Pharmacokinetics of Doxycycline after Parenteral Administration in the Houbara Bustard (Chlamydotis undulata)

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SUMMARY. Fourteen adult Houbara bustards (Chlamydotis undulata) infected with Chlamydia psittaci were administered doxycycline at 100 mg/kg body weight in seven intramuscular or subcutaneous injections at intervals of 7, 7, 7, 6, 6, and 5 days. Blood levels of doxycycline were measured after the first and seventh injections at the following intervals: 0, 12, 24, 36, 48, 72, 96, 120, 144, 168, and 180 hours. During these two periods, most of the birds showed plasma doxycycline levels in excess of 1 µg/ml, demonstrating effective doxycycline levels for 45 days. A multifactorial analysis of variance revealed no significant differences between males and females, between the two routes of administration, or among all four factors.

RESUMEN. Farmacocinética de la doxiciclina después de la administración parenteral en avutardas hubara (Chlamydotis undulata).

Se trataron 14 aves avutardas hubara (Chlamydotis undulata) infectadas con Chlamydia psittaci, con doxiciclina a una dosis de 100 mg/kg de peso corporal, en siete inyecciones vía intramuscular o subcutánea a intervalos de 7, 7, 7, 6, 6 y 5 días. Los niveles de doxiciclina en la sangre fueron medidos después de la primera y la séptima inyección a los siguientes intervalos: 0, 12, 24, 36, 48, 72, 96, 120, 144, 168 y 180 horas. Durante estos dos periodos, la mayoría de las aves mostraron niveles de doxiciclina mayores de 1 µg/ml, demostrando niveles efectivos de doxiciclina por 45 días. Un análisis multifactorial de varianza no reveló diferencias significativas entre hembras y machos, entre las dos rutas de administración o entre todos los factores.

Chlamydirosis was diagnosed in a flock of Houbara bustards (Chlamydotis undulata) in Saudi Arabia after several birds died. Captive breeding of these birds is part of the conservation program being conducted by the National Commission for Wildlife Conservation and Development in Saudi Arabia. Progeny bred at the National Wildlife Research Center are to be reintroduced into selected protected areas in the near future. To maximize the chances of the birds being released during the reintroduction program and to avoid the risk of spreading Chlamydia psittaci to the native wildlife (3), it was decided to attempt the eradication of C. psittaci from the breeding flock by means of therapeutic and sanitary measures.

Tetracyclines are recommended or even prescribed by law for the treatment of C. psittaci infections in several countries. As psittacosis is a zoonanthropotic disease and may be transmitted from psittaciformes to humans, broad experience has been gained with psittaciformes, mainly with the more widely kept species. In other species that are also known to spread psittacosis to humans, such as turkeys and ducks, pharmacological data are poorly documented. In the present study, we chose parenteral treatment with doxycycline, instead of the other tet-
racycline derivatives, for various reasons (see Discussion).

Generalization of the results with one avian species to the others should be done carefully and should take in account such parameters as body size and interspecies variations (4,20). The present study was thus set up to evaluate the toxicity of the drug, the serum concentration of doxycycline, and the time of elimination in Houbara bustards, before the 250 birds from the breeding unit were treated. No reports on tetacycline treatment in bustards or the related gruiformes were found in the accessible literature.

MATERIALS AND METHODS

Birds. Seven adult male and seven adult female Houbara bustards, all between 3.5 and 4.5 years of age, were used. All were of the subspecies undulata. All birds were healthy, and none had received any antibiotics during the 2 months before the study. Birds were randomly allotted into four isolation rooms at the quarantine station. The males weighed 1596 ± 149 g (1330–1765 g) (mean ± S.D. [range]) at the beginning of treatment, and the females weighed 1101 ± 89 g (985–1225 g). During the first sampling period in mid-October, the average maximum temperature was 31.8 C (31.5–32 C), and the average minimum temperature was 16.7 C (15–20 C); during the second sampling period in mid-November, average maximum and minimum temperatures were 27.1 C (25.5–29 C) and 9.4 C (5–12 C). The natural daylight period lasted about 13 hr. All the birds had free access to water and feed.

Doxycycline administration. Ten birds (five males, five females) were given a 2% doxycycline formulation (Vibraenös R; Pfizer GmbH, Karlsruhe, Germany) intramuscularly (IM) into the pectoral muscles. Four birds (two males, two females) were given the same formulation subcutaneously (SQ) at the base of the dorsal neck region. The dose was 100 mg/kg body weight (BW). Birds were weighed before each injection, and doses were calculated precisely. Seven injections were given over a period of 38 days at the following intervals: 7, 7, 7, 6, 6, and 5 days.

Blood-sample collection and bioassay. Blood samples were collected in two series: after the first injection and after the seventh. Blood (0.5 ml) was collected from the vena cutanea ulinartis. After the first injection, the sampling times were: 0 (control before injection), 12, 24, 36, 48, 72, 96, 120, 144, and 168 hr (just before the second injection), and 180 hr. After the seventh injection, the sampling times were: 0 (control before injection), 12, 24, 36, 48, 72, 96, 120, 144, and 168 hr. Individual blood samples were mixed with the buffer at a 1:2 ratio. Each 100 ml of buffer contained 0.1 M of a sodium-dihydrogen-phosphate solution (Merck, Darmstadt, Germany) with an added 1 g ammonium-oxalate-monohydrate (Merck). After pH was adjusted to 4.5 with hydrochloric acid, the buffer was boiled for 30 minutes at 100 C and kept in cold storage. The blood-buffer mixture was frozen at −20 C until samples were sent to the laboratory. Five birds that did not receive doxycycline were used as controls.

Laboratory technique. Doxycycline concentrations in the blood samples were determined using a modification of Bacillus cereus method for the determination of CTC in blood and feed (27). Two paper discs, each with a diameter of 6 mm, were dipped into each thawed blood sample and placed on the agar plates seeded with B. cereus var. mycoides (ATCC 11778, American Type Culture Collection, Rockville, Md.; concentration of B. cereus 2.5 × 106 spores/ml agar). The assays were incubated for 4 hr at 4 C and thereafter for 18 hr at 30 C. The zones of inhibition were compared with a standard curve in order to calculate the doxycycline concentration. The doxycycline standard curve ranged between 100 μg/ml and 0.0125 μg/ml.

Pharmacokinetic calculations. PK-Calc software, coupled with ESTRIP (24) was used to calculate the kinetic parameters from the mean plasma concentrations. The parameters obtained after each injection were the following: area under the curves (AUC), area under the moment curve (AUMC), mean residence time (MRT), plasma peak values (Cmax and Tmax), absorption (Tα, abs.) and elimination (Tε, el.) half times. A two-compartment open model with absorption phase was selected for each series.

Statistics. A multifactorial analysis of variance using a confidence level of 95% was performed for the two sampling periods for males, females, IM route, and SQ route (1).

RESULTS

Plasma concentration-time profiles were plotted using mean concentrations for each time period for the IM and SQ routes during the two sampling periods (Figs. 1 and 2). There were no significant differences between males and females, between the two routes, or between the four parameters. Because no sex difference was found, all values were grouped together for the pharmacokinetic evaluation. Table 1 shows calculated kinetic parameters. Very slight differences were observed between the first and last injection, either after SQ or IM administration. The extent of absorption, characterized by the AUC, was the only parameter that increased
at the end of the treatment period. Absorption half-life was prolonged only after multiple SQ injections. Generally speaking, the extent of absorption was the same after IM or SQ injections, but SQ administration resulted in a slightly more rapid elimination, correlated with a slower absorption. In view of the results of the samples collected for 8 days from day 38 onward, which were above 1 μg/ml even on the last day, it may be assumed that, on average, the minimum con-

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<th>Parameter*</th>
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<td>58.93</td>
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* AUC = area under the curve; AUMC = area under the moment curve; MRT = mean residence time; Cmax = plasma concentration peak value; Tmax = plasma concentration peak time; T1/2 abs. = absorption half-time; T1/2 el. = elimination half-time.

Concentration of 1 \( \mu \text{g/ml} \) was maintained in the plasma over the entire 45-day period.

Analysis of samples from control birds gave completely negative results.

None of the birds died during the treatment period. Body weights decreased considerably during the first week, probably due to the stress of handling once or even twice a day, and then increased again, indicating a good state of general health and good tolerance of the drug. An unimportant side effect was that the pigmentation of the iris became darker. This process was reversible after treatment ended.

Macroscopic changes in the muscles at the injection site were moderate, even after the seventh injection. A small hemorrhage was occasionally noticed, as was some doxycycline oozing from the administration site. The SQ injection site showed slight irritation, sometimes in the form of a thickening of the skin or mild inflammation.

**DISCUSSION**

The tetracyclines are considered cross-sensitive with regard to bacterial sensitivity to *C. psittaci*, but many pharmacological differences have been observed between the various derivatives. Chlortetracycline (CTC) used in mash or pelleted feeds is often poorly accepted, with the result that the generally accepted standard blood level of 1 \( \mu \text{g/ml} \) (28) is achieved only after a delay, if at all (17,18,19,21,22,29). Oxytetracycline (OTC) administered either orally or in the form of long-acting LA-200 (Pfizer GmbH, Karlsruhe, Germany) injections does not provide any advantages over CTC and may even cause local necrosis at the site of injection with prolonged application, although it does maintain effective blood levels for 2 or 3 days (8,25). The superior lipid-solubility of doxycycline provides better bioavailability and diffusion into the cells, where the initial bodies of *C. psittaci* develop (12). When administered IM, which is only possible with the European and Canadian preparation, at 75–100 mg/kg BW, efficient blood levels can be sustained for 6 or 7 days (9,18,19). The half-life times are species-specific and variable, and range in psittaciforms from approximately 10 hr to more than 20 hr (8). The half-life time is not fixed, rather, it decreases as the duration of treatment lengthens (for instance, from 27 hr to 12 hr over a 6-week course of application). This is probably due to drug enzyme induction (5,6). In contrast to the other tetracyclines, doxycycline is excreted in the feces without impact on the physiological intestinal flora (16). Immunosuppression is another side effect of all antibiotics, particularly the tetracyclines. A differentiation is made between an indirect transient increase in corticosteroid blood levels and a direct effect, which interferes with protein synthesis, phagocytosis, or antigen processing (23). The possible influence on the immune system should not be ignored, particularly as the microbiological agent is only inhibited by the treatment but still has to be eliminated actively by the immune system (11).

Little is known about the feeding behavior of the Houbara bustard, its tolerance to drugs, or
its acceptance of medicated feed. As a result of its adaptation to an arid environment, the Houbara bustard has developed a particular form of water metabolism, which is poorly understood and is characterized by irregular water consumption. This lack of data has led to the rejection of medication via drinking water. Treatment with long-acting injectable OTC LA-200 was rejected, because it requires an injection every 2 or 3 days (10) and thus excessive handling, and because of the possibility of local necrosis.

Parenteral treatment with doxycycline has been favored because of its longer-lasting efficacy, which reduces the number of times birds have to be handled. The injection intervals for the present study were chosen on the recommendation of Teichmann (26) and Jakoby (17,18). To date, no evidence of doxycycline resistance has been found in *Chlamydia* spp. isolated from pet birds (14). Gylstorff *et al.* (13) found no correlation between the highly variable individual doxycycline blood level and the clinical success of the treatment. This indicates that measuring blood levels alone does not allow for the effective evaluation of tissue concentrations. On the other hand, it has been proven that minimum inhibitory concentrations for avian *Chlamydia* strains in tissue cultures may be as low as 0.01–0.08 μg/ml (15).

SQ administration of the doxycycline appeared to result in serum levels comparable to those produced by IM application, despite slightly different absorption-elimination patterns. This was also noted by Black (2) with OTC in chickens and Dorrestein *et al.* (7) with doxycycline in pigeons. Peak values of doxycycline in blood after IM or SQ injection were between 5 and 10 μg/ml. In principle, adverse reactions cannot be excluded, particularly in birds that already have parenchymal lesions. Because of this quite high plasma peak level, the dosage should be reduced to approximately 80 mg/kg BW or even less, and SQ application might be generally more desirable. Further study is necessary to determine the exact dosage or whether longer intervals between injections are possible, particularly toward the end of the treatment period. The failure of blood levels to fall more rapidly during the treatment period could be interpreted as accumulation within the tissues. From the data reported here, it is not possible to draw any conclusions on the tissue levels or potential hazards (blood levels of 4–6 μg/ml for roughly 48 hr).

SQ injection is preferable, because of the large quantity of the drug that has to be injected (between 4 to 9 ml) and the risk of causing serious trauma to these wild birds, which are difficult to restrain. Moreover, some loss of the drug occasionally was observed after the IM injection.

The treatment was generally well tolerated. It cannot be determined from the data whether the weight loss during the first week of treatment was due entirely to the handling and not, at least in part, to the drug itself. A reversible change of color in the iris associated with tetracycline treatment is not described in the accessible literature.

The results of this experiment supported recommendations for treating Houbara bustards with doxycycline. Subsequently, all of the birds of the breeding unit were treated, at a dosage of 80 mg/kg BW of doxycycline administered SQ, which resulted in a radical decrease in mortality due to chlamydiosis.

REFERENCES


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