INFLUENCE OF EXPERIMENTALLY INDUCED TOXOPLASMOsis (TOXOPLASMA GONDII) ON THE PHARMACOKINETICS OF LEVOFLOXACIN

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The pharmacokinetics of levofloxacin was evaluated in Toxoplasma gondii infected and control mice. Single dose of levofloxacin (10 mg/kg b.w.) was administered orally to the T. gondii infected and control mice. The experimental infection was done by intraperitoneal administration of 200 µl of antigen solution (antigen solution concentrations were prepared under microscope at x40: 5, 10, and 20 trophozoites per microscopic field) per mouse from each concentration. Plasma concentrations of levofloxacin were determined by high performance liquid chromatography. Following the administration of levofloxacin, T. gondii infection resulted in significant reduction (p < 0.05) of the distribution half life (t₁/₂d) parameters and also in increase of the peak drug concentration (Cₘₚₚₚ) compared with in control group. Also the area under curve (AUC) and mean resistance time (MRT) values of levofloxacin in T. gondii infected group (4.72±1.03 and 6.63±0.71, respectively) were found smaller than in control group (6.12±0.72 and 8.46±0.27, respectively). The study results show that a clinician may have to suitably modify the dosage regimens of the levofloxacin while setting up therapy of bacterial infection or other disease.

Key words: mice, levofloxacin, T. gondii, pharmacokinetics.
Febrile conditions are known to markedly alter the pharmacokinetics of drugs in animals. These alterations in pharmacokinetic parameters during the course of a disease may influence the efficacy of antimicrobial therapy. To obtain the optimal efficacy of a drug, it is necessary to modify its dosage regimen on the basis of pharmacokinetic data of the drug obtained during the course of the disease (1, 7). Toxoplasmosis is an infection caused by a single-celled parasite called Toxoplasma gondii. The parasite is found throughout the world. Most infections by T. gondii are latent or asymptomatic, because the immune system usually hinders the parasite from causing illness. However, serious problems may occur in pregnant women. T. gondii infection occurs also in animals, and generally the clinical infection runs a similar course in most species. Toxoplasmosis is an important cause of abortions and stillbirths, particularly in sheep and sometimes in pigs and goats (2, 22). Therapy of toxoplasmosis in animals, unlike man, is seldom warranted. Current information on drug therapy is based on animal work, in vitro data on the treatment of T. gondii infections with clindamycin, macrolide antibiotics, sulphaizine and pyrimethamine (2, 5).

Levofoxacin is a fluoroquinolone antibiotic, an optical 5-(-) isomer of the racemic drug substance ofloxacin. It has a broad spectrum of in vitro activity against Gram-positive and Gram-negative bacteria, as well as certain other pathogens such as Mycoplasma, Chlamydia, Legionella, and Mycobacteria spp. (4, 8, 11, 12, 14, 15). It is a new quinoline antibacterial agent, approved for marketing by the US Food and Drug Administration in December 1996. Most of the available published data concerning levofloxacin were obtained during preclinical and clinical studies (7, 22).

However, there are no data on the pharmacokinetic profile of levofloxacin in a parasitic disease, and in our study we aimed to examine this subject. For this aim T. gondii infection was selected as a model. Besides, levofloxacin penetrates well into most body tissues and fluids including sputum, achieving concentrations that are generally higher than those in plasma (7, 9, 11, 22). The purpose of the present study is therefore to investigate the effect of T. gondii infection on plasma concentrations and pharmacokinetics of levofloxacin in mice.

Material and Methods

Animals. One hundred and twenty Swiss albino mice 30-35 g of b.w. were used in the study. They were assigned into two groups, consisting of 60 mice. Group 1 was used as control. Mice of the group were given only levofloxacin; they were not infected with T. gondii. Mice of group 2 were given levofloxacin orally and they were infected with T. gondii before drug application.

Infection. T. gondii trophozoites were injected intraperitoneally to 6 weeks aged Swiss albino mice; then, peritoneal fluid was collected from them after 48 h. Collected trophozoites were washed two times with sterile 0.9% saline solution by centrifugation at 1500 rpm for 5 min. Three antigen concentrations were prepared under microscope at x40: 5, 10, and 20 trophozoites per microscopic field. Minimum ten mice were injected intraperitoneally with 200 µl of the antigen per mouse from each concentration. After 48 h the growth control of T. gondii trophozoites from peritoneal fluids was tested (5).

Experimental design. Levofloxacin was provided in powdered form by Hoechst Marion Roussel. Blood samples (approximately 1 ml) were collected by
cardiac puncture into sterile glass test tubes with anticoagulant at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 18, and 24 h after levofloxacin administration. Five mice were used at each period. Plasma was obtained by centrifugation (1500 rpm for 15 min at room temperature) within 2 h from blood collection and stored at -20°C until analysis.

Plasma concentrations of levofloxacin were determined by high performance liquid chromatography (HP-1100 series, Agilent Chemstation Rev. A.08.03, Diodearray detector), as described by Gigosos et al. (21) Briefly, levofloxacin was extracted from plasma using methanol. The mobile phase was a mixture of buffer (pH: 3.5 orthophosphoric acid) and acetonitril (85/11, v/v). Ultraviolet absorbance measured at 280 nm was used for the detection, with the flow rate maintained at 1 ml/min. The recovery of levofloxacin was >90%. The limit of quantification of levofloxacin was 0.01 µg/ml.

**Pharmacokinetic analysis.** Plasma concentration time data were fitted to a 2-compartment open model with the first order absorption for kinetic analysis. Pharmacokinetic variables were calculated using a specific computer program based on equations described by Shumaker (PK CALC), and based on the equations described by Wagner (21). T<sub>max</sub> and C<sub>max</sub> values were determined by direct observation of the data.

**Statistical analysis.** Differences in pharmacokinetic data between T. gondii infected and control groups were analyzed for statistical significance by use of the student t-test. Differences of P < 0.05 were considered significant. All data in this study were tabulated as mean ± SEM.

**Results**

In the present study, we aimed to evaluate levofloxacin pharmacokinetics in control and T. gondii infected mice. The plasma-concentration-time profile and the pharmacokinetic parameters of levofloxacin after a single oral dose (10 mg/kg b.w.) is presented in Fig. 1 and Table 1. A statically significant (P < 0.05) difference between the control and T. gondii infected group was found for t<sub>1/2a</sub> only. The mean plasma drug concentration of >1.0 µg/ml was maintained for 24 h after drug administration. T. gondii infection resulted in significant reduction (P < 0.05) in the t<sub>1/2a</sub> parameter and also in increase in the peak drug concentration (C<sub>max</sub>) in T. gondii infected group compared with control group. Therefore the area under curve (AUC) and mean resistance time (MRT) values of levofloxacin in T. gondii infected group (4.72±1.03 and 6.63±0.71, respectively) were found smaller than in control group (6.12±0.72 and 8.46±0.27, respectively).
Fig. 1. Mean plasma concentration-time profile of levofloxacin control (●) and *T.gondii* infected (■) mice following oral administration of levofloxacin, 10 mg/kg b.w.

Table 1
Pharmacokinetic parameters of levofloxacin after oral administration of 10 mg/kg b.w. control and *T. gondii* infected mice

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Unit</th>
<th>Control group</th>
<th><em>T. gondii</em> infected group</th>
</tr>
</thead>
<tbody>
<tr>
<td>α</td>
<td>(h)</td>
<td>3.51±1.15</td>
<td>13.13±2.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2.64-5.28)</td>
<td>(10.98-15.75)</td>
</tr>
<tr>
<td>β</td>
<td>(h)</td>
<td>0.12±0.004</td>
<td>0.15±0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.12-0.13)</td>
<td>(0.14-0.18)</td>
</tr>
<tr>
<td>T₁/₂α</td>
<td>(h)</td>
<td>0.21±0.006 *</td>
<td>0.05±0.008 *</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.13-0.26)</td>
<td>(0.04-0.06)</td>
</tr>
<tr>
<td>T₁/₂β</td>
<td>(h)</td>
<td>5.65±0.14</td>
<td>4.54±0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5.45-5.86)</td>
<td>(3.77-5.14)</td>
</tr>
<tr>
<td>MRT</td>
<td>(h)</td>
<td>8.46±0.27</td>
<td>6.63±0.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(8.10-8.83)</td>
<td>(5.53-7.48)</td>
</tr>
<tr>
<td>AUC</td>
<td>(µg.h/ml)</td>
<td>6.12±0.72</td>
<td>4.72±1.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(5.30-7.27)</td>
<td>(3.42-6.23)</td>
</tr>
<tr>
<td>Tₘ₉ₖ</td>
<td>(h)</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.5-1.5)</td>
<td>(1.5-1.5)</td>
</tr>
<tr>
<td>Cₘ₉ₖ</td>
<td>(µg/ml)</td>
<td>1.290±0.24</td>
<td>1.610±0.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.120-1.750)</td>
<td>(1.050-2.010)</td>
</tr>
</tbody>
</table>

* P < 0.05 value with the same symbol are significantly different.

*T₁/₂α*-the distribution half life; *T₁/₂β*-the elimination half life; α-distribution rate constant; β-elimination rate constant; MRT-mean resistance time; AUC-area under the concentrations time curves; Tₘ₉ₖ-time to maximum concentration; Cₘ₉ₖ-maximum concentration.
Discussion

Clinical failures and the development of drug resistance during fluoroquinolone therapy have been recognized to occur most frequently after treatment of infections caused by organisms such as Staphylococcus aureus and Pseudomonas aeruginosa, which show only moderate susceptibility to currently available agents (6, 10, 12, 13, 20). Recent in vitro data specifically evaluating levofloxacin pharmacodynamics are consistent with published work concerning other fluoroquinolones. Published studies, which evaluated the pharmacokinetic profile of oral levofloxacin, have been conducted primarily in Japan and US (7, 22). These studies included healthy volunteers, volunteers with impaired renal function and patients with a variety of bacterial infections (2, 3). The pharmacokinetic profile of levofloxacin has been demonstrated to be very similar to that of the racemic mixture ofloxacin. The bioavailability of orally applied levofloxacin is similar to that of ofloxacin and approaches 100%. Levofloxacin is rapidly absorbed from the gastrointestinal tract with the time to maximum plasma concentrations ($t_{\text{max}}$) ranging from 0.8 to 2.4 h after administration of 50 to 100 mg of levofloxacin with or without food. Also in the present study we found that time to maximum plasma concentrations ($t_{\text{max}}$) in control and T. gondii infected groups was 1.5 h (7).

A linear, 2-compartment open model characterized by first-order elimination best describes the disposition of levofloxacin. Following the administration of single oral doses of levofloxacin 50-1000 mg to healthy volunteers, $C_{\text{max}}$ ranged from 0.6 to 9.4 mg/L and increased in a linear, dose-proportional fashion. The area under the plasma concentration-time curve (AUC) of levofloxacin ranging from 4.7 to 108 mg.h/L has also been demonstrated to increase in a linear, dose-proportional manner (2, 3).

Pharmacokinetic parameters of levofloxacin are variable with age, sex, race, renal and hepatic dysfunction, HIV infection, and some bacterial infections (17, 18). The pharmacokinetics of levofloxacin in patients with serious communicably-acquired infections (skin, respiratory tract or urinary tract) was examined in a clinical trial (17). In that study, results demonstrating that the pharmacokinetics of levofloxacin in patients with serious communicably-acquired infections are highly consistent with those observed in healthy individuals.

In another study (20) there were investigated the activities of levofloxacin against Mycobacterium tuberculosis both in vitro and in vivo, the pharmacokinetics of levofloxacin, and the effectiveness and safety of it in the treatment of pulmonary tuberculosis, with ofloxacin as control. There was no significant difference between the two formulations in the $T_{\text{max}}$, $T_{1/2}$, $C_{\text{max}}$, and AUC of ofloxacin and levofloxacin.

In the present study $C_{\text{max}}$ values were determined as 1.290±0.24, and 1.610±0.35 in control and T. gondii infected groups, respectively. Also AUC values were determined as 6.12±0.72 and 4.72±1.03 in control and T. gondii infected group, respectively. T. gondii infection induced marked alterations in the pharmacokinetics of levofloxacin as evidenced by a significantly lower value of $T_{1/2c}$ compared with control group. Also, MRT, AUC, and $T_{1/2}$ values decreased in T. gondii infected group, compared with control. Plasma drug concentrations in T. gondii infected group were found higher than control until 2 h and then the drug concentration rapidly decreased in T. gondii infected group compared with control group. This situation might be because of the faster absorption resulting from the T. gondii infection. T. gondii affects some
systems, tissues, and organs, especially immune system, eye, myocardium, pulmonary system, liver, spleen, and kidney.

The main aim of pharmacokinetic study is to compute suitable dosage regimens for use in clinical conditions (7, 22). In this study, the results showed that pharmacokinetic parameters of levofloxacin altered with *T. gondii* infection, especially $T_{1/2}$ value decreased significantly. Although AUC, MRT, and $T_{1/2}$ values were lower, $C_{\text{max}}$ values in *T. gondii* infected mice were higher compared with control mice. Finally, the clinician may have to suitably modify the dosage regimens of levofloxacin while setting up the therapy of infection.

References