The Effect of Vehicle on Corneal Penetration of Triturated Ketoconazole and Itraconazole

James P. Guzek, MD; Joann M. Roosenberg; David L. Gano, MD; Isak F. Wessels, MMed, FRCSE, FRCOphth, FACS

BACKGROUND AND OBJECTIVES: Triturated (crushed and suspended) ketoconazole has been recommended for the treatment of fungal keratitis when commercial antifungal eyedrops are unobtainable. The authors evaluated the in vivo corneal stromal concentration with different vehicles in the eyes of adult rabbits.

MATERIALS AND METHODS: Ketoconazole and itraconazole tablets were triturated to 20 mg/ml in four vehicles: polyvinyl alcohol (PVA), boric acid, olive oil, and balanced salt solution (BSS). Six eyes (de-epithelialized for better penetration) received one drop every 15 minutes for 2 hours. A yeast overlay bioassay of extracts determined the stromal concentration.

RESULTS: Itraconazole in BSS, olive oil, PVA, and boric acid produced inhibition zones of 17.3, 15.6, 15.4, and 13.2 mm, respectively. Ketoconazole produced inhibition zones of 35.9, 39.4, 41.8, and 44.7 mm, respectively. From a standard curve, the concentrations of ketoconazole in tissue were 512, 773, 1221, and 1492 μg/g, respectively.

CONCLUSION: The vehicle that is used to triturate antifungals affects the tissue concentration. This may have an impact on fungal keratitis therapy.


INTRODUCTION

Ketoconazole has been recommended topically and systemically for treating fungal keratitis. No specific ocular formulation is available, and pure drug or tablets have to be processed to obtain a suitable topical application. Trituration is a process of grinding tablets to a fine powder that is then suspended in a suitable liquid vehicle. Recommended vehicles include an equal volume mixture of boric acid and hydroxypropylmethylcellulose, or polyethoxylated castor oil. The influence of the specific vehicle on drug penetration and ultimate tissue concentration has not been reported. Personal experience using saline (the only vehicle available in Sri Lanka) yielded poor results for fungal keratitis (JPG, unpublished data, 1986). We investigated the stromal penetration of ketoconazole and itraconazole suspended in four common vehicles in vivo.
TABLE
Mean Diameters of Inhibition Zones (mm) With Triturated Ketoconazole and Itraconazole in Four Different Vehicles*

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Range</th>
<th>Confidence Interval (95%)</th>
<th>P Value</th>
<th>Calculated Tissue Concentration (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoconazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BSS</td>
<td>35.9</td>
<td>2.27</td>
<td>31–38</td>
<td>33.5–38.3</td>
<td>512</td>
<td></td>
</tr>
<tr>
<td>Olive oil</td>
<td>39.4</td>
<td>1.78</td>
<td>37–42</td>
<td>37.5–41.3</td>
<td>.27</td>
<td>773</td>
</tr>
<tr>
<td>Boric acid</td>
<td>41.8</td>
<td>2.26</td>
<td>39–45</td>
<td>39.4–44.1</td>
<td>.012</td>
<td>1221</td>
</tr>
<tr>
<td>PVA</td>
<td>44.7</td>
<td>2.67</td>
<td>39–48</td>
<td>41.9–47.5</td>
<td>.002</td>
<td>1492</td>
</tr>
<tr>
<td>Itraconazole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boric acid</td>
<td>13.3</td>
<td>1.32</td>
<td>11–15</td>
<td>11.6–15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVA</td>
<td>15.4</td>
<td>1.6</td>
<td>13–17</td>
<td>13.3–17.4</td>
<td>.11</td>
<td></td>
</tr>
<tr>
<td>Olive oil</td>
<td>15.6</td>
<td>1.16</td>
<td>14–17</td>
<td>14.4–16.8</td>
<td>.031</td>
<td></td>
</tr>
<tr>
<td>BSS</td>
<td>17.3</td>
<td>1.29</td>
<td>16–20</td>
<td>15.9–18.6</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*BSS = balanced salt solution; PVA = polyvinyl alcohol.
*Each vehicle was tested on six eyes and the results were averaged. The calculated tissue concentration was derived from a standard curve.

MATERIALS AND METHODS

Commercially available ketoconazole and itraconazole tablets were ground in a porcelain mortar with a pestle until a fine powder was obtained. The powder was suspended to a concentration of 2% (20 mg/ml) in four different vehicles: balanced salt solution (BSS), olive oil, 2% boric acid solution, and 1.4% polyvinyl alcohol (PVA).

The Institutional Review Board of Loma Linda University approved the protocol for this project, which complied with the requirements for animal-based research of the Association for Research in Vision and Optics (ARVO). Three adult, albino New Zealand rabbits weighing 5 to 7 pounds (2 to 3 kg) were allocated to each of the four treatment groups and the control group. Both eyes of each animal were used (ie, six eyes per solvent). After intramuscular anesthetic with 50 mg/kg of ketamine (Parke-Davis, Morris Plains, NJ) and 10 mg/kg of xylazine (Phoenix Pharmaceuticals, St. Louis, MO) was administered, the corneal epithelium was removed with a sterile #15 Bard Parker (Becton-Dickinson Acute Care, Franklin Lakes, NJ) blade under an operating microscope. When the animals had recovered 2 hours later, 1 drop of suspension was applied every 15 minutes for 2 hours (total of 9 drops). The control group received BSS drops. One hour after the last drop, the rabbits were killed with an overdose of intravenous pentobarbital. The corneas were excised aseptically, rinsed with BSS, and weighed. After pulverization in liquid nitrogen, a water extract was assayed for ketoconazole concentration using a yeast overlay technique, with the diameters of the zones of inhibition read after 40 hours of incubation. This assay was performed in duplicate.

A standard curve to compare tissue concentrations of ketoconazole with the zone of inhibition was derived by rehydrating freeze-dried corneas for 2 hours in known concentrations of pure ketoconazole. Despite our best efforts, pure itraconazole could not be obtained to construct a dilution curve.

RESULTS

The means, standard deviations, ranges, and 95% confidence intervals are listed in the table. Ketoconazole achieved mean zones of inhibition that ranged from 35.9 to 44.7 mm; the smallest zones occurred with BSS and the largest zones occurred with
PVA. The linear regression of the standard curve was good ($R^2 = 0.9856$). The slope of the regression line produced the following conversion formula:

$$\log(\text{Corneal concentration}) = 0.0562 \text{ (inhibition zone diameter)} - 1.2027$$

The zones of inhibition thus represent a range of tissue concentrations of ketoconazole, from 512 µg/g with BSS to 1492 µg/g with PVA. This threefold increase in concentration is statistically highly significant with the Student's $t$ test ($P = .002$). Olive oil seemed to be no different from BSS ($P = .27$). A suspension in boric acid achieved almost the same concentration as one in PVA.

The results for itraconazole are also represented in the table. The zones of inhibition were only half as large as those with ketoconazole, which could reflect either poorer solubility or relative insensitivity of the yeast organism. The least effect was found with boric acid, followed by PVA and olive oil. BSS was the best vehicle. The differences of the means and standard deviations are less marked than they were with ketoconazole, and statistical significance was attained between only BSS and boric acid with the analysis of variance test ($P < .001$).

**DISCUSSION**

The epithelium was removed to simulate the recommendation for increasing stromal penetration when treating fungal keratitis. The use of both eyes of each rabbit is contentious, as it risks crossover of the drug from one eye to the other, as well as appearing to not fully adhere to the protocol for the use of animals in research. However, the same drop was instilled in both eyes, which would not interfere with comparisons between different animals. Although the systemic drug concentration may have increased, its contribution to corneal stromal concentrations was considered to be minuscule in view of the far greater local concentrations achieved. The guidelines for animal care proscribe bilateral procedures only if animals are visibly distressed or incapacitated by the procedure, or if they are to survive the investigation. These animals were under experimental conditions for a maximum of 5 hours, during which time they did not display any change in behavior as compared with normal animals.

With ketoconazole, PVA achieved the greatest zones of inhibition and BSS achieved the worst. With itraconazole, BSS was the best vehicle and boric acid the worst. The reasons are not obvious, but possible factors include the following: with PVA (a more viscous agent), the drug may persist for a longer time on the surface of the cornea. However, this did not hold for itraconazole, nor did olive oil achieve a comparable effect. It is not uncommon for plant oils to be used as vehicles for drug delivery, but the persistence of oily substances on the cornea (especially after removal of the epithelium) has not, to the best of our knowledge, been reported. The low pH of boric acid (pH = 4.68) may have influenced the solubility of itraconazole. A more sensitive organism might have produced zones of inhibition as large as those with ketoconazole. The inability to relate the in vivo results to the actual drug concentration in tissue makes extrapolation risky, especially as to the significance of the differences using different vehicles with itraconazole.

Further investigation must be undertaken to establish the clinical relevance of these findings. Clearly, there is no simple recipe that will ensure good tissue penetration of triturated tablets. Whether the tissue concentrations achieved in vivo are sufficient to be clinically useful must be established, especially as minimal inhibitory concentrations of various agents used for treating fungal keratitis have not been reported from in vivo studies.

When conventional topical antifungals are not available, triturated ketoconazole or itraconazole may be beneficial, provided an appropriate vehicle is used. Because therapeutic failures follow inadequate drug concentrations, it is imperative that a vehicle that has been empirically confirmed to yield the highest drug concentration in tissue be selected. Such information may benefit the treatment of fungal keratitis in the often poorly equipped developing world. In the case of ketoconazole, BSS is significantly worse than boric acid or PVA, achieving less than one third of the concentration. In the case of itraconazole, BSS is better than boric acid. Awareness of the variability in the drug concentration in tissue as result of the vehicle used may help to avoid ineffective therapy.

**REFERENCES**


2. Komadin TG, Wilkes TKI, Shock JR. Treatment of Aspergillus fumigatus keratitis in rabbits with oral and


