EFFECT OF FEVER ON PHARMACOKINETICS OF OXYTETRACYCLINE IN CAMELS

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ABSTRACT
In this study, the effect of fever on the pharmacokinetics of long acting preparation of oxytetracycline (OTC) after intramuscular administration was investigated in camels. Injection of endotoxin lipopolysaccharide at a dose of 0.1 µg/kg in camels produced fever. Administration of long acting oxytetracycline at a dose of 10 mg/kg body weight intramuscularly to camels resulted in significantly higher concentration of antibiotic in febrile compared to normal camels. A higher mean plasma level, longer half-life and higher area under curve were observed in febrile compared to normal camels, suggesting favorable pharmacokinetic of oxytetracycline in febrile conditions. A high concentration of antibiotic in healthy and febrile condition 72 hours post-dosing would suggest persistent protection and efficacy for several days after a single intramuscular dose in camels.

KEYWORD: Camel, fever, Pharmacokinetics, intramuscular, oxytetracycline.

INTRODUCTION
Tetracyclines are one of the most extensively used antibiotics in the veterinary practice owing to its favourable pharmacokinetics and broad spectrum of antimicrobial efficacy. Tetracyclines are effective on gram-positive, gram-negative bacteria, Chlamydia, spirochetes and some protozoa (Prescott and Baggot, 1993). Oxytetracycline is a tetracycline with broad-spectrum antibacterial therapy, which normally requires some daily parenteral treatment (Roncada et al., 2000). It has been demonstrated that a long-acting formulation of OTC is the drug of choice for treatment of some acute and chronic diseases (Cornwell, 1980). Oxytetracycline is a valuable choice in both of systemic and localized or tissue infections due to its balanced distribution between the blood and tissues (MERCER et al., 1978; Grondel et al., 1987). The pharmacokinetics of long-acting preparations of OTC have been extensively studied in various animal species, including cattle (Craigmill et al., 2000), sheep (Nazawi et al., 1990), goat (Escudero et al., 1996), pigs (Escudero et al., 1996), fallow deer (Haigh et al., 1997) and camel (Al-Nazawi and Homeida, 2002). However, information on the pharmacokinetics of most of antibacterial in camel is limited. The objective of the present investigation is to determine the pharmacokinetic properties of long acting oxytetracycline in normal and febrile camels after intramuscular administration.

MATERIALS & METHODS
Animals
Eight clinically healthy males and females one humped camels (Camelus dromedarius) 3-6 years old and ranging in body weight from 220-320kg were used. They were kept in separate pens and allowed free access to hay and water. Drug administration
Animals were divided into two equal groups. Group 1, animals were given long acting oxytetracycline dihydrate (200mg/ml, Alamycin LA, Norbrook, UK) as a bolus i.m. injection at a dose of 10mg/kg body weight. The drug was also given to group 2 animals after induction of fever by endotoxin.

Induction of febrile state
Fever was induced by injecting lipopolysaccharide of E. coli (055: B) of (Difco Laboratories, Detroit, MI, USA) at a dose rate of 0.1µg/kg body weight intravenously (i.v.). A rise of rectal temperature of 1ºC 1/2 to 1 hour post injection is considered as febrile animals.

Collection of blood samples
Jugular blood samples were taken prior to and at 10, 20-, 30 minutes, 1, 2, 4, 6, 8, 16, 24, 72, 96 and 120 hours after administration of the drug. The blood samples were allowed to clot; serum is separated by centrifuged at 1200g for 5 minutes and stored at -20 ºC until analysis.

Analysis of oxytetracycline
Quantitation of oxytetracycline (OTC) in serum samples was accomplished using modification of the microbial inhibition assay (Nouws et al., 1990; Al-Nazawi and Homeida, 2002). Bioassay plates were prepared by placing 9.5g Mueller Hinton Medium (Difco Laboratories, Detroit, MI, USA) and 250ml H2O into 500ml Erlenmeyer flask which was autoclaved for 20 min solution then cooled to 50 ºC in a water-bath. One milliliter of a commercially available Bacillus cereus spore suspension (Difco Laboratories) was diluted in 50 ml of sterile saline and 0.4 ml of the diluted suspension is added to the media. After pouring and solidification of the media, 500 µl wells were cut into the solidified bioassay plates. Serum samples (500 µl) were placed directly into the wells without a cleanup step. Standards prepared using control serum was also added to each plate, and the plates are incubated overnight.
at room temperature (23 °C). Zones of inhibition were measured using micrometers and the results from the standards used to calculate the OTC concentration in each sample.

**Pharmacokinetic analysis**

The data from serum concentration of OTC in camels were analyzed using PKSolver (Zhang et al., 2010). Different models were used assuming noncompartamental, one compartment or two compartments kinetic model. Filtering and selection of the appropriate model was analyzed by examination of diagnostic parameters, taking r² as a measure of fitting accuracy. The Parameters calculated included area under the curve (AUC), distribution rate constants (a, b), y intercepts (A, B), Mean residence time (MRT), volume of distribution at steady state (Vss), half-life (t½), maximum concentration (Cmax) and time of maximum concentration (tmax). The area under curve was calculated by a linear trapezoid method.

**RESULTS**

Administration of endotoxin to camels at a dose of 0.1µg/kg body weight produced a consistent increase in rectal temperature with peak (41.6°C) at 3 hours after its injection. The comparison of oxytetracycline in serum of normal and febrile camels at various time intervals is represented graphically in Fig. 1. The drug appeared in serum at the same time. The maximum concentration was 1.7 µg/ml in febrile animals significantly higher than 0.8 µg/ml in normal animals. Interestingly, the time required to achieve the maximal plasma concentration was smaller in febrile than in normal animals (2.25 and 1.8 hours, respectively). The concentration of antibiotic was 0.22 µg/ml in normal and 0.42 µg/ml in febrile camels at 72 hours post antibiotic injection. The pharmacokinetic parameters of oxytetracycline in normal and febrile animals are given in Table 1. The values for elimination half-life (t½), area under curve (AUC), and mean residential time (MRT) were significantly higher in febrile camels as compare to normal camels.

**TABLE 1:** Pharmacokinetic parameters of oxytetracycline (10 mg/kg) given as a single intramuscular dose in normal or febrile camels.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Normal</th>
<th>Febrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>µg/ml</td>
<td>0.43</td>
<td>33.94</td>
</tr>
<tr>
<td>Alpha</td>
<td>1/h</td>
<td>0.027</td>
<td>0.69</td>
</tr>
<tr>
<td>B</td>
<td>µg/ml</td>
<td>0.51</td>
<td>1.00</td>
</tr>
<tr>
<td>Beta</td>
<td>1/h</td>
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<td>0.012</td>
</tr>
<tr>
<td>ka</td>
<td>1/h</td>
<td>2.023</td>
<td>0.75</td>
</tr>
<tr>
<td>Parameter</td>
<td>Unit</td>
<td>Value</td>
<td>Value</td>
</tr>
<tr>
<td>k10</td>
<td>1/h</td>
<td>0.020</td>
<td>0.045</td>
</tr>
<tr>
<td>k12</td>
<td>1/h</td>
<td>0.0012</td>
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</tr>
<tr>
<td>k21</td>
<td>1/h</td>
<td>0.022</td>
<td>0.198</td>
</tr>
<tr>
<td>Tmax</td>
<td>h</td>
<td>2.25</td>
<td>1.83</td>
</tr>
<tr>
<td>Cmax</td>
<td>µg/ml</td>
<td>0.89</td>
<td>1.7</td>
</tr>
<tr>
<td>AUC 0-t</td>
<td>µg/ml*h</td>
<td>34.34</td>
<td>49.5</td>
</tr>
<tr>
<td>AUC 0-inf</td>
<td>µg/ml*h</td>
<td>45.14</td>
<td>80.3</td>
</tr>
<tr>
<td>t1/2</td>
<td>h</td>
<td>33*</td>
<td>49*</td>
</tr>
<tr>
<td>MRT</td>
<td>h</td>
<td>46</td>
<td>71</td>
</tr>
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<td>Febrile</td>
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<tr>
<td>r obs-pre</td>
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<td>0.977</td>
<td>0.95</td>
</tr>
<tr>
<td>R²</td>
<td></td>
<td>0.99</td>
<td>0.97</td>
</tr>
</tbody>
</table>

*Based on noncompartamental analysis

**FIGURE 1:** The plasma concentrations versus time of oxytetracycline (10 mg/kg) given as a single intramuscular dose in normal or febrile camels. The data were analyzed by noncompartmental analysis (A) and 2 compartments model (B).
DISCUSSION

In this study, the pharmacokinetics of long acting preparation of oxytetracycline was estimated in normal and febrile camels. Fever was induced in camels by administration of endotoxins as previously described (Al-Dughaym, 2004). The serum oxytetracycline concentrations were constantly higher in febrile than in normal camels (Al-Nazawi and Homeida, 2002) and probably as a result of significant slower elimination and total body clearance observed in febrile camels. Significant increase in t1/2 obtained in febrile camels over that of normal camels suggests that oxytetracycline is removed from the body at slower rate in febrile condition compared to normal state. Endotoxin induced fever can result in various haemodynamic changes (Bradley, 1979; Al-Dughaym, 2004) including decrease in blood pressure, cardiac output, glomelular filtration, renal blood flow and decreased hepatic drug metabolism (Roth et al., 1997), all contributing to lower clearance rate and longer t1/2. The observed Cmax in this study indicates that the maximal plasma concentration is lower in camels compared with other animals. Cmax was 6.09 g/ml in sheep (Craigmill et al., 2000), 5.7 g/ml in calves (Kumar and Malik, 1998) and 4.4 g/ml in dogs (Kikuvi et al., 2001). This is due to the differences in the pharmaceutical formulation and slower absorption of the drug from the injection site. The present profile of long acting formulation in camels indicated lower Cmax compared with the non-long acting formulations; however, highly persistent plasma concentrations were detectable for about 3 days. Moreover, the plasma-drug concentration was higher in febrile camels. The higher AUC observed in febrile compared to normal camels would suggest a better clinical benefits of fever as it enhances the capacity of drug to penetrate cellular barriers (Booth and McDonald, 1988). Significantly, longer MRT in febrile condition compared to normal state may be due to cardiovascular changes associated with fever during which peripheral circulation increases (Pennington et al., 1975; El Korchi et al., 2001). Higher MRT indicated more residence of the drug in camels tissues and fluids, giving a clinical advantage of prolonged drug effect in feverish conditions. Rikihisa and Jiang have shown that in vitro concentrations of oxytetracycline above 0.01 µg/ml effectively suppress bacterial growth (Rikihisa and Jiang, 1988). In this study a mean concentration of 0.22 µg/ml and 0.42 µg/ml was maintained in normal and febrile states at 72 hours post antibiotic dosing, suggesting that dosing regimen of long acting oxytetracycline perform a sufficiently high and prolonged plasma concentrations in camels that may be effective in controlling chronic diseases and long standing infections.

REFERENCES


Fever on pharmacokinetics of oxytetracycline in camels


