AUREOMYCIN MILK AND SERUM CONCENTRATIONS IN DAIRY COWS

L. A. KANEGIS, G. TONELLI, AND HERBERT FALK
Lederle Laboratories Div., American Cyanamid Co.,
Pearl River, N. Y.

In the early pharmacodynamic investigations of aureomycin, sensitivity of a variety of microorganisms, including several commonly associated with bovine mastitis, was demonstrated (2, 7, 8, 9). These reports prompted more detailed studies, and in vitro sensitivity of organisms isolated from cases of mastitis were reported (5). Table 1 (5) shows the requirements in micrograms per milliliter for inhibition of growth of the organisms in these tests. With the exception of Pseudomonas aeruginosa, all organisms tested were highly susceptible to the action of aureomycin.

In vitro sensitivity of microorganisms to aureomycin is a useful guide, with certain limitations, to the drug level required to inhibit bacterial growth in the infected host. The concentrations of an antibiotic attained in the udder following infusion are subject to variation from animal to animal, and, indeed, from quarter to quarter in the same cow. Therefore, a study of individual quarter aureomycin concentrations in milk and the percentage frequency distribution of concentrations following a single infusion with 400 mg. aureomycin in ointment per quarter in 41 quarters of 12 cows was instituted to attempt to determine concentrations of drug that might be expected to be obtained by a certain dosage.

EXPERIMENTAL PROCEDURE

Twelve normal cows of various breeds were used in this test. Udder palpation and physical appearance of the milk by strip cup test showed freedom from

---

**TABLE 1**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inhibiting growth</th>
<th>Aureomycin HCl (γ/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Broth</td>
<td>Milk *</td>
</tr>
<tr>
<td>Strep. agalactiae</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Strep. dysgalactiae</td>
<td>1.25</td>
<td>1.25</td>
</tr>
<tr>
<td>Group C. Strep.</td>
<td>0.16 -- 2.5</td>
<td>0.63 -- 2.5</td>
</tr>
<tr>
<td>Strep. atheris</td>
<td>0.32</td>
<td>1.63</td>
</tr>
<tr>
<td>Staph. aureus (B Toxin)</td>
<td>0.63</td>
<td>1.25</td>
</tr>
<tr>
<td>Staph. aureus (A Toxin)</td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>Staph. aureus (Coagulase +)</td>
<td>0.63</td>
<td>0.63</td>
</tr>
<tr>
<td>Staph. aureus (Coagulase -)</td>
<td>0.02</td>
<td>0.16</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Coryne. pyogenes</td>
<td>1.25</td>
<td></td>
</tr>
<tr>
<td>Bacillus 5 Control</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Tests made in fresh skimmilk (not whey) with Bromeresol Purple.

clinical mastitis. The cows were milked at 12-hour intervals. In order that the experiment might approximate various field conditions, cows producing only 20 lb. of milk, as well as those producing up to 48 lb., were used.

Aureomycin crystalline ointment, containing 400 mg. of aureomycin (60 mg. per gram) in 7 g. of ointment in a collapsible tube, was used for udder infusion. Each quarter was infused with one tube of ointment immediately after the morning milking. Milk samples were taken immediately before treatment and every 12 hours thereafter through 120 hours, and blood samples were drawn at prescheduled hours through 60 hours. To obtain milk samples, the quarter was thoroughly cleaned with water and completely milked out into a clean enamel pail. The contents were weighed and stirred, and a sample was removed in a Pyrex tube and immediately frozen in dry ice. Blood samples were examined to determine if aureomycin is absorbed from the udder. Samples were assayed microbiologically for aureomycin content according to the method of Dornbush and Pelcak (3). It should be pointed out that any microbiological two-fold dilution assay has a relatively large error factor. Therefore, the final estimates should be viewed with this in mind.

RESULTS

In all cases very high concentrations of aureomycin were present in milk at 12 hours, and bacteriostatically effective concentrations persisted through 48

**Figure 1.**

*Mean concentrations of aureomycin in milk of cows receiving a single infusion of 400 mg. aureomycin per quarter in ointment.*
hours, even though the udders were milked out every 12 hours. Although each quarter received the same treatment, considerable variation in the concentration of drug was evident. The mean aureomycin concentrations in the milk are presented graphically in Figure 1.

Frequency distribution of concentrations of aureomycin in milk following a single infusion with 400 mg. aureomycin in ointment per tube, one tube per

TABLE 2

<table>
<thead>
<tr>
<th>Hr. quarters</th>
<th>Mean value</th>
<th>Concentration ranges of aureomycin in milk (γ/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (%) of quarters</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>12</td>
<td>37</td>
<td>122.5</td>
</tr>
<tr>
<td>24</td>
<td>39</td>
<td>50.4</td>
</tr>
<tr>
<td>36</td>
<td>40</td>
<td>11.8</td>
</tr>
<tr>
<td>48</td>
<td>41</td>
<td>3.9</td>
</tr>
<tr>
<td>60</td>
<td>41</td>
<td>1.8</td>
</tr>
<tr>
<td>72</td>
<td>41</td>
<td>0.85</td>
</tr>
<tr>
<td>84</td>
<td>29</td>
<td>0.55</td>
</tr>
</tbody>
</table>

* Aureomycin Crystalline Ointment for Udder Infusion, 60 mg./gram.

quarter, is shown in Table 2. At 12 hours, extremely high concentrations, ranging from 50 to 400 γ per milliliter, were present in 94.5 per cent of the quarters. At 24 hours, more than 50 per cent of the quarters contained concentrations between 10 and 50 γ per milliliter, and only 10 per cent were in a lower range (1.0 to 10.0 γ per milliliter). At 48 hours, concentrations of aureomycin, between 1.0 and 10 γ per milliliter still were present in 83 per cent of the quarters, with only slightly less than 5 per cent in lower ranges. On the basis of these figures, it can be stated that in the normal cow a single infusion of 400 mg. aureomycin per tube of ointment maintains bacteriostatically effective levels of aureomycin in milk for at least 48 hours.

Aureomycin serum concentrations following udder infusion of 400 mg.

TABLE 3

<table>
<thead>
<tr>
<th>Hr.</th>
<th>254</th>
<th>154</th>
<th>373</th>
<th>417</th>
<th>671</th>
<th>674</th>
<th>635</th>
<th>670</th>
<th>672</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>1</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>2</td>
<td>0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>&lt;0.05</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>0.1</td>
<td>**</td>
<td>**</td>
<td>&lt;0.05</td>
<td>0.1</td>
<td>&lt;0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>6</td>
<td>0.05</td>
<td>0.05</td>
<td>0.1</td>
<td>0.2</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>0.1</td>
</tr>
<tr>
<td>8</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
<td>0.8</td>
<td>0.4</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>18</td>
<td>0.1</td>
<td>0.2</td>
<td>0.4</td>
<td>0.4</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>0.2</td>
</tr>
<tr>
<td>24</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>0.2</td>
<td>0.1</td>
<td>0.05</td>
<td>0.1</td>
<td>0.05</td>
<td>0.2</td>
</tr>
<tr>
<td>36</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>48</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>60</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

** No sample taken.

* Aureomycin Crystalline Ointment for Udder Infusion, 60 mg./gram.
Aureomycin Milk and Serum Concentrations

Aureomycin are presented in Table 3. A peak aureomycin concentration in serum, varying between 0.1 and 0.8 \( \gamma \) per milliliter was reached in 12 hours. It is evident, therefore, that aureomycin is absorbed from udder to blood.

DISCUSSION

Several reports have appeared on the clinical use of aureomycin in the treatment of mastitis. Various dosage schedules have been used in the field, and, in the case of more than one infusion, at varying intervals (1, 4, 6, 10). Slanetz and Allen (10) and Bell and Jordan (1) have reported findings similar to ours in the infected cow. In infected herds, they found high inhibitory concentrations of aureomycin present for at least 48 hours following intramammary infusion of 200 mg. per quarter, even when the udder was milked at 12-hour intervals, and highly satisfactory results were obtained in the treatment of both streptococcic and staphylococcic mastitis with one or more such infusions.

The analysis presented in the study reported here can be used as a guide for dosage schedules for the treatment of the infected animal. The antibiotic concentrations shown are in milk and blood serum, not udder tissue. Deep-seated staphylococcic foci may not be reached by udder infusion and may require optimal intravenous dosage of 5 mg. per pound or more.

SUMMARY

A single infusion of 400 mg. aureomycin ointment per tube (60 mg. per gram) maintained high levels of aureomycin in milk for at least 48 hours from the time of infusion. A peak of aureomycin concentration in blood between 0.1 and 0.8 \( \gamma \) per milliliter was reached in 12 hours, indicating that aureomycin was absorbed through the mammary tissue.

REFERENCES