Therapeutic analysis of organic acids on experimental dermatophytosis in guinea pigs

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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 refractoriness 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 × 10^5 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.

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
Therapeutic analysis of organic acids on... 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 monocarboxylic 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 the skin 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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References