AN EXPERIMENTAL STUDY ON INTRASTROMAL INJECTION OF AMPHOTERICIN B IN *FUSARIUM SOLANI* KERATITIS IN RABBITS

BY

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3. TABLE OF CONTENTS

4. LIST OF FIGURES

5. LIST OF TABLES

6. ABSTRAK

Objektif:

Untuk membuat perbandingan keberkesanan diantara suntikan intrastromal amphotericin B 0.0005% (5μg/ml) dan suntikan intrastromal amphotericin B 0.15% (1.5mg/ml) serta untuk memastikan suntikan intrastromal amphotericin B adalah selamat di dalam ulser mata *'Fusarium solani'* pada mata arnab.

Tatacara:

Ulser mata diaruhkan melalui prosedur inoculasi yang telah ditetapkan untuk *Fusarium solani* ke atas lapisan kornea yang telah dikikis pada 18 ekor arnab. Kumpulan A (n=6) diberi rawatan titisan ubat amphotericin B 0.15% (kawalan) selama 12 jam sehari. Manakala kumpulan B (n=6) diberi rawatan suntikan intrastromal amphotericin B 0.15% dan kumpulan C (n=6) diberi rawatan suntikan intrastromal 0.0005% pada hari ke 3, 6, 9 dan 11. Selepas rawatan, pemeriksaan bersiri dijalankan untuk melihat perubahan pada saiz ulser, ketebalan ulser, infiltrasi stromal, 'hypopyon', dan lesi satelit selama 14 hari. Pada hari ke-14, enukleasi dijalankan dan dihantar ke makmal untul pemeriksaan patologi.

Keputusan:

Kumpulan yang diberi rawatan dengan suntikan intrastromal amphotericin B 0.0005% didapati lebih efektif berbanding dengan kumpulan yang diberi rawatan suntikan intrastromal 0.15% ($p<0.001$). Pemeriksaan bersiri menunjukan perubahan ketara pada saiz ulser ($p=0.01$) dan lesi satelit (0.02). Hasil pemeriksaan patologi

menunjukkan tiada fungi, ketebalan ulser setakat anterior dua per tiga, inflamasi sederhana dan minimal granulasi pada kumpulan yang diberi rawatan suntikan intrastromal 0.0005% amphotericin B. Tiada tanda-tanda dikompensasi pada kornea, 'punctate keratopathy' yang teruk atau 'corneal melting' yang dilihat pada kumpulan yang diberi rawatan suntikan intrastromal 0.0005%.

Kesimpulan:

Suntikan intrastromal Amphotericin B 0.0005% (5μg/ml) adalah efektif dan selamat pada kornea untuk rawatan ulser mata '*Fusarium solani*'. Adalah dicadangkan penggunaannya kepada pesakit yang tidak berkesan dengan rawatan ubat titisan. Ianya juga boleh mengurangkan kos serta mengurangkan masa rawatan di hospital.

7. ABSTRACT

Objective:

To compare the effectiveness between intrastromal injection of amphotericin B 0.15% (1.5mg/ml) and intrastromal injection of amphotericin B 0.0005% (5μg/ml) and the safety of intrastromal injection Amphotericin B 0.0005% in *Fusarium solani* keratitis in rabbits.

Methodology:

Fungal keratitis was induced with a standardized inoculum of *Fusarium solani* placed on the debrided cornea into the right eye of 18 New Zealand white rabbits. Rabbits in Group A (n=6) were treated with topical 0.15% amphotericin B every hourly for 12 hours daily (control), while rabbits in Group B (n=6) were treated with intrastromal injection 0.15 % (1.5mg/ml) of amphotericin B and Group C rabbits (n=6) were treated with intrastromal injection 0.0005% (5μg/ml) of amphotericin B. The intrastromal injection was given at day 3, 6, 9 and 11. Serial clinical examination was conducted at day 3, 6, 9, 11 and 14 to look at the changes in the size of the epithelial defect, the depth of the ulcer, stromal infiltration, hypopyon and the presence of satellite lesion. The infected eyes were enucleated on the day 14 and sent for histopathology evaluation.

Results:

The intrastromal injection of amphotericin B 0.0005% injection (group C) was found to be effective compared to intrastromal injection amphotericin B 0.15% ($p<0.001$) in treating *Fusarium* keratitis in rabbits. There was statistical significant difference in the size of epithelial defect ($p=0.02$) and satellite lesion ($p=0.02$) for the rabbits treated with intrastromal injection of amphotericin B 0.0005% compared to the intrastromal injection of amphotericin B 0.15%. Histopathological examination also revealed absence of fungal load, the depth of ulcer limited to anterior two third, moderate inflammatory responses and mild granulation tissue in the group treated with intrastromal injection amphotericin B 0.0005%. These histopathological findings were consistent with serial clinical observation. There was no evidence of corneal decompensation, severe punctate keratopathy and or corneal melting in the group treated with intrastromal injection amphotericin B 0.0005%.

Conclusion:

Intrastromal injection of amphotericin B 0.0005% (5μg/ml) was found to be effective and safe in treating *Fusarium solani* keratitis. Thus, after undergoing clinical trial it can be applied to the patient whose refractory to conventional therapy and can reduce the cost and shorten the hospital stay.

Chapter 1
Introduction

1.1 BACKGROUND

Fungal keratitis represents one of most difficult forms of microbial keratitis for ophthalmologist to diagnose and treat successfully. The key challenges include making the correct diagnosis based on the clinical characteristics of fungal keratitis, and the problems with the effectiveness of anti fungal drugs. The problems can be further complicated with late presentation associated with advanced infection, which may lead to poor vision and loss of vision.

Till now there is no precise guidelines for drug therapy have been established. There are several factors that were recognized to limit the efficacy of antifungal agents. One factor is the sensitivity of the infection to the specific antifungal therapy is not well established. Furthermore the penetration of antifungal agents into and through the cornea, bioavailability and delivery of the drug and toxicity of the antifungal agents are difficult to quantify.

In general, the antifungal agents are of low solubility and penetrate non-lipid layer poorly. The agents tend to be toxic at adequate therapeutic concentration lead to difficulty to determine the appropriate concentration. There is marked and noticeable variability between in vitro sensitivity patterns and in vivo efficacy. In addition, the pharmacokinetics, the effect of the immune response, and other drug interactions are not well understood.

The major group of antifungal agents are polyenes such as amphotericin B, natamycin, and nystatin; the azoles including clotrimazole, miconazole, ketoconazole and fluconazole. The current selection of antifungals is based on animal experiments, clinical experience, and published sensitivity data (O'Day et al, 1987). In vitro sensitivity testing of a particular isolate is rarely indicated. The data obtained from individual testing are difficult to interpret, and expensive to set up. In addition, by the time results are obtained, the clinical appearance of keratitis will determine whether it is responding to medical treatment or whether surgery is indicated. Recent study suggests that careful monitoring of infiltrate and antifungal testing may play a role in outcome of treatment (Vemuganti et al, 2002).

Although natamycin was found to be effective in treating *Fusarium solani* keratitis (Jones, et al, 1972), but there are recent concerns regarding natamycin resistance and inability to treat deep stromal keratitis. In addition, the cost is high. A 5 ml of natamycin cost around RM400, usually more that a bottle is needed to eradicate this highly virulent fungal infection, which brings the cost higher. For these reasons, topical amphotericin B remains a potent agent in treating *Fusarium* keratitis. It has wider spectrum of activity. Furthermore, topical Amphotericin B 0.15%, which is inexpensive, readily available and is sufficient to treat fungal keratitis without ocular toxicity from higher concentration (Wood et al, 1976).

In general, the clinical efficacy of an antifungal agent depends to a great extent on the concentration achieved in the target ocular tissue. It is depends on the molecular mass, concentration of the drugs, route of administration, the duration of contact with the target ocular tissue, and the ability of the compound to penetrate the eye (Thomas et al, 2003). Perhaps the intrastromal 0.0005% (5μg) injection of amphotericin B will improve the corneal concentration and increase the effectiveness in eradication of deep fungal ulcer.

1.2 FUNGAL KERATITIS

Corneal diseases are the major cause of vision loss and blindness, after cataract in overall importance (Whitcher et al, 2001). The World Health Organisation (WHO) has estimated that ocular trauma and corneal ulceration result in 1.5 to 2 million new cases of corneal blindness annually (Whitcher et al, 2001). In the warm climate developing agriculture-based countries; the commonest form of diseases affecting the cornea is fungal keratitis.

Fungal keratitis is an inflammation of cornea caused by fungal which is characterized by elevated areas, hyphae (branching) ulcers, irregular feathery margins, a dry rough texture, and satellite lesion. According to fungal morphology, fungal can be divided into four groups; nonpigmented filamentary fungi including *Fusarium spp* and *Aspergillus spp*; pigmented filamentary fungi including *Curvularia spp* and *Lasiodiplodia spp*; yeasts such as *Candida spp.* and filamentous non septated such as *Rhizopus* (mucormycosis).

The incidence of fungal keratitis varies geographically, but commonly occurs in warm, tropical climates. It is the major blinding eye disease in Asia (Khairallah et al, 1992, Srinivasan et al, 1991, Upadhyay et al, 1991) and leading cause of ocular morbidity. In two series of suppurative keratitis in North India, between 17 to 36% were of fungal etiology (Dunlop et al, 1994, Upadhyay et al, 1991). Similar finding was also found in similar studies conducted in Nepal (17%), Bangladesh (36%), Ghana (37%) (Upadhyay et al, 1991; Dunlop et al, 1994).

In a larger series from South Florida, fungal keratitis accounted for 20% of suspected microbial keratitis (Liesegang et al, 1980). In tropical regions, the most common organisms responsible for fungal keratitis are *Fusarium* and *Aspergillus* species (Leck et al, 2002). While, in temperate regions, where fungal keratitis is less common, yeast was isolated in 32%-72% of cases (Leck et al, 2002; Ritterband et al, 2006) and the incidence of fungal keratitis remains low (Coster et al, 1981; Asbell et al, 1982).

Fusarium species are most important etiology agent of fungal keratitis in South Florida; however in Asian series the predominant organisms seem to be *Aspergillus* species, reported in 35 to 47% (Khairallah et al, 1992, Srinivasan et al, 1991; Upadhyay et al, 1991).