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Pharmacokinetics of fosfomycin in chickens after a single intravenous dose and tissue levels following chronic oral administration

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Summary – This paper describes the pharmacokinetics of fosfomycin following a single iv bolus dose (10 mg/kg of body weight) in broiler chickens. Serial blood samples were collected up to 5 h post-administration. Fosfomycin serum concentrations were determined by a microbiological method, using Proteus mirabilis as the test microorganism. The serum concentration versus time curves after iv administration followed a biexponential decline. The main pharmacokinetic variables were $t_{1/2} = 23$ min, $t_{1/2} = 112$ min, $V_{D(area)} = 575 \pm 190$ mL/kg and $CL_b = 3.12 \pm 0.44$ mL/min/kg.

Tissue levels of fosfomycin in kidney, liver, lung, muscle, heart, fat, gizzard and serum were also determined after oral chronic administration of the drug in drinking water (150 μg/mL). During the oral chronic administration period, high FOS concentrations in serum were maintained (mean 6.1 ± 1.1 μg/mL), but a significant decline over time could be observed ($P < 0.05$). Fosfomycin was detected in all tissues except muscle, with mean concentrations ranging from 0.63 μg/g in fat to 13.48 μg/g in kidney. Twenty-four hours after the treatment was finished, fosfomycin levels were below the assay detection limit in all tissues tested.

Résumé – Pharmacocinétique de la fosfomycine administrée par voie iv et concentrations tissulaires après administration orale chronique chez le poulet. La pharmacocinétique de la fosfomycine a été étudiée chez le poulet de chair après administration par voie iv d’une dose unique de 10 mg/kg. Des échantillons de sang ont été prélevés pendant 5 heures suivant l’administration. Les concentrations sèreuses de fosfomycine ont été déterminées par une méthode microbiologique grâce à Proteus mirabilis comme microorganisme de test. Les courbes concentration versus temps en sérum après administration iv suivent une décroissance biexponentielle. Les principales variables pharmacocinétiques étaient $t_{1/2} = 23$ min, $t_{1/2} = 112$ min, $V_{D(area)} = 575 \pm 190$ mL/kg et $CL_b = 3.12 \pm 0.44$ mL/min/kg.

Les concentrations de fosfomycine dans les tissus (foie, poumon, muscle, cœur, graisse, estomac et sérum) ont également été déterminées après administration orale chronique du médicament dans l’eau d’alimentation (150 μg/mL). Au cours de la période d’administration orale chronique, des concentrations élevées de FOS ont été maintenues dans le sérum (moyenne 6.1 ± 1.1 μg/mL), mais une décroissance significative au cours du temps a pu être observée ($P < 0.05$). Fosfomycin a été détecté dans tous les tissus excepté le muscle, avec des concentrations moyennes variant de 0.63 μg/g dans la graisse à 13.48 μg/g dans le foie. Trente-quatre heures après le traitement, les niveaux de fosfomycine étaient inférieurs à la limite de détection de l’assay dans tous les tissus testés.

pharmacokinetics / fosfomycin / chicken / oral administration / intravenous administration / tissue levels
à l'utilisation de Proteus mirabilis. Les concentrations sériques en fonction du temps ont pu être décrites par une équation biexponentielle, dont les principaux paramètres étaient : $t_{1/2 \lambda_1} = 23$ min, $t_{1/2 \lambda_2} = 112$ min, $V_{\text{Darea}} = 575 \pm 190$ mL/kg and $C_{\text{area}} = 3,12 \pm 0,44$ mL.min$^{-1}$ kg$^{-1}$. Les concentrations tissulaires de fosfomycine dans le rein, le foie, le poumon, le muscle, le cœur, le tissu adipeux, le gésier et dans le sérum ont été mesurées après une administration orale chronique de la drogue dans l'eau de boisson (150 µg/mL). Les concentrations sériques de FOS restèrent élevées durant toute la durée de l'administration (6,1 ± 1,1 µg/mL), mais avec une diminution significative en fonction du temps. La fosfomycine a été détectée dans tous les tissus sauf dans le muscle, avec des concentrations moyennes comprises entre 0,63 µg/g dans le tissu adipeux, et 13,5 µg/g dans le rein. Vingt-quatre h après la fin du traitement, la concentration en fosfomycine se trouvait en dessous du seuil de détection dans tous les tissus prélevés.

pharmacocinétique / fosfomycine / poulet / administration orale / administration intraveineuse / concentration tissulaire

INTRODUCTION

Fosfomycin (FOS), chemically -(-)(1R,2S)-1,2-epoxypropyl-phosphonic acid, is an antimicrobial drug whose simple chemical structure considerably differs from the traditional structures of antimicrobial agents (fig 1). It has a broad in vitro antibacterial spectrum against both Gram-negative and Gram-positive bacteria (Goto, 1977; Woodruff et al, 1977, Neu and Kamirura, 1981). Its spectrum of activity and minimum toxicity makes FOS of therapeutic interest for small animal, cattle and poultry medicine (Escudero et al, 1991; Kobayashi and Baba, 1992; Piriz et al, 1992; Gutierrez and Rodriguez, 1993).

Although FOS is a valuable antimicrobial agent, there are only a few studies on its disposition after acute or chronic administration in domestic animals. Information is limited to human patients with impaired renal function, healthy human volunteers, dogs and rabbits (Dalet et al, 1977; Yamamura et al, 1978; Goto et al, 1981; Fernandez et al, 1986). The drug is rapidly cleared from the central compartment with elimination occurring mainly in the unaltered state through the kidney (Kawabata et al, 1978). FOS was not bound to plasma proteins and its volume of distribution was small.

In avian clinics, instances of infections caused by multiresistant Gram-negative or beta-lactamase Gram-positive microorganisms are becoming increasingly common. FOS may therefore represent an alternative for the prevention and treatment of these infections. Because pharmacokinetic evaluation is necessary to compare FOS to other available antibacterial agents used in the treatment of common poultry diseases, the purpose of this study was to define FOS disposition in chickens after iv administration. In addition, tissue levels were determined after oral administration of FOS in the drinking water.

MATERIALS AND METHODS

Intravenous administration

Four 14-day-old broiler chickens from the Animal Experimentation Service of the University of Zaragoza (Spain) were placed in individual cages.
1 week before experimentation. During this period, no clinical signs of disease were apparent. Animals were maintained under conditions of controlled temperature (25 ± 2 °C), light cycle (12/12 h) and humidity (45–65%). Food and water were supplied ad libitum. The (-)cis-isomer of FOS (Fyse SA, Spain), as a sodium salt, was administered as a single iv dose of 10 mg/kg of body weight, diluted in sterile saline through the humeral vein. Serial blood samples (1 mL) were collected at 0, 5, 15, 30, 45, 60, 90, 120, 180, 240 and 300 min post-injection, via an indwelling catheter inserted into the contralateral humeral vein. Blood samples were allowed to clot at 5 °C. Serum was then obtained by centrifuging at 3 000 g for 5 min and then stored frozen at −20 °C until analysis.

Oral administration

In this experiment, 20 14-day-old broiler chickens were placed on the floor in the same conditions of temperature, light cycle and humidity as described above. After an acclimation period of 1 week (21 days old), the (-)cis-isomer of FOS was administered in the drinking water (150 mg/L) for 5 consecutive days. During these 5 days, every morning between 7 and 8 am the drinking water containing FOS was removed, and the volume consumed was measured. Drug concentration was assayed to confirm stability of the drug over time. Before the change of the medicated water, blood samples (1 mL) from four chickens (sampled once) were taken at random daily for 5 days and assayed for FOS concentration. At the end of the treatment period, chickens were euthanised by pentobarbital administration, followed by exsanguination (eight animals in the morning of the sixth day and four animals 24 h after treatment termination). Samples of serum (0.5 mL), kidney, liver, lung, muscle, heart, fat and gizzard (up to 2 g) were taken. Each specimen was carefully weighed and kept at −20 °C until further assayed for FOS concentration.

Microbiologic assay

Samples from each tissue (2 g) were homogenised with 5 mL of 0.05 M Tris-hydroxymethylaminomethane buffer (pH 8.0) at 13 500 rpm for 3 min. After centrifugation (3 000 g for 5 min), the supernatants were collected and immediately assayed. Serum samples were assayed without undergoing the extraction process.

FOS concentration in the homogenates of the selected tissues and in the serum was assayed by an agar-well diffusion biological assay using Proteus mirabilis (ATCC 21100) as the test organism. Calibration curves for each tissue and for serum were constructed daily in the range 0.50–10 µg/mL by spiking pooled blank tissue homogenates or serum with a standard solution of FOS in saline (10 mg/mL). This method was linear at concentrations between 0.1 and 20 µg/mL (r² = 0.98). The quantitation limit was determined as 0.5 µg/mL both in serum and tissue homogenates. The detection limit was 0.25 µg/mL for tissue homogenates and 0.1 µg/mL for serum samples. Recovery was determined as 83 and 95% for tissue homogenates and serum, respectively. Inter- and intra-assay precisions, determined by analysis of control standards, were 7 and 5% for tissue homogenates and 5 and 4% for serum, respectively. All determinations were carried out in triplicate.

Data analysis

The following equation was used, describing the time course of FOS concentration in serum after iv administration:

\[ C = \sum_{i=1}^{n} C_i e^{-\lambda_i t} \] (1)

where \( C_i \) and \( \lambda_i \) are obtained by nonlinear least squares regression analysis, using a weighting factor of \( 1/c^2 \). The half-life in the distributive and post-distributive phases was calculated as

\[ t_{1/2} = \ln 2/\lambda_i \]

where \( \lambda_i \) is the slope of the distributive and post-distributive phases.

Total body clearance was calculated as follows:

\[ CL_b = \text{Dose/AUC} \]

where AUC is the area under the serum concentration versus time curve, calculated using the trapezoidal rule to the last data point and extrapolated to infinity by dividing \( C_{last} \) by \( \lambda_n \).
Finally, the volume of distribution based upon the AUC was calculated as

\[ V_{d(area)} = \frac{\text{Dose}}{\text{AUC} \times \lambda_a} \]

All results are presented as means ± SD, except for the half-lives, where the harmonic mean was calculated. The differences were analyzed by Student's unpaired t-test. \( P < 0.05 \) was considered to be statistically significant.

RESULTS

Intravenous administration

The time course of FOS concentration in serum after iv administration of a dose of 10 mg/kg of body weight to chickens was described by a biexponential decrease (fig 2). Table I shows the values obtained for the pharmacokinetic parameters established from the model. After iv administration, FOS had a slow distribution phase \( (t_{1/2\lambda_1} = 23 \text{ min}) \), which extended for approximately 100 min (fig 2). Thereafter, a log-linear elimination decrease began \( (t_{1/2\lambda_2} = 112 \text{ min}) \), and extended to 300 min. Volume of distribution, calculated by use of the area method, ranged from 311 to 733 mL/kg and the total body clearance from 2.65 to 3.69 mL.min⁻¹.kg⁻¹.

Oral administration and tissue levels

During the oral chronic administration period, high FOS concentrations in serum were maintained (mean ± SD: 6.1 ±...
Table I. Individual and mean pharmacokinetic parameters determined in four chickens after iv administration of fosfomycin (10 mg/kg body weight).

<table>
<thead>
<tr>
<th>Parameter (units)</th>
<th>Chicken</th>
<th></th>
<th></th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>$C_1$ (µg/mL)</td>
<td>34.5</td>
<td>39.1</td>
<td>27.1</td>
<td>35.7</td>
</tr>
<tr>
<td>$\lambda_1$ (min⁻¹)</td>
<td>0.0174</td>
<td>0.0320</td>
<td>0.0413</td>
<td>0.0291</td>
</tr>
<tr>
<td>$C_n$ (µg/mL)</td>
<td>6.7</td>
<td>8.1</td>
<td>20.5</td>
<td>8.7</td>
</tr>
<tr>
<td>$\lambda_n$ (min⁻¹)</td>
<td>0.0052</td>
<td>0.0047</td>
<td>0.0119</td>
<td>0.0036</td>
</tr>
<tr>
<td>$t_{1/2\lambda_1}$ (min)</td>
<td>39</td>
<td>22</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>$t_{1/2\lambda_n}$ (min)</td>
<td>132</td>
<td>148</td>
<td>62</td>
<td>191</td>
</tr>
<tr>
<td>AUC (µg.min.mL⁻¹)</td>
<td>3407</td>
<td>3090</td>
<td>2707</td>
<td>3773</td>
</tr>
<tr>
<td>$V_1$ (mL/kg)</td>
<td>232</td>
<td>202</td>
<td>193</td>
<td>217</td>
</tr>
<tr>
<td>$V_{\text{Darea}}$ (mL/kg)</td>
<td>561</td>
<td>694</td>
<td>311</td>
<td>733</td>
</tr>
<tr>
<td>$CL_n$ (mL.min⁻¹.kg⁻¹)</td>
<td>2.93</td>
<td>3.23</td>
<td>3.69</td>
<td>2.65</td>
</tr>
</tbody>
</table>

* Harmonic mean. $C_1$ and $C_n$ are the coefficients and $\lambda_1$ and $\lambda_n$ are the exponents that were derived by a nonlinear least squares regression of concentration data to eq (1). These terms are related to rate constants for the specific two-compartment pharmacokinetic model and were used to derive the following pharmacokinetic parameters: $t_{1/2\lambda_1}$ = distribution phase half-life; $t_{1/2\lambda_n}$ = terminal phase half-life; $V_1$ = volume of the central compartment; $V_{\text{Darea}}$ = apparent volume of distribution calculated by the area method; AUC = area under the serum concentration versus time curve calculated by the trapezoidal rule and extrapolated to infinity and $CL_n$ = body clearance.

Fig 3. Fosfomycin serum concentration (squares) and body weight (triangles) measured throughout oral administration of fosfomycin in drinking water (150 µg/mL) for 5 consecutive days. Values represent the mean (± SD) of four determinations.
1.1 μg/mL), but a significant decline over time (fig 3) could be observed ($P < 0.05$). The FOS concentration in the drinking water was measured daily, but chemical degradation was not apparent. In addition, water intake was recorded throughout FOS chronic administration but differences were not detected (mean 220 mL/kg of body weight; range 188–235 mL/kg of body weight). Throughout the FOS administration period chicken weight increase was linear and could be inversely correlated with FOS serum levels ($r = 0.93; P < 0.05$).

In this experiment, FOS tissue levels were determined after 5 consecutive days of administration in the drinking water (table II). On the morning of the sixth day (post-treatment day 0), FOS was detected in all tissues selected except in muscle, the highest concentration being in the kidney homogenates (13.5 ± 4.4 μg/g of tissue). Twenty-four hours after the treatment was finished, the FOS concentration in all tissues examined was below the detection limit of the assay (0.25 and 0.1 μg/mL in tissue homogenates and in serum, respectively).

### DISCUSSION

After iv administration of a dose of 10 mg/kg of body weight, FOS disposition in chicken was described adequately by a biexponential equation corresponding to a two-compartment model with first-order elimination from the central compartment. Experiments performed in other animal species led to a similar conclusion (Yaginuma et al, 1978; Fernandez et al, 1986). Nevertheless, a faster distributive phase was reported in these studies. The half-life in the post-distributive phase in chickens was 2 h, approximately, which agrees with that observed in mammals (Bouchet et al, 1988). The volume of distribution was determined as 575 ± 190 mL/kg, suggesting that FOS is mainly distributed in extracellular fluid and is not tissue-bound. The total body clearance (3.12 ± 0.44 mL.min⁻¹.kg⁻¹) was similar to the glomerular filtration rate in chickens (2.1 mL.min⁻¹.kg⁻¹) suggesting that, as described in other animal species, the kidney plays a key role in FOS elimination. To our knowledge, there are no previous reports concerning FOS pharmacokinetics in chickens. Our results indicate that the rate of FOS elimination in chickens is similar to that found in mammals.

In an attempt to evaluate the practical use of FOS, we determined the serum concentration and tissue levels of this drug after oral administration in drinking water for 5 consecutive days. Throughout the study a slow but significant decrease in FOS con-

### Table II. Serum and tissue concentrations (μg/mL or μg/g, respectively) determined after oral administration of fosfomycin in drinking water (150 μg/mL) for 5 consecutive days.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Mean ± SD*</th>
<th>Tissue-to-serum ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>4.80 ± 0.41</td>
<td>1.00</td>
</tr>
<tr>
<td>Fat</td>
<td>0.63 ± 0.02</td>
<td>0.13</td>
</tr>
<tr>
<td>Muscle</td>
<td>&lt; 0.5</td>
<td>—</td>
</tr>
<tr>
<td>Liver</td>
<td>2.55 ± 0.20</td>
<td>0.53</td>
</tr>
<tr>
<td>Kidney</td>
<td>13.48 ± 4.39</td>
<td>2.81</td>
</tr>
<tr>
<td>Lung</td>
<td>2.65 ± 0.45</td>
<td>0.55</td>
</tr>
<tr>
<td>Heart</td>
<td>1.67 ± 0.28</td>
<td>0.35</td>
</tr>
<tr>
<td>Gizzard</td>
<td>1.05 ± 0.36</td>
<td>0.22</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± SD of eight determinations.
centration in serum ($P < 0.05$) was observed that could be correlated with the recorded weight gain in these animals ($r = 0.93; P < 0.05$), suggesting that in fast-growing species, such as chickens, weight and/or body composition may be an important factor in determining FOS disposition. However, other factors such as an impaired intestinal permeability to FOS cannot be ruled out (Ishizawa et al, 1991).

To the end of the treatment period, FOS was detected in serum and in selected tissues, except in muscle. After a 24-h withdrawal period, the serum and tissue FOS concentrations were below the detection limit of the assay. Except for the kidney, all tissues examined showed FOS levels below those observed in serum. To our knowledge, only one report has been presented describing MIC (Minimum Inhibitory Concentration) of FOS (0.4–60 μg/mL) for clinical isolates from poultry (Frost et al, 1974). Information regarding FOS antimicrobial activity usually comes from clinical isolates from human patients (Gobernado et al, 1987) and other animal species (Escudero et al, 1991; Kobayashi and Baba, 1992; Piriz et al, 1992; Gutierrez and Rodriguez, 1993). Based on this partial information, we could observe potentially antibacterial concentrations (larger than 1.0 μg/mL) for common agents causing infections in domestic fowl including Pasteurella, Staphylococcus aureus, Escherichia coli, Salmonella spp, Shigella spp, and Yersinia enterocolitica, in all tissues examined except in muscle and fat. Well-perfused organs, such as liver or lung, had intermediate concentrations compared to those found in serum. These findings support the argument that FOS was only slightly distributed into the intracellular space. FOS is highly water-soluble and, consequently, its transfer across biological membranes may be difficult. In contrast, its low level of protein binding and its low molecular weight facilitate diffusion into extracellular fluid. The sum of these opposite factors seems to negatively affect the FOS diffusion ability to tissues such as muscle and fat. In contrast, kidney FOS concentration was approximately three-fold higher than that found in serum, suggesting that this organ is capable of accumulating important amounts of this drug (Yaginuma et al, 1978; Bouchet et al, 1988).

To our knowledge, FOS has not been approved for use in chickens in the European Community. Nevertheless, our findings suggest that oral administration of FOS in drinking water at a dose of 150 μg/mL for 5 consecutive days, provides potentially therapeutic concentrations of the drug in chickens.

ACKNOWLEDGMENTS

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