Comparative pharmacokinetics of sulfamethazine after intravenous administration in bovine (Bos taurus) and buffalo (Bubalis bubalis) calves

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Sulfamethazine is a sulfonamide that presents a broad spectrum of activity, including gram-positive and gram-negative bacteria, Chlamydia spp. and some protozoa. This drug has been reported to be highly efficacious in the treatment of pneumonias, diarrheas and coccidiosis in cattle, as commonly susceptible micro-organisms are Bacillus spp., Brucella spp., Listeria monocytogenes, Nocardia spp., Streptococcus spp., Escherichia coli, Chlamydia spp., Pneumocystis carinii, Cryptosporidium spp., and Toxoplasma spp. (Lindsay et al., 1996; Lindsay & Dubey, 1999; Oliveira et al., 2000; Spoo & Riviere, 2001). However, Leptospira spp. and Pseudomonas spp. are resistant (Prescott, 2000).

The pharmacokinetic (PK) behavior of sulfamethazine in ruminant species is characterized by a relatively high bioavailability after oral administration (58% in sheep), a small volume of distribution (0.24–0.50 L/kg) and an elimination half-life, which oscillated between 2 and 11 h after intravenous administration. The PK behavior of this drug depends on age, sex and time of day of distribution and approximately 14 h after oral administration. The PK behavior of this drug depends on age, sex and time of day of administration. The PK behavior of this drug depends on age, sex and time of day of administration. The PK behavior of this drug depends on age, sex and time of day of administration. The PK behavior of this drug depends on age, sex and time of day of administration. The PK behavior of this drug depends on age, sex and time of day of administration.

In the past, the therapeutic recommendations applied to a single ruminant species were extrapolated to the others because no important differences among cattle, sheep, goats and buffalo were recognized. However, a different metabolic behavior along the ruminant species (Elshelihk, 1997) and physiological differences between bovines and buffaloes (such as corporal composition, hepatic metabolism or renal excretion) have been described (Mason, 1974; Groves, 1989). The aim of our work was to study the possible inter-species differences in the PK behavior and pharmacokinetic/pharmacodynamic (PK/PD) integration of sulfamethazine after intravenous administration in buffalo (Bubalis bubalis) and bovine (Bos taurus).

The experiment was performed in five male buffaloes and six male bovine calves (3–4 months old and weighing 120 ± 15 kg). A complete clinical and hematological evaluation was performed throughout the study. The animals were placed in boxes and were given alfalfa hay and had access to water ad libitum. The study was approved by Institutional Animal Use Committee. A sodium sulfamethazine formulation was utilized in the PK study as a 30% injectable saline solution (Allignani Hnos. SRL, Santa Fe, Argentina; Batch 05–01).

Both groups received a single 60 mg/kg (0.20 mL/kg) dose of sulfamethazine. The drug was administered intravenously into the right jugular vein. Blood samples (4 mL) were taken in heparinized sterile syringes and centrifuged at 2000 g for 15 min within 60 min after collection.

Sulfamethazine was extracted using disposable C18 cartridges (Sep-Pak Cartridges; Water Associates Inc., Milford, MA, USA), which were previously conditioned with 5 mL of methanol, followed by 3 mL of water (pH 3.0: acetic acid). All samples were applied to the cartridges and then sequentially washed with 5 mL of water and eluted with 3 mL of acetonitrile concentrated to dryness under a stream of nitrogen. Sulfamethazine concentrations were quantified using HPLC/UV according to a modification of a method previously described by Löschler et al. (1990). An integrated HPLC system (Konik 500 B; Konik Instruments, Instrumentación Analítica SRL, Buenos Aires, Argentina), with UV detection was used. Separation was accomplished using a reverse-phase column (Water SPHERISORB RP C18 5 μm, 25 × 0.4 cm; Precolumn RP C18, Water Associates Inc.). The liquid phase was acetonitrile: acetic acid solution pH 3.0 (8:92) (Sigma-Aldrich Corporation, St Louis, MO, USA); with a 1.5 mL/min flow, a 270 nm and 35 °C oven temperature.

Linear calibration curves were obtained between 0.30 and 300 μg/mL concentration range (bovine: R² > 0.997; buffalo: R² > 0.994). Limit of quantification (LOQ) were 0.36 and 0.50 μg/mL for bovine and buffalo, respectively. Precision was...
between groups were performed using a Mann–Whitney software package (SPSS Inc., Chicago, IL, USA). Comparisons using PCNONLIN V4.0 software package (Statistical Consultants administration were subjected to a noncompartmental analysis up to 2 months was assessed. AUC bovines (30.34 mL/h t similar to half-life (9.37 h) reported by Atef et al. 1992), and other ruminant species (Bulgin et al., 1998) described a comparatively low extent of acetylation of sulfamethazine in ruminants depended mainly on the extent of the metabolism (Nouws et al., 1988). Jain et al. (2000) described a comparatively low extent of acetylation of sulfamethazine and they suggested its safe use in buffalo calves without much risk of toxicity.

Sulfonamides are classified as short-, intermediate- and long-acting according to plasma concentration–time profile. These drugs are considered to be short-acting if blood concentration after one therapeutic dose remains above 50 µg/mL for <12 h. Intermediate-acting sulfonamides are considered to maintain this plasma concentration between 12 and 24 h after administration and long-acting sulfonamides show concentrations of 50 µg/mL or more for at least 24 h after dosing (Spoor & Riviére, 2001). Sulfamethazine plasma concentrations oscillated from 235 to 67.87 µg/mL for <12 h. Therefore, in cattle, this drug could be classified as an intermediate-acting sulfonamide. On the other hand, in our study, buffaloes showed plasma concentrations from 235 to 67.87 µg/mL at 0 and 6 h, that remained above 50 µg/mL only for 10 h (9.98 ± 2.22 h); thus, it behaves as a short-acting sulfonamide. In contrast, Mody and Malik (1997) classified sulfamethazine as an intermediate-acting drug in buffaloes.

PK/PD modeling is a good alternative for selecting a rational dosage regimen. Sulfonamides could be considered as time-dependent drugs. There is evidence from disease model studies to indicate that the time for which concentration exceeds MIC (t > MIC) is an important determinant of the outcome of therapy. In periods when concentrations decrease below MIC regrowth of organisms occurs. Therefore, it is recommended that t > MIC should be achieved for a whole and not only for some proportion of the inter-dose interval (Frimodt-Moller, 2002). We have included the calculation of weighted AUC

This species. An explanation for clearance differences could be found in the metabolic characteristics of these species, due to the elimination of sulfamethazine in ruminants depended mainly on the extent of the metabolism (Nouws et al., 1988). Jain et al. (2000) described a comparatively low extent of acetylation of sulfamethazine and they suggested its safe use in buffalo calves without much risk of toxicity.

### Table 1. Pharmacokinetic parameters after intravenous administration of sulfamethazine (60 mg/kg) in cattle (n = 6) and buffaloes (n = 5)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Bovine</th>
<th>Mean</th>
<th>SD</th>
<th>Buffalo</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI (mL/h/kg)*</td>
<td>30.34</td>
<td>6.39</td>
<td>45.31</td>
<td>10.63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vd (ao) (L/kg)</td>
<td>0.311</td>
<td>0.041</td>
<td>0.383</td>
<td>0.120</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vd (ao) (L/kg)</td>
<td>0.317</td>
<td>0.035</td>
<td>0.399</td>
<td>0.121</td>
<td></td>
<td></td>
</tr>
<tr>
<td>λ (h⁻¹)**</td>
<td>0.090</td>
<td>0.013</td>
<td>0.112</td>
<td>0.009</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t1/2 (h)***</td>
<td>7.46</td>
<td>1.05</td>
<td>6.17</td>
<td>0.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRT (h)</td>
<td>10.48</td>
<td>1.77</td>
<td>8.44</td>
<td>1.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (µg/h/mL)*</td>
<td>2009</td>
<td>387</td>
<td>1365</td>
<td>310</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P = 0.014–0.015, **P = 0.017–0.018, ***P = 0.037.
AUC, area under the plasma concentration–time curve from time zero to infinity; CI, total plasma clearance (CI = Dose/AUC); Vd(ao), volume of distribution at steady state (Vd(ao) = D/β-AUC); Vd(dos), volume of distribution at time zero (Vd(dos) = CI/MRT); λ, rate constant; t1/2, terminal half-life (t1/2 = 0.693/λ); MRT, mean residence time from time zero to infinity (MRT = AUMC/AUC).

![Sulfamethazine plasma concentration vs. time in bovine and buffalo calves](image.png)

**Fig. 1.** Sulfamethazine plasma concentration [mean (SD)] vs. time curves after intravenous administration of a 60 mg/kg dose in (■) bovine (n = 6) and (□) buffalo (n = 5) calves.
The values obtained for calculated PK/PD ratios \( t > \text{MIC} \) and \( \text{WAUC} \) are present in Table 2. MIC values used in this work were 4 \( \mu g/mL \) which is the \( \text{MIC}_{90} \) value for \( Staphylococcus aureus \) strains isolated from bovine mastitis (Oliveira et al., 2000), and 8, 32 and 128 \( \mu g/mL \) which has been described by Prescott (2000). This author indicates that MIC of 8–32 \( \mu g/mL \) is a reasonable definition of susceptibility and MIC of \( \geq 64–128 \mu g/mL \) can be interpreted as evidence of resistance to sulfonamides. According to the data shown in Table 2, important differences between bovine and buffalo exist for micro-organisms that have a MIC value <32 \( \mu g/mL \). Hence, a different dosage regimen should be used in these species; however, further studies are necessary to establish an optimal dosage regime.

ACKNOWLEDGMENTS

We wish to thank Mr Rogelio Allignani from Laboratorios Allignani Hnos SRL (Santa Fe, Argentina), for providing sulfamethazine for HPLC standard and clinical experiences. The authors wish to thank Mr Mariano Díaz-Flores for his technical assistance, Mr Santiago Cano for his advices and the staff of the Servicio de Préstamo Interbibliotecario, Facultad de Veterinaria, for helping.

REFERENCES


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Table 2. Pharmacokinetic/pharmacodynamic parameters after intravenous administration of sulfamethazine (60 mg/kg) in bovine (n = 6) and buffalo (n = 5) calves

<table>
<thead>
<tr>
<th>t &gt; MIC (h)</th>
<th>WAUC (h)</th>
</tr>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Cattle</td>
<td>Buffalo</td>
</tr>
<tr>
<td>4 ( \mu g/mL )</td>
<td>42.68 ± 6.26*</td>
</tr>
<tr>
<td>8 ( \mu g/mL )</td>
<td>34.94 ± 5.29**</td>
</tr>
<tr>
<td>32 ( \mu g/mL )</td>
<td>19.44 ± 3.47**</td>
</tr>
</tbody>
</table>

*P = 0.017–0.018, **P = 0.030.

⁠¹Oliveira et al., 2000.

⁠²Prescott, 2000.

†Time the drug concentration remains over the MIC. WAUC, weighted AUC.

\[ \text{WAUC} = (\text{AUC}/\text{MIC})(t > \text{MIC}/(t > \text{MIC})_{\text{max}}) \]

T he values obtained for calculated PK/PD ratios \( t > \text{MIC} \) and \( \text{WAUC} \) are present in Table 2. MIC values used in this work were 4 \( \mu g/mL \) which is the \( \text{MIC}_{90} \) value for \( Staphylococcus aureus \) strains isolated from bovine mastitis (Oliveira et al., 2000), and 8, 32 and 128 \( \mu g/mL \) which has been described by Prescott (2000). This author indicates that MIC of 8–32 \( \mu g/mL \) is a reasonable definition of susceptibility and MIC of \( \geq 64–128 \mu g/mL \) can be interpreted as evidence of resistance to sulfonamides. According to the data shown in Table 2, important differences between bovine and buffalo exist for micro-organisms that have a MIC value <32 \( \mu g/mL \). Hence, a different dosage regimen should be used in these species; however, further studies are necessary to establish an optimal dosage regime.