Corneal Pharmacokinetics of Topical Clarithromycin

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Purpose. To evaluate the pharmacokinetics of topically applied clarithromycin, a new macrolide antibiotic, at various concentrations in a rabbit model.

Methods. Clarithromycin in dosages of 10, 20, and 40 mg/ml was administered topically every 2 hours for 48 hours to three groups of 16 New Zealand albino rabbits. Both corneas were treated. Right corneas were deepithelialized with n-heptanol. At 6, 12, 24, and 48 hours, tissue concentrations were determined in four animals from each group.

Results. Tissue drug concentrations increased with drug dosage and duration of therapy. Drug concentrations were significantly higher at 48 hours than at 6 hours, 12 hours, and 24 hours for both epithelialized and deepithelialized eyes in the 20 mg/ml and 40 mg/ml treatment groups (all \( p < 0.0015 \)). A steady state concentration was not achieved in any group. Tissue drug concentrations were higher in deepithelialized corneas for each dose after 6 hours, although differences were not significant (all \( p > 0.059 \)). Highest mean drug concentration at 48 hours was 241 \( \mu \)g/g in animals receiving 40 mg/ml of clarithromycin. After 6 hours, tissue concentrations in some groups were above MIC90 for many Chlamydia sp., Staphylococcus sp., and Streptococcus sp., and by 12 hours, concentrations were greater than MIC90 in all groups for many nontuberculous mycobacteria.


Clarithromycin, a new broad-spectrum macrolide antibiotic, has been used orally for treatment of acute maxillary sinusitis, pharyngitis, bronchitis, pneumonia, and skin infections. A topical preparation has been used experimentally to treat Mycobacterium fortuitum keratitis in an animal model.1 We examined the corneal pharmacokinetics and tissue concentrations of clarithromycin in rabbits after topical dosing to investigate the potential value of this agent for other corneal infections.

MATERIALS AND METHODS. Forty-eight disease-free New Zealand albino rabbits, divided randomly into three groups of 16 animals each, were used. The cornea of the right eye of each animal was de-epithelialized to determine whether an intact epithelium affects corneal drug levels. We instilled one drop of topical proparacaine onto the right eye of each animal. A 6-mm disk of filter paper soaked in n-heptanol was placed onto the anesthetized right eye for 10 seconds and removed. The cornea was then rinsed with balanced salt solution to remove residual n-heptanol. Fluorescein was used to verify the presence of an adequate epithelial defect of at least 4 mm at four time points: immediately after n-heptanol use, and at 12 hours, 24 hours, and 48 hours. The epithelial defects remained stable over the course of the experiment.

We used three dosages of clarithromycin: 10, 20, and 40 mg/ml. Purified clarithromycin in powder form was dissolved in 2 ml of methanol and then brought to a total volume of 50 ml using 0.01 M phosphate-buffered saline at pH 6.5 for each dose group. The final concentration of methanol was 4%. Animals in each group received the same concentration of drug in both eyes.

Each application of clarithromycin consisted of one drop (approximately 50 \( \mu \)l) placed directly onto the cornea. The antibiotic was placed every 2 hours for 48 hours. At 6, 12, 24, and 48 hours, corneas were harvested from four animals from each group. Animals were killed using pentobarbital (100 mg/kg), and a central corneal button was removed using a 10-mm trephine. Corneas were frozen immediately at \(-70^\circ\text{C}\).

To determine tissue concentrations of clarithromycin, a standardized bioassay method was performed.5 Each cornea was ground in 1 ml of phosphate-buffered saline for 1 minute, and this suspension was mixed with an equal amount of acetonitrile. After 1 hour, the samples were centrifuged, and 30 \( \mu \)l of the suspension was added to paper disks. The organism Micrococcus luteus, ATCC number 9341, was then plated onto antibiotic medium number 11. Disks containing clarithromycin samples were placed onto this culture and incubated at 30°C for 18 to 20 hours.
Zones of inhibition were measured using an agar diffusion assay read by a computerized Optomax (Boston, MA) image analyzer (series 4). Concentrations for each cornea were determined in triplicate, averaged for each cornea, and compared to a standard curve. All assays were performed on masked specimens.

To compare results to known MIC90 levels, the corneal drug concentrations were converted from units of microgram per gram of corneal tissue to microgram per milliliter of corneal tissue suspension. Conversions were calculated by the following formula:

\[
\text{tissue concentration (\mu g/g)} \times \text{individual corneal mass (g)} \div \text{extraction buffer volume (2 ml)} = \text{suspension concentration (\mu g/ml)}.
\]

Toxicity of the drug to the ocular surface was judged using the method developed by Draize, which grades the degree of conjunctival redness, chemosis, discharge, corneal opacification, and iris damage on a scale with a maximum of 110 points.

Statistical analysis was performed using one sample paired t-test assuming that the data were normally distributed. One-way analysis of variance was used to compare means across concentration groups and time intervals. The least squared difference method was used to make pairwise comparisons.

This study conforms to guidelines in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**RESULTS.** Tissue drug concentrations increased for each dose of drug over time in both epithelialized and deepithelialized eyes (Figs. 1, 2; Table 1). Tissue drug concentration at 48 hours was significantly higher than tissue concentrations at 6 hours, at 12 hours, and at 24 hours, only for deepithelialized eyes (P < 0.0083). A steady state concentration was not achieved in any group.

Clarithromycin concentrations were compared between right and left eyes within each group and were consistently higher in deepithelialized corneas in all groups after the 6-hour time point (Table 1). In group 1 (10 mg/ml) at 6 hours, however, the tissue drug concentrations in the epithelialized eyes were significantly higher than in the deepithelialized eyes (P = 0.007). No other comparison between epithelialized and deepithelialized eyes in any group reached a level of statistical significance (P > 0.059).

Tissue drug concentrations were compared between treatment groups at each time point separately for epithelialized and deep epithelialized eyes. At 6 hours, differences between group 1 (10 mg/ml) and group 3 (40 mg/ml) were significant for the deep epithelialized eyes (P = 0.0122) but not for the epithelialized eyes (P = 0.1325). At 48 hours, differences between group 1 (10 mg/ml) and group 3 (40 mg/ml) were also significant for the deep epithelialized eyes (P = 0.0122) but not for the epithelialized eyes (P = 0.0908). Comparisons between groups 1 and 2 and between groups 2 and 3 were not significantly different for either epithelialized or deep epithelialized eyes at other time points.

After 6 hours, drug levels in group 2 (20 mg/ml) and group 3 (40 mg/ml) were above MIC90 for most Chlamydia sp. (≤0.125 \mu g/ml), Staphylococcus sp. (0.125 \mu g/ml), and most Streptococcus sp. (0.015 to 0.25 \mu g/ml); and by 12 hours, levels in all groups...
TABLE 1. Clarithromycin Concentration in Deepithelialized Versus Epithelialized Corneas

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/ml)</th>
<th>Time After First Dose (hours)</th>
<th>Drug Concentration* (µg/g)†</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>Deepithelialized Eyes</td>
<td>Epithelialized Eyes</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
<td></td>
<td>1.35 (±0.40)</td>
<td>9.31 (±0.86)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td></td>
<td>29.59 (±13.31)</td>
<td>26.71 (±8.96)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td></td>
<td>47.27 (±17.88)</td>
<td>40.49 (±11.60)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td></td>
<td>128.13 (±28.61)</td>
<td>47.03 (±8.05)</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td></td>
<td>3.72 (±0.89)</td>
<td>8.29 (±1.51)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td></td>
<td>22.68 (±6.82)</td>
<td>21.25 (±2.51)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td></td>
<td>64.71 (±37.98)</td>
<td>27.43 (±10.16)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td></td>
<td>160.87 (±24.10)</td>
<td>143.61 (±24.39)</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td></td>
<td>8.04 (±2.56)</td>
<td>13.61 (±2.67)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td></td>
<td>35.89 (±7.36)</td>
<td>25.22 (±6.49)</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td></td>
<td>23.42 (±10.86)</td>
<td>24.56 (±2.02)</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td></td>
<td>241.42 (±23.91)</td>
<td>110.33 (±31.88)</td>
</tr>
</tbody>
</table>

*All values represent the mean of data from four corneas unless otherwise specified (± standard error of the mean).
†Microgram of drug divided by gram of corneal tissue.
‡Statistical analysis performed using one sample t-test to compare µg/g levels in epithelialized corneas with µg/g levels in deepithelialized corneas.
§Statistical analysis performed using one sample t-test to compare concentrations at 6 and 48 hours for each group.
‖Based on data from three corneas.
¶Based on data from two corneas.

were greater than MIC90 for many nontuberculous mycobacteria.6,7 All the groups had a 0/110 Draize score of the ocular surface in the epithelialized eye by 12 hours. At 24 hours, three of four animals in group 3 had a score of 6/110. The other groups remained at 0/110 for the duration of the experiment. The deepithelialized eyes in all groups had conjunctival hyperemia extending onto the palpebral conjunctiva with mild to moderate chemosis and mild mucus production, which was attributed to persistent epithelial defects. There was no evidence of stromal change in any of the animals.

DISCUSSION. Clarithromycin is a new macro-lide antibiotic with advantages over erythromycin, including two to four times the antimicrobial activity against Staphylococcus sp. and Strept. sp. (including Strept. pneumoniae) and activity against nontuberculous mycobacteria.6,7 Streptococcus and Staphylococcus spp. that are resistant to erythromycin tend to be resistant to clarithromycin as well.6 The pharmacokinetics of clarithromycin tend to be superior to erythromycin, with higher tissue concentrations and a longer elimination half-life (5 to 7 hours), thereby requiring less frequent oral dosing.4,5

The macrolide antibiotics exert their effects by binding to the 50S subunit of the 70S ribosome, thus preventing RNA-dependent protein synthesis. These drugs are considered bacteriostatic but may become bactericidal against certain organisms if present in sufficiently high concentrations.6 These drugs are more effective at an alkaline pH.5 The drug is basic and lipophilic with good tissue penetration and high volumes of distribution.

An oral formulation of clarithromycin was approved by the Food and Drug Administration in October 1991 for use against susceptible gram-positive organisms (Strept. pyogenes, Strept. pneumoniae), gram-negative organisms (Hemophilus influenzae, Moraxella catarrhalis), and nontuberculous mycobacteria, and it has been used clinically to treat acute maxillary sinusitis, pharyngitis, bronchitis, pneumonia, and skin infections. Laboratory studies in a rabbit model showed that topical clarithromycin may also be useful against Mycobacterium fortuitum keratitis.8

Clarithromycin is active against anaerobic organisms, gram-positive bacteria, C. trachomatis, Bordetella pertussis, Moraxella catarrhalis, Legionella pneumophila, and Neisseria gonorrhoea. The 14-OH metabolite of clarithromycin is active against H. influenza.5 This drug has a synergistic effect with existing regimens, including ethambutol and tetracycline, against M. avium and has been shown to enter leukocytes and macrophages readily.5,8 Intracellular pathogens such as Chlamydia sp., Legionella sp., and Mycobacterium sp. are thus susceptible to this agent. Clarithromycin is 10 times more

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active than azithromycin against mycobacteria, especially \textit{M. avium}, \textit{M. chelonae} (50 isolates had MIC $\leq 0.25$ \textmu g/ml), and \textit{M. fortuitum}.\textsuperscript{5,7}

Macrolides are rarely active against \textit{Pseudomonas} sp. or methicillin-resistant \textit{Staph. aureus} and \textit{Staph. epidermidis}.\textsuperscript{5} Recent studies, however, have shown an interaction between clarithromycin and the biofilms produced by \textit{Ps. aeruginosa}, thereby improving the penetration of other antibiotic agents.\textsuperscript{10}

Clarithromycin provides better coverage of \textit{Streptococcus} sp. than the aminoglycosides and may be more reliable against \textit{Streptococcus} sp. than the variable results found with topical ciprofloxacin. The prevalence of \textit{Streptococcus} sp. isolates that are resistant to ciprofloxacin has risen dramatically since this drug has become commercially available; in some studies, as many as 25\% of isolates are resistant (Hwang DG, unpublished data, 1993). The cephalosporins are commonly used alone or in combination with aminoglycosides for presumed bacterial keratitis, but they do not provide adequate coverage of \textit{Neisseria sp.}, \textit{Chlamydia sp.}, or \textit{Mycobacterium} sp. Clarithromycin could provide an alternative treatment for some cases of keratitis caused by these organisms.

Clarithromycin may be useful for many selected forms of keratitis based on sensitivity testing, but it is limited as a first-line, broad-spectrum agent because \textit{Ps. aeruginosa} is a common cause of bacterial keratitis.\textsuperscript{5} Its usefulness as a synergistic agent against the biofilms of \textit{Ps. aeruginosa} warrants further investigation. Treatment failures in \textit{M. chelonae} have been reported with ciprofloxacin, and clarithromycin may provide a viable therapeutic alternative.\textsuperscript{11} The ability of clarithromycin to penetrate tissues and achieve high concentrations may provide a bactericidal effect against certain organisms in contrast to the bacteriostatic effect of erythromycin. In rabbit corneas, we have shown good tissue penetration and MIC\textsubscript{90} levels in excess of those necessary to eradicate many ocular pathogens.

Tissue levels appeared to be higher in deep epithelialized eyes, but significant differences were not identified when epithelialized and deep epithelialized eyes were compared within each group at each time point. Comparison of treatment groups 1 (10 mg/ml) and 3 (40 mg/ml) revealed significant dosage differences in deep epithelialized eyes but not in epithelialized eyes at the 6- and 48-hour time points. Information about drug toxicity is incomplete because steady state drug levels were not achieved and the effect of higher drug concentration in the cornea could not be determined.

\textbf{Key Words}
clarithromycin, pharmacokinetics, keratitis, antimicrobial agent, cornea

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\textbf{References}