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Comparative pharmacokinetics of an ampicillin/sulbactam combination administered intramuscularly in lactating sheep and goats

E Escudero*, A Espuny, MS Vicente, CM Cárceles

Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, University of Murcia, Campus de Espinardo, 30 071 Murcia, Spain

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Summary — The pharmacokinetic behaviour of an ampicillin/sulbactam (2:1) combination was studied after intramuscular administration of a single dose (20 mg/kg body weight: 13.33 mg/kg of ampicillin and 6.67 mg/kg of sulbactam) to six sheep and six goats. The objective was to determine whether there were differences between sheep and goats in the disposition profiles of ampicillin and sulbactam. The disposition curves for both drugs were best described by a biexponential equation (one-compartment open model with first order absorption) in both sheep and goats. The maximum plasma concentrations of ampicillin and sulbactam were similar in both sheep (10.61 ± 6.36 and 9.17 ± 3.82 mg/L respectively) and goats (11.02 ± 2.69 and 9.25 ± 0.85 mg/L respectively), in spite of the fact that the ampicillin dose was twice that of sulbactam. The time of the peak plasma concentration for both drugs was also similar in both sheep and goats. The elimination half-life of ampicillin was 0.81 ± 0.17 h in sheep and 0.71 ± 0.12 h in goats, and that of sulbactam was 1.02 ± 0.36 h in sheep and 1.13 ± 0.18 h in goats. The rate of drug removal from the body was faster in sheep than in goats and consequently the area under the curve was greater for goats. It was concluded that the similarity in the disposition and elimination of both drugs in sheep and goats indicated that the combination preparation could be administered at the same dosing rate in both species.

pharmacokinetics / ampicillin / sulbactam / sheep / goat

Résumé — Comportement pharmacocinétique d'une association d'ampicilline–sulbactam (2:1) administrée par voie intramusculaire chez la brebis et la chèvre. Le comportement pharmacocinétique d'une association d'ampicilline–sulbactam (2:1) administrée par voie intramusculaire à la dose unique de 20 mg/kg (correspondant à 13,33 mg/kg d'ampicilline et 6,67 mg/kg de sulbactam) a été étudié chez six brebis et six chèvres. Le but de cet essai a été de déterminer les différences possibles entre ces deux espèces, en ce qui concerne le profil de disposition de l'ampicilline et du sulbactam. Les
courbes de concentrations plasmatiques des deux principes actifs ont été ajustées avec une équation biexponentielle (modèle monocompartmental ouvert avec une phase d'absorption de premier ordre) chez les deux espèces. Les concentrations maximales de l'ampicilline et du sulbactam étaient similaires chez la brebis (10,61 ± 6,36 et 9,17 ± 3,82 mg/L respectivement) et la chèvre (11,02 ± 2,69 et 9,25 ± 0,85 mg/L respectivement) bien que la dose d'ampicilline administrée ait été deux fois supérieure à celle du sulbactam. Les temps d'apparition du pic de concentration plasmatique étaient similaires pour les deux principes actifs chez les deux espèces. Les demi-vies d'élimination de l'ampicilline et du sulbactam ont été respectivement de 0,81 ± 0,17 et 1,02 ± 0,36 heure chez la brebis et de 0,71 ± 0,12 et 1,13 ± 0,18 heure chez la chèvre. Pour l'ampicilline, la clairance corporelle et l'aire sous la courbe des concentrations plasmatiques étaient significativement différentes entre la brebis et la chèvre (p < 0,05). En conclusion, la similitude de disposition et d'élimination des deux drogues chez la brebis et la chèvre indiquait que leur association peut être utilisée aux mêmes doses chez les deux espèces.

pharmacocinétique / ampicilline / sulbactam / brebis / chèvre

INTRODUCTION

Sulbactam is a new semisynthetic compound which inhibits β-lactamases irreversibly. Sulbactam has been shown to extend the in vitro spectrum of β-lactam antibiotics to a number of resistant bacteria, although in itself it has little intrinsic antibacterial activity.

Ampicillin is combined with sulbactam in a 2:1 ratio. Combinations of ampicillin and sulbactam have been found to demonstrate increased effectiveness against an experimental model of acute systemic infection in mice (English et al, 1986; Retsema et al, 1986) and against infection due to a nafcillin-resistant strain of S aureus in a rabbit endocarditis model (Washburn and Durack, 1981). The combination was also found to be effective in the treatment of ampicillin-resistant pneumatic Pasteurellosis under clinical and experimental conditions in feedlot and housed calves (Bentley and Cummins, 1987; Bryson et al, 1978; Farrington et al, 1987; Grimshaw et al, 1987) and in the treatment of experimentally induced Klebsiella pneumoniae lung infection in foals (Hoffman et al, 1992).

Even though a large amount of information is available on the pharmacokinetics of ampicillin used alone (Nouws et al, 1982; Oukessou and Toutain, 1992), and it has a potentially significant therapeutic use in combination with sulbactam in veterinary practice, only limited data are available on its pharmacokinetics in ruminant species (Grimshaw and Colman, 1987; Montesissa et al, 1994). The objective of this study was to compare the absorption and elimination rates of ampicillin and sulbactam in sheep and goats following intramuscular injection of a single dose (20 mg/kg) of an ampicillin/sulbactam (2:1) combination using high performance liquid chromatography to measure the plasma levels of both drugs.

MATERIALS AND METHODS

Animals

Six Murciano-Granadina goats weighing 43,5–52 kg and six Segureña sheep weighing 52–58,5 kg were used. All animals were adult females, milk-producing (in lactation phase), and in good health. The animals were stabled and fed an antibiotic-free diet for at least 30 days preceding the study. Water was available ad libitum.

Study design

An aqueous solution of ampicillin/sulbactam (2:1 ratio) was administered to the animals intramuscularly at a dosage of 20 mg/kg body weight (13,33 mg/kg of sodium ampicillin and 6,67 mg/kg of sodium sulbactam; generously supplied by
The combination was injected in the semimembranosus muscle and blood samples were collected at 0 h (immediately prior to treatment), and 0.04, 0.08, 0.17, 0.25, 0.33, 0.42, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10 and 12 hours after the drug administration. Blood samples (5 mL) were taken from the opposite jugular vein in heparinized Vacutainer® tubes. The plasma was separated by centrifugation at 1500 g for 15 minutes and stored at −70 °C until being assayed.

**Analytical method**

The plasma concentrations of ampicillin and sulbactam were analysed by high performance liquid chromatography using a Gilson HPLC equipped with a Spectra-Physics Datajet CH1 integrator according to the method of Blum et al (1989), with extraction described by Rudrik and Bawdon (1981). A 350-μL plasma sample was deproteinated with 2 mL acetonitrile. The samples were mixed and centrifuged at 1500 g for 5 min, and the supernatant was decanted into 3 mL dichloromethane in a screw-cap tube. The specimens were mixed and centrifuged again as previously described. Samples (20 μL) of the upper aqueous layer were injected onto a column and precolumn 7-μm μBondapack C18 (Macherey-Nagel, Düren, Germany) and scanned by a UV detector set at 229 nm and 0.02 absorbance units for ampicillin and 313 nm and 0.05 absorbance units for sulbactam. The mobile phase for ampicillin was 4% acetonitrile plus 96% phosphate buffer 0.1 M with pH adjusted to 6.1; the flow rate was 2.8 mL/min and the retention time was 9 min. For sulbactam, the mobile phase was 12% acetonitrile plus 88% phosphate buffer 0.1 M (pH = 6.1) and 4 mL of tetrabutylammonium hydroxide (solution 20% in water) was added per 1 L of mobile phase; the flow rate was 2.2 mL/min and the retention time was 2.1 min. In the sulbactam assay, the plasma samples were previously derivatized (Bawdon and Madsen, 1986) using the imidazole reagent (8.5 g of imidazole in 20 mL of distilled water with 5 N HCl added to bring the solution to pH 6.8, and the volume adjusted to 40 mL with water.) The assay was validated by measuring concentrations of known amounts of ampicillin and sulbactam in sheep and goat plasma. The assayed values varied less than ± 2.1% from the calculated values. The assay was linear from 100–0.5 mg/L. For ampicillin the between-batch and within-batch recoveries were 96.3 ± 0.6 and 97.7 ± 0.9%, and those of sulbactam were 97.2 ± 0.7% and 98.4 ± 0.8% respectively.

**Pharmacokinetics**

The plasma concentration–time data after intramuscular administration were fitted to bi-, tri- and tetraexponential equations by the retroprojection method (Gibaldi and Perrier, 1982), and the PKCALC computer program (Shumaker, 1986) was used to obtain the best estimates for the parameters of these equations. Refinement of the estimates was carried out using the PCNONLIN computer program. The data were analysed on an individual animal basis and using a weighting of 1/concentration. The Akaike’s information criterion (AIC) (Yamaoka et al, 1978) was used to select the best fitting of the plasma concentration–time data to a one-, two- or three-compartment model.

The pharmacokinetic parameters were calculated from the final equations obtained (Gibaldi and Perrier, 1982) and the area under the plasma concentration–time curve (AUC) was calculated using the trapezoidal logarithmic method with extrapolation to infinite time. Standard symbols, equations and definitions for plasma pharmacokinetic studies were used (Aronson et al, 1988).

**Statistical analysis**

The usual statistical parameters were obtained and the Kolmogorov–Smirnov test was employed to verify the normality of the data and to test for between-animal differences in the parameters. Parametric (Students t test) and non-parametric (Mann Whitney test) tests were used to test the parameters for significant differences (p < 0.05) between sheep and goats, and between ampicillin and sulbactam within each species (Powers, 1990). The statistical computer program Statgraphics (Manugistics Inc, version 7.0, USA) was used.

**RESULTS**

The mean plasma concentrations of ampicillin and sulbactam at the sample collec-
tion times for sheep and goats are plotted in figures 1 and 2 respectively. In both species, the plasma levels of sulbactam were above the ampicillin plasma levels for the last phase of the curves. The ampicillin and sulbactam plasma concentration vs time data after intramuscular administration of the combination preparation were best fitted to a one-compartment open model with first order absorption in both species. The pharmacokinetic parameters (mean ± SD) based on compartmental pharmacokinetic analysis and noncompartmental methods are presented in tables I and II for sheep and goats respectively.

The Mann Whitney test and the Student's t-test performed on pharmacokinetic parameters after intramuscular administration revealed significant differences between ampicillin and sulbactam. In sheep, a significantly slower relative total plasma clearance (CI/F) was found for sulbactam compared to ampicillin (p < 0.05) but no significant differences existed between their apparent elimination half-lives associated with the terminal slope of a semilogarithmic concentration–time curve (t_{1/2,az}). In goats, the elimination rate constant (\lambda_z), the corresponding half-life (t_{1/2,az}) and the relative body clearance (CI/F) were significantly different (p < 0.05). The elimination for ampicillin was faster than that of sulbactam. The apparent distribution volume calculated by the area method (V_z/F) was significantly (p < 0.05) higher for sulbactam than for ampicillin.

There were significant differences (p < 0.05) between sheep and goats only for ampicillin in some of the pharmacokinetic parameters. These were the apparent dis-
Table I. Pharmacokinetic parameters (mean ± SD) of ampicillin and sulbactam in sheep \((n = 6)\) after intramuscular administration at a dose of 13.33 mg/kg of ampicillin and 6.67 mg/kg of sulbactam.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ampicillin</th>
<th>Sulbactam</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_a) ((h^{-1}))</td>
<td>12.80 ± 4.48</td>
<td>14.66 ± 5.47</td>
<td>NS</td>
</tr>
<tr>
<td>(\lambda_z) ((h^{-1}))</td>
<td>1.02 ± 0.49</td>
<td>0.79 ± 0.25</td>
<td>NS</td>
</tr>
<tr>
<td>(t_{1/2ka}) ((h))</td>
<td>0.11 ± 0.09</td>
<td>0.09 ± 0.06</td>
<td>NS</td>
</tr>
<tr>
<td>(t_{1/2\lambda z}) ((h))</td>
<td>0.81 ± 0.17</td>
<td>1.02 ± 0.36</td>
<td>NS</td>
</tr>
<tr>
<td>(C_{max}) ((mg/L))</td>
<td>10.61 ± 6.36</td>
<td>9.17 ± 3.82</td>
<td>NS</td>
</tr>
<tr>
<td>(t_{max}) ((h))</td>
<td>0.35 ± 0.15</td>
<td>0.32 ± 0.11</td>
<td>NS</td>
</tr>
<tr>
<td>(V_z/F) ((L/kg))</td>
<td>1.32 ± 0.25</td>
<td>1.32 ± 0.26</td>
<td>NS</td>
</tr>
<tr>
<td>AUC ((mg.h.L^{-1}))</td>
<td>12.92 ± 1.69</td>
<td>15.56 ± 3.78</td>
<td>NS</td>
</tr>
<tr>
<td>MRT ((h))</td>
<td>1.35 ± 0.46</td>
<td>1.53 ± 0.52</td>
<td>NS</td>
</tr>
<tr>
<td>MAT ((h))</td>
<td>1.01 ± 0.54</td>
<td>0.70 ± 0.46</td>
<td>NS</td>
</tr>
<tr>
<td>CL/F ((L.h^{-1}.kg^{-1}))</td>
<td>1.05 ± 0.16</td>
<td>0.46 ± 0.13</td>
<td>(P &lt; 0.05)</td>
</tr>
</tbody>
</table>

\(k_a\): Absorption rate constant (first-order), \(\lambda_z\): Elimination rate constant, \(t_{1/2ka}\): Absorption half-time, \(t_{1/2\lambda z}\): The apparent elimination half-life associated with the terminal slope \((\lambda_z)\) of a semilogarithmic concentration–time curve, \(t_{max}\): The time to reach maximum concentration following drug administration, \(V_z\): The apparent volume of distribution calculated by the area method. AUC: The area under the plasma concentration–time curve from zero to infinity. MRT: Mean residence time. MAT: Mean absorption time. CL/F: The total body clearance of drug/systemic bioavailability.

Table II. Pharmacokinetic parameters (mean ± SD) of ampicillin and sulbactam in goats \((n = 6)\) after intramuscular administration at a dose of 13.33 mg/kg of ampicillin and 6.67 mg/kg of sulbactam.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ampicillin</th>
<th>Sulbactam</th>
<th>Level of significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_a) ((h^{-1}))</td>
<td>8.65 ± 3.24</td>
<td>9.59 ± 4.11</td>
<td>NS</td>
</tr>
<tr>
<td>(\lambda_z) ((h^{-1}))</td>
<td>1.00 ± 0.18</td>
<td>0.63 ± 0.11</td>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>(t_{1/2ka}) ((h))</td>
<td>0.20 ± 0.09</td>
<td>0.09 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>(t_{1/2\lambda z}) ((h))</td>
<td>0.71 ± 0.12</td>
<td>1.13 ± 0.18</td>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>(C_{max}) ((mg/L))</td>
<td>11.02 ± 2.69</td>
<td>9.25 ± 0.85</td>
<td>NS</td>
</tr>
<tr>
<td>(t_{max}) ((h))</td>
<td>0.43 ± 0.23</td>
<td>0.34 ± 0.12</td>
<td>NS</td>
</tr>
<tr>
<td>(V_z/F) ((L/kg))</td>
<td>0.78 ± 0.14</td>
<td>1.18 ± 0.08</td>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>AUC ((mg.h.L^{-1}))</td>
<td>17.67 ± 2.77</td>
<td>18.18 ± 2.82</td>
<td>NS</td>
</tr>
<tr>
<td>MRT ((h))</td>
<td>1.34 ± 0.16</td>
<td>1.72 ± 0.28</td>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>MAT ((h))</td>
<td>1.04 ± 0.16</td>
<td>0.87 ± 0.17</td>
<td>NS</td>
</tr>
<tr>
<td>CL/F ((L.h^{-1}.kg^{-1}))</td>
<td>0.75 ± 0.12</td>
<td>0.36 ± 0.05</td>
<td>(P &lt; 0.05)</td>
</tr>
</tbody>
</table>

distribution volume, calculated by the area method \((V_z/F)\), the area under the concentration–time curve from zero to infinity (AUC) and the relative body clearance \((CI/F)\) of ampicillin. The AUC value was greater and the clearance consistently smaller in goats.
than in sheep, but the distribution volume was unexpectedly higher in sheep than in goats.

DISCUSSION

When an antibiotic is to be administered as one component of a combination, such as ampicillin with the β-lactamase inhibitor sulbactam, it is particularly important that the inhibitor should have a pharmacokinetic behaviour similar to that of the antibacterial component for each route of administration.

The ampicillin and sulbactam plasma concentration vs time data after an intramuscular administration of the combination preparation were best fitted to a one-compartment open model with first order absorption in sheep and goats. This is in agreement with the findings of previous studies of ampicillin carried out in humans (Foulds, 1986; Ripa et al, 1990) and various animal species (Oukessou and Toutain, 1992; Montesissa et al, 1994), however Nouws et al (1982) could not adequately fit the intramuscular and subcutaneous plasma concentrations of five parenteral ampicillin formulations in ruminant calves to linear compartment models. The disposition of sulbactam administered in combination with ampicillin has also been described by a one-compartment model with first order absorption in humans (Foulds, 1986; Ripa et al, 1990) and in sheep and calves (Montesissa et al, 1994).

The mean residence time (MRT), the statistical parameter analogous to half-life, reflected similarities in the persistence of both drugs in the bodies of the sheep and goats. The difference between the elimination half-lives of ampicillin and sulbactam in goats was due to a difference in the rate of excretion of the drugs, which was reflected in the clearance values. But the difference in the relative systemic body clearance of both drugs in sheep was not reflected by a difference in the half-life values. The non-compartmental analysis revealed smaller species-related differences and showed that the mean residence time (MRT) and mean absorption time (MAT) were similar in sheep and goats and were consistent with their efficacy in combination. For ampicillin, Oukessou and Toutain (1992) found similar values for MRT (1.54 ± 0.91 h) and MAT (1.17 ± 0.91 h) in sheep.

In both species, ampicillin and sulbactam reached similar peak concentrations ($C_{\text{max}}$) despite the fact that different doses were administered. The times to reach the peak concentration ($t\_{\text{max}}$) were also similar. Montesissa et al (1994) reported similar $C_{\text{max}}$ and $t\_{\text{max}}$ for ampicillin and sulbactam in sheep and calves except that the peak concentration of sulbactam was reached 2.4-times earlier in calves than in sheep.

Renal excretion of unchanged ampicillin is the major route of elimination (Baggot, 1992). In the present study, the relative systemic clearances of ampicillin ($1.05 ± 0.16 \text{ L.h}^{-1}.\text{kg}^{-1}$ in sheep and $0.75 ± 0.12 \text{ L.h}^{-1}.\text{kg}^{-1}$ in goats) and sulbactam ($0.46 ± 0.13 \text{ L.h}^{-1}.\text{kg}^{-1}$ in sheep and $0.36 ± 0.05 \text{ L.h}^{-1}.\text{kg}^{-1}$ in goats), were higher than the glomerular filtration rates (inulin clearance in sheep and goats has been estimated to be $0.14 \text{ L.hr}^{-1}.\text{kg}^{-1}$). This suggested active renal tubular secretion, as also occurs in humans (Foulds et al, 1985). This has been confirmed in horses where oral administration of probenecid (a competitive inhibitor of renal tubular secretion of organic anions) produced a significant reduction in the clearance rate together with an increase in the elimination half-life of ampicillin (Sarasola and McKellar, 1992). The influence of concomitant administration of probenecid on ampicillin plasma levels and on the bioavailability in calves (Ziv and Horsey, 1979) and pigs (Galtier and Alvinerie, 1979; Galtier and Charpenteau, 1979) has been also
described. This has been shown for sulbactam in humans (Foulds et al, 1983).

The plasma levels of a drug are, at least in part, an index of the drug’s potential therapeutic efficacy. It is therefore very important to evaluate the extent to which a drug formulation affects the potential therapeutic results, i.e., the likelihood of a dose achieving plasma drug levels equal to or higher than the MIC of the most susceptible pathogens involved (Nouws et al, 1982). It has been demonstrated that the ampicillin plasma levels even depend on the intramuscular injection site in calves (Marshall and Palmer, 1980) and in dwarf goats during endotoxin-induced fever (Groothuis et al, 1980).

From the plasma drug concentrations after the intramuscular administration into healthy sheep and goats in this study, it may be inferred that an ampicillin/sulbactam combination administered intramuscularly at a dose of 13.33 plus 6.67 mg/kg bodyweight, should be effective (ampicillin concentrations over 0.5 µg/mL, Girard et al, 1987) for at least 5 h. Furthermore, ampicillin produced a post-antibiotic effect, i.e., a persistent suppression of bacterial growth after a short exposure to antimicrobial agents. A post-antibiotic effect of ampicillin lasting 1–4 h with Gram-positive bacteria has been demonstrated (Bundtzen et al, 1981).

Even though the differences in disposition kinetics of both drugs were statistically significant, the same intramuscular dosing rate of this antimicrobial combination can generally be used in sheep and goats.

ACKNOWLEDGMENTS

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