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Original Article

The Effect of Monomycin and Gentamycin Sulfate on Growth of *Promastigotes* of *Leishmania* Under In Vitro Conditions

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Abstract

Introduction: Given the prevalence of leishmaniasis in some parts of Iran and the medical problems caused by it and considering some reports on the effectiveness of monomycin in treating the disease, this study was performed to determine the effect of this drug on *Leishmania* major promastigotes in vitro.

Materials and Methods: The study was conducted experimentally. Drug films containing 15% monomycin sulfate and 0.5% gentamicin sulfate were prepared using ethyl cellulose and hydroxypropyl methyl cellulose (HPMC) with the aim of developing a treatment for cutaneous leishmaniasis. In order to investigate the mechanism of drug release and its effect on parasites, the modified Novy-MacNeal-Nicolle (NNN) medium was used for the amplification of the parasites and the growth inhibition zone around the drug release areas was measured. The studied environments included environments containing drug discs, environments without drug discs, and environments with placebo discs. The rates of dead and live parasites were determined in these environments and statistically compared using chi-square test.

Results: It was revealed that the growth of parasite colonies was inhibited in the plates containing drug films. In 3-cm halo around the drug film, no parasite growth was observed and promastigotes were observed to be dead and degenerated. However, in the plates containing placebo and medium without film, parasitic colonies were observed. The survival rate of the parasites in placebo and drug-free plates was 96% and it was 7% in the drug plate (P<0.000).

Conclusion: Monomycin and gentamicin sulfate drug discs have good potential for gradual release. They affect the growth of the parasite and can be used in humans.

Keywords: In vitro, Growth, Promastigotes of Leishmania, Monomycin, Gentamycin

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Introduction

Leishmania parasite has many pathogenic and non-pathogenic species. So far, more than 20 species of pathogens have been reported in humans (1,2). These species cause a wide range of clinical manifestations. The cutaneous form of leishmaniasis is the most common and it is estimated that 1.5 million new cases of the disease are caused by different species of *Leishmania* in different parts of the world every year (3,4). This type of disease does not cause death or disability in normal people (5). However, for a variety of reasons, including the length of the illness, the appearance of discomfort, and the development of secondary infections, it should be controlled (6). Today, the most important treatment used for various types of leishmaniasis is the use of pentavalent antimonials (7,8). Considering the resistance of some cases to this drug, lack of response to treatment, and various side effects of this drug, efforts are made to develop new forms of the drug that can heal the wound faster, have the fewest side effects, and do not leave a scar after healing (9,10). Monomycin and gentamicin are aminoglycosides that are an important class of antibiotics used in the treatment of bacterial infections. They inhibit protein synthesis in bacteria by binding to ribosomes and in addition to antibacterial effect, they have antiparasitic effect (11). In this regard, various studies have identified the effect of monomycin on promastigotes of *Leishmania* parasite in vitro and positive results have been obtained regarding

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the effect of the drug on the parasite in these conditions. Drug films containing 15% monomycin sulfate and 0.5% gentamicin sulfate were prepared and the mechanism of drug release, the duration of drug release from the film, and the biological effects of the drug on the parasite were investigated in vitro. Accordingly, the proliferation of promastigotes in plates containing culture medium was investigated.

Materials and Methods

An experimental design was used to conduct this study. The standard *Leishmania* major strain was used in this study. The parasite was transferred from the stock culture to the modified Novy-MacNeal-Nicolle (NNN) medium to grow (12,13). The most suitable stage of the parasite in the culture medium for keeping it alive and freezing is the logarithmic phase of the parasite, so a concentration of 1×10^6 promastigotes/mL was prepared. Then, 1% to 8% sterile glycerol was added. After mixing the above-mentioned materials, Eppendorf vials were divided into volumes of 2 mL and gradually frozen. The vials were first transferred to 4°C, stored at -20°C for 24 hours, and then transferred to a -96°C freezer. Each vial was taken out of the freezer, melted at 25°C for further experiments, and transferred to fresh media. Plates containing modified NNN medium were prepared for amplification and adaptation of Leishmania parasite. The cultured promastigotes were diluted with RPMI 1640 liquid medium after counting. Using a sampler, diluted promastigotes were poured into plates containing culture medium and spread as a smear on the plate surface. The plate was covered with parafilm and placed at 28°C. After 10 to 14 days, the plate surface was tested for the presence of parasite colonies. By confirming the ability of the parasite to multiply in such an environment in the next stage of the study, the parasite was moved from the active culture medium to the triple plates of the first group containing the drug disk of the second group of plates (14).

Plates containing placebo and the third group of normal plates without disc were kept in the same conditions to determine the growth and multiplication of the parasite. The surface of each plate after storage for specific time periods was examined using wet sampling method and direct microscopic observation and the status of the parasite in terms of growth and proliferation or death and degeneration was examined. Additionally, the rates of dead and live parasites were determined and statistically analyzed using chi-square test.

Results

Dead and degenerated promastigotes were observed in plates containing drug discs up to a radius of 3 cm and there was no evidence of parasite growth and proliferation. In slides prepared from the surface of the culture medium of plates containing placebo disc and plates without disc, the parasite multiplied according to previous observations. Therefore, it was possible to observe the amplified colonies of promastigotes on the plate surface. The distribution of parasites by plate type is presented in Table 1. It was revealed that out of every 100 promastigotes counted in plates containing placebo disc and drug-free discs, 96% of parasites were active and 4% of the parasites were dead. This condition was reversed on plates containing drug film and lower number of live parasites were observed and about 93% of promastigotes were found to be dead or degenerated. A significant difference was found between the plates containing the drug disc and the two plates containing the placebo disc and the control in the survival rate ($P<0.000$). However, no difference was found between the placebo and control groups. On the other hand, the survival rate of parasites in plates containing placebo discs and control plates was about 14 times higher compared to the drug group.

Discussion

Based on the results of this study, 93% of promastigotes cultured in plates containing drug discs are found to be dead and degenerated after one week. In the study conducted by El-On, *Leishmania* promastigotes were exposed to 100 µg/mL of paromomycin in RPMI 1640 medium. The medium containing the parasite and drug was kept at 28°C and only a small number of promastigotes were able to survive (15). In another study by the same researcher, *Leishmania* protozoa isolated from patients were cultured in a medium containing 100 µg/mL of paromomycin and it was observed that 85% to 99.5% of promastigotes were dead after one day of contact with the drug. Leishmaniasis is one of the most common parasitic diseases in the tropics. There is still no definitive treatment for this disease, and systemic treatment of leishmaniasis has its own complications and costs; therefore, this treatment should be considered in specific cases (16). Monomycin and gentamicin are aminoglycosides that are an important class of antibiotics used in the treatment of bacterial infections. They inhibit protein synthesis in bacteria by binding to the ribosome, and in addition to antibacterial effect, they have antiparasitic effect (17). In this study, in order to make the drug more effective, a new form of the drug was prepared and the release of the active substance from its base film was investigated. This

Table 1. Distribution of Dead and Alive *Leishmania* Parasites by Plate Type

Plate Type	Condition of the Parasite Life	
	Dead (%)	Alive (%)
Placebo	4	96
Drug-free	4	96
Drug	93	7

study also showed that the combination of drug-based compounds has good potential for very gradual drug release and the duration of release in this study is suitable for controlling the growth of *Leishmania* promastigotes in consecutive days. The film prepared in this study is a new form of the drug, the gradual release of which causes the parasite to be in constant contact with the drug, and as a result, it has a great effect on eradicating the parasite. Therefore, according to the preliminary results of this ongoing study on the animal model, this treatment system can be adapted to humans and it can be predicted that this treatment method can be considered as one of the easy treatment methods with the least complications. Therefore, it is suggested that this study be performed with different doses of the drug to determine the most effective dose against *Leishmania* major promastigotes and to design studies on animal models.

Conflict of Interests

The authors declare that they have no conflict of interest.

Ethical Issues

In this study, ethical considerations have been fully observed.

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Authors' Contribution

YG did writing and editing of the manuscript, and YF designed the study. BKA, AZ, and ES did data collection and statistical analysis.

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