Intraocular penetration of dicloxacillin in experimental animals

Raymond E. Records

Adult rabbits were given single intravenous injections of 25 mg. per kilogram of sodium dicloxacillin. At intervals of time ranging from 15 minutes to 8 hours after injection, primary or secondary aqueous humor was aspirated from each eye. At the same time, a specimen of cardiac blood was withdrawn. Other animals were given subconjunctival injections of 50 mg. of dicloxacillin. Aqueous humor was aspirated one and 4 hours after injection. Assay of intraocular fluids and serum for antibiotic activity was by the disc-plate inhibition method with Sarcina lutea FDA 1001 as test organism. Following intravenous injection, dicloxacillin failed to penetrate the intact blood-aqueous humor barrier. However, excellent penetration was obtained into secondary aqueous humor, the resulting high dicloxacillin concentrations remaining in the intraocular fluids for some 4 to 8 hours. High antibiotic concentrations were present in primary aqueous humor one and 4 hours after subconjunctival injection of dicloxacillin. Although dicloxacillin is highly bound to plasma protein, high titers are attainable in secondary aqueous humor. The presence of two chlorines on the isoxazolyl group which enhances movement of this compound across membranes in other areas (gut) also facilitates movement across the disturbed blood-aqueous humor barrier into the intraocular fluids.

Dicloxacillin (3-[2,6-dichlorophenyl]-5-methyl-4-isoxazolyl penicillin sodium monohydrate) is a new member of the isoxazolyl family of semisynthetic penicillins. Structurally, it differs from penicillin G by the substitution of an isoxazolyl side chain (Fig. 1). Dicloxacillin is dissimilar to cloxocillin and oxacillin by the presence of two chlorine constituents on the isoxazolyl group.1 The molecular weight as a monohydrate is 510.3. Dicloxacillin is soluble to the extent of 15 Gm. per 100 ml. H₂O at 25° C.; it is only slightly soluble in common organic solvents and is somewhat more stable in acid solutions than either oxacillin or cloxocillin. One milligram of dicloxacillin is equivalent to approximately 1,164 Oxford units of penicillin.2

The antibacterial spectrum of this compound is similar to the other isoxazolyl penicillins. Dicloxacillin strongly induces the production of penicillinase by some strains of Staphylococci, although penicillinase activity is inhibited to some degree by the presence of dicloxacillin.2 In several reports, dicloxacillin was found to be more active against penicillin G resistant Staphylococci than either cloxocillin or oxacillin.3 4

In the human, dicloxacillin is 95 to 96 per cent bound to plasma protein, the highest protein binding of any penicillin.5 Absorption from the human gut, although somewhat variable, is the most complete of any of the isoxazolyl penicillins. Blood
levels following oral administration of dicloxacillin are 2 to 4 times higher than blood levels produced by similar doses of oxacillin.1-3 In many individuals, blood levels observed following oral administration are as high as those obtained following intramuscular injection. Although excellent absorption from the gastrointestinal tract is the rule, some individuals show little or no absorption following oral administration.1-3 Excellent clinical results have been reported with the use of dicloxacillin in a wide range of severe Staphylococcal infections in man.3-5

This study was undertaken to determine if this new antibiotic, which is highly effective against penicillinase producing Staphylococcus aureus, would reach therapeutically useful concentrations in the intraocular fluids of experimental animal eyes.

**Methods and materials**

The dicloxacillin used in this study was supplied as the dry powdered drug for laboratory investigation with each milligram containing 900 μg of dicloxacillin.* All doses and results were calculated on the basis of micrograms of pure dicloxacillin. The dry powdered drug was weighed and mixed with sterile distilled water to form a solution for intravenous injection containing 100 mg per milliliter of dicloxacillin activity.

Adult rabbits of either sex weighing 3 to 5 kilograms each were divided into 10 groups with at least 8 animals in each group. Topical anesthesia was obtained by instilling two drops of 0.5 per cent proparacaine hydrochloride in each eye prior to experimental procedures. No systemic anesthetic agent was used. Each animal in Groups 1 through 8 was given a single, rapid intravenous injection of 25 mg per kilogram of sodium dicloxacillin. Injections were given in a marginal ear vein. A light source placed below the ear provided an excellent view of the venous wall during the actual injection. Extravasation of the injected solution outside the vein was easily seen and if observed, the animal was discarded from the study.

Subconjunctival injections were performed by dissolving 150 mg of dicloxacillin powder in 1 ml of sterile distilled water. One-third milliliter (50 mg) was then injected beneath the bulbar conjunctiva of both eyes of each animal in Groups 9 and 10.

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*Furnished by Brian Bevan, M.D., of Bristol Laboratories.
Table I. Mean aqueous humor level

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>Plasma</th>
<th>Primary aqueous</th>
<th>Secondary aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0:15</td>
<td>32.56 ± 1.52 *</td>
<td>0</td>
<td>27.37 ± 5.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>24.0-35.0 (9)</td>
<td></td>
<td>0-50.0 (8)</td>
</tr>
<tr>
<td>2</td>
<td>0:15</td>
<td>29.89 ± 4.20</td>
<td></td>
<td>17.0-50.0 (9)</td>
</tr>
<tr>
<td>3</td>
<td>0:30</td>
<td>18.89 ± 2.39</td>
<td>0</td>
<td>12.0-35.0 (9)</td>
</tr>
<tr>
<td>4</td>
<td>0:30</td>
<td>18.83 ± 1.46</td>
<td></td>
<td>12.0-35.0 (10)</td>
</tr>
<tr>
<td>5</td>
<td>1:00</td>
<td>2.44 ± 0.66</td>
<td></td>
<td>10.0-30 (10)</td>
</tr>
<tr>
<td>6</td>
<td>2:00</td>
<td>0.74 ± 0.17</td>
<td></td>
<td>0.0-1.4 (10)</td>
</tr>
<tr>
<td>7</td>
<td>4:00</td>
<td>0</td>
<td></td>
<td>13.90 ± 1.31</td>
</tr>
<tr>
<td>8</td>
<td>8:00</td>
<td>0</td>
<td>0.53 ± 0.22</td>
<td>10.0-14.0 (10)</td>
</tr>
<tr>
<td>9</td>
<td>1:00</td>
<td></td>
<td>181.00 ± 19.92</td>
<td>70.0-250.0 (10)</td>
</tr>
<tr>
<td>10</td>
<td>4:00</td>
<td></td>
<td>50.70 ± 9.67</td>
<td>13.0-100.0 (10)</td>
</tr>
</tbody>
</table>

*Mean aqueous humor level in micrograms per milliliter ± standard error of mean over range of values obtained (number of animals).
†Subconjunctival injection.

Primary aqueous humor was aspirated from the anterior chamber via a 27 gauge disposable needle inserted through clear, avascular cornea 1 mm. central to the limbus. A secondary aqueous humor was produced in other animals by a rapid aspiration of primary aqueous humor. Following this procedure, the anterior and posterior chambers of the eye will fill with secondary aqueous humor, a fluid in which many constituents of plasma are present. This provides an experimental model of the alteration in the "blood–aqueous barrier" found with severe intraocular inflammation. Cardiac blood was obtained by percutaneous cardiac puncture, allowed to clot, and the serum separated by centrifugation.

Assay of serum and aqueous humor was by the disc-plate inhibition method with Sarcina lutea FDA 1001 as test organism. Nine milliliters of BBL 01-076 seed agar containing 0.03 ml. of a suspension of Sarcina (adjusted to a 34 per cent light transmission through a 5,300 A filter) was placed in a sterile Petri dish. A 6 mm. filter paper disc containing 0.03 ml. of aqueous humor or plasma was then placed on the agar surface. The plates were then incubated for 20 hours at 37° C. Zones of inhibition about the disc were measured and compared with those produced by a known dicloxacillin standard. The antibiotic content of each specimen was then calculated. All statistical results are expressed as the arithmetic mean ± the standard error of the mean.

Results

A single intravenous injection of 25 mg. per kilogram of dicloxacillin resulted in peak plasma levels 15 minutes after injection. These high levels declined rapidly and dropped below useful concentrations between one and 2 hours. No antibiotic activity was detectable in plasma 4 or 8 hours after injection (Table I).

Dicloxacillin failed to cross the blood-
aqueous humor barrier in normal rabbit eyes. No antibiotic activity was detectable in the primary aqueous humor of any animal (Table I). Dicloxacillin penetrated the disturbed blood-aqueous humor barrier easily, producing secondary aqueous humor concentrations nearly as high as those present in the plasma 15 and 30 minutes after injection. The secondary aqueous humor levels declined at a slower rate than the corresponding plasma concentrations. This resulted in a much higher antibiotic titer in the intraocular fluids than in the plasma between 30 minutes and 8 hours following intravenous injection.

Subconjunctival injection of a single 50 mg. dose resulted in extremely high dicloxacillin levels in primary aqueous humor which were still present 4 hours after injection.

No ill effects were noted in any animal following intravenous dicloxacillin injection or after aqueous humor aspiration. No ocular abnormalities were observed with the exception of a tiny corneal opacity at the site of the needle tract.

Immediately following subconjunctival injection, a large conjunctival bleb was present. This rapidly decreased in size until at 4 hours only a slight chemosis of the conjunctiva was noted. The only abnormalities observed in any animal were an occasional petechial conjunctival hemorrhage and occasionally a narrow (1 mm. wide) crescent of superficial corneal edema just anterior to the margin of the conjunctival bleb. Neither the conjunctival hemorrhages nor the crescent of corneal edema were noted in any animal 24 hours after the subconjunctival dicloxacillin injection.

Histologic section of representative eyes enucleated 48 hours after subconjunctival injection revealed only scattered inflammatory cells with slight edema of the episcleral tissues. Necrosis of conjunctiva, episclera, or sclera was not observed in any specimen.

**Comment**

The sensitivity of various common bacteria to dicloxacillin is shown in Table II. A concentration of 0.50 μg per milliliter would inhibit growth of most gram-positive cocci including penicillinase producing *Staphylococcus aureus*. This latter organism has been implicated as the pathogen responsible for many cases of postoperative endophthalmitis. Dicloxacillin levels in secondary aqueous humor far exceed those needed to inhibit growth of *Staphylococcus aureus*. Such extremely high antibiotic levels are present in secondary aqueous humor for at least 4 hours following a single intravenous injection. An entirely satisfactory growth-inhibiting concentration of 0.53 μg per milliliter is still present at 8 hours.

We are unaware of previous studies dealing with the intraocular penetration of dicloxacillin. Oxacillin, another member of the isoxazolyl penicillin family, required an intravenous dose of 75 mg. per kilogram to produce therapeutically useful levels in secondary aqueous humor of rabbits. Like dicloxacillin, oxacillin did not cross the intact blood-aqueous humor barrier. In another report, oxacillin was found not to penetrate the intact human blood-aqueous humor barrier. Oxacillin, which is 93 percent bound to plasma protein, failed to penetrate into secondary aqueous humor of rabbits until a dose of 75 mg. per kilogram was given. Dicloxacillin, which is slightly

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**Table II. Sensitivity of various common organisms to dicloxacillin**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Average minimum inhibiting concentration (μg/mL*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diplococcus pneumonia</td>
<td>0.15</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>0.55</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> S1</td>
<td>0.30</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> R1</td>
<td>0.35</td>
</tr>
<tr>
<td>Streptococcus fecalis</td>
<td>50.00</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>&gt;100.00</td>
</tr>
<tr>
<td>Salmonella species</td>
<td>&gt;100.00</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>&gt;100.00</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>&gt;100.00</td>
</tr>
</tbody>
</table>

*Modified after Bennett, Naumann, Knott, and various other sources.

*S* = Nonpenicillinase-producing.

*R* = Penicillinase-producing.
more bound to plasma protein (95 per cent), crosses the disturbed blood–aqueous humor barrier easily. Addition of two chlorines to the isoxazolyl group, the only chemical difference between oxacillin and dicloxacillin increases absorption from the gut by 2 to 4 times. Movement across the disturbed blood–aqueous humor barrier is apparently also enhanced by dichlorination of the side chain.

Although great care must be taken in extrapolating animal data to the clinical situation, the information obtained from this study is highly favorable. Investigation of the penetration of dicloxacillin into human secondary aqueous humor is now underway.

REFERENCES


