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IN VIVO AND *IN VITRO* EFFECT OF CLARITHROMYCIN ON *TOXOPLASMA GONDII*

TOXOPLASMA GONDII ÜZERİNE KLARİTROMİSİNİN *IN VIVO* VE *IN VITRO* ETKİSİ

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SUMMARY

In recent years a number of studies have been carried out on the treatment of toxoplasmosis with semi-synthetic macrolides. The purpose of this study was to determine the in vivo and in vitro effect of clarithromycin, a semi synthetic macrolide, on Toxoplasma gondii. For in vivo study, different doses of clarithromycin were administered intraperitonally by gavage to mice infected with T. gondii. It was observed that the best result was achieved with the application of 300 mg/kg clarithromycin by gavage per day as compared with other doses (Mann Whitney U test, p< 0.05). Different doses of clarithromycin were added onto monolayer HEP-2 cells infected with T. gondii. Fifty mg/L clarithromycin was determined to be more effective that the other doses (Mann Whitney U test, p< 0.05). According to in vivo and in vitro trial results, clarithromycin was found to inhibit the growth of T. gondii.

ÖZET

Son yıllarda toksoplazmozun semi-sentetik makrolitlerle tedavisi ile igili bir çok çalışma yapılmıştır. Bu çalışmada, semi-sentetik bir makrolit olan klaritromisinin Toxoplasma gondii üzerine in vivo ve in vitro etkileri araştırılmıştır. In vivo olarak, klaritromisin farklı dozlarda intraperitoneal ve gavaj yolu ile T. gondii ile infekte farelere verilmiştir. Diğer dozlarla karşılaştırıldığında, en iyi sonucun gavaj yolu ile 300 mg/kg/G'lık uygulama ile elde edildiği saptanmıştır (Mann Whitney U test, p< 0.05). Klaritromisin farklı dozlarda T. gondii ile infekte monolayer HEP-2 hücrelerine eklenmiş ve 50 mg/L'lik dozun daha etkili olduğu belirlenmiştir (Mann Whitney U test, p< 0.05). In vivo ve in vitro deneme sonuçlarına göre, klaritromisinin T. gondii'nin üremesini inhibe ettiği saptanmıştır.

INTRODUCTION

In the standard treatment of toxoplasmosis, prymethamine and sulphadiazine are used together to produce a synergistic effect. However, adverse side effects such as skin lesions associated with sulphanamides and hematologic lesions associated with prymethamidine are often seen during this treatment. Therefore, studies are beingcarried out on alternetive drugs and drug combinations which might replace pyrimethamine-sulphadiazine in the treatment of toxoplasmosis (1, 2).

The bacteriostatic agents of the macrolide group affect microorganisms by reversibily connecting to 50S ribosoma subunits and inhibiting the protein synthesis (3). The most important and oldest known macrolide is erythromycin.

Clarithromycin (6-0 methylerythromycin) and azythromycin, on the other hand, are newly developed semi-synthetic erythromycin derivations. These two semi-synthetic macrolides are effective against not only bacteria but also protozoa such as *T. gondii*, *Cryptosporodium* spp. and *Plasmodium* spp. (4). In *in vitro* studies carried out, it has been asserted that clarithromycin does not produce a morphological change on *T. gondii*, but affects it by reducing the number of host cells infected with parasites and the number of parasites in these cells (1, 2. 5). The purpose of this study was to investigate whether there was any difference between the effects created by administering clarithromycin, a semi-synthetic macrolide, to mice intraperitoneally and by gavage, and whether the *in vitro* effect worked on the *T. gondii* located in HEP-2 cells.

MATERIALS AND METHODS

Toxoplasma gondii: Tachyzoites of the virulent TR-RH strain maintained through serial intraperitoneal (i.p.) passages were used. For experimental infections, tachyzoites were harvested from mouse peritoneal fluids 72 h postinfection and purified by centrifugation. The parasites were counted in a hemocytometer, and their numbers were adjusted to 2 x 10^6/ml with saline.

***In vivo* studies:** A total of 45 white laboratory mice, each weighing 20-25 g, were used. The mice were divided into two main groups. In the first main group, there were 5 subgroups of 4 mice each receiving clarithromycin (Abbott, 500 mg, flacon) intraperitonally. In the other main group, there were 5 different subgroups of 5 mice each receving clarithromycin by gavage. The mice were administered in doses of 100, 200, 300 and 600 mg/kg/day clarithromycin intraperitonally. The last group (controls) were given 1 ml of physiological solution. After 12 hours, each mouse was inoculated with 0.5 ml aliquots of 2x10^5/ml *T. gondii*. The mice were administered the same doses of 100, 200 and 300 mg/kg/day clarithromycin 5 times with 24 hour intervals. The group which received 600 mg/kg/day clarithromycin received only single dose. The control mice were given 1 ml of physiological solution 5 times with 24 hour intervals. The same procedure was also applied to the mice which received the drug by gavage. All the mice were observed until they died, and the times of death were recorded.

***In vitro* studies:** *In vitro* studies were carried out on 24 well tissue culture plate (2 cm^2/Gibco). To each well, 1 ml RPMI 1640 culture medium containing 10^4 HEP-2 cells, 10% FCS (fetal calf serum), 100 U/ml penicillin and 100 mg/ml streptomycin were added and then incubated in a incubator providing an environment with 5% CO_2 for 48 hours at 37° C. After observing that the cells developed a monolayer, each chamber was inoculated with 4x10^5 *T. gondii* trophozoite (6). Four hours after the inoculation, 0.2 ml physiological solution was placed in six wells. Then 1, 5, 10 and 50 mg/L clarithromycin dissolved in 0.2 ml physiological solution was added to the other wells (each dose to 6 wells). After the plates were incubated for 48 hours, the supernatant inside the chambers were collected into Eppendorf and the *T. gondii* trophozoites in each were counted.

Statistical analysis: The difference between clarithromycin doses were evaluated by means of One Way Anova, whereas the doses were compared with one another by Mann Whitney U test.

RESULTS

***In vivo* experiments:** Data related to administration of clarithromycin to mice intraperitonally are shown in Figure 1. No significant difference between the doses of clarithromycin was found (p>0.05). The fact that the mice which received inraperitoneal clarithromycin died before the control group was not significant.

Data related to the administration of clarithromycin to mice by gavage are shown in Figure 1. There is a significant difference between the clarithromycin doses administered and those applied in the control group (p<0.05). The most effective dose was 300 mg/kg/day (p<0.05).

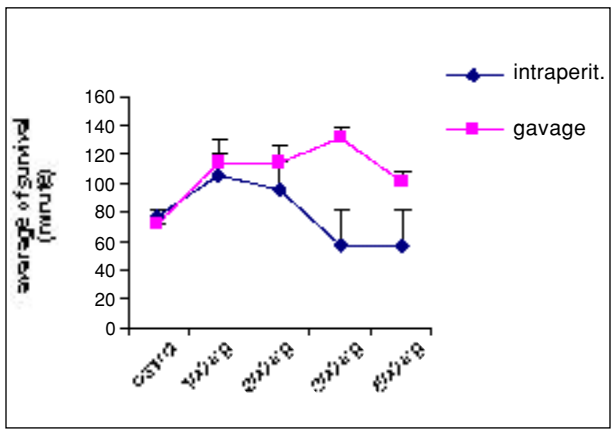

Figure 1. The effect of clarithromycin on *T. gondii in vivo*

***In vitro* experiments :** When different doses of clarithromycin were applied to *in vitro T. gondii* trophozoites, significant differences were determined between the doses (p<0.05). When the doses were compared with one another, 50 mg/L was found to be more effective than the other doses (p<0.05) (Figure 2).

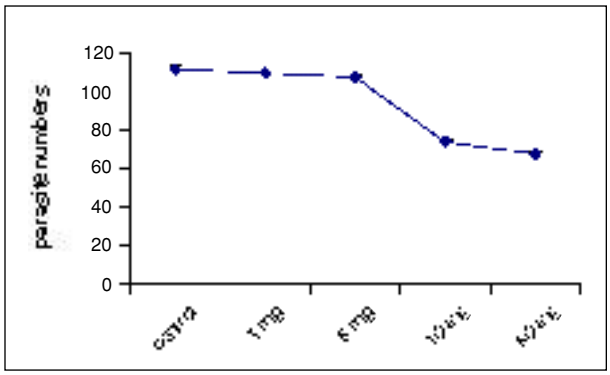

Figure 2. The effect of clarithromycin on *T. gondii in vitro*

DISCUSSION

Clarithromycin is administered intraperitonally, by gavage or subcutaneously to mice alone or in combination with other drugs in the treatment of acute and chronic experimental toxoplasmosis infections (7-9). In the *in vivo* section of this study, similar to the doses used in other studies (10), the mice infected with *T. gondii* were received intraperitoneally 100, 200, 300 and 600 mg/kg/day clarithromycin, and it was observed that these mice died before the control group which received physiological solution. When clarithromycin is administered perorally, the bioavailability is 55% since the drug is metabolized at a considerably high rate (3). Because clarithromycin was given intraperitoneally in this study, the bioavailability of the drug fully occurred. Thus, this application might have brought about the toxic side effects of the drug and drug toxicity which developed before toxoplasmosis killed all the mice.

In the treatment of toxoplasmosis developed in animal models, drugs are often administered by gavage (7, 9, 11). Chang et al. (9) administered clarithromycin (A-56268) to mice with toxoplasmosis by gavage, and investigated the effect of the drug under *in vivo* conditions. They applied 200 and 300 mg/kg/day clarithromycin as well as a single dose of 600 mg/kg/day to mice by gavage and reported that 300 mg/kg/day clarithromycin dose was effective ($p<0.01$) (9). The data obtained in the present study were compatiple with this data, 300 mg/kg/day being the most effective dose of statistical significance ($p<0.05$).

In their study where clarithromycin was applied *in vivo*, Derovin et al. (11) determined that there was no lengthening in the life span of the mice given 500 mg/kg/day of the drug by gavage whereas the life span of those given 200 mg/kg/day of the drug lengthened. In a similar study carried out with a virulent strain, Araujo et al. (7) reported a 20% increase in the life span of infected mice. Paralle to the data obtained in both studies in this study, the group given 200 mg/kg/day of clarithromycin had longer life span; however, 300 mg/kg/day was found to be the most effective dose.

In a study Khan et al. (12) carried out on mice, administration of 200 mg/kg/day of clarithromycin did not lengthen the life span of the animals. The authors reported that there was a significant difference only when the drug was used with trovafloxacin ($p>0.05$). In the present study however, a single 200 mg/kg/day dose of clarithromycin lengthened the life span of the mice ($p<0.05$)

Romand et al. (13) investigated the *in vivo* and *in vitro* effect of clarithromycin against *T. gondii*. In the *in vivo* section of the study, they reported a significant difference in the life span of the mice when 200 mg/kg/day clarithromycin by gavage was used ($p<0.01$). These data are in parallel to the results of the present study. It has been determined *in vitro* that inhibition occurred with a dose of 1 mg/L, the lowest dose used in the present study, but the best inhibition was obtained with 50 mg/L ($p<0.05$). In the *in vitro* section of a study done by Romand et al. (13) using the MRC-5 cell line, doses of 0.1, 0.5, 2 and 10 mg/L clarithromycin were used and maximum effect was obtained with the *in vitro* administration of high doses of clarithromycin. Similarly, Derouin et al. (5) reported in an *in vitro* study they carried out with the immunoenzymatic method using the MRC-5 cell chain that clarithromycin inhibited the reproduction of *T. gondii* even at concentrations as low as 0.05 mg/L, but asserted that maximum inhibition occurred at high concentrations such as 40 mg/L.

Chang et al. (8), who investigated *in vitro T. gondi* reproduction in rat periton macrophages, found the IC50S for clarithromycin as μM(=10.5 mg/L). In the presen study, however, it was observed that this dose led to an average 39% reduction in the number of parasites.

In this *in vivo* study where clarithromycin was applied intraperitonally and by gavage, it was observed that the intraperitoneal application was not appropriate and the gavage method should be preferred. Moreover, it is believed that the results obtained in the *in vitro* section of this study would contribute to the establisment of effective doses of clarithromycin for treatment of toxoplasmosis with success.

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