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A dosage regimen of oxytetracycline for budgerigars *(Melopsittacus undulatus)*

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Introduction

Oxytetracycline, a broad-spectrum antibiotic, is commonly used as an effective antibacterial agent in pet birds. While information on the pharmacological properties of oxytetracycline or long-acting oxytetracycline in some birds has been reported[2,5,14,16], data on the pharmacokinetics and tolerability of oxytetracycline in budgerigars are limited. The recommended dose of oxytetracycline for pet birds is often based on clinical experience and pharmacokinetic data collected from other species[3].

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

Materials and Methods

Birds and chemicals

The experiments were carried out in 97 one-year-old clinically healthy budgerigars weighing an average of 31.5 g, and purchased from Sapporo Kotori Shokai (Sapporo). The birds were housed in cages at 23°C. Feed and water were supplied *ad libitum*.

Oxytetracycline hydrochloride (Terramycin injectable solution) was obtained from Sankyo Co. Ltd. (Tokyo).

Serum oxytetracycline concentration

Oxytetracycline hydrochloride was injected into the birds' pectoral muscle at the dose of 50 mg/kg (0.03 ml/30 g bird), 100 mg/kg (0.06 ml/30 g bird) and 150 mg/kg (0.09 ml/30 g bird). Serum oxytetracycline concentration was measured after a single intramuscular (IM) administration. Because of the difficulty encountered in collecting multiple blood samples from a single bird, a population of birds was used and data were combined for analysis. Three groups of 24 budgerigars were divided further into 6 groups of 4 birds. Group I birds received a dose of 50 mg/kg, those of group II received 100 mg/g and those of group III received 150mg/kg. Venous blood samples (0.6 ml) were collected once from 4 birds in each group at one of the following times : 1, 2, 4, 6, 12 and 18 hr after administration of 50 mg/kg or 100 mg/kg and 1, 2, 6, 12, 18 and 24 hr after administration of 150mg/kg dosage. Blood was collected from the jugular vein while the birds were under anesthesia. Each bird was anesthetized with 0.5 mg/bird of xylazine (Bayer Ltd., Tokyo) and 1.25 mg/bird of ketamine (Sankyo Co. Ltd., Tokyo). The blood samples were centrifuged

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and the serum was assayed for oxytetracycline by bioassay.

Serum oxytetracycline concentration was determined using a modification of the bioassay described by Yonezawa[18]. *Bacillus stearothermophilus var. calidolactis* purchased from the Japan Dairy Technical Association (Tokyo) was the test organism. The assay medium was made of commercial agars (Mil-P No. 1, No. 2 and No. 3, Kyokuto Pharm. Co. Ltd, Tokyo) according to the manufacturer's instructions. Six standards, ranging from 0.5 to 16 μg of oxytetracycline/ml, were prepared in sterile 0.1 M potassium dihydrogen phosphate buffer (pH 4.5). Paper discs (Toyo Roshi Co. Ltd., Tokyo) containing 20 μl of each standard solution or each serum sample were placed on the surface of the agar containing the test organisms. After incubation for 18 hr at 55°C, the diameter of the zone was measured and the oxytetracycline concentration in each sample was determined. The diameter of the zone of inhibition was measured to the nearest 0.1 mm. Activity in each sample was determined from the average of at least 2 replicates.

Tolerability assays

Five groups, S, T50, T100, T150 and a control group of 5 birds were used. All birds of group T50 were injected into the left pectoral muscle with 50 mg/kg of oxytetracycline hydrochloride every 12 hr for 5 days. Group T100 birds were treated with 100 mg/kg of the drug every 12 hr for 5 days. In group T150, the birds were treated with 150 mg/kg of the drug every 12 hr for 5 days. In group S, the birds were treated in the same way as those in group T150, except that physiological saline was used instead of oxytetracycline. Untreated healthy budgerigars served as the control. Venous blood samples (0.8 ml) were collected at 24 hr after the treatment ended. All blood samples were collected under anesthesia as described previously. Budgerigars were weighed before and after treatment. Statistical analysis between pre- and post-treatment body weight was performed using Student's t test.

Blood analysis

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CPK) and uric acid were determined by following usual laboratory methods. All the results were expressed as mean $\pm$ SE. The significance of differences between experimental groups was determined by Duncan's multiple range test.

Histopathology

After the blood collection, two randomly chosen birds from each group were euthanatized and the following organs were collected: brain, eyes, pituitary, lungs, heart, spleen, kidneys, adrenal glands, pancreas, liver, gizzard, small and large intestine, pectoral muscle, leg muscle, sciatic nerve and bone marrow. The tissue samples were fixed in 10% neutral-buffered formalin solution and embedded in paraffin wax, and sections were prepared for staining with haematoxylin and eosin.

Results

Serum oxytetracycline concentration

Following IM administration of oxytetracycline hydrochloride at 50, 100 and 150 mg/kg, serum peak concentrations of 6.7, 10.7 and 13.5 μg/ml, respectively, were obtained at 1 to 2 hr following injection (Fig. 1-3). Mean serum oxytetracycline concentrations with the

50 mg/kg dose exceeded about 5 μg/ml for 2 hr. With the 100 mg/kg dose, mean serum oxytetracycline concentration exceeded 5 μg/ml for 12 hr and the concentration exceeded about 5 μg/ml for 18 hr using the 150 mg/kg dose.

Tolerability assays

In all groups, LDH and uric acid values did not change significantly (Table 1). AST, ALT and CK in group T150 were significantly higher than those in the control group or those in group S. CK in group T150 was significantly higher than that in group T50, whereas AST and ALT showed no significant differences among the oxytetracycline-treated groups.

Body weight in the groups treated with 50 mg/kg to 150 mg/kg revealed no significant decrease (Table 2).

Histopathological findings

No prominent histopathological alterations were detected in any of the tissue samples except for the pectoral muscle, where necrosis and hemorrhage, including infiltration of macrophages and heterophils, were observed. Cellular infiltration was prominent in the muscle of oxytetracycline-treated birds.

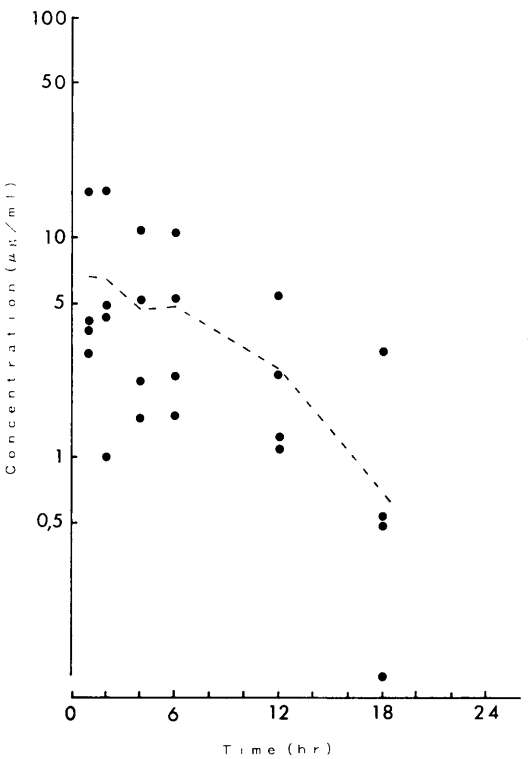

Fig. 1. Serum concentration-time profiles of oxytetracycline following a single intramuscular administration of 50 mg/kg of oxytetracycline hydrochloride to healthy budgerigars. The dotted line represents the mean of four independent birds.

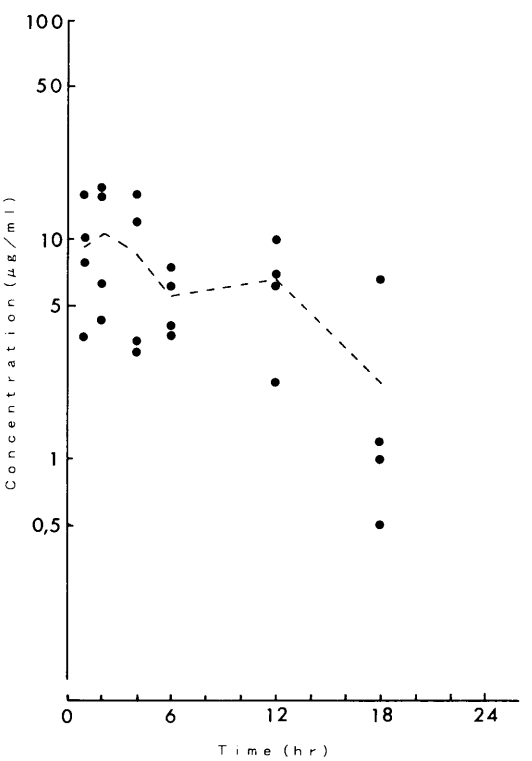

Fig. 2. Serum concentration-time profiles of oxytetracycline following a single intramuscular administration of 100 mg/kg of oxytetracycline hydrochloride to healthy budgerigars. The dotted line represents the mean of four independent birds.

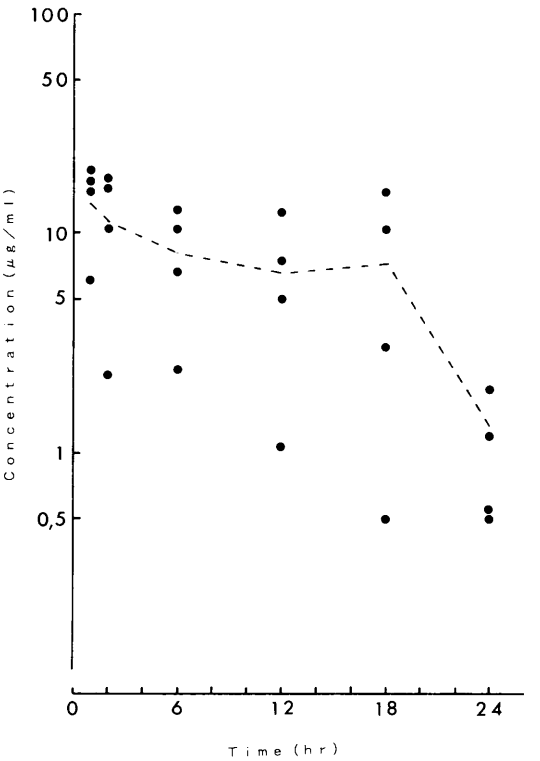

Fig. 3. Serum concentration-time profiles of oxytetracycline following a single intramuscular administration of 150 mg/kg of oxytetracycline to healthy budgerigars. The dotted line represents the mean of four independent birds.

Table 1. Blood chemical values (mean±S. E.) in budgerigars at 24 hr after treatment

	Control (n=5)	S (n=5)	T 50 (n=5)	T 100 (n=5)	T 150 (n=5)
AST (IU/L)	348^{a} ±57	726^{ab} ±110	1451^{bc} ±649	1040^{bc} ±465	1926^{c} ±106
ALT (IU/L)	8.6^{a} ±1.9	21.6^{a} ±7.8	89.6^{ab} ±40.0	43.0^{ab} ±12.9	76.6^{b} ±27.5
LDH (IU/L)	313^{a} ±55	273^{a} ±54	354^{a} ±158	479^{a} ±98	551^{a} ±116
CK (IU/L)	1567^{a} ±340	1879^{a} ±840	2395^{a} ±1071	2755^{ab} ±356	4343^{b} ±766
Uric acid (mg/dl)	7.5^{a} ±1.5	8.3^{a} ±1.7	8.9^{a} ±4.0	7.0^{a} ±1.3	8.4^{a} ±1.3

Control: Untreated normal birds.
S: Injection of 0.09 ml/30 g bird of physiological saline every 12 hr for 5 days.
T50: Injection of 50 m/kg of oxytetracycline hydrochloride every 12 hr for 5 days.
T100: Injection of 100 mg/kg of oxytetracycline hydrochloride every 12 hr for 5 days.
T150: Injection of 150 mg/kg of oxytetracycline hydrochloride every 12 hr for 5 days.
Means in each colomn followed by the same superscript are not significantly different ($P<0.05$).

Table 2. Body weight (mean±S. E.) of budgerigars before and after oxytetracycline treatment

Body weight (g)	S (n=5)	T 50 (n=5)	T 100 (n=5)	T 150 (n=5)
Pre-treatment	33.4±1.4	31.2±3.5	28.8±1.4	32.2±0.8
Post-treatment	33.4±1.5	28.6±2.5	28.4±1.4	29.6±2.2

S: Injection of 0.09 ml/30 g of physiological saline every 12 hr for 5 days.
T50: Injection of 50 mg/kg of oxytetracycline hydrochloride every 12 hr for 5 days.
T100: Injection of 100 mg/kg of oxytetracycline hydrochloride every 12 hr for 5 days.
T150: Injection of 150 mg/kg of oxytetracycline hydrochloride every 12 hr for 5 days.

Discussion

Tetracyclines have a wide antibacterial spectrum; the minimum inhibitory concentration (MIC) varies widely between different species of bacteria. The average MIC for oxytetracycline for avian strains of *Pasteurella* spp. varied from 0.08 to 4.0 μg/ml[7]. Some bacteria have developed resistance to oxytetracycline. In 309 strains of *E. coli* isolated from pet birds imported to Japan, 70.6 percent were resistant to oxytetracycline[12]. In those sensitive strains, the MIC varied from 1.56 to 6.25 μg/ml. The minimum serum concentration of 5 μg/ml was chosen in the present experiment because the serum concentration 5 μg/ml is considered to be therapeutic for the majority of microorganisms that show susceptibility to oxytetracycline[1,16]. With the 100 mg/kg dose, mean serum oxytetracycline concentration exceeded 5 μg/ml for 12 hr and the concentration exceeded about 5 μg/ml for 18 hr using the 150 mg/kg dose; however, oxytetracycline concentrations with the 50 mg/kg dose exceeded 5 μg/ml for only 2 hr. In order to treat infected birds, a dosage regimen of 100 to 150 mg/kg every 12 hours is considered to be effective. However, one has to determine the MIC for the organisms causing the infection before using such a dosage regimen.

AST and LDH activity and uric acid concentration in the control birds were compatible with those reported previously in healthy budgerigars[8,13]. This suggests that the values found in control birds might be regarded as normal.

In the present experiment, AST, ALT and CK were determined to be sensitive parameters of adverse effects of oxytetracycline injection. Enhanced AST activity has been observed in avian species following IM injection of various drugs[5,6,9,10]. Measuring CK activity after an IM injection has been reported to be a sensitive method to determine local toxicity[15]. Recently, ALT has been considered to be a good indicator of muscular injury[9,10]. The high AST, ALT and CK activities observed in birds injected with oxytetracycline may be due to injury inflicted on the pectoral muscles, because no apparent histopathological changes were detected in any other organs examined. Enhanced LDH activity has been reported to be induced by injury to various organs[11,17], but in this study, LDH was not significantly elevated in any of the treatment groups. The level of uric acid was not a sensitive parameter for determination of the adverse effects of oxytetracycline.

Compared with birds treated with 50 mg/kg of oxytetracycline hydrochloride, the average value for CK activity was markedly higher in the birds treated with 150 mg/kg dose. Obviously, the degree of tissue damage depended on the increase in the dosage rate.

Body weight in the groups treated with 50 mg/kg to 150 mg/kg revealed no significant decrease. Therefore the dosage regimen of 50 to 150 mg/kg for 5 days was seen to

have comparatively little toxic effect. Very few toxicological studies on tetracycline, oxytetracycline, doxycycline and minocycline have been performed on birds. The acute toxicity (LD_{50}) dose of doxycycline given in the crop of one-week-old chickens was 2500 mg/kg[4].

As the recommended dose of oxytetracycline for budgerigars has been based on clinical experience and data extrapolated from other species, it is important that the dosage regimen be based on pharmacokinetic data, as shown in this study.

Summary

A dosage regimen and tolerability to oxytetracycline hydrochloride were evaluated in budgerigars. Serum oxytetracycline was determined using a bioassay. Following intramuscular administration at 50, 100 and 150 mg/kg of body weight, serum peak concentrations were obtained 1 to 2 hr following injection. Mean serum oxytetracycline concentrations wth the 50 mg/kg dose exceeded about 5 μg/ml for 2 hr. With the 100 mg/kg dose, the concentration exceeded 5 μg/ml for 12 hr and the concentration exceeded about 5 μg/ml for 18 hr using the 150 mg/kg dose. As the minimum serum concentration of 5 μg/ml is considered to be therapeutic, an intramuscular dosage regimen of 100 to 150 mg/kg of every 12 hr was recommended as effective against many of the common bacterial infections in budgerigars. Serum AST, ALT, LDH, CK and uric acid values as well as body weight were determined after intramuscular injection of oxytetracycline hydrochloride every 12 hr for 5 days, then tissue samples from various organs were examined histologically. AST, ALT and CK activities in oxytetracycline-treated birds were significantly elevated. The most prominent adverse effect was muscular damage at the injection site. A dosage regimen of 50 to 150 mg/kg every 12 hr for 5 days was considered to have little toxic effect.

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要　　約

セキセイインコにおけるオキシテトラサイクリンの投与量と投与間隔を検討した。血清濃度の測定には生物検定法を用いた。50 mg/kg, 100 mg/kg または 150 mg/kg の塩酸オキシテトラサイクリンを胸筋に筋肉注射後，1-2 時間で最高血中濃度に達した。その後 100 mg/kg 投与では 12 時間にわたって 5 μg/ml 以上の血中濃度が維持され，150 mg/kg 投与時には 18 時間にわたって 5 μg/ml 以上が維持された。有効血中濃度は 5 μg/ml と考えられているので，100 mg/kg または 150 mg/kg を 12 時間毎に 1 日 2 回投与することによりセキセイインコの多くの感染症の治療に有効であると思われた。50 mg/kg，100 mg/kg，150 mg/kg の塩酸オキシテトラサイクリンあるいは生理食塩水を 1 日 2 回 5 日間筋肉内注射後，血清 AST，ALT，LDH，CK 活性および尿酸濃度を測定した。また無処置鳥の値も測定した。実験前後の体重も測定し，さらに全身臓器を病理組織学的に検索した。無処置群に比べてオキシテトラサイクリン注射群で AST，ALT，CK 活性値の上昇を認めた。注射の影響としては胸筋注射部位の筋肉障害が主なものであった。50-150 mg/kg を 12 時間毎に 1 日 2 回 5 日間投与する方法は比較的毒性が少ないと思われた。