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Therapeutic Efficacy of Moxifloxacin and Amphotericin B Combination in Experimental Candida Endophthalmitis

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Abstract: Fungal endophthalmitis is a rare but sight-threatening disease. Despite an expanding range of fungal pathogens, there are only few therapeutic agents for its treatment available. The current study compared *in vivo* efficacy of intravitreal injection of Moxifloxacin, liposomal amphotericin B (Amp-B) and Voriconazole monotherapies and combination treatment against Candida albicans in an exogenous endophthalmitis model in rabbit eyes Endophthalmitis was induced by intravitreal injection of *C. albicans* in rabbits` eyes with simultaneous intravitreal drug injection. Rabbits were divided into 6 prophylactic treatment groups, each group with 9 rabbits. Group 1 (control group) received sterile balanced salt solution, group 2 (Mox group) moxifloxacin, group 3 (Amp-B group) liposomal Amp-B, group 4 (Vor group) Voriconazole. Group 5 combined (Mox+ Amp) moxifloxacin and liposomal Amp-B and group 6 combined (Mox+Vor) moxifloxacin and Voriconazole. Clinical scoring was performed by assessing the cornea, conjunctiva, iris and vitreous after 48 hours of therapy. Quantitative analysis of microorganisms, were performed. Therapy groups were compared according to the clinical and microbiological analysis scores. The lowest *C. albicans* growth count was found in the combined groups; (Mox+Amp) group and (Mox+Vor) group followed by (Amp-B group) then (Vor group) and highest count was found in (Mox group) and (control group).Total clinical scores were significantly different between treatment groups and the control group ($p < 0.001$). Clinical scores of the (Mox+Amp), (Mox+Vor), (Amp-B) and (Vor) groups were found to be significantly lower when compared with the control group and Mox groups. The inhibitory activity of Amp-B and that of Vor was slightly enhanced when combined with Mox as shown in clinical score and colony count. Conclusion: The knowledge of the pharmacodynamic interactions between fluoroquinolones and antifungal agents mjght guide selection of drugs for patients with concurrent bacterial and fungal infections.

Key words: Amphotericin B · Moxifloxacin · Candida Endophthalmitis · Voriconazole · Rabbit

INTRODUCTION

Invasive Candida infections contribute to significant morbidity and mortality in patients with healthcare-associated infections. They represent a major burden on the public health system and are challenging to diagnose and treat [1].

Candida endophthalmitis arises in two discrete ways: Exogenous form signifies that the infection was introduced into the eye from the "outside," either by trauma, eye surgery, or extension of corneal infection (keratitis). Fungi are directly inoculated into the aqueous and/or vitreous.

The endogenous form follows candidemia, with hematogenous seeding of the eye. Fungi usually first seed the highly vascular choroid, then infection typically progresses through the retina into the vitreous. The aqueous is sometimes involved as well [2].

Candida endophthalmitis is sight-threatening ocular infection that needs early diagnosis and effective antifungal treatment. Patients at risk of invasive fungal infections are also at risk for developing bacterial infections. Therefore, these patients often receive antifungal therapy concomitantly with antibacterial agents as the fluoroquinolones [3].

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Amphotericin B (Amp-B) has been the gold standard in antifungal chemotherapy for a long time. New antifungal drugs or drugs potentiating the activity of current antifungals may improve treatment outcomes. Newly available drugs include those in the echinocandin class, such as caspofungin, micafungin and anidulafungin, as well as the newer generation triazoles, such as voriconazole, posaconazole, isavuconazole, ravuconazole and albaconazole [3].Topoisomerase enzyme subtypes are targets for new antifungal drug research [4]. The bactericidal activity of fluoroquinolone antibiotics is based on inhibition of topoisomerase 2. Fourth-generation fluoroquinolones such as moxifloxacin inhibit topoisomerase 2 and topoisomerase 4 simultaneously [4]. Fluoroquinolones are broad-spectrum antibacterial agents that act on DNA gyrase (topoisomerase II) and topoisomerase IV, resulting in inhibition of DNA replication, recombination and transcription and ultimately bacterial death [5]. Although fluoroquinolones have no intrinsic antifungal activity, high levels of topoisomerase I and II have been reported in pathogenic fungi [6-8], offering a potential mechanism of interaction between fluoroquinolones and antifungal agents [9]. Reports indicate that many fluoroquinolones potentiate antifungal activity of current antifungal drugs such as Amp-B and fluconazole [10-13].

The aim of this study was to investigate *in vivo* susceptibilities of Candida spp. to moxifloxacin alone, in comparison with moxifloxacin and amphotericin B combination and moxifloxacin and Voriconazole combination.

MATERIALS AND METHODS

Amphotericin B: Liposomal Amphotericin B (Ben Venue Laboratories, Inc., (AmBisome; Astellas Pharma, Deerfield, Illinois) for Intravenous infusion, available as 50mg /vial was used. Aseptically 500ml of sterile water was added to lyophilized powder of amphotericin B vial to yield a preparation containing 1mg amphotericin B/10 ml to be used. i.e. 10 microgram Liposomal Amphotericin B / 0.1ml.

Moxifloxacin: Moxifloxacin hydrochloride solution (Avelox; Bayer AG, Leverkusen, Germany) for intravenous infusion, available as 400 mg/250 mL, was used.

Aseptically 150ml was added to the intravenous solutions of moxifloxacin to yield a preparation containing 1mg moxifloxacin/1ml to be used i.e 100 microgram moxifloxacin/0.1 ml.

Voriconazole: (Pfizer Pharmaceuticals) lyophilized powder vials were used each vial contains 200mg voriconazole. The powder was reconstituted aseptically by adding 19 ml sterile water to have total volume of 20 ml of voriconazole with concentration 10mg/ml i.e. 1mg/0.1 ml.

Balanced Salt Solution (BSS): (BSS) (Alcon Laboratories) is a sterile physiologically balanced salt solution of sodium chloride (NaCl).

Inocula: *Candida albicans* strain was cultivated in Sabouraud's dextrose agar (SDA) culture plate and then inoculated into normal saline solution. A suspension containing 1 million organisms per milliliter (10^6 CFU/mL) was prepared by making serial dilutions and testing for viability on SDA plates. The concentration of *C. albicans* was adjusted using McFarland's tube [3].

Rabbits: Endophthalmitis was induced by intravitreal injection of *C. albicans* in right eye of 54 New Zealand rabbits weighing 2–2.5 kg with simultaneous intravitreal drug injection. Rabbits were divided into 6 prophylactic treatment groups, each group with 9 rabbits. Group 1 (control group) received 0.1 mL of sterile (BSS) 0.9% NaCl, group 2 (Mox group) 100 µg moxefloxacin /0.1 mL, group 3 (Amp-B group) 10 µg liposomal Amp-B/0.1 mL, group 4 (Vor group) Voriconazole 50 µg/0.1 ml. Group 5 (combi group) (M+ A) both 100 µg moxefloxacin/0.1 mL and 10 µg liposomal Amp-B/0.1 ml intravitreally and group 6 (M+V) both 100 µg moxefloxacin/0.1 mL and Voriconazole 50 µg/0.1 ml.

Clinical scoring was performed by assessing the cornea, conjunctiva, anterior chamber, iris and vitreous after 48 hours of therapy. Quantitative analysis of microorganisms, were performed. Therapy groups were compared according to the clinical and microbiological analysis scores.

Pupils were dilated using 1% tropicamide and 2.5% phenylephrine hydrochloride. The rabbits were anaesthetized with intramuscular injection of ketamine hydrochloride (50 mg/kg) and xylasine hydrochloride (0.5 mg/kg). Eyelids and eye lashes were scrubbed with 10% (*v*/*v*) povidone iodine and an eye speculum was placed in position. The ocular surface was sterilized with 5% povidone iodine. Following topical anesthesia with 0.5% (*v*/*v*) proparacain, 0.1 mL of fungal suspension (10^5 CFU of *C. albicans*) was injected into the midvitreous via pars plana (2 mm posterior to limbus) of all rabbits using a tuberculin syringe. Next, 0.1 mL of aqueous humour was withdrawn through a limbal paracentesis using another tuberculin syringe to normalize intraocular

Table 1: Clinical infection grading scale

Score	Conjunctiva	Cornea	Iris	Vitreous
0	Normal	Clear	Normal	Clear
1	Mild edema	Focal edema	Mild Hyperemia	Vitreous haze
2	Edema, mild hyperemia, slight exudate	Diffuse edema	Marked Hyperemia	Fundus details not clear
3	Edema, mild hyperemia, heavy exudate	Opaque	Synechia, irregular pupil	No red reflex

pressure. 0.2 ml was withdrawn in case of the combi groups. Intravitreal injection of BSS or drugs according to treatment groups was performed just after paracentesis via pars plana. All eyes were examined for signs of inflammation at 48 h. by a masked observer. The conjunctiva, cornea, anterior chamber, iris and vitreous were examined and graded separately according to the severity of inflammation based on a score from 0 (no inflammation) to 3 (severe inflammation) as listed in (Table 1). The total clinical inflammation score for each eye was calculated by addition of these four scores. After the examination, vitreous aspirates of 0.1 mL were obtained with a 22-gauge syringe for microbiological analysis and inoculated on the surface of SDA plates and spread it by a sterile glass-spreader evenly and allowed to grow for 48 h at 37°C. Colonies identified as *C. albicans* were counted and recorded for each eye (colony counts).

Statistical Analysis: Data management and statistical analysis were done using SPSS vs.25. Numerical data was summarized as median and ranges. Comparison between different groups were done using Kruskal Wallis test. All P values were two sided. P value less than 0.05 was considered significant.

RESULTS

Results were available for the 54 rabbits. There were nine eyes in each of; the control group, the Moxifloxacin group, the Amp-B group, the Voriconazole group, the combined Amp-B Moxifloxacin group and combined Voriconazole moxefloxacin group.

Clinical Scores: The total clinical infection scores values are shown in (Table 2) and (Fig. 1). The difference in clinical scores between control group and other groups was statistically significant (Kruskal–Wallis test, p <0.001). The control group and Mox group had higher scores compared to the Amp-B, Voriconazole and combined groups; Amp with Mox and Vor with Mox (p < 0.03 for all comparison). There was no statistically significant difference between the control and Mox groups (p =1), between the Amp-B, Voriconazole and combined groups ($p=1$) and between the two combined group Amp-B with Mox and Vor with Mox (p=1). The tow combined groups (Amp + Mox) and (Vor + Mox) shoed clinically the lowest score.

Table 2:Total clinical score of the treatment groups

		Controls	Mox	Amp	Vor	Amp + Mox	Vor + Mox	P value
Total clinical score	Median	10	10	5	4	3	3	<0.001
	Range	6 - 12	7 - 12	1 - 6	1 – 6	1 - 7	2 - 5	
	Mean	9	10	4	4	3	3	

Mox= moxifloxacin, Amp= Amphotericin-B, Vor= Voriconazole

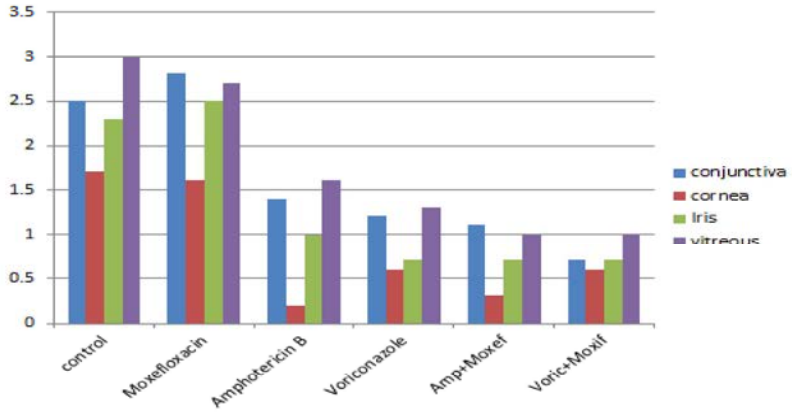

Fig. 1: Mean clinical infection scoring of the 6 groups of treatment

Table 3: The colony forming unit counts of vitreous aspirates

		Control	Mox	Amp	Vor	Amp+Mox	Vor+Mox	P value
(CFU) count	Median	412	235	35	40	14	29	<0.001
	Range	103 - 480	158 - 274	9 - 67	9 - 89	7 - 74	10 - 76	
	Mean	384	226	36	49	21	31	

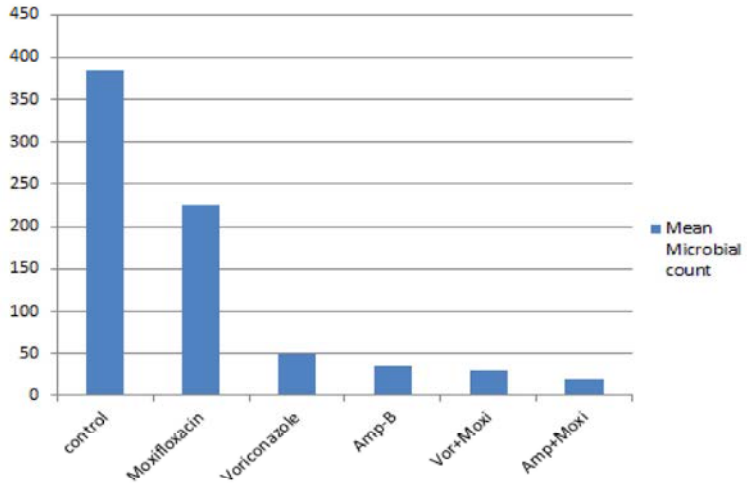

Fig. 2: Microbial Count of the 6 groups of treatment

Microbiological Findings (Colony Counts): The mean and (minimum-maximum, median), colony forming units (CFU) counts of vitreous aspirates was 384 (103-480, 412) in control group, 226(158-274, 235) in Moxifloxacin group, 36 (9-67, 35) in Amphotericin B group, 49(9-89, 40) in Voriconazole group, 21(7-74, 14) in Amp+Mox and 31(10-76, 29) in Vor + Mox group (Table 3).

The difference in colony counts among groups was statistically significant (P <0.001). The control and Mox group had higher numbers of colony counts compared with Amp-B, Vor. And combined groups; Amp with Mox and Vor with Mox (P<0.043 for all comparisons). There was no statistically significant difference between the control group and the Mox group, between Amp-B, Vor and the combined groups and between the two combined groups Amp-B with Moxifloxacin and voriconazole with Moxifloxacin (P=1 for all comparisons)

Combined groups of Amp-B with Mox and Vor with Mox had the least colony count followed by Amp-B group then Voriconazole group and the biggest count in Mox group and control group (Fig. 2).

DISCUSSION

Contradictory results such as synergy or indifferent effect have been reported about the interactions between quinolones and antifungal drugs in different studies [14].

It is obvious from the study design that intravitreal drug injections were used as a prophylactic treatment, as they are given simultaneously with the *C. albicans* inoculation. There were no much reported studies of the interactions between antifungal agents and other fluoroquinolones that are administered concomitantly with antifungal therapy [15].

In the present study no statistically significant difference between the control and Mox groups in terms of colony counts and clinical scores has been found. Thus, moxifloxacin when used alone showed statistically non-significant *in vivo* antifungal activity. However, the mean colony count in Mox group; 226 was less than control group; 384.

Quinolones with a cyclopropyl moiety at position 1 of the quinolone ring (ciprofloxcacin, clinafloxacin and moxifloxacin) were shown to have immunomodulating activity through effects on cytokines. They induce production of interleukin-2 (IL-2), IL-3, granulocyte macrophage colony-stimulating factor (GM-CSF) and interferon-gamma (IFN-γ) [16, 17] and inhibit production of tumor necrosis factor alpha (TNF-α) and IL-8 [18]. Prophylactic moxifloxacin treatment 4 days before the intratracheal challenge with *C. albicans* to cyclophosphamide-injected mice was shown to significantly reduce pneumonia, weight loss and mortality [18].

Yudum *et al.* [3] in a study of antifungal efficacies of moxifloxacin, with antifungal drugs in experimental Candida albicans endophthalmitis in rabbits has found a synergistic effect in the *in vitro* tests but failed to demonstrate increased *in vivo* efficacy of a combination therapy. On the contrary, Theodouli *et al*. [15] in a comparative *in vitro* study between ciprofloxacin, moxifloxacin and levofloxacin and antifungal agents, has found that, ciprofloxacin had stronger synergistic interactions with antifungal agents than levofloxacin and moxifloxacin did. Synergistic activities were found between levofloxacin or moxifloxacin with caspofungin against C. albicans, but antagonistic interactions were found between levofloxacin or moxifloxacin with fluconazole against C. albicans.

In our work, the difference between the Amp-B and control groups was statistically significant, proving *in vivo* efficacy of Amp-B. The difference between the Amp-B and (Amp +Mox) combined group for the same parameters was not statistically significant. However, Mean Colony count in (Amp +Mox) group 21 was less than 36 in Amp group. The same results were shown with Voriconazole; the difference between the Vor and control groups was statistically significant, proving *in vivo* efficacy of Vor. The difference between the Vor and (Vor + Mox) combined group for the same parameters was not statistically significant. However, Mean Colony count in (Vor + Mox) group 23 was less than 48 in Vor group.

It seems that the inhibitory activity of Amp-B and Voriconazole were slightly enhanced when combined with Mox this is the same as many reports that certain quinolones potentiate the activities of various antifungal agents *in vivo* [12, 14, 19, 20].

The mechanisms of these interactions are not well understood. Fluoroquinolones, by themselves, do not possess a significant antifungal growth inhibitory activity. However, fluoroquinolones have the capacity to bind to fungal topoisomerase [15]. Thus, fluoroquinolones may inhibit fungal DNA replication and thereby exhibit an antifungal effect. Since this effect is only apparent when fluoroquinolones are combined with antifungal agents, it might be possible that antifungal agents may alter fungal cell membrane permeability and thereby increase intracellular concentrations of fluoroquinolones. This could explain the synergistic interactions between antifungal agents and fluoroquinolones. Furthermore, fluoroquinolones may also enhance the activity of antifungal agents resulting in synergistic interactions by: (i) increasing intracellular levels of antifungal agents, as fluoroquinolones are effluxed via ATP-binding cassette (ABC) transporters [15, 21] (ii) co-operating with amphotericin B molecules in forming pores in the fungal membrane, [21, 22] as fluoroquinolones and amphotericin B are also amphoteric molecules [22] and (iii) increasing the penetration of antifungal agents or increasing the sensitivity of glucan synthase to echinocandins. These hypotheses warrant further study.

CONCLUSIONS

The present study demonstrated none statistically significant but still clinically positive *in vivo* interactions between fluoroquinolones (Moxifloxacin) and antifungal agents (Amp-B and Voriconazole) against C. albicans

The knowledge of the pharmacodynamic interactions between these agents is important. The choice of the best combination among the fluoroquinolones and an antifungal agent could potentially improve the outcome of a patient with concurrent bacterial and fungal infections.

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