Comparative pharmacokinetics of intravenous cephalexin in pregnant, lactating, and nonpregnant, nonlactating goats

L. AMBROS
V. KREIL
L. TARRAGONA
A. MONFRINOTTI
R. HALLU &
M. REBUELTO

Farmacología, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Argentina


The aims of this study were to describe and compare the pharmacokinetics of a single dose of cephalexin (10 mg/kg) after its intravenous (i.v.) administration to five goats in three different physiological status: nonpregnant nonlactating (NPNL), pregnant (P) and nonpregnant lactating (L). Blood samples were collected at predetermined times, and plasma concentrations of cephalexin were measured by microbiological assay. Relevant pharmacokinetic parameters were calculated using noncompartmental analysis. Statistical comparison was performed applying the nonparametric ANOVA. No significant differences were found for cephalexin pharmacokinetic parameters between the L and the NPNL group. Median V_dss was significantly lower in pregnant goats (0.09 [0.07–0.10] L/kg) compared with NPNL goats (0.16 [0.14–0.49] L/kg). Median total Cl and V_dl were significantly lower in pregnant goats (0.25 [0.19–0.29] L/h/kg and 0.11 [0.10–0.13] L/kg, respectively) than in lactating goats (0.40 [0.32–0.57] L/h/kg and 0.20 [0.14–0.23] L/kg, respectively). Median AUC_0–¥ was significantly higher in pregnant goats (37.79 [34.75–52.10] µg·h/mL) than in lactating goats (25.11 [17.44–31.14] µg·h/mL). Our study showed that even though some pharmacokinetic parameters of cephalexin are altered in pregnant and lactating goats, these differences are unlikely to be of clinical importance; therefore, no dose adjustment would be necessary during pregnancy and lactation.

(Paper received 11 May 2010; accepted for publication 27 July 2010)

Luis Ambros, Farmacología, Facultad de Ciencias Veterinarias, Universidad de Buenos Aires, Argentina. E-mail: ambros@fvet.uba.ar

INTRODUCTION

Currently, limited data are available regarding the effects of pregnancy and lactation on the disposition of drugs. Several complex physiological changes occurring during pregnancy may affect drug’s absorption, distribution, biotransformation and excretion, i.e. increased maternal plasma volume and fat content, increased cardiac output, hypalbuminemia, cytochrome induction or inhibition and enhanced glomerular filtration rate (Dawes & Chowienczyk, 2001; Weiner et al., 2005). Moreover, placental selective transfer and metabolizing activity, and foetal development may also contribute to alterations in drug disposition (Dawes & Chowienczyk, 2001; Weiner et al., 2005). During lactation, the mammary gland is transformed from an inactive organ to an active exocrine gland, developing a highly vascularized tissue, which implies a new regional blood flow distribution and various carrier-mediated systems by which xenobiotics may be excreted into the milk (McManaman & Neville, 2003). It is important to investigate the effects of altered physiology during gestation and lactation on the plasma concentrations and pharmacokinetics of drugs, because these changes could jeopardize clinical outcome.

Beta-lactam antibiotics, such as penicillins and cephalosporins, exhibit a time-dependent antimicrobial activity, that is, death of bacteria is related to drug concentration and length of time bacteria are exposed to the drug (Craig, 1998; Drusano, 2004; McKellar et al., 2004). Previous studies have demonstrated that both pregnancy and lactation may modify plasma concentrations of some antibiotics, including beta-lactams, macrolides and fluoroquinolones, (Soback et al., 1994; Bengtsson et al., 1997; Courtin et al., 1997; Ambros et al., 2007; Marin et al., 2007), thus, therapeutic efficacy may become unpredictable. However, dosage regimens for antibiotics in pregnant and lactating individuals are currently based on pharmacokinetic studies performed on nonpregnant, nonlactating individuals.
Cephalexin, a first-generation cephalosporin, possesses excellent activity against many gram-positive micro-organisms such as staphylococci and streptococci. The minimum inhibitory concentration (MIC) of cephalexin against susceptible gram-positive bacteria isolated from cattle ranges between 0.5 and 0.75 µg/mL (Gentilini et al., 2000). The pharmacokinetic properties of cephalexin have been evaluated in various species and routes of administration, as in beef cattle (Waxman Dova et al., 2008) cow and buffalo calves (Garg et al., 1990, 1992), dogs (Carli et al., 1999; Rebuelto et al., 2005), cats (Thornton & Martin, 1997), horses (Villa et al., 2002; Davis et al., 2005) and ponies (Lees et al., 1990); however, no studies have neither described the pharmacokinetics of this drug in goats nor compared the effects of different reproductive status, i.e. pregnancy and lactation, on its disposition. The aims of this study were to describe and compare the pharmacokinetics of a single dose of cephalexin after its intravenous (i.v.) administration to goats in three different physiological status: nonpregnant non-lactating (NPNL), pregnant (P) and nonpregnant, lactating (L).

**MATERIALS AND METHODS**

**Animals**

Five Criollas goats of 2–4 years of age throughout different physiological status (NPNL, P and L) were used in this experiment, according to a protocol approved by our Institutional Animal Care and Use Committee.

Mean (SD) weights the day of each study were 38.7 ± 6.1, 42.7 ± 6.9 and 38.0 ± 6.6 kg for the NPNL, P and L status, respectively. Pregnant goats were studied between gestational days 115 and 118. Lactating goats were in the 28–31 days postpartum lactation period, in their first or second lactation. Daily milk production ranged between 190.0 and 280.0 mL per day. All animals were in good health throughout the study, as determined by physical examination and haematological analyses. During acclimatization for 3 weeks, and the subsequent treatment periods, the animals were kept outside, were they grazed on natural pasture and had access to water ad-libitum.

**Experimental design**

All animals in the three reproductive status received a single dose (10 mg/kg) of cephalexin (Cephalexina Richet; Richet, Buenos Aires, Argentina) in the right jugular vein. administered as a bolus. In all experiments, heparinized blood samples were collected from the left jugular vein at 0, 0.08, 0.17, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 h after the i.v. administration. Blood samples were centrifuged at 2500 g, for 10 min. The serum was stored at 4 °C until analysis.

**Analytical method**

Plasma concentrations of cephalexin were determined by microbiological assay (Bennet et al., 1966) using Micrococcus luteus ATCC 9341 as test organism. Standard curves of the drug were prepared in free antibiotic pooled from goat serum. Each sample was measured in triplicate. Inhibition zones around the sample wells were measured with a digital caliper and compared with inhibition zones produced by the standards. The quantification limit was 0.39 µg/mL. The inter- and intra-assay coefficients of variation were <7.1 and 6.0%, respectively; the method was between 0.39 and 100.0 µg/mL (r was no <0.989 in all determinations).

**Pharmacokinetic analysis**

Plasma levels of cephalexin were subjected to noncompartmental analysis using PCnonlin V 4.0 software package (SCI Software, Lexington, KY, USA). Relevant pharmacokinetic parameters were calculated according to classical equations (Gibaldi & Perrier, 1982). The apparent terminal rate constant (λz) was determined by linear regression of the last 4–5 points on the terminal phase of the logarithmic plasma concentration–time curves. The elimination half-life (t1/2z) was calculated as ln 2/λz. The area under the curve from 0 to the last measurable concentration (AUC0–last) was calculated by the linear trapezoidal rule and extrapolated to infinity (AUC0–inf) according to AUC0–last + Cz/λz, where Cz is the last measured concentration. The mean residence time (MRT) was calculated as AUMC/AUC0–inf, where AUMC is the area under the curve of the product of time and the plasma drug concentration vs. time from time zero to infinity. Total body clearance (Cl) was calculated as the ratio of the administered dose (D) to AUC0–inf, and the apparent volume of distribution (Vz) estimated as the ratio of Cl to λz. Volume of distribution at the steady-state (Vds) was calculated as D/AUMC/AUC2.

**Statistical analysis**

The statistical comparison between cephalexin blood concentrations and pharmacokinetic parameters calculated after the i.v. administration of cephalexin to NPNL, P and L goats was performed applying the nonparametric ANOVA (Friedman test), when significant differences (P ≤ 0.05) were identified, Dunns post hoc test was used for comparing groups.

**RESULTS**

All animals were in good health throughout the entire study. No adverse reaction that could be related to the drug administration was registered.

Mean cephalexin serum concentrations vs. time curve and median (range) pharmacokinetic parameters following single i.v. administration to goats at the three different reproductive status are presented in Fig. 1 and Table 1, respectively. Extrapolated AUC(last–inf) was between 0.8 and 2% of the observed AUC(AUC0–last). No significant differences were found for cephalexin pharmacokinetic parameters between the L and the NPNL group. Median Vds was significantly lower in pregnant goats.
Successful antimicrobial treatment depends on appropriate plasma and tissue concentrations. Modifications of the pharmacokinetics of antimicrobial agents raise a major concern, as not only the outcome of therapy may be impaired, but also selection of resistant bacteria may occur if antibiotic concentrations are insufficient to provide full antibacterial activity (Courvalin, 2008; Roberts et al., 2008). Physiological changes in some processes involved in drug’s absorption and disposition may be altered during pregnancy and lactation. In this study, we describe and compare the pharmacokinetics of cephalexin following a single i.v. administration to goats in three different reproductive status. The microbiological assay used in this experiment was appropriate for the quantification of cephalexin concentrations, as no active metabolite has been identified for this drug; therefore, serum concentration data and pharmacokinetics correlate accurately with total antimicrobial activity of the drug (Waxman Dova et al., 2008).

To our knowledge, this is the first report describing cephalexin pharmacokinetics in goats; therefore, comparison with the results obtained in other pharmacokinetic studies in this species under similar conditions is not possible. Our plasma concentration–time data showed that the drug was rapidly eliminated, with a short median elimination half-life of 0.36 h and median high clearance values of 0.35 L/h/kg in NPNL goats. The total body clearance calculated in this study is higher than the physiological glomerular filtration rate of goats (2.58 mL/kg/min). As cephalexin is primarily eliminated by the kidneys, this data suggests that in goats both glomerular filtration and tubular secretion are involved in the excretion of cephalexin; in addition, the high degree of ionization of this drug in the urine precludes distal nephron reabsorption of the drug. The half-life calculated in our study is shorter than those described for horses (1.59 h, Villa et al., 2002; 2.02 h, Davis et al., 2005) and buffalo calves (3.17 h, Garg et al., 1990) following i.v. administration. However, it is possible that a lower limit of quantification than that of the assay used in the present study (0.39 µg/mL) would have detected a more prolonged half-life. The small value of volume of distribution

Table 1. Pharmacokinetic parameters calculated by noncompartmental analysis after the intravenous administration of cephalexin (10 mg/kg) to five goats. Data are presented as median (range)

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Nonpregnant, nonlactating</th>
<th>Pregnant</th>
<th>Lactating</th>
</tr>
</thead>
<tbody>
<tr>
<td>λz (h⁻¹)</td>
<td>1.94 (0.87–2.33)</td>
<td>2.30 (1.58–2.50)</td>
<td>2.47 (1.60–3.21)</td>
</tr>
<tr>
<td>t1/2_z (h)</td>
<td>0.36 (0.30–0.79)</td>
<td>0.29 (0.27–0.42)</td>
<td>0.40 (0.32–0.57)</td>
</tr>
<tr>
<td>AUC0–∞ (µg h/mL)</td>
<td>28.80 (20.53–35.84)</td>
<td>37.79* (34.75–52.10)</td>
<td>25.11 (17.44–31.14)</td>
</tr>
<tr>
<td>MRTz (h)</td>
<td>0.46 (0.38–1.01)</td>
<td>0.36 (0.29–0.52)</td>
<td>0.35 (0.25–0.52)</td>
</tr>
<tr>
<td>Cl (L/h/kg)</td>
<td>0.35 (0.28–0.49)</td>
<td>0.25* (0.19–0.29)</td>
<td>0.40 (0.32–0.57)</td>
</tr>
<tr>
<td>Vd (L/kg)</td>
<td>0.18 (0.16–0.32)</td>
<td>0.11* (0.10–0.13)</td>
<td>0.20 (0.14–0.23)</td>
</tr>
<tr>
<td>Vdss (L/kg)</td>
<td>0.16 (0.14–0.49)</td>
<td>0.09** (0.07–0.1)</td>
<td>0.13 (0.11–0.20)</td>
</tr>
</tbody>
</table>

λz, apparent terminal rate constant; t1/2_z, terminal half-life; AUC0–∞, area under the plasma concentration–time curve from time zero to infinity; MRTz, mean residence time; Cl, total body clearance; Vd, apparent volume of distribution; Vdss, volume of distribution at the steady-state.

*Significantly different (P ≤ 0.05) from lactating group. **Significantly different (P ≤ 0.05) from nonpregnant, nonlactating group.
calculated in the present study is not surprising and indicates limited diffusion of the drug, typically related to the hydrophilic properties of beta-lactams.

Our data showed that pregnancy, but not lactation, significantly affected the distribution of i.v. cephalaxin in goats. Cephalexin plasma concentrations in pregnant goats were higher than those measured in the other L and NPNL groups. \( V_{\text{dz}} \) were significantly lower in pregnant when compared with the NPNL group, whereas for the elimination-related pharmacokinetic parameters, as \( C_l \) and \( \lambda_z \), no differences were found between these two groups. However, significant differences were found for some pharmacokinetic parameters when comparing pregnant vs. lactating goats. Clearance and \( V_{\text{dz}} \) were significantly lower, and \( AUC_{0-\infty} \) significantly higher in pregnant than in lactating animals. The significant increases in both \( C_l \) and \( V_{\text{dz}} \) observed in lactation compared to pregnancy may have accounted for the lower serum concentrations of cephalexin during lactation.

Different results were expected regarding cephalexin disposition in pregnant animals. During pregnancy, the increases in the body water content creates a new compartment in which hydrophilic drugs may distribute and increases in the cardiac output and changes in its distribution enhances the renal blood flow and glomerular filtration rate. Therefore, lower plasma concentrations, enhanced volume of distribution and renal clearance for hydrophilic drugs that are primarily excreted with no modification by the kidneys may be expected when they are administered during pregnancy (Dawes & Chowienczyk, 2001; Weiner et al., 2005). The increase in cephalexin plasma concentrations in pregnant animals observed in our study may be because of the fact that, as dose was calculated as mg/kg body weight, the pregnant uterus and foetus may have played a role of an extracompartment, in which cephalexin is not freely distributed, as its high ionization in blood and low liposolubility impairs its passage through membranes. In addition, the stage of pregnancy may also have accounted for our results, as physiological changes because of pregnancy are most marked during the third trimester, i.e. days 100–150 in the goat. In a previous report, it has been shown that the steady-state volume of distribution of gentamicin was significantly increased from mid to end of pregnancy and plasma clearance was increased by about 150% at the end of pregnancy in ewes (Oukessou & Toutain, 1992).

Despite the differences in \( V_{\text{dz}} \) and \( C_l \) between pregnant and lactating animals, neither the \( t_{1/2} \) nor the MRT, both pharmacokinetic parameters describing the persistence of a drug in the body, exhibited significant differences between the three groups. The lack of difference in \( C_l \) and \( \lambda_z \) between the pregnant and NPNL goats and the balance in the increases of both \( C_l \) and \( V_{\text{dz}} \) in the lactating group accounted for this lack of difference.

The influence of the reproductive status in the pharmacokinetics of drugs is not yet clearly defined. Conflicting results have been reported on the changes related to pregnancy and lactation. Our findings are in good agreement with what has been previously reported for penicillin G following i.v. administration to ewes and cows in late pregnancy and in early lactation. This study demonstrated that penicillin G serum concentrations and \( AUC \) were lower, and both clearance and volume of distribution at steady-state were higher, in lactating compared to pregnant animals (Bengtsson et al., 1997). Similar to our study, these authors found no differences in either penicillin MRT or elimination half-life in cows; however, MRT was significantly shorter in lactating than in pregnant ewes (Bengtsson et al., 1997). A previous study on ceftriaxone pharmacokinetics following i.v. administration to dairy goats (Courtin et al., 1997) reported a significantly lower \( AUC \) and a higher clearance for lactating goats compared with nonlactating goats; however, similar to our study, no differences in volume of distribution at steady-state were found. The findings of our study differ from those reported for gentamicin in mares, in which there were no differences in the plasma concentrations and pharmacokinetic parameters calculated for this drug during late pregnancy and early lactation (Santschi & Papich, 2000), and to those reported for penicillin in ewes, in which there was an increase in clearance and volume of distribution at steady-state in pregnant when compared with nonpregnant animals (Oukessou et al., 1990). However, in agreement with our study, the latter found that, during lactation, penicillin plasma clearance and apparent volume of distribution were significantly higher than during pregnancy, but not compared to the control animals, whereas MRT was similar to those of the control period and pregnancy (Oukessou et al., 1990). A previous report characterizing the pharmacokinetics of ceftriaxone in lactating ewes found that the kinetic parameters calculated for the lactating animals were similar to those recorded in a previous report for nonlactating sheep (Goudah et al., 2006). When erythromycin was administered by the i.v. and intramuscular routes to lactating and nonlactating goats, \( AUC \) and MRT were significantly higher and clearance and elimination half-life significantly lower in the lactating than in nonlactating animals (Ambros et al., 2007). Difloxacin elimination half-life following i.v. administration to lactating goats reported by Marín et al. (2007) was shorter and clearance was higher than those previously reported for nonlactating goats. Norfloxacin elimination half-life and MRT were lower in lactating ewes than those calculated for the same animals 1 month after weaning (Soback et al., 1994).

For time-dependent antimicrobial agents, as beta-lactams, the shape of the disposition curve is important, as it reflects the time during which the plasma concentrations remain above the MIC. Previous reports have determined that the maximal bactericidal efficacy for cephalosporins is approached when plasma concentrations are greater than the MIC of the pathogen for 60–70% of the dosing interval, whereas a bacteriostatic effect is observed when \( T > \text{MIC} \) is 30–40% of the dosing interval (Craig, 1998; Drusano, 2004). In our study, the disposition curves were almost superimposable, even though considering the higher levels of the pregnant group. Cephalexin MICs against gram-positive pathogens as streptococci and staphylococci have been reported within a range of 0.5–0.75 \( \mu \text{g/mL} \) (Gentilini et al., 2000). In the present study, cephalexin serum concentrations remained above the MIC of gram-positive pathogens for 2.2 and 1.5 h after the
administration to goats in the pregnant, nonpregnant nonlactating and lactating status, respectively, thus, $T > MIC$ was similar in the three groups, suggesting a similar clinical outcome. This low $T > MIC$ means no practical application in goats, as dosage intervals of 2–3 h would be needed for clinical success; however, intramuscular or subcutaneous routes of administration could provide longer $T > MIC$s.

Our study showed that even though some pharmacokinetic parameters of cephalixin are altered in pregnant and lactating goats, these differences are unlikely to be of clinical importance, considering $T > MIC$ as a surrogate marker of cephalixin antibacterial activity. These results suggest that in this species, no adjustment in the dosage or the frequency of administration of cephalixin is necessary when used for treating infectious diseases caused by susceptible gram-positive bacteria during pregnancy or lactation, even though the currently recommended dosages are derived from studies in nonpregnant, nonlactating animals. Further trials are warranted to confirm our observations.

CONFLICT OF INTEREST STATEMENT

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

ACKNOWLEDGMENT

This study was supported by a grant of Secretaría de Ciencia y Técnica, Universidad de Buenos Aires, Argentina, Project 01/V026.

REFERENCES


