The Dynamics of Clindamycin-2-Phosphate in vivo and its Transfer to Tissues

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Studies were conducted on clindamycin-2-phosphate, a preparation of clindamycin CLDM developed for injection, and the following results were obtained:

1) The concentration of CLDM phosphate and lincomycin (LCM) in sera were measured in rabbits following continuous intravenous infusion of 20 mg/kg over a 60 minute period, and the results were compared in a crossover test.

2) The concentration of CLDM phosphate in the oral tissues of rabbits following continuous intravenous drip infusion of 20 mg/kg over a 60 minute period was found to be a maximum in the submaxillary gland (21.1 µg/g), followed by the liver, submaxillary lymph nodes, sera and tongue. The concentration of LCM was found to be the highest in the submaxillary gland (20.2 µg/g), followed by the submaxillary lymph nodes, tongue, sera and liver.

3) The metabolism of CLDM phosphate in vivo was investigated using TLC. CLDM phosphate was found to metabolize to CLDM N-demethyl CLDM in vivo.

4) The peak concentration of CLDM phosphate in human sera following intravenous drip infusion of 600 mg over a 60 minute period averaged 11.9 µg/ml, while the average concentration of LCM in sera under identical circumstances was approximately twice that of CLDM phosphate.

(Key Words: Clindamycin-2-phosphate, Lincomycin, Blood Level, Tissue Concentration, Metabolite)

INTRODUCTION

Clindamycin-2-phosphate is an antibiotic produced by the addition of phosphate to clindamycin through an ester linkage and it has been developed for both intramuscular and intravenous injection. The chemical name is 7 (S)-chloro-7-deoxylincomycin-2-phosphate, it has a molecular formula of C_{18}H_{34}O_{8}N_{2} SCLP and a molecular weight of 504.97. It appears as a white crystal powder that is readily soluble in water. While the drug in itself has little antibacterial action, it is readily hydrolyzed in vivo to clindamycin by phosphatase. Clindamycin, in turn, is metabolized to N-demethylclindamycin and clindamycin sulfoxide, two metabolites with considerable antibacterial potency.

Clindamycin (CLDM) has a broad antibacterial spectrum against gram-positive cocci and anaerobic bacteria similar to that of lincomycin (LCM), and its antibacterial potency is believed to be 4-8 times that of LCM (2, 5). In the present investigation, the dynamics of clindamycin-2-phosphate (CLDM phosphate, Nippon Upjohn) were compared with those of LCM in vivo.

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EXPERIMENTAL MATERIALS AND METHODS

1. Serum concentration in rabbits

In four healthy normal New Zealand White (NZW) rabbits, concentrations of CLDM phosphate and LCM in sera were compared following continuous intravenous drip infusion of 20mg/kg of each antibiotic in a crossover test.

CLDM phosphate and LCM (20mg/kg each) were administered through a vein in the ear by means of continuous intravenous drip infusion over a period of 60 minutes. Blood samples were obtained 1, 2, 3, and 4 hours after starting the infusion.

Concentrations of both antibiotics in the blood were measured by bioassay using Micrococcus luteus ATCC 9341 as the test organism in a thin layer cup method. A standard curve was established by serial dilution of CLDM. The standard and samples were diluted with 0.15 M phosphate buffered solution (0.15 M P.B.S.).

2. Concentrations of antibiotics in the oral tissues

Eight healthy normal NZW rabbits weighting 2.5—3kg were divided into two groups of four animals each and administered 20mg/kg each of CLDM phosphate and LCM through the veins in the ear by continuous infusion over a 60 minute period. The rabbits were sacrificed by bleeding under Ravonal anesthesia at 1, 2, 3, and 4 hour intervals following the start of the infusion, and the tongue, submaxillary gland, submaxillary lymph nodes and liver were removed. The organs were emulsed and diluted 10 times with 0.15 M P.B.S. A standard curve was prepared for CLDM.

3. Drug metabolism in vivo

CLDM phosphate metabolites were measured in the submaxillary glands, tongue, liver, sera and urine of rabbits following intravenous injection of CLDM phosphate. CLDM phosphate (20mg/kg) was administered through the veins of the ear by means of continuous drip infusion over a 60 minute period. The rabbits were sacrificed at the completion of the infusion by bleeding to permit removal of the submaxillary glands, tongue and liver, and to collect serum samples. Tissue homogenate and sera were emulsed with 0.15 M P.B.S., and the pH was adjusted to 8.0 with sodium hydroxide. After extraction with ethyl acetate, the samples were concentrated and redissolved in a small amount of metanol for measurement.

Intravenous drip infusions of 600mg of CLDM phosphate dissolved in 250ml of 5% glucose were administered to human patients over a period of 60 minutes, and serum and urine samples were obtained. Serum samples were collected 0.5, 1, and 2 hours following the start of the infusion, and urine was collected within the first 2 hours.

Bioautograms were prepared for measurement by thin layer chromatography (TLC). The carrier used was a spot film of silica gel (Tokyo Kasei) and the developing solvent was either an 8:5:1 mixture of ethyl acetate: acetone: water or an 18:5:2 mixture of methyl ethyl ketone: acetone: water.

4. Concentration in human sera
Intravenous drip infusions of 600mg of CLDM phosphate and 600mg of LCM dissolved in 250ml of 5% glucose were administered over a 60 minute period to six and five patients respectively.

In all patients, serum concentrations of the two antibiotics were measured in chronological sequence following prophylactic administration for oral surgical procedures. Measurements were carried out 0.5, 1, 2, 3, 4, 5, and 6 hours following the start of CLDM phosphate administration and 5 hours following the start of LCM administration.

EXPERIMENTAL RESULTS

1. Sera concentrations in rabbits

Serum concentrations of CLDM phosphate and LCM following continuous intravenous drip infusion of 20mg/kg of each antibiotic over a 60 minute period were measured in four NZW rabbits by a crossover test. Results are summarized in Tables 1 and 2. Peak concentrations of CLDM phosphate averaging 6.98\(\mu g/ml\) were recorded 1 hour after the start of the infusion, and they gradually declined to 1.84\(\mu g/ml\) after 2 hours, 2.08 \(\mu g/ml\) after 3 hours, and 0.94\(\mu g/ml\) after 4 hours.

Peak concentrations of LCM averaging 10.75\(\mu g/ml\) were recorded 1 hour after the start of administration and they gradually declined to 2.39\(\mu g/ml\) after 2 hours, 1.60\(\mu g/ml\) after 3 hours, and 1.00\(\mu g/ml\) after 4 hours.

Table 1 Serum concentration in rabbits CLDM phosphate, 20mg/kg I.V. for 60 min.

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<thead>
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<tr>
<td>A</td>
<td>5.8</td>
<td>5.1</td>
<td></td>
<td></td>
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<tr>
<td>B</td>
<td>6.5</td>
<td>2.0</td>
<td>1.36</td>
<td>1.35</td>
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<tr>
<td>C</td>
<td>5.4</td>
<td>1.45</td>
<td>0.80</td>
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<tr>
<td>D</td>
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<td>1.07</td>
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<td>mean</td>
<td>6.98</td>
<td>1.84</td>
<td>2.08</td>
<td>0.94</td>
</tr>
<tr>
<td>(S.D.)</td>
<td>(2.20)</td>
<td>(0.36)</td>
<td>(2.02)</td>
<td>(0.35)</td>
</tr>
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</table>

\(\mu g/ml\)

Table 2 Serum concentrations in rabbits LCM, 20mg/kg I.V. infusion for 60 min.

<table>
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<td>9.6</td>
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<td>B</td>
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<td>0.67</td>
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<tr>
<td>C</td>
<td>12.2</td>
<td>3.13</td>
<td>2.30</td>
<td>1.10</td>
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<tr>
<td>D</td>
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<tr>
<td>mean</td>
<td>10.75</td>
<td>2.39</td>
<td>1.60</td>
<td>1.00</td>
</tr>
<tr>
<td>(S.D.)</td>
<td>(2.53)</td>
<td>(1.20)</td>
<td>(0.81)</td>
<td>(0.55)</td>
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</table>

\(\mu g/ml\)
2. Antibiotic concentrations in the oral tissues of rabbits

Concentrations of CLDM phosphate in the oral tissues of rabbits following continuous intravenous drip infusion of 20 mg/kg of CLDM phosphate over a 60 minute period were measured and are shown in Table 3 and Figure 1. Average concentrations in sera peaked at 1/2 hour (5.0 µg/ml), maintained a consistent level up to 1 hour, and subsequently decreased gradually to a concentration of 1.05 µg/ml which was maintained up to 4 hours. Antibiotic concentrations in the tongue peaked at 4.37 µg/g 1 hour following administration and gradually declined to a concentration of 1.06 µg/g which was maintained for 4 hours. The highest antibiotic concentration among the various tissues examined occurred in the submaxillary glands where drug levels peaked at 21.1 µg/g 1 hour after administration, declined rapidly up to 2 hours, and then decreased gradually to a level of 6.3 µg/g which was maintained for 4 hours.

Table 3 Serum and tissue concentrations CLDM phosphate, 20 mg/kg I.V. for 60 min.

<table>
<thead>
<tr>
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<tr>
<td>tongue</td>
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<td>3.49</td>
<td>2.96</td>
<td>1.06</td>
<td></td>
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<tr>
<td>submaxillary gland</td>
<td>21.1</td>
<td>8.7</td>
<td>7.0</td>
<td>6.3</td>
<td></td>
</tr>
<tr>
<td>submaxillary lymph nodes</td>
<td>7.0</td>
<td>5.8</td>
<td>3.70</td>
<td>3.10</td>
<td></td>
</tr>
<tr>
<td>liver</td>
<td>12.0</td>
<td>7.6</td>
<td>3.81</td>
<td>3.52</td>
<td></td>
</tr>
<tr>
<td>serum</td>
<td>5.0</td>
<td>4.60</td>
<td>1.70</td>
<td>1.27</td>
<td>1.05</td>
</tr>
</tbody>
</table>

(standard = CLDM) µg/g & µg/ml

![Figure 1](image_url)

Fig. 1 Rabbit CLDM phosphate 20 mg/kg I.V. 60 min. infusion
The concentration in the submaxillary lymph nodes reached a
maximum of 7.0μg/g after 1 hour, followed by a gradual decline. The
concentration in the liver reached a maximum of 12.0μg/g after 1 hour,
followed by a gradual decline.

The maximum concentration in each tissue was reached after 1 hour,
and was highest in the submaxillary gland, followed by the liver, submaxillary
lymph nodes and the tongue in that order. After 2 hours, antibiotic con-
centrations in all tissues were higher than the concentrations in the sera.

Drug concentrations in the oral tissues of rabbits following continuous
intravenous drip infusion of 20mg/kg LCM for 60 minutes are shown
in Table 4 and Fig. 2. The average concentration in the sera reached
a maximum of 10.7 μg/ml after 30 minutes, followed by a gradual decrease
to 0.52μg/ml after 4 hours. The concentration in the tongue reached a peak
of 7.6μg/g within 1 hour, followed by a rapid decline until after 2 hours
when the concentration declined sharply, reaching an undetectable levels
after 3 hours. The drug concentrations in the submaxillary glands reached
a maximum of 20.2μg/g which was the highest concentration of antibiotic
after 1 hour observed in all of the tissues examined, followed by a gradual
decline to a level of 3.94μg/g after 4 hours. The concentration of antibiotic
in the submaxillary lymph nodes reached a maximum of 8.0μg/g after
1 hour, followed by a gradual decline to 4.17μg/g after 4 hours. The
concentration in the liver reached a peak level of 5.8μg/g after 4 hours,
followed by a rapid decline for 3 hours, and reduction to an undetectable
level after another 4 hours.

![Graph showing drug concentrations in different tissues](image)

**Fig. 2** Rabbit LCM 20mg/kg I.V. 60 min. infusion
Table 4  Serum and tissue concentrations LCM, 20mg/kg I.V. infusion for 60 min.

<table>
<thead>
<tr>
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<th>1h</th>
<th>2h</th>
<th>3h</th>
<th>4h</th>
</tr>
</thead>
<tbody>
<tr>
<td>tongue</td>
<td>7.6</td>
<td>1.39</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>submaxillary gland</td>
<td>20.2</td>
<td>10.6</td>
<td>5.6</td>
<td>3.94</td>
<td></td>
</tr>
<tr>
<td>submaxillary lymph nodes</td>
<td>8.0</td>
<td>8.2</td>
<td>5.1</td>
<td>4.17</td>
<td></td>
</tr>
<tr>
<td>liver</td>
<td>5.8</td>
<td>1.11</td>
<td>0.60</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>serum</td>
<td>10.7</td>
<td>7.0</td>
<td>1.94</td>
<td>1.47</td>
<td>0.52</td>
</tr>
</tbody>
</table>

μg/g & μg/ml

In each tissue, the peak concentrations were reached within 1 hour. The tissue with the highest concentration of antibiotic was the submaxillary gland, followed in descending order by the submaxillary lymph nodes, tongue and liver.

3. Metabolism in vivo

The presence of metabolites was investigated in vivo in rabbits by means of TLC following continuous intravenous drip infusion of 20mg/kg of CLDM phosphate over a period of 60 minutes.

Fig. 3 shows the bioautogram prepared for a culture medium using Micrococcus luteus ATCC 9341 as the test strain after development with ethyl acetate, acetone, and water (8:5:1) as the solvent. After 0.5 and 1 hour following the start of the intravenous infusion, one inhibitory circle with an Rf value corresponding to that of CLDM was noted.

Fig. 4 is a bioautogram prepared with methylethyl ketone: acetone: water (18:5:2) as the solvent. In the sera and submaxillary gland 0.5 hour following the intravenous injection of CLDM phosphate, and in the submaxillary gland 1 hour after injection, antibacterial activity was noted at an Rf corresponding to that of CLDM. No activity was detectable in the tongue or liver.
Fig. 4  TLC bioautograms  
adsorbent: silicagel (spot film)  
solvent: methylethylketone: acetone: water (18:5:2)  
test organism: Micrococcus luteus ATCC 9341

Fig. 5 shows a bioautogram prepared with methylethyl ketone, acetone, and water (18:5:2) as the solvent. Zero to two hour urine and serum samples were obtained from a patient 0.5, 1, and 2 hours following intravenous injection of 600mg of CLDM phosphate. One inhibitory circle was evident from serum samples obtained at each time interval an Rf corresponding to that of CLDM. In the Zero to two hour urine sample, two inhibitory circles, one corresponding to the Rf of CLDM and the other to that of N-demethyl CLDM, were noted.

Fig. 5  TLC bioautograms  
adsorbent: silicagel (spot film)  
solvent: methylethylketone: acetone: water (18:5:2)  
test organism: Micrococcus luteus ATCC 9341
4. Concentration in human sera

A continuous drip infusion of 600mg of CLDM phosphate was administered over a 60 minute period to measure the antibiotic concentration in sera chronologically. The results are shown in Table 5 and Fig. 6. The average serum concentration in six subjects reached 11.9µg/ml after 1 hour, followed by a gradual decrease to a level of 1.63µg/ml which was maintained up to 6 hours.

Table 5 Serum levels of CLDM phosphate 600mg/60 min. I.V. infusion

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<th>5h</th>
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<tr>
<td>K.K.</td>
<td>14.0</td>
<td>21.0</td>
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<td>4.40</td>
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<td>G.M.</td>
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<tr>
<td>Y.I.</td>
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<td>3.10</td>
<td>1.75</td>
<td>1.20</td>
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<tr>
<td>M.M.</td>
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<td>1.95</td>
<td></td>
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<tr>
<td>K.I.</td>
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<td>4.40</td>
<td>3.40</td>
<td>3.00</td>
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<tr>
<td>Y.K.</td>
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<td>13.2</td>
<td>8.5</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Average          8.5  11.9  6.1  3.18  2.58  2.75  1.63

(standard = CLDM) µg/ml

In addition, 600mg of LCM was administered to patients by intravenous drip infusions over a 60 minutes period to measure the drug concentration in sera. The results are shown in Table 6 and Fig. 7. Average drug concentrations in the sera of five patients reached a peak of 24.4µg/ml after 0.5 hour, maintained high values up to 1 hour, decreased rapidly until 2 hours following termination of the intravenous infusion, and gradually declined to a level of 3.40µg/ml after 5 hours.
Table 6  Serum levels of LCM 600mg/60 min. I.V. infusion

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<td>M.N.</td>
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<tr>
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<tr>
<td>Y.K.</td>
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<tr>
<td>Average</td>
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<td>22.0</td>
<td>8.3</td>
<td>6.0</td>
<td>5.0</td>
</tr>
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</table>

**DISCUSSION**

CLDM phosphate is an antibiotic developed for administration by injection with a relatively mild antibacterial potential in its original form. This compound, however, is believed to be metabolized in vivo to CLDM, N-demethyl CLDM, and CLDM sulfoxide (1, 4, 7).

In the present investigation, 20mg/kg of CLDM phosphate was administered to rabbits by continuous drip infusion over a 60 minute period to measure CLDM concentrations in sera. A peak value of 6.98μg/ml was obtained following termination of the drip infusion. Four hours after the start of administration, the concentration of CLDM in sera was maintained at approximately 0.94μg/ml. In a crossover test, 20mg/kg of LCM was administered by continuous drip infusion over a 60 minute period.
to measure the concentration of LCM in sera. A peak concentration of 10.75μg/ml was reached following drip infusion, a level somewhat higher than that achieved with CLDM phosphate. Because of individual variations, a comparison of the levels of the two drugs by the t-test showed no significant differences.

Measurement of concentration of both CLDM and LCM in the oral tissues of rabbits indicated that the most favorable transfer was to the submaxillary glands. In particular, the concentration of CLDM transferred to the submaxillary glands reached a drug level 4—6 times of that achieved in sera. The concentration of transferred CLDM was higher in each of the tissues measured than in sera for up to 4 hours following administration. The concentration of LCM in the submaxillary gland and lymph nodes remained elevated up to 4 hours following administration but became undetectable after 3 hours in the tongue and 4 hours in the liver.

Tissue concentrations of CLDM were higher than those of LCM, and CLDM demonstrated consistently better maintenance levels.

Measurement of the metabolites of the two antibiotics in vivo following administration of 20mg/kg of each to rabbits by a continuous intravenous drip infusion over a 60 minute period showed the presence of only CLDM in the sera, submaxillary glands, tongue and liver. Following oral administration of CLDM, however, the presence of N-demethyl CLDM was also detected in sera and the oral tissues (3). In the present investigation, CLDM and N-demethyl CLDM were detected in the urine following administration of CLDM phosphate to patients.

The concentration of CLDM in sera was measured in six adult males following intravenous drip infusion of 600mg of CLDM phosphate over a period of 60 minutes. A peak value of 11.9μg/ml was obtained immediately following completion of the infusion. This level was similar to the 10.2μg/ml level reported by Sawae and Takii (6). The concentration of LCM in sera was measured following intravenous drip infusion of 600mg of LCM over a 60 minute period in five adult males. An average concentration of 24μg/ml was recorded after 30 minutes of infusion and this dropped to an average of 22.0μg/ml by the time of completion of the infusion. When peak concentrations of the two drugs were compared, LCM demonstrated a consistently higher peak than CLDM phosphate. Elevated concentrations for both drugs were maintained for 5—6 hours.

Gram-positive bacteria such as staphylococci, streptococci, and various anaerobes are organisms frequently associated with infection of the oral cavity. LCM and CLDM are effective against these organisms, and, consequently, are often the drugs of choice in oral infections. The CLDM phosphate used in the present investigation maintained a lower concentration in the sera than LCM but a higher concentration in oral tissues. In view of the antibacterial potency that is 4—8 times that of LCM, together with its marked capacity for concentration in the oral tissues, CLDM phosphate should be preferred over LCM for use by oral surgeons.
REFERENCES
2) Kosakai N et al: Sensitivity of various recent isolated pathogenic bacteria to clindamycin and lincomycin.
7) The Upjohn Company: Metabolism of clindamycin-2-phosphate.