Therapeutic analysis of organic acids on experimental dermatophytosis in guinea pigs

Shokri, H.[1]*; Asadi, F.[2]

[1]Faculty of Veterinary Medicine, University of Mazandaran, Amol, Iran. [2]Department of Biochemistry, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

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Correspondence
Shokri, H.
Faculty of Veterinary Medicine, University of Mazandaran, Amol, Iran.
Tel: +98(121)2271054
Fax: +98(121)2271054
E-mail: hshokri@umz.ac.ir

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Abstract

The purpose of this study was to determine the therapeutic efficacy of citric and tartaric acids on experimental dermatophytosis in guinea pigs. In this study, the susceptibility of *Trichophyton mentagrophytes* var. *mentagrophytes* to citric and tartaric acids was assessed using the macrodilution broth method. In the animal experiments, a cream consisting of the mixed acids was topically applied once per day for ten consecutive days, beginning five days following infection. Animals in a positive control group were treated with 1% clotrimazole cream. The results of this study showed that application of the mixed acid cream led to a significant improvement in guinea pigs suffering from dermatophytosis ($P < 0.05$). A similar improvement in local symptoms was achieved in the animals treated with 1% clotrimazole cream. These results indicate that organic acids such as citric and tartaric acids can potentially act as topical antifungal agents and may represent a valuable new approach in dermatophytosis.

Introduction

Dermatophytes are pathogenic fungi that have a high affinity for keratinized structures, such as nails, skin and hair, and can cause superficial infections known as dermatophytosis in both humans and animals (yahyaraeyat *et al*., 2009). These fungi affect millions of individuals annually and have prompted a serious public health concern, due to prolonged treatment of the disease and its refractivity to therapy (Havlickova *et al*., 2008). Dermatophytosis is a nonfatal infection but it is difficult to eradicate, often necessitating long-term treatment. Although antifungal agents such as clotrimazole, griseofulvin and, more recently, allylamines and triazoles have been used to treat dermatophytosis, resistance to the various drugs is frequently observed (Martinez-Rossi *et al*., 2008). This is mainly because different tineas (produced by diverse etiological agents) tend to be empirically treated with the same drugs (Weitzman and Summerbell, 1995). As a consequence, the dermatomycoses tend to persist, greatly diminishing the quality of life of the person suffering from the infection (Seebacher, 2003). Since one of the strategies to combat antifungal resistance is treatment with the appropriate antifungal agent when the etiological agent is known, new antifungal agents that selectively inhibit a single fungal species are urgently required (Mock *et al*., 1998). In the search for powerful antimicrobial agents, the biological activity of organic acids has been previously investigated. Several studies reported the antimicrobial effect of organic acids, such as formic and propionic acids (Cherrington *et al*., 1990), lactic acid (Dibner and Buttin, 2002; Mc William and Stewart, 2002), medium-chain fatty acids (Marounek *et al*., 2003), Benzoic acid (Flores *et al*., 2008) and citric and acetic acids (Lee *et al*., 2002). Despite the antibacterial activity of organic acids, little information has been found about the inhibitory effect of these acids against fungal pathogens (Aboellil *et al*., 2010). In a study conducted by Shokri (2011), citric acid demonstrated more fungistatic and fungicidal activities than those of tartaric acid against *Trichophyton mentagrophytes*, *Candida albicans*, *Aspergillus fumigatus* and *Malassezia furfur*. As an alternative to antifungal therapy, citric and tartaric acids have been suggested for local treatment of dermatophyte infection. In this context, the main objective of the present study was to determine the therapeutic efficacy of citric and tartaric acids on guinea pigs with dermatophytosis.

Materials and Methods

In vitro antifungal activity

Trichophyton mentagrophytes var. *mentagrophytes* was isolated from cattle with dermatophytosis and stored at - 80°C in the Mycology Research Center, Faculty of Veterinary Medicine, Tehran, Iran. The test compounds including citric and tartaric acids were obtained as reagent-grade powders

from their respective manufacturer (Merck Co., Darmstadt, Germany). In vitro fungistatic and fungicidal concentrations were determined using the broth macrodilution technique, following the procedure outlined by Shokri (2011). Serial dilutions of citric and tartaric acids were prepared from stock solutions ranging from 50% to 0.75%. Each tube was inoculated with 10 µl of the fungal suspensions (2.5×10^6 cell/ml), incubated at 30°C for seven days and monitored for visible growth following gentle vortexing of the tubes. The minimum inhibitory concentrations (MICs) were determined by inspection of the dose-response curves and defined as the lowest concentration of the test compound that reduced growth below 100% of that in the control samples.

Animal infection

The guinea pigs were randomly divided into three groups, each comprising five animals; a treatment group, and positive and negative control groups. Infection was established following the procedure of Odds *et al*. (2004). Each animal's back was shaved using electric clippers and an approximately 2.5 cm^2 area was marked. The following day, the marked site was lightly abraded with sandpaper and infected with 50 µl of the inoculum (10^6 cells). The suspension was gently rubbed into the skin with a sterile cotton-tipped swab until the fluid was no longer visible. The infection was initiated under an occlusive dressing and then this area was covered with a sterile adhesive bandage and held in place with extra adherent tape. The bandage was held in place for five days prior to antifungal treatment. The control animals were divided into two groups; one group received treatment with 1% clotrimazole cream and the other received no treatment.

Drugs and Treatment

A mixed acid cream was prepared in our laboratory using citric acid (0.18 g/g), tartaric acid (0.18 g/g), eucerin (3.6 g/g), stearic acid (0.18 g/g) and malic acid (0.09 g/g, as a carrier). Clotrimazole cream (1%) was purchased from Daru Pakhsh Co., Tehran, Iran. The creams were applied uniformly on the entire infected site using sterilized spatulas and administered in 0.5 g doses once per day for ten consecutive days. Experimental dermatophytosis initiated in the dorsal skin of guinea pigs causes Kerion Celsi-like lesions at advanced stages of infection. In the present study, topical treatment of the drugs was started on day five following infection, when the lesions were barely detectable. Culture studies were done to assess the efficacy of treatment.

Evaluation of infection

Culture studies were performed to assess the efficacy of each treatment. Two days following the final treatment, all animals were sacrificed under ether anesthesia and ten skin sections were obtained from each treated site. Each section was implanted onto a Sabouraud glucose agar plate containing chloramphenicol and cyclohexamide (Merck Co., Darmstadt, Germany), and the plates were incubated at 30°C for ten days. The treatment was assessed as effective if no fungal growth was detected following incubation.

Statistical Analysis

Student's t-test was used to assess statistical differences between the treatment groups. Probabilities of 5% were judged to be statistically significant.

Results and Discussion

In recent years, the proliferation of new classes of drugs, such as allylamines (e.g terbinafine) and active azoles (e.g. itraconazole and clotrimazole), represents a noteworthy trend in dermatophytosis therapy (Weitzman and Summerbell, 1995). However, treatment with both azoles and terbinafine for prolonged periods of time requires regular laboratory monitoring of liver function (Zapata Garrido *et al*., 2003). Moreover, these antifungal agents may undergo interactions with other medications (Huang *et al*., 2004). For many years, Griseofulvin was the only available antifungal treatment for dermatophytosis, and is still the preferred drug for treatment. However, concerns have been raised with respect to its resistance and toxicity (Huang *et al*., 2004). Totally, a greater number of cured cases of dermatophytosis, reduced adverse effects, a decrease in drug interaction and the lower cost of the antifungal topical agents, such as azoles, make them a favorable choice for management of superficial fungal infections, including dermatophytosis. However, the spread of drug resistant pathogens is one of the most serious threats to the successful treatment of dermatophytic infections. In this context, new antifungals derived from organic acids have received considerable attention as potential alternatives to existing drugs for the treatment of dermatophytosis, where a topical therapy is required. Citric and tartaric acids have been proposed for the local treatment of dermatophytic infections due to *T. mentagrophytes*, the main fungal agent in animal's dermatophytosis. *In vitro* results have shown that both acids individually (MIC value of 5%) and in combination (MIC value of 5%) exhibit antifungal activities towards different pathogenic fungi, especially *T. mentagrophytes* var. *mentagrophytes* (Shokri, 2011). Specifically, aqueous solutions containing at least 2.5% citric acid and 2.5% tartaric acid exerted a fungicidal effect against *T. mentagrophytes.* Previous studies have reported the germination and outgrowth of microbial spores, as well as the inhibition of growth of both bacterial and fungal

cells, caused by a variety of organic acids (Sofos and Busta, 1981). Benzoic acid has been utilized as the most common preservative agent against pathogenic microorganisms (Brul and Coote, 1999). Many mono- and dicarboxylic acids and hydroxylated analogs with a carbon chain, such as formic, acetic, propionic, butyric, lactic, malic, tartaric and citric acids, exhibit strong antimicrobial activities (Dibner, 2003). The antifungal activity of these acids has been attributed to an ability to penetrate the microbial cell wall. Inside the cell, the acid molecules then dissociate, reducing the pH below the target value. As a consequence, the cell wall tries to eliminate the protons (H^+ ions) released by the acids. This is an energy consuming process, which exhausts the fungal metabolism and results in the eventual death of the fungal cell. Since these early indications of antifungal activity of such acids, clinical studies have been required to confirm their effectiveness in the field. Therefore, a mixed preparation of acids was selected for local treatment of *T. mentagrophytes* infection in the present study. In our experiment, the rise of the first clinical changes was recorded on day 5 after the inoculation; the finding mentioned complies with the results published in literature (Uchida *et al*., 1991; Itoyama *et al*., 1997). Erythema had expanded over the entire infected sites in the infected animals, with a marked inflammatory response of the skin and intense scale formation. For therapeutic efficacy, once a day topical treatment with a 2.5% cream of mixed acids as well as 1% clotrimazole (the reference drug) cream was started on the fifth day postinfection and continued for 10 days. In animals treated with 2.5% mixed acid cream once per day following inoculation, no lesions developed. The results of the cultivation examination were negative in acid- treated mice on day 12 following infection and indicated no viable fungi. A similar improvement of local symptoms and negative mycological analysis were observed when animals were treated with 1% clotrimazole cream. Up to now, no study has been carried out on therapeutic efficacy of organic acids, in particular citric and tartaric acids, on animals with dermatophytosis. Several investigators reported that other organic materials have anti-dermatophytic activities, but their potencies in the treatment of dermatophytosis are different (Arika *et al*., 1990; Aguilar-Guadarrama *et al*., 2009). These discrepancies can be attributed to several factors that may influence the effectiveness of the drugs tested, including the presence of organic material, mixed infections of *T. mentagrophytes* with other fungi or bacteria, and the presence of fungi in biofilms (Haesebrouck *et al*., 2007). In comparison with the negative control group, the mixed acid treatment markedly reduced the local symptoms of swelling, inflammation and scale formation after ten days of treatment once per day. The results of this study indicate that the efficacy of mixed acids can be attributed to their fungicidal activity and a long period of cutaneous retention of the acid cream after topical application. Therefore, it is proposed that a once daily application of a mixed acid preparation holds significant potential for the treatment of dermatophytosis in animals.

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