Pharmacokinetics and Tolerability of Chloramphenicol in Budgerigars (Melopsittacus undulatus)

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ABSTRACT. Pharmacokinetics and tolerability of chloramphenicol (CP) were evaluated in budgerigars. Following intramuscular administration of CP at 100 and 200 mg/kg of body weight, serum peak concentrations of 35.3 and 90.7 μg/ml, respectively, were obtained 0.25 hr following injection, and these values declined with a terminal half-life of 2.5 and 2.7 hr, respectively. Based on these results, an intramuscular dosage regimen of 100 mg/kg of CP 3 times a day or of 200 mg/kg twice daily was recommended as effective against many of the common bacterial infections in budgerigars. Red blood cell count, packed cell volume, hemoglobin, total plasma protein, serum AST, ALT, LDH and CK values were determined after intramuscular injection of either CP or physiological saline 2 or 3 times a day for 5 days. The birds were weighed before and after treatment. Tissue samples from various organs were examined histologically. The most prominent adverse effect was muscular damage at the injection site. A dosage regimen of 200 mg/kg twice daily for 5 days was considered to be safe.—key words: budgerigar, chloramphenicol, dosage regimen, pharmacokinetics, tolerability.


Chloramphenicol, a broad spectrum antibiotic, is commonly used in pet birds as an effective antibacterial agent, especially against Salmonella infections [10]. In birds, chloramphenicol induces toxic effects similar to those observed in mammals. It has been reported that large doses of chloramphenicol produce reversible anemia and inappetence in chickens, turkeys and ducks [13, 14, 17-19]. While information on the pharmacological properties of chloramphenicol in chickens and ducks has been reported [4, 5, 20, 21], data on the pharmacokinetics and toxicity of chloramphenicol in budgerigars are limited [2].

The purpose of the present study was to determine an appropriate dosage regimen of chloramphenicol in budgerigars and also to document any adverse effects of such administration.

MATERIALS AND METHODS

Birds and chemicals: The experiments were carried out in 56 one-year-old clinically healthy budgerigars, weighing an average of 35.7 g, and purchased from Sapporo Kotori Shokai (Sapporo). The birds were housed in cages at room temperature. Feed and water were supplied ad libitum.

Chloramphenicol sodium succinate was obtained from Sankyo Co., Ltd. (Tokyo). The drug was dissolved in distilled water (100 mg/ml) for use in the experiments.

Pharmacokinetic analysis: Chloramphenicol solution was injected into the birds-pectoral muscles at a dose of 100 mg/kg or 200 mg/kg. Serum chloramphenicol concentrations were measured after single intramuscular (IM) administration. Because of difficulties encountered in collecting multiple blood samples from a single bird, data were obtained from a population of birds and combined for analysis. Two groups of 18 budgerigars each were further divided into 6 groups of 3 birds each. Group I birds received a dose of 100 mg/kg and those of group II received 200 mg/kg. Venous blood samples (1.0 ml) were collected once from three birds of each group at one of the following times: 0.25, 1, 2, 4, 8 and 12 hr after drug administration. Blood samples were collected from the jugular vein while the birds were under anesthesia. The anesthetic (0.05 ml/bird) contained a 1:1 mixture of 2% xylazine (Bayer) and 5% ketamine (Sankyo). The blood samples were centrifuged and serum was assayed for chloramphenicol by HPLC.

Pharmacokinetic parameters, except the area under the serum concentration time-curve (AUC), were calculated according to accepted method [11]. Confidence intervals were determined according to Ichihara [6]. The average and SE of the AUC were calculated, as described by Bailar [1].

Determination of chloramphenicol concentration: Serum chloramphenicol concentrations were measured using a modification of the HPLC method described by Kushida et al. [9]. Chloramphenicol was extracted into diethyl ether and the extracts were analyzed on a HPLC apparatus (Model 655, Hitachi) equipped with a 4.6 x 25 cm SORBAX ODS column (Dupont) and a 295 nm UV detector (Model 638-30, Hitachi). Analyses were performed using a mobile phase of methyl alcohol, acetonitrile and 0.02 M KH₂PO₄ 5:15:80 (v/v/v) at a flow rate of 1.2 ml/min.

Tolerance assays: Four groups of 5 birds each were used. All birds of group C1 were injected in the left pectoral muscle with 100 mg/kg of chloramphenicol 3 times a day for 5 days. Group C2 birds were treated with 200 mg/kg of the drug twice daily for 5 days. In group C3, the birds were treated in the same way as those in group C2, except that the dose rate was 300 mg/kg. The birds in the control group were injected with 0.09 ml/bird of physiological saline twice daily for 5 days. Injection volume and interval were the same as those used in group
C3. Venous blood samples (0.8 ml) were collected 24 hr after the treatment ended. All blood samples were collected under anesthesia as described above.

Budgerigars were weighed before and after treatment. Statistical analysis between pretreatment and posttreatment body weights was performed using Student's t-test.

Blood chemical analysis: Red blood cell (RBC) counting, hemoglobin (Hb), packed cell volume (PCV), total plasma protein (TP), serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and creatine kinase (CK) were determined following usual laboratory methods. All the results were expressed as mean ± SE. The significance of differences between control and experimental groups was determined by Student's t test.

Histology: Two birds from the control group and group C3 were killed after blood collection, and samples from the following organs were collected: brain, eye, pituitary, lung, heart, spleen, kidney, adrenal gland, pancreas, liver, gizzard, large and small intestine, pectoral muscle, leg muscle, sciatic nerve and bone marrow. Tissues were fixed in 10% neutral-buffered formalin and embedded in paraffin, sectioned at 4 μm and stained with haematoxylin and eosin (HE).

RESULTS

Pharmacokinetic analysis: Following administration of the 2 mentioned doses of chloramphenicol, serum concentration data were fitted to a 1-compartment model described by the following equation:

\[ C_p = C_0 e^{-K_e t} \]

where \( C_p \) is the concentration of chloramphenicol at time \( t \), \( C_0 \) is the extrapolated serum concentration at time 0, \( K_e \) is the 1st-order rate constant of elimination and \( e \) represents the base of natural logarithm (Table 1). Rapid absorption of chloramphenicol occurred at the IM injection sites. Following IM administration of 100 to 200 mg/kg, serum chloramphenicol concentrations of 35.3 μg/ml and 90.7 μg/ml, respectively, were obtained at 0.25 hr, and those values declined with a terminal half-life of 2.5 and 2.7 hr respectively. Mean chloramphenicol concentration at 8 hr after the administration of 100 mg/kg was 5.0 μg/ml, and that at 12 hr after administration of 200 mg/kg was 4.7 μg/ml (Fig. 1).

Tolerability assays: PCV decreased in groups C1 and C3 (Table 2). There was no significant difference in RBC, Hb, TP, AST, ALT, LDH and CK between the control group and the treated groups, even though AST and ALT showed a tendency to rise in the chloramphenicol-treated birds. Body weight decreased significantly in group C3 (Table 3).

Microscopic finding: No histological alterations were detected in any of the tissues examined except for the pectoral muscle, where necrosis, hemorrhages and infiltration by macrophages and heterophils were observed.

![Fig. 1. Serum concentration-time profiles of chloramphenicol following a single intramuscular administration of 100 mg/kg (○) or 200 mg/kg (●) to healthy budgerigars. Each value represents an average of three independent experiments.](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>100 mg/kg</th>
<th>200 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₀</td>
<td>μg/ml</td>
<td>41.9 [34.0-51.6]</td>
<td>91.5 [68.4-122.3]</td>
</tr>
<tr>
<td>Kₑ</td>
<td>hr⁻¹</td>
<td>0.272 [0.238-0.307]</td>
<td>0.261 [0.214-0.308]</td>
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<tr>
<td>T₁/₂</td>
<td>hr</td>
<td>2.5 [2.3-2.9]</td>
<td>2.7 [2.3-3.2]</td>
</tr>
<tr>
<td>Cₘₐₓ</td>
<td>μg/ml</td>
<td>35.3 [27.7-42.9]</td>
<td>90.7 [55.3-126.0]</td>
</tr>
<tr>
<td>Tₘₐₓ</td>
<td>hr</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>AUC</td>
<td>μg·hr/ml</td>
<td>157.2±10.0</td>
<td>357.8±25.0</td>
</tr>
</tbody>
</table>

C₀: Extrapolated serum concentration at time 0.
Kₑ: First-order rate constant of elimination.
T₁/₂: Half-life of the drug.
Cₘₐₓ: Maximum serum drug concentration.
Tₘₐₓ: Time of peak serum concentration.
AUC: Area under the serum concentration time curve.
a) 95% confidence interval.
b) SE.

Cellular infiltration was prominent in the muscles of chloramphenicol-treated birds (Fig. 2).

DISCUSSION

Since absorption of chloramphenicol after IM administration occurred very rapidly, an estimate of the rate constant of absorption with our sampling procedure was impossible to be determined. The rapid absorption of chloramphenicol after intramuscular administration was also reported in Chinese spot-billed ducks [4]. The
Table 2. Blood chemical values (mean±SE) in budgerigars at 24 hr after treatment

<table>
<thead>
<tr>
<th></th>
<th>Control (n=5)</th>
<th>C1 (n=5)</th>
<th>C2 (n=5)</th>
<th>C3 (n=5)</th>
</tr>
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<tbody>
<tr>
<td>RBC (×10⁶/mm³)</td>
<td>539.4±27.2</td>
<td>534.2±56.2</td>
<td>486.4±60.7</td>
<td>492.0±43.1</td>
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<tr>
<td>PCV (%)</td>
<td>56.8±1.6</td>
<td>47.4±3.7</td>
<td>49.6±4.8</td>
<td>49.6±2.7</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>18.9±1.7</td>
<td>16.8±1.5</td>
<td>17.5±2.3</td>
<td>16.4±1.7</td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>3.2±0.2</td>
<td>3.2±0.1</td>
<td>3.2±0.3</td>
<td>2.6±0.4</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>726.0±145.5</td>
<td>1564.0±602.6</td>
<td>1366.0±321.3</td>
<td>1172.0±220.4</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>21.6±7.9</td>
<td>44.4±10.4</td>
<td>49.4±17.0</td>
<td>42.8±14.0</td>
</tr>
<tr>
<td>LDH (IU/l)</td>
<td>273.2±54.1</td>
<td>256.8±74.8</td>
<td>220.8±33.3</td>
<td>266.8±32.0</td>
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<tr>
<td>CK (IU/l)</td>
<td>2487.6±840.2</td>
<td>4615.0±1488.9</td>
<td>5233.8±1963.1</td>
<td>3130.4±724.3</td>
</tr>
</tbody>
</table>

C1: Injection of 100 mg/kg of chloramphenicol 3 times a day for 5 days.
C2: Injection of 200 mg/kg of chloramphenicol twice daily for 5 days.
C3: Injection of 300 mg/kg of chloramphenicol twice daily for 5 days.
Control: Injection of 0.09 ml/bird of physiological saline twice daily for 5 days.
a) Significant difference (P<0.05) from control.

Table 3. Body weight (mean±SE) of budgerigars before and after chloramphenicol treatment

<table>
<thead>
<tr>
<th>Body weight (g)</th>
<th>Control (n=5)</th>
<th>C1 (n=5)</th>
<th>C2 (n=5)</th>
<th>C3 (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>33.4±1.4</td>
<td>36.6±2.4</td>
<td>33.0±1.9</td>
<td>32.8±1.0</td>
</tr>
<tr>
<td>Posttreatment</td>
<td>33.4±1.5</td>
<td>34.0±1.8</td>
<td>32.0±1.6</td>
<td>28.5±0.8</td>
</tr>
</tbody>
</table>

C1: Injection of 100 mg/kg of chloramphenicol 3 times a day for 5 days.
C2: Injection of 200 mg/kg of chloramphenicol twice daily for 5 days.
C3: Injection of 300 mg/kg of chloramphenicol twice daily for 5 days.
Control: Injection of 0.09 ml/bird of physiological saline twice daily for 5 days.
a) Significant difference (P<0.05) from pretreatment body weight.

The half-life of chloramphenicol following an IM injection of 100 mg/kg or 200 mg/kg was slightly longer than that reported in budgerigars [2].

During the varied regimen studied in the present experiment, the administration of 100 mg/kg to budgerigars every 8 hr provided serum concentrations greater than 5 µg/ml after injection, and administration of 200 mg/kg every 12 hr provided serum concentrations greater than about 5 µg/ml. A chloramphenicol serum concentration of about 5 µg/ml is generally considered effective for therapeutic purposes [4, 16]. However, since MIC of chloramphenicol against some organisms is greater than 5 µg/ml [3, 12], MIC for the organisms causing infection before antibiotic therapy must be determined. A dosage regimen of 100 mg/kg of chloramphenicol 3 times daily or 200 mg/kg twice daily is considered to be more effective in infected budgerigars than the dose of 50 mg/kg twice daily adopted by Clark et al. [2], who obtained a low chloramphenicol level (approximately 0.7 µg/ml).

The average AST, ALT and CK activity in the chloramphenicol-treated budgerigars was not significantly higher than that of the control group. Compared with normal untreated budgerigars in the previous papers [7, 15], AST and ALT values of chloramphenicol-treated birds were considered to be extremely high. The most likely source of AST and ALT elevation in the present study was injury inflicted on the pectoral muscles, because no histological changes were detected in any of the other organs examined. Enhanced CK activity has been observed in budgerigars following IM injection of gentamicin [8]. However, it was not possible to compare the CK activity observed in the present study with that reported in previous papers [7, 15], since CK activity was not reported in previous studies. The average ALT and CK activity found in group C2 was higher than that in groups C1 or C3. This discrepancy may be attributed to experimental error.

PCV decreased significantly in the group treated with 100 mg/kg of chloramphenicol 3 times a day or 300 mg/kg twice daily; however, RBC and Hb values did not show any significant changes, and no histological alterations were detected in the bone marrow. Moreover, the groups treated with 200 mg/kg or 100 mg/kg revealed no significant decrease of body weight. The dosage regimen

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of 200 mg/kg for 5 days was considered to be safe. Studies in ducks demonstrated that a dose of 500 mg/kg/day was slightly toxic, but a duck given 1,500 mg/day died in 14 days [13]. The average chloramphenicol LD₅₀ in 4-week-old chickens proved to be 3,100 mg/kg [18]. Compared to man, avian species may be very resistant to the effects of chloramphenicol.

An intramuscular dosage regimen of 200 mg/kg of chloramphenicol twice daily for 5 days was recommended as effective against many of the common bacterial infections in budgerigars. The only adverse effect found in our study was muscle injury at the injection site which may be considered as a negligible effect.

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REFERENCES