Mycolase II: an enzyme antifungal agent interacts with the polyene antibiotic pimaricin in the treatment of keratomycosis

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Keywords: mycolase II, pimaricin, interaction keratomycosis, rabbits, candida albicans

Abstract

An animal model of keratomycosis was used to study the interaction between the new enzyme antifungal compound mycolase II and the polyene antifungal antibiotic pimaricin.

Well established corneal infection caused by an ocular pathogenic candida albicans on New Zealand white male rabbits were treated with 3% mycolase II, 5% pimaricin and a combination of 3% mycolase II and 5% pimaricin respectively.

The rates of resolution of the corneal lesions for each group of eyes treated by the various drugs were determined and the results were analysed by computer using a two-way analysis of variance to determine the interaction or independence of 3% mycolase II in combination therapy with 5% pimaricin in rabbit keratomycosis. The analysis of variance showed a significant level of positive interaction after each period of treatment. (P <0.001).

Introduction

Mycolase II, is a mixture of enzymes of fungal origin containing various carbohydrases, including chitinase and laminarinase (3). 3% mycolase II has been shown to be effective therapeutically used as topical drops on well established candida keratitis in rabbits (9). Mycolase II, containing a chitinase, acts by selectively digesting the cellwall of fungi (3). Most true fungi have been shown to contain chitin in their cell wall (2).

Pimaricin (natamycin), on the other hand, is a polyene antifungal compound isolated from Streptomyces natalensis (13) (Fig. 1). It has a wide spectrum of antifungal activity and has been used for topical ocular therapy in oculomycosis as a 5% suspension in saline (7, 10). Pimaricin binds to the sterol which is present in the fungal plasma membrane, thereby interfering with its permeability (6). Pimaricin is of the same family as amphotericin B, but is less toxic systemically and topically and is a better alternative to the amphotericins.

The combination of mycolase II and pimaricin in the therapy of mycotic infections of the eye would seem to promise a potent weapon against the ocular
pathogenic fungi. The present study was undertaken in order to test the potential of this combination in the therapy of candida keratitis in rabbits.

Materials and methods

The method of corneal microtrephination and inoculation of the 100% corneal infective dose (C.I.D.100) of various ocular pathogenic fungi described by the present author was used (11). After corneal microtrephination under general anaesthesia, the two corneas of each rabbit were inoculated with an ocular pathogenic Candida albicans (ER 1646) at a dilution of 10\(^4\) yeasts/ml. This dilution was found to produce 100% corneal lesions 48 hours after inoculation in a different series of experiments (10, 11).

Mycolase II and pimaricin interaction

To study the interaction of 3% mycolase II in combination therapy with 5% pimaricin, a factorial experiment design was used (1). Fourteen New Zealand white (NZW) male rabbits weighing 2–3 kg were used. After corneal microtrephination and inoculation of the corneas with candida albicans (ER 1646) the eyes were left for 48 hours before treatment with various drugs was started. For treatment, the eyes were divided into four groups of seven, randomly allocated. The first group of eyes received no treatment at all (gpa), the second group received treatment with 3% mycolase II alone (gpb). The third group (gpc) received treatment with 5% pimaricin alone while the fourth group (gpd) received treatment with the drug combination; 3% mycolase II and 5% pimaricin concurrently. Each drug and drug combination was given hourly as drops for ten consecutive hours daily for a period of sixteen days.

Readings of the rates of corneal infection were taken at forty-eight hours after inoculation and at eight days, twelve days, fourteen days and sixteen days during treatment (11). The experiments were terminated after sixteen days of therapy. The results were analysed by computer using a two-way analysis of variance (8).

Mycology

Forty-eight hours after inoculation, swabs and scrapings were taken from sixteen eyes for mycological studies. Two weeks later, these were repeated for sixteen eyes again (10 treated, 6 untreated). Three weeks later, three of the treated eyes were enucleated for histopathological studies.

Results

Mycolase II and Pimaricin interaction

Table 1 shows the percentage rates of infection of corneal lesions of fourteen rabbits 48 hours after inoculation and at different periods of treatment with various combinations of 3% mycolase II and 5% pimaricin.

Analysis of variance showed a significant level of interaction after each period of treatment between the two drugs (p < 0.001). Because the effects of these two drugs were not additive, a second analysis of t-tests was carried out to determine their mode of interaction (Table 2). The result showed that there was a positive interaction between the effect of 3% mycolase II and the effect of 5% pimaricin when these two drugs were combined in therapy in this animal model of oculomycosis. The combined effect was somewhat less than the sum of each alone.

Mycology

Swabs and scrapings taken from sixteen eyes 48 hours after inoculation with candida albicans all grew the pathogen after 36 hours incubation in sabouraud’s agar. After two weeks of therapy, all the eyes which were receiving treatment (10 eyes) were negative for the fungus while the six eyes which received no treatment still grew the pathogen. Three eyes which were enucleated from the treated groups of rabbits and examined histopathologically showed some neovascularization and inflammatory cells in the cornea but revealed no yeasts or pseudohyphal forms.