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THE PHARMACOKINETICS AND PHARMACODYNAMICS OF FLUMETHASONE IN CAMELS

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ABSTRACT

The pharmacokinetics (PK) and pharmacodynamics (PD) of flumethasone was evaluated in 6 healthy camels after a single intravenous bolus doses of 5 µg/kg body weight. The PD was performed by applying PK/PD modeling using cortisol, circulating lymphocytes, neutrophils and plasma glucose as biomarkers. Plasma flumethasone and cortisol concentrations were measured by validated liquid chromatography/mass spectrometry methods (LC-MS/MS). Plasma flumethasone *versus* time concentration were fitted by nonlinear regression and were best described by a two compartment model. The PK parameters (mean ± SD) were; terminal elimination half-life was 10.45 ± 0.65 h, total body clearance was 115.8 ± 7.99 ml/h/kg and volume of distribution at steady state was 1631.6 ± 116.03 ml/kg. The PD parameters showed that flumethasone is a very potent steroidal anti-inflammatory drug as reflected by the estimated low IC_{50} of flumethasone for cortisol and lymphocytes.

Key words: Camel, flumethasone, pharmacodynamics, pharmacokinetics

Flumethasone is a synthetic corticosteroid structurally similar to dexamethasone but with an additional fluorine atom at position 6. Corticosteroids are widely used in veterinary medicine to treat various lameness conditions (Ferguson and Hoenig, 1995). Despite the wide use of injectable flumethasone in race camels in The United Arab Emirates, yet there are no pharmacokinetics (PK) or pharmacodynamics (PD) reports in camels or in large animals. The aim of the present study was to develop a sensitive liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the detection of flumethasone and cortisol in camel plasma and to apply it for the evaluation of the PK and PD of flumethasone in camels after intravenous (i.v.) administration. Another goal of the study was to advise on a withdrawal period before camel racing following a therapeutic dose of flumethasone.

Materials and Methods

Animals and drug administration

Six clinically healthy male race camels 4-6 years old and body weight ranging in from 300-400 kg were used and kept in open pens. Good-quality hay and lucerne were fed once daily and water was provided *ad libitum*. This study was approved by the ethical committee in the Veterinary Department, Ministry of Agriculture. Flumethasone (Fluvet, 0.5 mg/mL; Zoetis, Mexico) was administered as a bolus intravenous (i.v.) injection (jugular vein) at a dose of 5.0 µg/Kg body weight (manufacturer recommended dose for bovine). Two blood samples were collected for analytical purposes in heparinised vacuum tubes from the opposite jugular vein at time 0 (predose) and at 5, 10, 15, 30, 45, 60 min and at 1.5,2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, 24 h timed after drug administration. A 3 mL blood sample was collected for the estimation of glucose, WBC, lymphocytes and neutrophils as reported previously (Al Katheeri *et al,* 2004a). Seven mL blood samples were collected for the quantification of plasma flumethasone and cortisol concentrations. Plasma was separated by centrifugation (2000 g for 10 min) and was stored at -20°C. Plasma samples were assayed within 10 days.

Analysis of plasma flumethasone

Flumethasone was extracted from plasma (1.0 mL) by solid phase extraction (C18) as reported previously (Al Katheeri *et al,* 2004a; 2004b). Cortisol-D3 was used as internal standard. The concentration of flumethasone and cortisol in plasma were measured by a validated liquid chromatography/mass spectrometry (LC/MS/MS). The LC system used was Agilent 1200 series with autosampler and column compartment

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(Agilent, USA). The chromatographic separation was performed using an Agilent Zorbax ZDB C18 column (3.5 m × 2 mm × 50 mm, Santa Clara, CA) linked to a Phenomenex pre-column filter (4 × 2 mm, Torrance, CA) operating in gradient mode at 35°C. The mobile phases were 0.1% formic acid (solvent A) and methanol (solvent B). A linear gradient was run at 0.3 mL/min, with 40% solvent B at the start (t = 0 min), increasing to 90% solvent B at t = 4 min. The gradient was then returned to 40% solvent B at t = 4.20 min and stabilised until t = 7.3 min before starting the next injection. The temperature of the autosampler tray and of the column compartment was set at 10 and 35°C, respectively. Ten µl was used for injection. Mass spectrometric analysis was performed on a 5500 Q-Trap mass spectrometer (ABSciex, Foster City, CA, USA) equipped with a turbo ion spray interface for electrospray ionisation (ESI) operated in positive ion mode. The source-dependent parameters were optimised using flow injection analysis (FIA) of flumethasone (20 ng/ml) into the mass spectrometer at 10 µL/min. Ion spray voltage was set at 5000 V and source temperature was at 550°C. Curtain gas, gas 1, and gas 2 were medium, 50, and 60 psi, respectively. Declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) used for each analyte were established and optimised during the tune procedure. Protonated molecules were used as precursor ions with selected reaction monitoring of the following transitions for flumethasone, cortisol and cortisol-D_3, respectively: 411–253 m/z, 363–121 m/z and 366–121 m/z.

The analytical method was validated at the beginning of the experiment. Linear calibration curves ($r > 0.999$) were obtained for flumethasone (0.5-20 ng/mL) and cortisol (0.5-50 ng/mL). The inter- and intra-assay coefficient of variations of the quality control samples (1.5, 7.5 and 15 ng/mL; n=9) were less than 9% and the accuracy was less than 15%. The limit of quantification of both analytes was 0.5 and ng/ml. The limit of detection (LOD) was 0.15 ng/ml.

Pharmacokinetic and pharmacodynamics analysis

Pharmacokinetic analysis of plasma flumethasone concentrations for each animal was performed using least – square nonlinear regression analysis program (WinNonLin Standard edition, version 4.0.1, Pharsight, Sunnyvale, CA, USA). One-, two- and three-compartment models were tested for the best fit to the i.v. administration data.

Weighting was achieved according to the variance modes: $var(t) = 1 / Y_{observed\ 2}$ where var (t) is the variance of the residual error of drug concentration at time t and Y observed is the observed drug concentration at time t. The best fit was based on Akaike (1976) and Schwarz (1978) criteria, analysis of residual plots and correlation matrix. The PK-PD surrogates, the reduction of plasma cortisol concentration and the number of lymphocytes and the increase of plasma glucose concentration and neutrophils number were calculated. Calculations were performed on individual data using least-square nonlinear regression analysis (WinNonLin Standard edition, version 1.5, USA). An indirect response model describing the PD effect of the drug with the mechanism producing the effect was used (Dayneka *et al,* 1993; Al Katheeri *et al,* 2004a; Al Katheeri *et al,* 2004b).

Results and Discussion

The mean serum concentration–time curves following the i.v. flumethasone administration are shown in Fig 1. Estimated PK parameters are summarised in Table 1. There are no reports on the PK of flumethasone in camels or in large species to compare our results with. However, flumethasone PK in camels was characterised by a long terminal elimination half-life, large volume of distribution and slow systemic clearance. Fluorinated compounds are reported to have increased lipophilicity (Wakefield, 2000) which might have contributed to the large volume of distribution and decreased clearance.

Table 1. Pharmacokinetic parameters of flumethasone following intravenous administration to 6 healthy male camels at a dose of 5.0 µg/Kg body weight. Data are expressed as mean and standard deviation.

Variable	Mean	Standard deviation
AUC (µg h^{-1} ml^{-1})	44.50	7.50
$t_{½\alpha}$ (h)	0.20	0.12
$t_{½\beta}$ (h)	10.40	1.59
VC (ml kg^{-1})	482.67	117.89
ClT (ml kg^{-1} h^{-1})	115.08	19.54
Vss (ml kg^{-1})	1631.64	284.21
AUMC (µg h^{-2} ml^{-1})	645.11	185.62

t½a= half–life of distribution phase; t½b= half–life of elimination phase; AUC= area under the curve to infinity; Vss= volume of distribution at steady state; ClT= total body clearance; Vc= volume of central compartment; AUMC= area under first moment curve.

Dexamethasone, a fluorine containing drug was also reported to have large volume of distribution, decreased clearance and long terminal elimination half-life (Al Katheeri *et al,* 2004a; Al Katheeri *et al,*

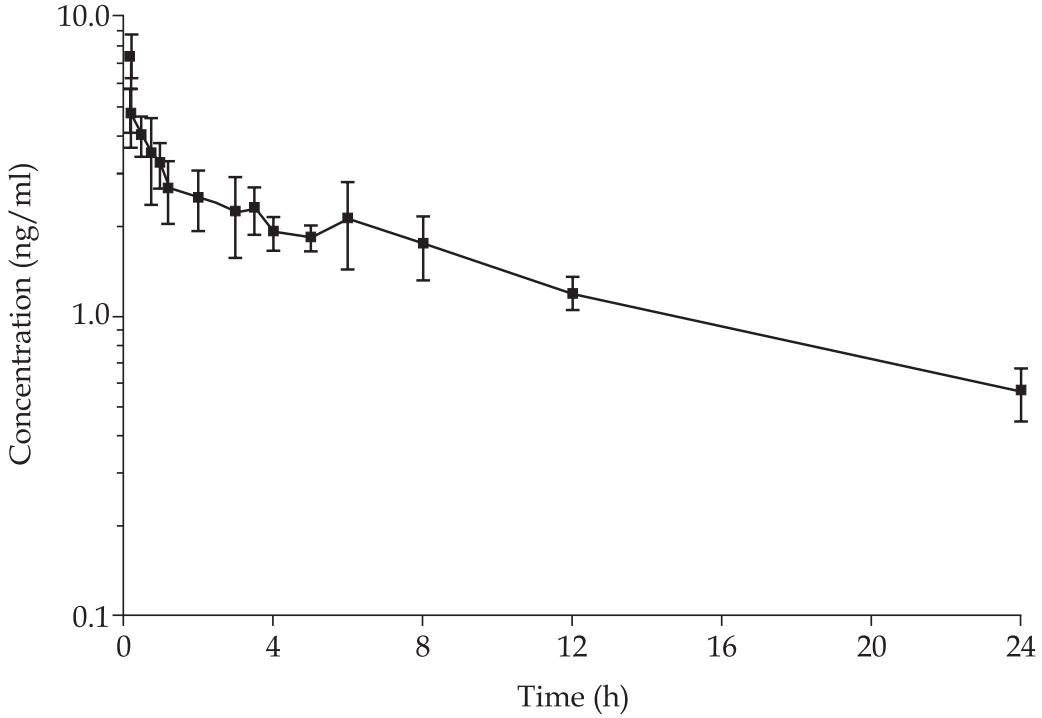

Fig 1. Flumethasone plasma concentrations-time profile of 6 male camels after an i.v. dose of 5.0 µg flumethasone/ Kg body weight. Values are presented as means ± standard deviation.

2004b). The estimated IC_{50} of flumethasone for cortisol and lymphocytes were 4.52 ± 1.33 and 3.57 ± 1.69 ng/ml, respectively. The EC_{50} for neutrophils and glucose were 33.5 ± 7.93 and 4.23 ± 0.82 ng/ml, respectively. The PD parameters showed that flumethasone is a very potent steroidal anti-inflammatory drug as reflected by the estimated low IC_{50} of flumethasone for cortisol and lymphocytes.

The plasma concentration of flumethasone at 24 h post administration was 0.56 ± 0.11 ng/ml. This means that in some animals, the plasma concentration of flumethasone would still be theoretically detectable 48 h post administration as reflected by the LOD of the method (0.1 ng/ml) and the long elimination terminal half-life (10.40 ± 1.59 h). Due to the large variation of pharmacokinetic parameters in animals, camel owners are therefore advised to withhold flumethasone use for a period of 2-3 days before racing.

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