inflammatory cytokines that have been shown to be stimulated by fungal infections [16, 17]. Furthermore, topical administration of sertaconazole nitrate was also found to reduce the content of the cytokines, IL-2, TNFα, and IFNγ in inflamed murine tissue. Presumably the decreased release of cytokines mediates the reduced inflammatory response observed in the oxazolone model of contact hypersensitivity (Fig. 4a).

Skin and vaginal fungal infections are frequently characterized by itching and burning [2, 3, 5, 20], and the presence of itching is significantly associated with the severity of topical fungal infections [10]. Pruritus (itching) has been characterized as an unpleasant sensation which elicits the urge to scratch and can range in severity from acute to intractable [38]. Pruritus can lead to a vicious itch-scratch cycle resulting in disrupted skin integrity and subsequent decreased barrier resistance to infections [7]. Pruritus is initiated by stimulation of unmyelinated small diameter sensory neurons, C-fibers, which conduct pain, itch and stinging sensations [29]. Activation of small diameter sensory neurons induces the release of neuropeptides such as Substance P and calcitonin gene-related peptide substances triggering nociceptive responses (itching, stinging, pain) [28]. The release of these substances from peripheral terminals in the skin, in turn, act on peripheral blood vessels and immune cells producing an inflammatory response (i.e. neurogenic inflammation) that is characterized by erythema, edema, warmth and hypersensitivity [15]. Of the anti-fungal agents evaluated, only fluconazole and sertaconazole nitrate were found to significantly inhibit neurogenic inflammation, thus suggesting these anti-fungal compounds may have putative anti-itch activity. Furthermore, using a murine model of Substance P-induced itch we demonstrate that sertaconazole nitrate significantly reduced the scratching response compared to vehicle. These results demonstrate that sertaconazole can modulate the nociceptive responses that occur during fungal infection. Furthermore, the anti-inflammatory activity may reduce the scratching-induced secondary inflammation, preventing the disrupted skin barrier function.

The results of this study are consistent with reports that a sertaconazole 2% solution produced a 39.8% inhibition of croton oil-induced edema in the ears of Sprague-Dawley rats [1]. In contrast to other anti-fungal agents examined, clinically relevant concentrations of sertaconazole nitrate were found to elicit effective anti-inflammatory activity against a variety of dermal inflammation models. Of the agents evaluated, only Butoconazole and Fluconazole were found to significantly reduce dermal inflammation in irritant and neurogenic dermatitis models respectively, whereas sertaconazole significantly reduced edema in all dermal inflammation models. Differences in anti-inflammatory activity between anti-fungal agents have also been demonstrated by other investigators. Itraconazole, but not fluconazole, has been shown to inhibit the 5-lipoxygenase pathway and formation of leukotrienes in human polymorphonuclear leukocytes [31]; however, we found that sertaconazole has no direct effect on the inhibition of 5-lipoxygenase (data not shown). Fluconazole and ketoconazole failed to inhibit cytokine gene expression in human lymphoid cells, while itraconazole and miconazole produced a slight inhibition [14]. In another study, topical flutrimazole and ketoconazole creams (2% each) inhibited carrageenan-induced rat paw edema by approximately 40% [21]. In a clinical study, miconazole was partially effective in suppressing symptoms of inflammation in patients with inflamed bacterial or mycotic skin infections, but the effect was less than that produced by hydrocortisone for the majority of the trial duration [22]. Taken together, although the eight compounds (butoconazole, ciclopirox olamine, fluconazole, miconazole nitrate, sertaconazole nitrate, terconazole, tioconazole and ketoconazole) examined all have excellent fungicidal activity these results demonstrate that they differ in activity against dermal inflammation and itch.

Sertaconazole nitrate is a broad-spectrum anti-fungal agent with demonstrated activity against dermatophytes, yeast, and some gram-positive bacteria [8, 9, 26]. The drug is well suited for topical administration as evidenced by its good safety profile, low systemic absorption, and long-lasting cutaneous retention [26]. Clinical safety and efficacy of sertaconazole nitrate on seborrheic dermatitis, versicolor, cutaneous candidiasis or dermaphytosis was recently reviewed by Torres et al. [33]. These studies have demonstrated that sertaconazole has comparable or superior efficacy to other imidazole anti-fungal agents such as miconazole, sulconazole, clotrimazole and ketoconazole, with a better clinical safety profile. In addition, the anti-inflammatory properties of anti-fungal agents, such as sertaconazole, may contribute to the efficacy of the drug in the treatment of cutaneous fungal conditions, particularly in those cases associated with pronounced inflammation, as evidenced by the presence of erythema,

pruritus, and scaling. Because of such symptoms, topical corticosteroids are often added to anti-fungal therapy to provide rapid symptomatic relief [2]; however, the potential for steroid-induced skin atrophy can limit the therapeutic use of an anti-fungal/steroid combination therapy [35]. Thus, the presence of an anti-inflammatory and anti-itch action by sertaconazole nitrate may contribute to symptom relief and may obviate the need to add a topical corticosteroid in some patients.

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