RESEARCH ARTICLE

IJAVMS

Pérez *et al.*, IJAVMS, Vol. 6, Issue 6, 2012: 424-435
DOI: 10.5455/ijavms.20130111041914

Pharmacokinetics and Bioavailability of Calcium Fosfomycin in Post Weaning Piglets after Oral Administration

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Rec.Date: Nov 23, 2012 10:19

Accept Date: Dec 21, 2012 16:19

Abstract

Calcium fosfomycin pharmacokinetics and bioavailability were studied in post weaning piglets after oral administration at 30 mg/kg of bodyweight (BW)). Fosfomycin plasma concentrations were measured by high-performance liquid chromatography MS/MS. The $T_{1/2}$ was of 1.80 ± 0.89 h. C_{max}, T_{max} and bioavailability were 3.60 ± 0.96 µg/mL, 3.00 ± 0.00 h and 20.0 ± 1.85 %, respectively. The area under the fosfomycin concentration:time curve in plasma $AUC_{(0-\infty)}$ was 45.48 ± 9.20 µg h/mL. Different authors have determined a minimum inhibitory concentration ranging from 0.25 - 0.5 µg/mL for *Streptococcus sp.* and *Escherichia coli*, respectively. Considering these minimum inhibitory concentrations of sensitive bacteria to fosfomycin, taking into account fosfomycin is a time-dependent antimicrobial and according to the values of fosfomycin plasma concentration vs. time profiles observed in this study, it was determined that effective plasma concentrations of fosfomycin for sensitive bacteria can be obtained following oral administration of fosfomycin at a dose of 30 mg/kg in piglets.

Keywords: calcium fosfomycin, post weaning piglets, pharmacokinetics, bioavailability.

Introduction

Fosfomycin (cis-1,2-epoxyphosphonic acid) is a bactericidal broad-spectrum antibiotic that is not structurally related to other classes of antimicrobial agents. It acts inside the bacterial cytoplasm[1] by inhibition of cell wall and early murein/peptidoglycan synthesis in proliferating bacteria [2]. Fosfomycin inhibits an initial step in peptidoglycan synthesis, which is triggered by uridine diphosphate N-acetyl-glucosamine-enol-pyruvyl-transferase and its co-enzyme, phosphonole-pyruvate [1,2], causing bactericidal activity against Gram positive and Gram negative bacteria [3]. So, due to its mechanism of action and when compared with other antibiotics, fosfomycin *in vitro* activity has a broader spectrum of action than penicillins and semi-synthetic cephalosporins [4]. On the other hand, due to its particular chemical structure and mode of action [5], cross-resistance with other antibiotics has not been reported [3].

Fosfomycin tends to form salts easily due to its acidic nature. Orally (PO), it is used as a calcium salt, whereas intravenously (IV), intramuscularly (IM) and subcutaneously (SC) as the more water-soluble disodium salt. This salt cannot be used PO, due to its degradation at the acidic pH of the stomach. Fosfomycin-tromethamine salt is highly hydro-soluble and offers a good bioavailability in humans after oral administration [1,5] and in weaning piglets after intramuscular injection [6].

The low toxicity and potential efficacy of fosfomycin are the main factors that contribute to it use in humans and animals [7]. It is also widely used in animal production due to its rapid effect, good tolerance and absence of side effects [8].

Different analytical methods for determination of fosfomycin in biological matrices have been described in the literature [9,10,11,12,13]. Most of them are time consuming and include a derivatization step for the analysis. Currently, HPLC MS/MS is the method of choice for xenobiotics determination. It use has been described for fosfomycin determination in serum of humans [14], chickens [15,16] and piglets [6] and in chicken tissues [17].

Pharmacokinetics (PK) profiles of the various derivates of fosfomycin have been described in humans [18,19,20], chickens [8,16], rabbits [21], cows [22], dogs [12] and horses [13]. Soraci et al. [6] have studied disodium fosfomycin PK in weaning piglets (21-25 days old) after intramuscular administration. Weaning is a critical period for piglets and it is characterized by a transient drop in food intake, associated with a state of under-nutrition. This affects different aspects of the physiology and metabolism of the animal [23] and it is frequently associated with infectious diseases [24]. Over decades many antibiotics have been used to reduce pathogen infection in pigs, and consequently, some bacteria have become resistant [23]. In clinical practice, fosfomycin represents a potential alternative for the treatment of infections caused by resistant bacteria in weaning piglets. Particularly in piglets, fosfomycin is indicated to treat a wide variety of bacterial infections (*Haemophilus parasuis*, *Streptococcus suis*, *Pasteurella multocida*, *Bordetella brochiseptica*, *Staphylococcus hyicus*, *Escherichia coli*), associated to stress and/or to different virus diseases [25].

The systemic bioavailability of antibiotics may affect the time over which the bacteria are exposed to toxic concentrations [26]. Important differences in bioavailability (F) have been found after oral administration in relation to the various derivatives of fosfomycin salts, such as disodium fosfomycin (41-85%), calcium fosfomycin (20%) and trometamol fosfomycin (34-41%) [5,19]. Furthermore, the intramuscular administration of disodium fosfomycin offers a more predictive route of dose absorption than oral administration. It may be associated with two facts a) absorption from the gastrointestinal tract is a saturable process associated to the phosphate system and b) there is a degradation of disodium fosfomycin in acid gastric pH [12]. Beyond that intramuscular route is more predictive for dose absorption, oral administration is useful for the treatment of infectious intestinal diseases, especially when the drug has a poor bioavailability.

Considering that the characterization of PK of antibiotics in plasma can be used to predict and optimize their antimicrobial efficacy [27] and that the effective action of antibiotics depends on a sustained and sufficient drug concentration at the site of action [28], it would be relevant to study the pharmacokinetics and bioavailability of calcium fosfomycin in swine. Previous investigations have focused on the study of these parameters in humans and broiler chickens. However, information in swine

is not available. In clinical practice, fosfomycin represents a potential alternative for the treatment of porcine infections caused by resistant bacteria; therefore, PK studies of calcium-fosfomycin are necessary for a rational use of this drug in pig production. The objective of this study was to define the plasmatic disposition and absolute bioavailability of calcium fosfomycin in weaning piglets after IV and PO administration.

Materials and methods

This work was performed at the Laboratory of Toxicology and at the experimental farm of the Faculty of Veterinary Sciences, UNICEN, Tandil, Buenos Aires, Argentina.

Plasma disposition of calcium fosfomycin was evaluated in six piglets (three males and three females) following a single PO dose of 30 mg/kg. To determine the absolute bioavailability of calcium fosfomycin, another group of six piglets, received 15 mg/kg of disodium fosfomycin by IV route. The study was carried out following the rules of ethical approval by the experimental ethics committee of the Faculty of Veterinary Sciences, UNICEN, Tandil, Buenos Aires, Argentina.

Antibiotic

Sterile powdered disodium and calcium fosfomycin were supplied by Bedson S.A., Laboratories (Pilar, Buenos Aires, Argentina).

Animals

Twelve weaning piglets 25-28 days old (10 ± 1.5 kg b.w.) were used in this trial. Animals were weighted, identified and housed in pens in weaning rooms.

Administration

Animals were divided in two groups of six animals (three males and three females). One group received an intravenous dose of 15 mg/kg b.w. of disodium fosfomycin. The other group received an oral dose of calcium fosfomycin (30 mg/kg b.w.), via an orogastric tube. The antibiotic was previously dissolved in sterile distilled water and it was administered at final volume of 1.5 mL (IV) and 10 mL (PO). Doses were administered after 6 hours of fasting.

Sampling

To minimize the stress and facilitate blood sampling, a permanently heparinized, long catheter was placed in the left external jugular vein of each piglet, according to the method of Matte [29] modified by Soraci et al. [30]. For this, after 6 hours of solid fasting, animals underwent a sedated state (to reduce stress during clamping), through the combined use of diazepam-ketamine at 2 mg/kg and 15 mg/kg, respectively, via IM. After 20 minutes, animals were supine placed. The neck was disinfected with a commercial solution of povidone-iodine. Subsequently, a 1.5 cm incision was conducted in the middle of the neck, at skin level, at a point located between the tip of the breastbone and the base of the lower jaw and 1 cm inside of the trachea. The incision involved only skin and subcutaneous tissue. With the aid of blunt scissors the area was divulsed, exposing easily the external jugular vein. The blood vessel was exposed to the lips of the wound and tied in a knot cranial simple linen thread. A small cut in the vein allowed the introduction of a nasogastric sterilized tube. The probe was inserted about 10 cm toward the heart and connected to the vessel wall with a simple knot linen thread. 300 µl of dead space was filled

with an anticoagulant solution containing 250 units of heparin and the tube was closed at one end with the plug of the adapter. A U-stitch permitted the closure of the wound. Three milliliter blood samples were collected after discarding the upper 0.5 mL of heparinized blood. Sampling times were: 0, 5, 10, 15, 30 and 45 min and 1-4, 6, 8, 12 h. Blood samples were immediately centrifuged. The plasma was recovered, identified and frozen at -20 ºC until analyzed within 4 days.

Drug assay

Analytical procedure

Determination of calcium fosfomycin in plasma was carried out in triplicate by a high-performance liquid chromatography mass-mass spectrometry (HPLC-MS/MS) according to the method used by [6,15,16].

Instruments

The HPLC-MS/MS system (Thermo Electron Corporation), consisted of a Finnigan Surveyor auto sampler and a Finnigan Surveyor MS quaternary pump. The detector was a Thermo Quantum Discovery Max triple quadrupole mass spectrometer, equipped with a ESI source. Nitrogen used as nebulizer and sheath gas was obtained through a nitrogen generator from Peak Scientific (Inchinnan). Data processing was done using Xcalibur software, also from Thermo. A Turbo Vap workstation (Caliper) with bath temperature and air flow control was used for solvent evaporation.

Mass Spectrometer conditions

The mass spectrometer was operated in negative ionization mode. The tuning parameters were optimized with 10 µg mL^{-1} individual aqueous fosfomycin and fudosteine solutions. A syringe pump directly infused the solutions into the ion source at 10 µl min^{-1}, while the mobile phase was delivered from the LC pump through a T connection to give the corresponding chromatographic flow rate. Spray voltage was set to -3800 eV, capillary temperature was 350 ºC. Argon 99,99% purity was used for collision induced dissociation (CID) at 1.6 m Torr in the collision cell. Source CID energy was set to -8 eV. Fosfomycin and fudosteine detection and quantification were achieved by single reaction monitoring of transitions m/z 137→79 with optimized collision energy of 25, and 178→91 with optimized collision energy of 14, respectively. The precursor ions of m/z 137 and 178 are selected in the first quadrupole (Q1). After de Q2 collision-induced fragmentation (with a partial fragmentation of the parent ion of m/z 137), the produced ions of m/z 79 and 91 are detected in Q3, and also the parent ion of m/z 137, which acts, in this case, as a daughter ion, to reach the needed 4 identifications points.

Chromatographic conditions

Separation was achieved on a Phenomenex Luna CN (cyano) (411, Madrid Avenue Torrance, CA90501–1430, USA), stationary phase, 150 mm x 4.6 i.d., 5 µm column. The mobile phase consisted of acetonitrile:water 20:80 working in isocratic mode, at a flow rate of 250 µl min^{-1}. The column was maintained at 30 ºC. Samples in the auto sampler were kept at 10 ºC. Sample injection volume was 20 µl and chromatographic run time was 6 min. Quantification was achieved by calculating area ratio between fosfomycin and it IS fudosteine, as the assay response. Validation parameters as well as their acceptance range were in accordance with international guidelines[31,32], as previously demonstrated by[6].

Validation parameters

Validation parameters and acceptance ranges, were in accordance with international guidelines[31,32]. Quantification was achieved by calculating fosfomycin area as the assay response. Calibration curves, performed by drug free plasma extracts in the range of 0.1 µg/mL to 50 µg/mL, prepared under the same conditions, were performed in quintuplicates, and assayed within one week, in order to assess Linearity by Hartley's Test. Least square linear regression was used for curve fitting. QC samples fortified at 3 levels were processed in triplicates on 4 separate days, in order to assess Accuracy and Precision of the method. The accuracy was expressed as relative error (RE) and it was required to be ±15% (except for the limit of detection, for which 20% is accepted). Within-day precision (repeatability) was calculated by the mean coefficient of variation (CV) which was required to be less than 15% for all concentrations (except for the limit of detection, for which 20% is accepted). Between days precision (intermediate precision) was expressed as between days coefficient of variation, which was calculated using the following equation:

$$CV_{bd} = \frac{SD_{bd}}{\mu}$$

Being:

µ: average media

SDbd= between day standard deviation (calculated as the square root of between days variance)

Between days variance was obtained after substracting the contribution of within day variability, using the following equation

$$SD^2_{bd} = SD^2(\mu) + \frac{n-1}{n} SD^2_{wd}$$

Being:
SD2(µ): variance of every day mean
n : number of observations per day
SD2wd: average within day variance.

Lower limit of quantification was defined as the lowest concentration at which both precision and accuracy were less than or equal to 20%, and it was obtained by analyzing fortified tissues at the lower level of the calibration curve, in 5 replicates, on three different days. Recovery of fosfomycin following extraction was calculated by comparing the fosfomycin mean peak area of QC samples with the values obtained for post-extraction, spiked samples, which represented 100% recovery. Selectivity was determined by analyzing plasma from 6 healthy piglets from different farms, which had never received antimicrobial treatment. To determine fosfomycin stability, stock solutions kept at 4ºC were tested regularly in order to assure a constant concentration throughout the study. To evaluate bench top stability during in-day manipulation, fosfomycin standard solutions (within the range of calibration curve) were kept at room light and temperature for 6 hours. Aliquots were taken every hour and injected into HPLC-

MS/MS system. Mean peak area ratios were compared with those obtained for a freshly prepared solution. Stability of fosfomycin in serum extracts was also evaluated. Samples obtained from chicken treated with fosfomycin were extracted and quantified. These samples were left inside the autosampler (at 10ºC) and re quantified every 5 days for a period of 2 weeks. The decision limit (CCα) is defined as the limit above which it can be concluded, with an error of probability of α, that a sample contains the analyte. The detection capability (CCβ) is defined as the lowest concentration of analyte at which the method is able to detect and quantify contaminated samples with a statistical certainty of 1- β[32].

Data analysis

The analysis of PK parameters of individual plasma disposition in animals was carried out using a non-compartmental method and fitting the concentration-time data to an appropriate model by means of a pK Solutions 2.0 computer program (Summit Research Services, Asland, OH, USA). The non-compartmental models have grown steadily in use. They can be used to determine in a simple and rapid way (without deciding on a particular compartmental model) certain PK parameters which are useful in the pharmacokinetic-pharmacodynamic (PK/PD) studies of antibiotics[33]. The area under the curve (AUC) for fosfomycin was estimated by the method of trapezoids[34]. Volume of distribution and body clearance were calculated by classical methods[35]. Equation 1 determines the area under the curve from zero to infinity. The half-life time ($T_{1/2\beta}$) is obtained by the equation 2. The absolute bioavailability of calcium fosfomycin is calculated with the equation 3. The body extraction ratio (E_{body}), a numerical value between 0 and 1, which can be regarded as the percentage of the drug being cleared by the entire body during a single passage through the different clearing organs contributing to de body clearance, was calculated using Equation 4.

Equation 1: $AUC_{(0-\infty)} = AUC_{(0\text{-}Clast)} + C_{last}/\lambda_z$
Where λ_z represent the slope of the last phase.
Equation 2: $T_{1/2\beta} = 0.693/\lambda_z$

Equation 3: $(AUC_{PO}/AUC_{IV}) \times (Dose_{IV}/Dose_{PO}) \times 100$
Where, AUC_{PO} is the AUC before oral administration of calcium fosfomycin;
AUC_{IV} is the AUC before the intravenous administration of disodium fosfomycin; and
$Dose_{IV}$ and $Dose_{PO}$ are oral and intravenous doses, respectively.

Equation 4: E_{body} = Body clearance / Cardiac output
Where, Cardiac output (mL/kg/min) = 180 x Body weight $(kg)^{-0.19}$

Results

All validation parameters were within the range of acceptance as evidenced by Table 1. Table 2 indicates the results for accuracy, Table 3 shows repeatability (within day precision) and intermediate precision (between day precision) and Table 4 expresses the recovery of the method. In regard to stability, no significant differences in concentrations ($\alpha < 0.05$) were observed neither between stock fosfomycin solution kept at 4ºC for 4 months, nor for a 10 μg mL^{-1} fosfomycin solution left on bench top for 6 hours, compared to freshly prepared ones. Evaluation of drug stability in plasma samples, showed no significant differences ($\alpha < 0.05$) between freshly prepared samples and those kept in the autosampler for 2 weeks. The stability of fosfomycin in analytical conditions and in the biological matrix allowed us to simplify analytical procedures.

Table 1. Fosfomycin plasma validation parameters - Summary.

PARÁMETER	ACCECPTANCE CRITERIA	VALUES
Lineality - Linear range 0.1-50 µg/mL	<0.995	0.998
Lineality - Hartley's Test	FMAX<FTABULADO	24.56<25.20
Intermediate Precision (CV%)	< 15	3.02-4.94
Accuracy (ER %)	< 15	0.68-0.73
% R	80-20	94.93-106.18
Limit of quantification	CV % = < 20 ER % = < 20	0.10 ppm CV % = 18.00 ER % = 10.00
CCα (µg/kg)	-	0.04
CCβ (µg/kg)	-	0.08

Table 2. Accuracy

Concentration (ppm)	Obtained average concentration (ppm)	Standard Deviation	Accuracy ER (%)
5	5,03	0,24	0,68
10	9,82	0,29	1,72
20	20,14	0,81	0,73

Table 3. Estimation of repeatability (within day precision) and intermediate precision (between day precision) for blank serum samples spiked at 10 µg/ml (obtaining a final concentration of 0.125µg/ml to be injected into HPLC system).

	Day 1	Day 2	Day 3	Day 4	Mean (µ)	SD^2 (µ)
Mean	0.124	0.118	0.123	0.125	0.122	$9{,}66 \times 10^{-6}$
SD	0.004	0.005	0.002	0.006		
CV%	3.22	4.23	1.62	4.80		1.94

Within day precision: 3.47 Between day precision: 9.88

Table 4. Recovery of the method

Nominal Concentration (ppm)	Obtained Concentration (ppm)	Recovery (%)
5	4,969	99,38
5	4,845	96,90
5	5,061	101,22
5	5,094	101,88
5	4,829	96,58
10	18,985	94,93
10	20,119	100,60
10	20,123	100,62
10	19,511	97,56
10	19,571	97,86
40	40,371	100,93
40	39,927	99,82
40	40,768	101,92
40	42,083	105,21
40	42,473	106,18

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Table 5 lists the kinetic parameters observed after IV and PO administration and Figure 1 shows the mean plasma levels of fosfomycin after IV and PO administration of 15 mg/kg and 30 mg/kg, respectively.

After IV administration, the apparent volume of distribution by the area method (Vd_{area}) was 273 ± 40.7 mL/kg, the mean elimination half-life ($T_{½}\beta$) was 1.54 ± 0.4 h. After PO administration of a 30 mg/kg b.w dose, the mean peak concentration (C_{max}) observed was 3.60 ± 0.96 µg/mL with a calculated T_{max} 3.00 ± 0.00 h. F (%) was 20.0 ± 1.85 %. The $T_{½}\beta$ was 1.80 ± 0.89 h. E_{body} was 0.02.

Table 5. PK parameters of fosfomycin obtained in piglets after intravenous (IV) and oral (PO) administration of a single dose of 15 mg/kg and 30 mg/kg, respectively.

PARAMETERS	IV MEAN ± SD	PO MEAN ± SD
$T_{½}\beta$ (h)	1.54 ± 0.40	1.80 ± 0.89
$AUC_{0-\infty}$ (µg.h/mL)	111.05 ± 22.6	45.48 ± 9.20
Vd_{area} (mL/Kg)	273.0 ± 40.7	-
Cl_b (mL/h/kg)	140.0 ± 39.6	-
MRT (h)	3.5 ± 1.4	-
T_{max} (h)	-	3.0 ± 0.00
C_{max} (µg/mL)	-	3.6 ± 0.96
F (%)	-	20.0 ± 1.85

Where;
AUC: Area under the plasma concentration-time curve.
MRT: mean residence time.
Vd_{area}: volume of distribution.
Cl_b: Clearance.
C_{max}: the maximum concentration after the oral dose.
T_{max}: time after the oral dose.
F: Bioavailability

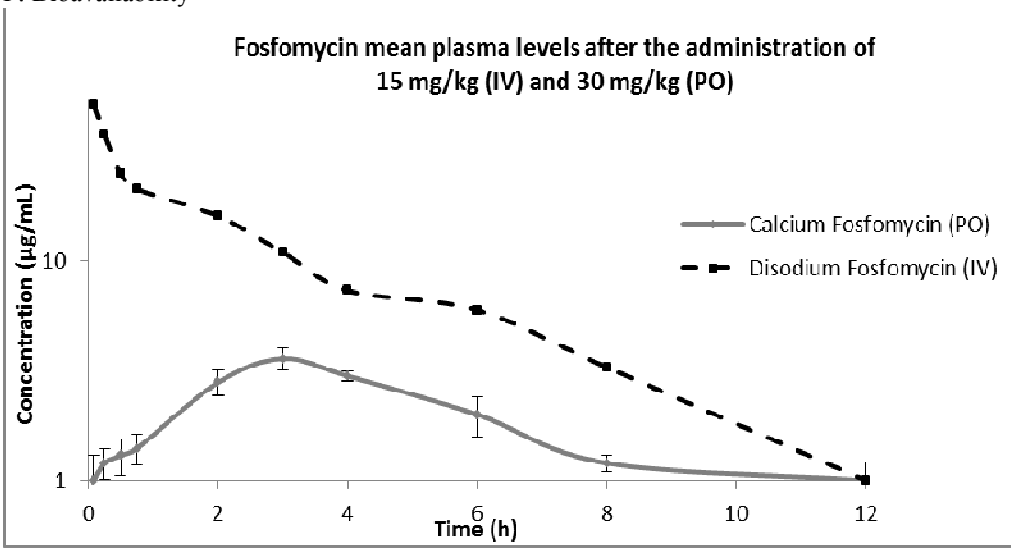

Fig. 1. Mean plasma levels of fosfomycin after IV and PO administration of 15 mg/kg and 30 mg/kg, respectively.

PHARMACOKINETICS OF CALCIUM FOSFOMYCIN IN WEANING PIGLETS

Discussion

Oral PK of fosfomycin was described in humans (calcium fosfomycin and trometamol fosfomycin) [18,19]. In chickens [8] and rabbits [21] studies on oral treatment were carried out after chronic administration.

The disodium fosfomycin dose used for the IV study was of 15 mg/kg. The same dose was used by [6], an intermediate dose between the ones tested in horses (10 and 20 mg/kg), broilers [8], and cattle (20 mg/ kg) [22] and lower than the doses documented for broiler chickens (40 mg/kg) [16] and dogs (40 and 80 mg/kg) [12].

PK parameters of disodium fosfomycin after a single IV administration of 15 mg/kg b.w. were similar to those found by [6].

Plasma clearance (Cl_b), the most important PK parameter due to its role in the determination of the dosage rate [36], was 140.0 ± 39.6 mL/kg/h (2.33 $mL.min^{-1}.kg^{-}1$), slightly higher although similar to the Cl_b value found in piglets (131.50 ± 30.07 mL/kg/h) by [6]. The Cl_b value is comparable to the glomerular filtration rate of a weaning piglet (1.30 - 1.73 $mL.min^{-1}.kg^{-1}$) [37]. This is expected considering that fosfomycin is a polar compound of low molecular weight which is excreted unmetabolized by the kidney. The value was higher than the ones found in other mammals [12, 13, 22] and broiler chickens [16]. The value found for the body extraction ratio (E_{body}), was of importance because an E_{body} of 0.05 or lower is generally desirable to develop a drug for oral administration with a not too high dosage regimen [36].

The short $T_{1/2\beta}$ values were similar to those found for the disodium fosfomycin PK in piglets (1.85 ± 0.19 h; IM - 15 mg/kg) [6], horses (1.23 ± 0.08 h; IV- 10 mg/kg; 1.34 ± 0.01 h; IV- 20 mg/kg; and 1.54 ± 0.07 h; IM - 10 mg/kg; 1.57 ± 0.02 h; IM - 20 mg/kg [13], dogs (1.28 ± 0.06 h; IV - 40 mg/kg and 1.30 ± 0.08 h; IM - 80 mg/kg) [12], broiler chickens (1.4 h; IV - 40 mg/kg and 1.1 h; IM - 10 mg/kg) [16] and cattle (1.33 ± 0.3 h; IV - 20 mg/kg and 2.17 ± 0.4 h; IM - 20 mg/kg) [22]. Calcium fosfomycin $T_{1/2\beta}$ was longer than the one found by [16] in broiler chickens (1.30 h; PO - 40 mg/kg).

Vd_{area} was moderate. This can be explained by its negligible binding to plasma proteins [12], and its marginal distribution into cells and the extracellular space fluid [38,39].

The bioavailability of calcium fosfomycin after PO administration was low. Taking this into account it is expected that a largest percentage of the administered drug (80%) will be retained in the gut, by the food, with an important local activity.

The low bioavailability is also accompanied by an early T_{max} and a low C_{max}. It is important to note that T_{max} and C_{max} are hybrid variables influenced both by the rate of drug elimination and absorption, and they should not be used as a state of the drug absorption [40]. Furthermore, it must be taken into account that this work was performed in young pigs and the PK parameters of drugs in post weaning piglets may markedly differ from those in adult animals. In this regard, drug bioavailability may vary between young and adult animals [41], due to the morpho-physiological changes that occur in the intestine, such as a temporary reduction in the processes of absorption, villus atrophy, crypt depth increase, reduction of digestive enzymes concentration and affection of intestinal mucus quality and quantity [42].

Fosfomycin is considered a typically time-dependent antimicrobial drug (%T > MIC) [1,12,22] and it is accepted that, for some time-dependant antimicrobials, the area under the concentration-time curve divided by the MIC_{90} (AUC/MIC_{90}) ratio is the PK/PD predictor of clinical efficacy [26,28]. The AUC/MIC_{90}

ratio documented for macrolides and tetracyclines is 25 [13,28]. In horses, [13], documented for *Streptococcus sp.* (MIC_{90}: 0.25 µg/mL), fosfomycin AUC/MIC_{90} ratios of 996 and 1260, after a SC dose of 10 and 20 mg/kg, respectively, AUC/MIC_{90} ratios of 460 and 896 for an IM dose of 19 and 29 mg/kg, respectively. Different authors have determined a fosfomycin MIC_{90} ranging from 0.25 µg/mL (*Streptococcus sp.*) to 0.5 µg/mL (*E. coli*), respectively [22,43]. Soraci et al [6] found for fosfomycin administered by the IM route, AUC/MIC_{90} ratios of 396 for *Streptococcus sp.* and 198 for *E. coli*. The PO AUC value obtained in this study for calcium fosfomycin was 45.48 ± 9.20 µg h/mL, and the AUC/MIC_{90} ratios for *Streptococcus sp.* and *E. coli* were 182 and 91, respectively. These values are higher than 25. Therefore, the ratios obtained in this study seem to be large enough to suggest an acceptable *in vivo* efficacy of calcium fosfomycin in weaning piglets.

Considering fosfomycin is a time-dependent antimicrobial, taking into account the MIC_{90} of sensitive bacteria to fosfomycin, and according to the values of fosfomycin plasma concentration vs. time profiles, we can conclude calcium fosfomycin is a good option to treat some sensitive bacteria, especially intestinal microorganisms, following PO administration of 30 mg/kg in weaning piglets.

References

1. Popovic M, Steinort D, Pillai S, Joukhadar C. Fosfomycin: an old, new friend? Eur J Clin Microbiol Infect Dis 2010, 29(2)127-142.

2. Kahan FM, Kahan JS, Cassidy PJ, Kropp H. The mechanism of action of fosfomycin (phosphonomycin). Ann N Y Acad Sci 1974, 235, 364-386.

3. Gobernado M. Fosfomicina. Rev Esp Quimioter 2003, 16 (1):15-40.

4. Mata J, Rodriguez A, Gallego A. Fosfomycin: in vitro activity. Chemother 1977, 23(1)23-24.

5. Patel SS, Balfour JA, Bryson HM. Fosfomycin tromethamine. A review of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy as a single-dose oral treatment for acute uncomplicated lower urinary tract infections. Drugs 1997, 53(4), 637-656.

6. Soraci AL, Pérez DS, Martínez G, Dieguez S, Tapia MO, Amanto F, Harkes R, Romano O. Disodium fosfomycin pharmacokinetics and bioavailability in post weaning piglets. Res Vet Sci 2011, 90(3), 498-502.

7. Gallego A, Rodríguez A, Mata JM. Fosfomycin: pharmacological studies 1974, 10, 161-168.

8. Aramayona JJ., Bregante MA, Solans C, Rueda S, Fraile LJ, Garcia MA. Pharmacokinetics of fosfomycin in chickens after a single intravenous dose and tissue levels following chronic oral administration. Vet Res 1997, 28(6):581-588.

9. Pianetti GA. Determinação cromatográfica da Fosfomicina em amostras biológicas. Cad Farm 1997, 13, 129.

10. Loste A, Hernández E, Bregante MA, García MA, Solans C. Development and validation of a gas chromatographic method for analysis of fosfomycin in chicken muscle samples. Chromatogr 2002, 56, 3-4.

11. Petsch M, Mayer-Helm BX, Sauermann R., Joukhadar C, Kenndler E. Determination of fosfomycin in pus by capillary zone electrophoresis. J Chrom A 2005, 1081(1), 55-9.

12. Gutierrez OL, Ocampo CL, Aguilera JR, Luna J, Sumano, LH. Pharmacokintics of disodium fosfomycin in mongrel dogs. Res Vet Sci 2008, 85(1):156-161.

13. Zozaya DH, Gutiérrez OL, Ocampo CL, Sumano LH. Pharmacokinetics of a single bolus intravenous, intramuscular and subcutaneous dose of disodium fosfomycin in horses. J Vet Pharm Ther 2008, 31(4)321-327.

14. Li L, Chen X, Dai X, Hui Chen M, Zhong D. Rapid and selective liquid chormatographic/tandem mass spectrometric method for the determination of fosfomycin in human plasma. J Chrom B 2007, 856, 171-177.

15. Dieguez S, Soraci A, Tapia O, Carciochi R, Pérez D, Harkes R, Romano O. Determination of antibiotic fosfomycin in chicken serum by liquid chromatography-tandem mass spectrometry. J Liq Chrom Rel Technol. 2011, 34(2)116-128.

16. Soraci AL, Pérez DS, Tapia MO, Martínez G, Dieguez S, Buronfosse-Roque F, Harkes R, Colusi A. Romano O. Pharmacocinétique et biodisponibilité de fosfomycine chez le poulet de chair. Rev Méd Vét 2011, 162(7)358-363.

17. Pérez DS, Soraci AL, Dieguez SN, Tapia MO. Determination and withdrawal time of fosfomycin in chicken muscle, liver and kidney. Int J Poult Sci 2011, 10(8): 644-655.

18. Kirby WM. Pharmacokinetics of fosfomycin. Chemother 1977, 23, 141-151.

19. Segre G, Bianchi E, Cataldi A, Zannini G. Pharmacokinetic profile of fosfomycin trometamol (Monuril). Eur Urol 1987, 13, 56-63.

20. Vargas E, Pacheco E, Beneit JA. Antibióticos (V): Misceláneos: Fosfomicina. In: Farmacología y su proyección a la clínica. Velázquez BL. Ed. Oteo. isa ed. Madrid. 1987, 840-841.

21. Fernández Lastra C, Mariño EL, Dominguez-Gil A. Phosphomycin levels in serum and interstitial tissue fluid in a multiple dosage regimen in rabbits. Arzneim Forsch 1987, 37(8):927-929.

22. Sumano LH, Ocampo CL, Gutierrez OL. Intravenous and intramuscular pharmacokinetics of a single-day dose of disodium fosfomycin in cattle, administered for 3 days. J Vet Pharm Ther 2007, 30(1)49.

23. Dirkzwagera A, Veldmana B, Bikkera P. A nutritional approach for the prevention of post weaning syndrome in piglets. Anim Res 2005, 54, 231-236.

24. Nabuurs MJA, Hoogendoorn A, van der Molen EJ, van Osta ALM.Villous height and crypt depht in weaned and unweaned pigs, reared under various circumstances in the Netherlands. Res Vet Sci 1993, 55, 78-84.

25. Martineau GP. Maladies d'elevage des porcs. Editions France Agricole. 1997, 174- 209.

26. Toutain PL., Bousquet-Mélou A. and Martinez M. AUC/MIC: a PK/PD index for antibiotics with a time dimension or simply a dimensionless scoring factor? J Antimicr Chemother 2007, 60, 1185-1188.

27. del Castillo J, Elsener J, Martineau GP. Strategies metaphylactiques parvoie orale chez le porc en croissance. Meta-analyse et modélisation appliquées aux tetracyclines. J Rech Porc France 1997, 29, 39-46.

28. Toutain PL, del Castillo RE, Bousquet-Mélou A. The pharmacokinetic-pharmacodynamic approach to a rational dosage regimen for antibiotics. Res Vet Sci 2002, 73, 105-114.

29. Matte JJ. Dévéloppement d'une méthode rapide et non-invasive de cathétérisme jugulaire chez le porc: un outil de recherche accesible à l'industrie. J Rech Porc France 1997, 29, 67-72.

30. Soraci AL, Amanto F, Pérez DS, Martínez G, Dieguez SN, Vega G, Tapia MO. Metodología de cateterismo yugular en lechones de destete. Analecta Vet 2010, 30, 12-15.

31. U. S. Department of Health and Human Services, Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (CVM), Guidance for Industry, Bioanalytical Method Validation. 2001.

32. European Commission Decision, Implementing Council Directive Concerning the performance of analytical methods and the interpretation of the results. Official Journal of the European Communities, L221, 2002/657/EC, 23-33. 2002.

33. Riviere JE Comparative Pharmacokinetics: Principles, Techniques and Applications: Iowa State University Press, Ames. 1999, pp. 148-167.

34. Baggot D. Principles of drug disposition in domestic animals. In: Baggot, D. (Ed.), The Basics of Veterinary Clinical Pharmacology. W.B. Saunders, Philadelphia, PA. 1977, pp. 1-22.

35. Gibaldi M, Perrier B. Pharmacokinetics. New York. Dekker, School of Pharmacy, University of Washington. Seattle, WA and School of Pharmacy. University of Arizona, Tucson, AZI. 1975, pp. 494.

36. Toutain PL and Bousquet-Mélou A. Plasma clearance. J Vet Pharm Ther 2004, 27, 415-425.

37. Eskild-Jensen A, Jacobsen L, Christensen H, Frøkiaer J, Jørgensen HS, Djurhuus JC, Jørgensen TM. Renal function outcome in unilateral hydronephrosis in newborn pigs. II. Function and volume of contralateral kidneys. The J Urol 2001, 165, 205-209.

38. Eskild-Jensen A, Thomsen K, Rungø C, Ferreira LS, Fogt Paulsen L, Rawashdeh YF, Nyengaard JR, Nielsen S, Djurhuus JC, Frøkiær J. Glomerular and tubular function during AT1 receptor blockade in pigs. Am. J Physiol - Renal Physiol 2007, 292, 921-929.

39. Soraci AL, Pérez DS, Martínez G, Amanto F, Tapia MO, Dieguez S, Fernández Paggi MB. Fosfomycin concentrations in epithelial lining fluid in weaning piglets. J Vet Pharm Ther 2012, 35, 4, 406-409.

40. Toutain PL and Bousquet-Mélou A. Bioavailability and its assessment. J Vet Pharm Ther. 2004, 27, 455-466.

41. Nouws JFM. Pharmacokinetics in immature animals: a review. J An Sci Nutr Res Rev 1995, 8, 137-164.

42. Corpet DE. Mécanismes de la promotion de croissance des animaux par les additifs alimentaires antibiotiques. Rev Méd Vét 2000, 151, 99-104.

43. Fernández P, Herrera I, Martinez P, Gómez L, Prieto J. Enhancement of the susceptibility of *Staphylococcus aureus* to phagocytosis after treatment with fosfomycin compared with other antimicrobial agents. Chemother. 1995, 41, 45-49.