Investigation of *in vitro* Antileishmanial Activity of Moxifloxacin, Linezolid and Caspofungin on *Leishmania tropica* Promastigotes

Leishmania tropica Promastigotları Üzerine Moksifloksasin, Linezolid ve Kaspofunginin *in vitro* Antileishmanial Etkisinin Araştırılması

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ABSTRACT

Objective: This study aimed to evaluate the potential *in vitro* anti-leishmanial activities of moxifloxacin, linezolid and caspofungin against *Leishmania tropica*.
Methods: *In vitro* effects of all agents were studied by using the microdilution method. For this purpose, serial dilutions of the aforementioned agents were prepared in concentrations between 4096 µg/mL-0.008 µg/mL. Afterwards, promastigotes incubated in suitable medium were counted with the hemocytometer and adjusted as having a last concentration of $2.5x10^6$ cells/mL in wells containing medium+antibiotic or antifungal. After incubation live promastigotes were counted with the hemocytometer and inhibitor concentrations (IC_{50}) were determined by comparing with the control that contained no antibiotics or antifungal.
Results: IC_{50} values of moxiloxacin, linezolid and caspofungin were found as 194.7 µg/mL, 896 µg/mL and 235.7 µg/mL, respectively.
Conclusion: As a result, moxifloxacin was found to be effective in lower concentrations than the other studied agents against *L. tropica* promastigotes. *(Turkiye Parazitol Derg 2013; 37: 1-3)*

Key Words: *Leishmania tropica*, antileishmanial activity, moxifloxacin, linezolid, caspofungin

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ÖZET

Amaç: Bu çalışmada, *Leishmania tropica* üzerine moksifloksasin ve linezolid ile kaspofunginin, potansiyel anti-leishmanial etkilerinin *in vitro* olarak araştırılması amaçlandı.
Yöntemler: Tüm ajanların *in vitro* etkisi mikrodilüsyon yöntemiyle araştırıldı. Bu amaçla moksifloksasin, linezolid ve kaspofunginin 4096 µg/mL-0.008 µg/mL arasındaki konsantrasyonlarda seri dilüsyonları yapıldı. Ardından uygun besiyerinde inkübe edilen promastigotlar hemositometre ile sayıldı ve besiyeri+antibiyotik veya antifungal içeren kuyucuklardaki son konsantrasyonları $2.5x10^6$ hücre/mL olacak şekilde ayarlandı. İnkübasyondan sonra canlı promastigotlar hemositometre ile sayıldı ve ajanların %50 inhibitör konsantrasyonları (IK_{50}) kontrollerle karşılaştırılarak belirlendi.
Bulgular: Moksifloksasin, linezolid ve kaspofunginin *in vitro* IK_{50} değerleri sırasıyla 194.7 µg/mL, 896 µg/mL ve 235.7 µg/mL olarak bulundu.
Sonuç: Moksifloksasinin, *L. tropica* promastigotlarına karşı çalışılan diğer ajanlara göre daha düşük konsantrasyonlarda etkili olduğu sonucuna varıldı. *(Turkiye Parazitol Derg 2013; 37: 1-3)*

Anahtar Sözcükler: *Leishmania tropica*, antileishmanial activite, moxifloxacin, linezolid, caspofungin

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INTRODUCTION

Leishmaniasis is an important tropical disease which influences 20 million people in 80 countries worldwide and 350 million people are at risk. *Cutaneus leishmaniasis* (CL) is being reported in many areas of our country, especially from Southeast Anatolia, Mediterranean and Aegean Regions of Turkey (1). Currently, the, first choice of treatment for the disease is still pentavalent antimony compounds. Recently, increase in the number of resistant cases for these compounds and inefficacy of the treatment in immunosuppressive individuals have been observed. It is determined that pump mediated multiple drug resistance has a part in resistance development (2). Alternative treatment options have been investigated, because current drugs have only limited effect on leishmaniasis and are toxic and expensive (3, 4).

Nowadays, effects of intracellularly active antibiotics and antifugal agents have been researched on *Leishmania* amastigotes and promastigotes (5, 6). Quinolones are synthetic antibacterial drugs and nalidixic acid is a prototype antibiotic of this class. It acts by inhibiting DNA topoisomerase type II (girase) or topoisomerase type IV, that are responsible for DNA replication, recombination and repair in bacteria. Moxifloxacin is a broad spectrum fluoroquinolone and is active against Gram-positive, Gram-negative and atypical pathogens. In addition,it can be taken once daily.

Linezolid is the first oxazolidinone derivation that is used clinically. It deteriorates the tRNA binding site by bonding the 50S ribosomal subunit and therefore formation of 70S initiation complex is prevented (7). Echinocandins are semisynthetic lipopeptide compounds which inhibit 1,3-β-glucan synthesis, an important component of thefungus cell wall. They show selective toxicity because mammalian cells do not include 1,3-β-glucan. The most known member of this group is caspofungin and others are also available (8).

In this study, we aimed to evaluate the potential in-vitro antileishmanial activities of moxifloxacin, linezolid and caspofungin against *Leishmania tropica* (MHOM/TR/10/CBU52).

METHODS

Parasite

In our study, *L. tropica* promastigotes (MHOM/TR/10/CBU52), isolated in Manisa were used.

Agents and Methods

In this study, *in vitro* effects of moxifloxacin (Bayer, Turkey), linezolid (Pfizer, Turkey) and caspofungin (Merck Sharp & Dohme, Turkey) were studied by using the microdilution method according to Clinical Laboratory Standards Institute (CLSI) recommendations (9). For this purpose, serial dilutions of mentioned agents were prepared in concentration between 4096 µg/mL-0.008 µg/mL. Afterwards, promastigotes that had been incubated in RPMI-1640 medium (Sigma), including 5% fetal-calf serum (FCS), were counted with the hemocytometer and adjusted as having a final concentration of $2.5x10^6$ cells/mL in wells containing 200 µL RPMI+5% FCS +antibiotic or antifungal. Microplates were incubated for 48 hours in 27°C. Live promastigotes were counted with the hemocytometer after 48 hours and inhibitor concentrations (IC_{50}) were determined by comparing with the control which does not contain antibiotics or antifungal. Amphotericin B (Sigma) (100 µg/mL- 0.0002 µg/mL), that is used for CL treatment, was used as control. The procedure was performed in triplicate and mean values of the results were calculated.

RESULTS

IC_{50} values of moxiloxacin, linezolid and caspofungin were found as 194.7 µg/mL, 896 µg/mL and 235.7 µg/mL, respectively. IC_{50} value of amphotericin B was detected as 0.026 µg/mL. IC_{50} values of studied agents and the number of live promastigotes are shown in Table 1.

DISCUSSION

Cutaneus leishmaniasis is common in many regions of the world, including our country. Today, the increasing number of patients with immune deficiency increases the incidence of opportunistic *Leishmania* infections. Use of pentavalent antimony compounds, that is the first choice of leishmaniasis treatment, was restricted due to several side effects and resistance development (10). Thus, new treatment options are being considered.

One of the groups among the alternative treatment choices is antifungal agents. Amphotericin B is the most commonly used one in this group and it was approved by Food and Drug Administration (FDA) for the treatment of visceral leishmaniasis. Efficacy of azoles such as ketoconazole, itraconazole and flucon-

Table 1. The *in vitro* effects of various agents on *L. tropica*

Agents	IC_{50} (µg/mL)	Concentrations (µg/mL)	Number of Promastigots ($x10^4$)
Moxifloxacin	194.7	4096	0
		2048	0
		512	1
		256	12
		128	25
		16	35
Linezolid	896	4096	6
		2048	14
		512	25
		256	30
		128	35
		16	35
Caspofungin	235.7	4096	0
		2048	0
		512	6
		256	16
		128	20
		16	30
Amphotericin B	0.26		
Growth control: $35x10^4$ cell/mL			

azole that inhibit ergosterol synthesis in fungus was tested on leishmania species (11). Caspofungin is a new antifungal agent and prevents fungus cell wall synthesis. A limited number of studies were made researching the efficacy of caspofungin against some species of protozoa. For example, it is found that caspofungin is effective in 250 mg/L concentration against *Acanthomoeba* species (12). In the present study, efficacy of caspofungin was investigated in concentrations between 4096-0.008 µg/mL on *L. tropica,* and the IC_{50} value was found as 235 µg/mL. Studies on *in vivo* efficacy of caspofungin in different *Leishmania* species will establish a better evaluation of possibility for using this agent in leishmaniasis treatment.

There are a limited number of studies evaluating the efficacy of linezolid on protozoa. In a study regarding to efficiency of linezolid on *Plasmodium falciparum*, protein synthesis inhibitor drugs such as doxycycline and azithromycin were used as controls and antimalarial effects of these antibiotics was attributed to being active against prokaryote organelles such as mitochondria and apicoplast. However, it was found that linezolid is not as efficient as others (13). In an immunodeficient patient wtih acute granulomatous *Acanthamoeba* encephalitis, combination therapy with linezolid, meropenem, moxifloxacin and fluconazole were found effective in survival (14). In the present study, the IC_{50} value of linezolid, studied in concentrations between 4096-0.008 µg/mL, was found as a very high value of 896 µg/mL. The low efficacy of linezolid against *Leishmania* was attributed to the different ribosome structure between parasites and bacteria.

Studies investigating the efficacy of fluoroquinolon antibiotics in treatment of clinical leishmaniasis, are available (5, 15). It was stated that DNA topoisomerase enzymes of trypanosomatide parasites (*Leishmania* spp. and *Trypanosoma* spp.) are potential targets in terms of selective inhibition. These enzymes have significant structural and biochemical differences compared to their homologues present in humans (5, 10). It was also found that topoisomerase II inhibitors are effective against *Trypanosoma cruzi* and *L. donovani* amastigotes (16). In our study, IC_{50} value of moxifloxacin was found 194.7 µg/mL and this is the lowest value among the studied agents. It was reported that some newly synthesized fluoroquinolone derivations are effective against *Toxoplasma gondii* and blood phases of *P. falciparum* (17). Also, it was detected that fluoroquinolones are efficient against *Leishmania* species in animal models and human macrophages cell lines (10, 15, 16). Van Der Vliet et al. (15) reported a suppurative *Pseudomonas aeruginosa* otochondritis along with CL ulceration and it is determined that ciprofloxacin is effective for treatment of this infection. Hence, fluoroquinolones can be used both for *Leishmania* infections and for secondary bacterial infections that may occur.

CONCLUSION

Moxifloxacin was found to be effective in lower concentrations than the other studied agents against *L. tropica* promastigotes and it was considered that it can be used as an alternative treatment agent. Evaluation of the in-vivo effects of linezolid, caspofungin and especially moxifloxacin is required for providing more detailed information.

Conflict of Interest

No conflict of interest was declared by the authors.

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