Small Ruminant Research 102 (2012) 208–212

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Pharmacokinetics of sodium and trihydrate amoxicillin after intravenous and intramuscular administration in llamas (*Lama glama*)

Verónica E. Kreil[a,*], Luis Ambros[a], Laura Montoya[a], Ruben Hallu[a], Marcela Rebuelto[a], Guillermo Bramuglia[b]

[a] Farmacología, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Chorroarín 280, CP 1427 Buenos Aires, Argentina
[b] Departamento de Farmacología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 954, CP 1407 Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 20 April 2011
Received in revised form 18 July 2011
Accepted 25 July 2011
Available online 9 September 2011

Keywords:
Llamas
Pharmacokinetics
Antibiotics
Amoxicillin
Infectious diseases

ABSTRACT

The pharmacokinetics of amoxicillin following a single intravenous (i.v.) and intramuscular (i.m.) administration of a conventional sodium formulation and following a single i.m. administration of a trihydrate long acting suspension was investigated in adult llamas. On phase 1, in a cross-over design, six llamas received sodium amoxicillin (20 mg/kg) by the i.v. and i.m. routes. On phase 2, six llamas received i.m. trihydrate amoxicillin suspension (15 mg/kg). Amoxicillin plasma concentrations were determined using a microbiological assay. Significant differences were found for mean peak plasma concentration (40.4 ± 12.1 versus 3.04 ± 1.02 μg/ml), mean elimination half-life (0.86 ± 0.3 versus 9.96 ± 3.1 h) and mean residence time (1.37 ± 0.5 versus 15.1 ± 4.1 h) following sodium and trihydrate i.m. amoxicillin, respectively. Bioavailability was similar for both sodium (1.44 ± 0.2) and trihydrate (1.14 ± 0.4) formulations. The results of the pharmacokinetic/pharmacodynamic indices of time above the minimal inhibitory concentrations ($T > MIC$) suggest that 20 mg/kg sodium amoxicillin would produce a good therapeutic outcome in infections due to susceptible bacteria administered every 8–12 h, while for 15 mg/kg trihydrate amoxicillin, higher doses or more frequent intervals may be needed for treating low susceptibility bacteria in llamas.

1. Introduction

The llama (*Lama glama*) is one of the four recognized New World members of the camelid family and is a popular work, productive and companion animal in at least five South American countries, USA and Europe. Currently, in our country there are no drugs approved for use in llamas, and veterinarians extrapolate drug dosage regimens from other species (sheep, goat, cattle). Such extrapolation may not be appropriate, resulting in ineffective therapy and/or serious adverse reactions (Lashev and Pashov, 1992).

Several infectious diseases have been described in camelids (Fowler, 1996). Gram negative (*Escherichia coli*) diarrhea is an important disease in llama neonates that, if not treated early, may progress to a severe illness. Broad-spectrum antibiotics with good activity against Gram negative bacteria should be given in the onset of diarrhea (Whitehead and Anderson, 2006). Amoxicillin (AMX) is a broad-spectrum bactericidal semisynthetic betalactam drug, currently approved and widely used as antimicrobial drug in many species. AMX molecular structure and pharmacokinetic characteristics are similar to those of ampicillin. However, AMX has advantages over ampicillin such as more rapid bactericidal effect, better absorption after oral administration (Prescott, 2006), lower toxicity, and less-irritating effects after intramuscular and subcutaneous injection. AMX is active against

* Corresponding author. Tel.: +54 11 45248459; fax: +54 11 45248459.
E-mail address: kreil@fvet.uba.ar (V.E. Kreil).

0921-4488/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.smallrumres.2011.07.015

many Gram positive and Gram negative bacteria including non-penicillinase-producing staphylococci, *Staphylococcus aureus*, beta-hemolytic streptococci, *Fusobacterium necrophorus* and some Enterobacteriaceae, although it is ineffective against *Pseudomonas aeruginosa*, *Bacteroides fragilis* and *Klebsiella pneumoniae* (Prescott, 2006). Beta-lactam class antibiotics are best described as having a time-dependent bactericidal activity. The time that the plasma concentrations of the drug remain above the minimal inhibitory concentrations (MIC) of the pathogen organism ($T > MIC$) is the pharmacokinetic/pharmacodynamic (PK/PD) index that better predicts its antibacterial efficacy (Craig, 1998; Drusano, 2004). Therefore, variations in the plasma concentrations may affect the clinical outcome when beta-lactams, such as AMX, are used for treating susceptible bacterial infections.

Accurate pharmacokinetic information on the disposition of antibiotics is required to provide effective antimicrobial treatment and avoid resistance production. Several antimicrobial agents have been studied in llamas and alpacas, as ampicillin (Kreil et al., 2001), trimethoprim–sulfamethoxazole (Chakwenya et al., 2002) and enrofloxacin (Gandolf et al., 2005) in alpacas, gentamicin (Dowling et al., 1996), ampicillin, tobramycin, trimethoprim, sulfamethoxazole, enrofloxacin and ceftiofur in llamas (Christensen et al., 1996) and ceftiofur in llamas and alpacas (Drew et al., 2004). The pharmacokinetics of AMX has been studied in several domestic species, including desert sheep and Nubian goats (Elsheikh et al., 1999), sheep (Craigmill et al., 1992; Fernandez et al., 2007), goats (Craigmill et al., 1992; Cárceles et al., 1995; Escudero et al., 1998) and cows (Archimbault and Boutier, 1981). However, to our knowledge, no studies concerning the pharmacokinetic disposition of AMX have been reported in llamas. So, the objective of the present study was to determine the pharmacokinetics of AMX following a single intravenous (i.v.) and intramuscular (i.m.) administration of a conventional sodium formulation and following a single i.m. administration of a trihydrate long acting suspension to adult llamas.

2. Materials and methods

2.1. Experimental animals

Experimental animals were 12 healthy adult female llamas, whose mean weight was 128.0 ± 24 kg and 129.2 ± 23 kg, in phase 1 ($n = 6$) and 2 ($n = 6$), respectively. Llamas were determined to be clinically normal before each study, based on history, physical examination and haematologic evaluation. All llamas stayed in a shed during the experience with access to green food and water ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee of the Faculty of Veterinary Science, University of Buenos Aires, Argentina.

2.2. Dosage form

An aqueous sodium AMX solution (Amoxidal®, Roemmers Laboratories, Buenos Aires, Argentina, 200 mg/ml) was used for i.v. and i.m. administration (phase 1). For i.m. depot administration (phase 2), an AMX trihydrate salt suspension (Clamoxyl® L.A., Pfizer Laboratories, Buenos Aires, Argentina, 153 mg/ml) was used.

2.3. Experimental design

On phase 1, each of the six animals received 20 mg/kg of sodium AMX solution by the i.v. and i.m. routes in a randomized cross-over design with a 2-week washout period between treatments. On phases 2, 6 llamas received an i.m. injection of 15 mg/kg of a trihydrate AMX salt suspension. The i.v. administration was injected into the right jugular vein and the i.m. administrations were injected into a bare region of the gluteus.

2.4. Blood sampling

The same blood sampling schedule was used following i.v. and i.m. administration of sodium AMX solution. Heparinized samples (2 ml) were taken via left jugular venipuncture at 0.08, 0.16, 0.33, 0.5, 0.66, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12 y 24 h postdosing. For trihydrate AMX salt suspension, heparinized samples were taken via jugular venipuncture at 0.33, 0.5, 0.66, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, 12, 14, 24, 27, 30, 33, 36, 38 and 48 h postdosing. Blood samples were maintained under refrigeration pending centrifugation at 3000 × *g* for 10 min and the supernatant plasma was refrigerated at 4 °C until analysis within 4 days post extraction.

2.5. Amoxicillin determination

AMX plasma concentrations were determined by microbiological bioassay (Bennet et al., 1966) using *Bacillus subtilis* ATCC 6633 as test microorganism. The standard curve was prepared in llama normal plasma between 0.1 and 50 μg/ml AMX concentrations the same day the blood samples were collected. Each sample was plated in triplicate and each standard dilution was repeated four times. The limits of detection and quantification of the method were 0.1 μg/ml in both cases (Shah et al., 1992). The limit of quantification was the lower concentration used for the pharmacokinetics analysis. The method was linear between 0.1 and 50 μg/ml ($r = 0.989$). The inter-assay and intra-assay coefficients of variation and the bias of the assay were <7%, <10% and 9.8%, respectively, for all tested concentrations (LLQ bias was of 13.8%).

2.6. Pharmacokinetic analysis

Individual AMX concentration versus time curves obtained after the i.v. and i.m. administrations were analyzed by non-linear methods using TOPFIT 2.0 (Gustav Fisher, Jena, Germany). The peak concentration in plasma (C_{max}) and the time to C_{max} (T_{max}) after i.m. administration were estimated by observed data from tabulated plasma concentrations. Other pharmacokinetic parameters were calculated according to classical equations (Gibaldi and Perrier, 1982). The terminal elimination rate constant (λ) was determined by linear regression of four or five points in the terminal phase. The area under the curve from 0 to the last measurable concentration ($AUC_{0\text{-last}}$) was calculated by the linear trapezoidal method, and extrapolation to infinity was obtained by dividing the last observed concentration by λ. The percentage of AUC extrapolation was calculated as $(AUC_{0\text{-inf}} - AUC_{0\text{-last}})/AUC_{0\text{-inf}}$. The elimination half-life ($t_{1/2\lambda}$) was calculated as $0.693/\lambda$. The mean residence time (MRT) was calculated as $AUMC/AUC_{0\text{-inf}}$, where AUMC is the area under the curve of the product of time and the plasma drug concentration versus time from time zero to infinity. The mean residence time for absorption (MRT_{abs}) was calculated as $MRT_{i.m.} - MRT_{i.v.}$ for the i.m. administration of sodium AMX, whereas for the AMX trihydrate administration the MRT_{abs} was calculated as each $MRT_{i.m.}$ − mean $MRT_{i.v.}$. Total body clearance (Cl) was calculated as the ratio of the administered dose (D) to $AUC_{0\text{-inf}}$ and the apparent volume of distribution (Vd_z) was estimated as the ratio of Cl to (. Volume of distribution at the steady state (Vd_{ss}) was calculated as Cl × MRT. Absolute bioavailability (f) was calculated as $f = AUC_{0\text{-inf(i.m.)}}/AUC_{0\text{-inf(i.v.)}}$ for the sodium AMX administration, and $AUC_{0\text{-inf(i.m.)}} \times Dose_{i.v.}$/mean $AUC_{0\text{-inf(i.v.)}} \times Dose_{i.m.}$ for the trihydrate AMX administration. The results are expressed as mean ± standard deviation (SD).

Time that AMX concentrations remained above MIC ($T > MIC_{90}$) was calculated graphically for both i.m. treatments for the previously reported MIC_{90} values of several microorganisms isolated in related species: *E. coli* and *Pasteurella multocida* (4 μg/ml, Post et al., 1991; Sato et al., 2005), *S. aureus* (0.5 μg/ml, Watts and Salomon, 1997), and coagulase negative staphylococci (0.8 μg/ml, Moroni et al., 2005), and *Mannheimia haemolytica* (0.188 μg/ml, Delis et al., 2010) obtained from Collection de l' Institut Pasteur (Paris, France) and was expressed in hours.

2.7. Statistical analysis

A computerized program (GraphPad Prism, 5.0) was used to identify the presence of differences between parameters calculated after