IN VITRO PENETRATION OF FOSFOMYCIN IN RESPIRATORY CELLS

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SUMMARY

Infectious diseases of the respiratory system are important in intensive swine production. Fosfomycin is a widely used antibiotic for the treatment of swine lung infections. The aim of this research was to study the penetration of fosfomycin in an in vitro model of respiratory cells (HEp-2 cells). Cell cultures were subjected to four treatments: a) negative control; b) disodium fosfomycin (280µg/mL); c) disodium fosfomycin (130µg/mL) and d) calcium fosfomycin (130µg/mL). Intracellular concentrations of fosfomycin were analysed by high-performance liquid chromatography tandem mass spectrometry (HPLC MS/MS). Concentrations in HEp-2 cells incubated with 280µg/mL of disodium fosfomycin ranged from 0.74 to 2.79µg/mL (Tmax: 12 hours). When incubated with the same formulation of fosfomycin at a concentration of 130µg/mL, intracellular concentrations ranged between 0.31 and 1.60µg/mL (Tmax: 12 hours). Calcium fosfomycin reached intracellular concentrations that varied between 0.46 and 1.11µg/mL (Tmax: 8 hours). Fosfomycin concentrations exceeded the MIC90 for the most important pathogens in swine respiratory infections (Streptococcus spp.; 0.25µg/mL). Therefore, it is apparent that fosfomycin is an alternative for the treatment of intracellular respiratory infections in pigs.

INTRODUCTION

The swine industry provides one of the most important sources of animal protein. Worldwide, the pork industry is the largest in production and consumption, followed by poultry meat and beef. In pig production, weaning is considered as a critical period for piglets. It is characterised by a decrease in food intake that leads to a status of under nutrition, affecting other aspects of animal physiology and metabolism (Dirkzwagera *et al.*, 2005). During this period, animals are more susceptible to infectious diseases (Nabuurs *et al.*, 1993).

Dysentery and respiratory syndromes are the most frequent conditions that appear in this period and they are one of the most significant problems in modern intensive swine production systems (Gardner and Hird, 1990; Hopper *et al.*, 1992; Stevenson *et al.*, 1993; Galina *et al.*, 1994; Kay *et al.*, 1994; Albina *et al.*, 1995; Cooper *et al.*, 1995; Done and Paton, 1995; Van Reeth *et al.*, 1996 ; Martineau, 1997; Christensen *et al.*, 1999; Thanawongnuwech *et al.*, 2000; Carr, 2001; Cloutier *et al.*, 2003; Došen *et al.*, 2007). The main sites of infection (biophase) of the pathogens responsible for these disorders are the interstitial and intracellular fluid. In this regard, it should be noted that most of enteric diseases are caused by intracellular organisms, such as salmonella spp. (facultative intracellular) and *Lawsonia intracellularis* (obligate intracellular) (Pluske *et al.*, 1996; Pedersen et al, 2008). Both the intracellular and interstitial fluids are also the pulmonary biophase of facultative organisms such us *Mycoplasma hyopneumoniae, Bordetella bronchisceptica, Salmonella choleraesuis, Pasteurella multocida* and Streptococcus spp.

Different antibiotics have been used for decades to reduce pathogen infection in pigs (irrational use). For this reason, many bacteria have become resistant to the most frequently used antimicrobials (Dirkzwagera *et al.*, 2005; Mathew *et al.*, 1998; Rood *et al.*, 1985). Among them, fosfomycin (cis-1, 2-epoxyphosphonic acid) is an intensive production, widely used antibiotic (Serrano, 2002). It is a broad-spectrum antibiotic, structurally unrelated to other classes of antimicrobial agents. It inhibits cell wall synthesis as it interferes with peptidoglycan production at an earlier stage than beta-lactams or glycopeptide antibiotics (Gobernado, 2003; Kahan *et al.*, 1974; Lin, 1976; Popovic *et al.*, 2009). When compared with other antibiotics, fosfomycin has a broader *in vitro* spectrum of action than penicillin and semi-synthetic cephalosporins (Mata *et al.*, 1977), and cross-resistance has not been reported (Gobernado, 2003). The use of fosfomycin in animals and humans has been proposed because of its low toxicity and potential efficacy (Gallego *et al.*, 1974), being also widely used in animal production due to its rapid effect, good tolerance and lack of side effects (Aramayona *et al.*, 1997; Carramiñana, 2004).

The pharmacokinetics of fosfomycin has been described in humans (Gallego *et al.*, 1974; Damaso *et al.*, 1990; Falagas *et al.*, 2008,), rabbits (Fernandez Lastra *et al.*, 1986, 1987),

broilers (Aramayona *et al.,* 1997, Soraci *et al.,* 2011), cattle (Sumano *et al.,* 2007), horses (Zozaya *et al.,* 2008), dogs (Gutiérrez, 2008) and pigs (Soraci *et al.,* 2010). Its chemical structure supports different salts: sodium, calcium and tromethamine (Pérez-Valazco and Chávez Hernández Velasco, 1997; Serrano, 2002). This is due to its acidic nature that allows the rapid formation of salts. The fosfomycin-calcium salt formulation is used orally, whereas the more water-soluble disodium salt can be used intravenously. Fosfomycin-tromethamine salt is highly hydro-soluble and offers a good oral bio-availability in humans (Borsa *et al.,* 1988; Patel *et al.,* 1997; Popovic *et al.,* 2009). Perez *et al.,* (2011) have established a fosfomycin withdrawal period of three days for broiler chicken muscle, liver and kidney and two days for pig tissues.

It has been demonstrated that, besides being a bacterial inhibitor, fosfomycin has other properties such as inhibition of bacterial adhesion to epithelial cells, penetration of wells in biofilms of exo-polysaccharide, and protection against nephrotoxicity caused by drugs such as cisplatin, cyclosporine, aminoglycosides, vancomycin, teicoplanin, amphotericin B and polymyxin (Gobernado, 2003). Martínez *et al.,* (2011) demonstrated that fosfomycin has a protective effect on HEp-2 cells when they are incubated with the mycotoxin deoxinivalenol. Morikawa *et al.,* (1996) and Honda *et al.,* (1998) have shown that fosfomycin has immune-modulatory effects on lymphocytes. Similarly, Krause *et al.,* (2001) studied the effect of fosfomycin on neutrophil function and showed that the destruction of micro-organisms is increased when incubated with this antibiotic.

Fosfomycin exhibits a time dependent killing, so it kills bacteria when its concentrations remain constantly above the Minimum Inhibitory Concentration (MIC) (Aliabadi and Lees, 1997; Toutain *et al.,* 2002). In this regard, for an antibiotic to be effective against relevant pathogens, it is essential to reach concentrations higher than the MIC at the site of action (Nix *et al.,* 1991; Schentag and Ballow, 1991; Toutain *et al.,* 2002). Fosfomycin is indicated for the treatment of a variety of porcine bacterial pathogens *(Haemophilus parasuis, Streptococcus suis, Pasteurella multocida, Bordetella bronchiseptica, Staphylococcus hyicus* and *Escherichia coli)* associated with stress and/or several viral diseases (Martineau, 1997), and its MIC90 for most of the important pathogens in swine production has been established as 0.25-0.5µg/mL (Fernández *et al.,* 1995, Sumano *et al.,* 2007).

Although fosfomycin is widely used in clinical practice in the pork industry, its use in pigs is given on the basis of its potential clinical efficacy. However, there are no studies showing that this antibiotic reaches adequate concentrations in the biophase where the most important pathogens operate. According to this background, the aim of this work was to determine fosfomycin concentration in the intracellular fluid of HEp-2 cells (as a model of fosfomycin penetration into respiratory cells). It was hypothesised that the antibiotic reaches concentrations above the MIC90 for most of the micro-organisms which are relevant in swine production systems and to determine the relationship between intracellular and serum fosfomycin concentrations in weaning piglets.

MATERIALS AND METHODS

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

Antibiotic

Calcium and disodium fosfomycin (98.9% of purity) were from Bedson Laboratory, Pilar, Buenos Aires, Argentina.

To estimate the amount of drug that reaches plasma and is in condition to penetrate into the cell culture, calcium fosfomycin was used at 130µg/mL. This dose was estimated considering a therapeutic dose of 30mg/kg body weight (BW) of calcium fosfomycin for pigs, an average post-weaning piglet BW of 15kg, a 4.6% of BW volume of blood where the antibiotic is dissolved (690mL) and a bioavailability (F%) of 20 (Pérez *et al.,* unpublished data). Disodium fosfomycin was used at 130 and 280µg/mL. It was considered that a therapeutic dose of 15mg/kg BW Piglets BW and BW volume of blood were similar to calcium fosfomycin experiments, except for the F%, which was 86 (Soraci *et al.,* 2010). Stock solution of fosfomycin salts (280µg/mL for disodium fosfomycin and 130µg/mL for calcium fosfomycin) were prepared in physiological saline solution (PSS). The 130µg/mL disodium fosfomycin solution was prepared from the 280µg/mL stock solution.

Cell line

For these experiments, HEp-2 cells were used. HEp-2 cells are laryngeal cells of human origin and are widely used for *in vitro* experiments with respiratory pathogens (Roblin *et al.,* 1992; Reddy and Kummar, 2000, Reddy and Hayworth, 2002). For culture, cells were seeded in culture flasks and grown in Eagle's Minimal Essential Medium (E-MEM), supplemented with 20% of foetal bovine serum (FBS). At 24 hours, the medium was replaced (E-MEM with 10% FBS) to allow cell division to continue up to a complete confluent monolayer. Subcultures were performed when cell monolayers were 100% confluent (1.2×10^6 cells/well). Cells were maintained in the incubator at 37°C and 5% CO_2.

Experimental design

Four different experiments were performed. In all cases, the culture medium was removed and cell cultures were washed with PSS to eliminate residual Phosphate buffered saline (PBS), since phosphates of the buffer might interfere with the detection of fosfomycin by HPLC-MS/MS. Cells were incubated from five minutes to 24 hours at 37°C and 5% CO_2, depending on the experiment: a) HEp-2 cells were incubated with PSS, in absence of fosfomycin (negative control); b) cell cultures were incubated with 2mL of the 280µg/mL stock solution of disodium fosfomycin; c) HEp-2 cells were incubated with 2mL of disodium fosfomycin at 130µg/mL and d) HEp-2 cells were incubated with 2mL of 130µg/mL stock solution of calcium fosfomycin. The results were corroborated by six replicates.

Cell cultures were observed under an inverted microscope at 40X to determine if the monolayer was intact and if cell detachment, cytoplasmic 'dots' (sign of intoxication) and loss of cell membrane were not present. In any case, cells were not affected during the incubation period.

Determination of fosfomycin concentrations in HEp-2 cells

After each incubation time (five, 10, 15, 30 and 45 minutes and one, two, three, four, six, eight, 12, 18 and 24 hours), the supernatant was collected and each well containing HEp-2 cells was washed twice with High-performance Liquid Chromatography (HPLC) water. Supernatants were centrifuged at 3,500rpm for six minutes to obtain the extracellular fosfomycin. Pellet (sloughed cells) was restored to the corresponding well. After the addition of 2mL of HPLC water to the wells, culture plates were sealed and sonicated for 30 minutes to break the cells and release fosfomycin to the HPLC water. Then, the content of each well was centrifuged at 10,000rpm at 4°C, with 1mL filtered through nylon filters of 0.22µm, placed in vials, and intra and extracellular concentrations of fosfomycin were determined by HPLC-MS/MS.

To estimate the influence of HEp-2 cells intracellular water on fosfomycin intracellular concentrations, we based our experiment on Kiem and Shentag (2008) work. We took into account the HEp-2 cell count in a well plate (1.2×10^6 cells) and the mean cell volume (2.75×10^{-6}). The processed data obtained from Xcalibur software was recalculated considering the intracellular volume of water. The degree of penetration of fosfomycin into HEp-2 cells was determined by comparing the AUC_{0-t} of HEp-2 cells with the AUC_{0-t} of serum.

Instruments

The HPLC-MS/MS system was from Thermo Electron Corporation (San Jose, CA, USA), consisting 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 an electrospray ionisation (ESI) source. Nitrogen used as a nebuliser and sheath gas was obtained through a nitrogen generator from Peak Scientific Ltd. (Inchinnan, Scotland). Data processing was done using Xcalibur software, also from Thermo.

Mass spectrometer conditions

The mass spectrometer was operated in negative ionisation mode. The tuning parameters were optimised with 10µg/mL individual aqueous solutions of fosfomycin directly infused in the ion source by means of a syringe pump at 10µl/min, with influence of mobile phase delivered from the LC pump through a T-connection to give the corresponding chromatographic flow rate. The spray voltage was set to -3,800eV, the capillary temperature was 350°C, and argon 99.999% purity was used for collision induced dissociation (CID) at 1.6m Torr in the collision cell. Source CID energy was set to -8eV. Fosfomycin detection and quantification were achieved by single reaction monitoring of transitions m/z 137->79 with an optimised collision energy of 25.

Chromatographic conditions

Separation was achieved on a Phenomenex CN (cyano) stationary phase, 75mm x 4.6i.d., 5µm column. The mobile phase consisted of acetonitrile:water (20:80), working in isocratic mode, at a flow rate of 100µl/min. The column was maintained at 30°C, while the samples in the auto sampler were at 10°C. Sample injection volume was 20µl, chromatographic run time was eight minutes and fosfomycin retention time was six minutes.

RESULTS

The results were analysed by analysis of variance (ANOVA), using InStat3 software. Intracellular concentrations of the antibiotic in HEp-2 cells incubated with 280µg/mL of disodium fosfomycin ranged from 0.74 to 2.79µg/mL with a Tmax of 12 hours (see Figure 1). Figure 2 shows the intracellular and extracellular concentrations of fosfomycin, when cells were exposed to 280µg/mL dose, from zero to 24 hours. When HEp-2 cells were incubated with the same formulation of fosfomycin at a concentration of 130µg/mL, intracellular concentrations ranged between 0.31 and 1.60µg/mL, with a

Figure 1 – *Disodium fosfomycin intracellular kinetic in HEp-2 cells (280µg/mL)*

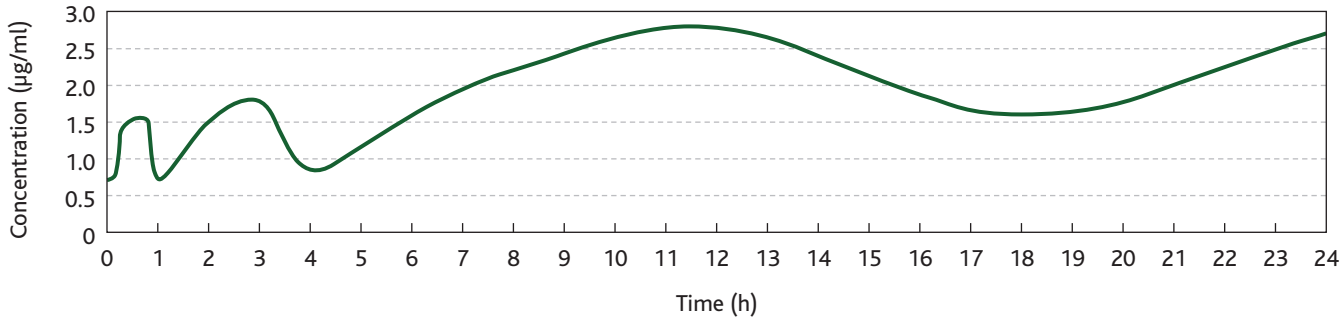


Figure 2 – *Disodium fosfomycin kinetics in HEp-2 cells (280µg/mL)*

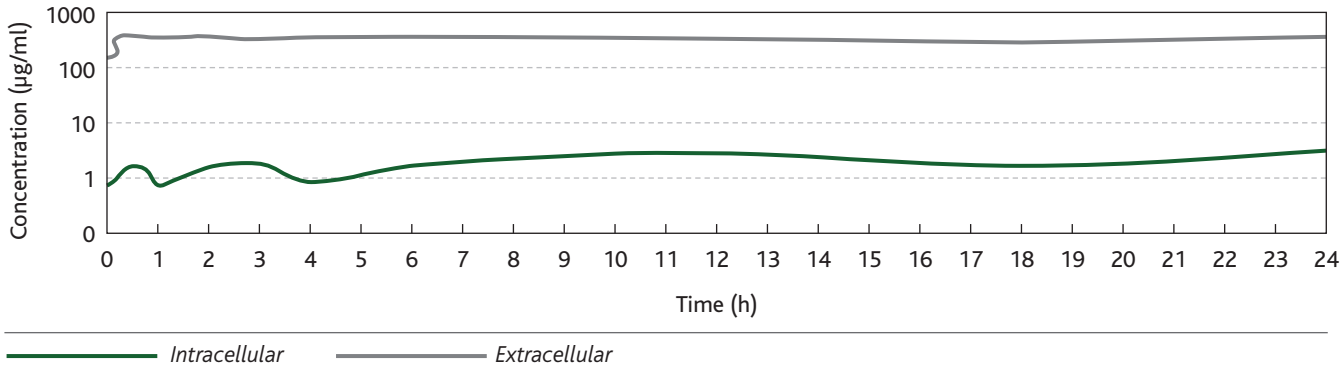


Figure 3 – *Disodium fosfomycin intracellular kinetics in HEp-2 cells (130µg/mL)*

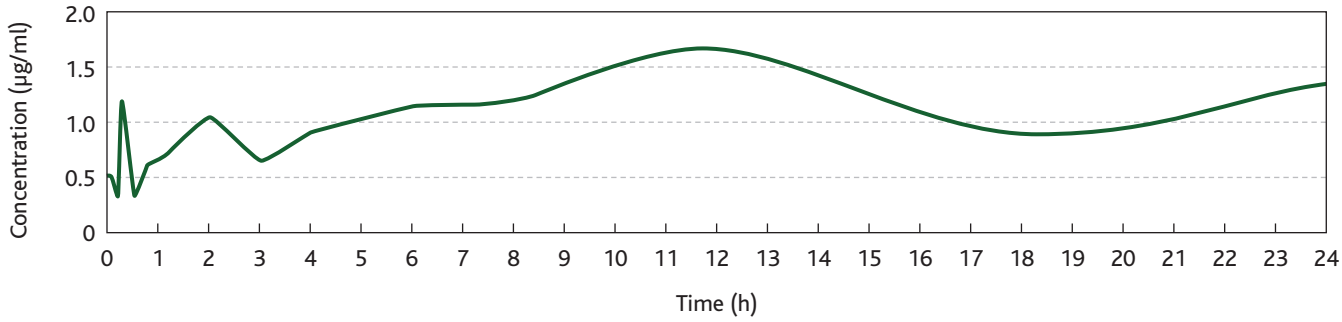
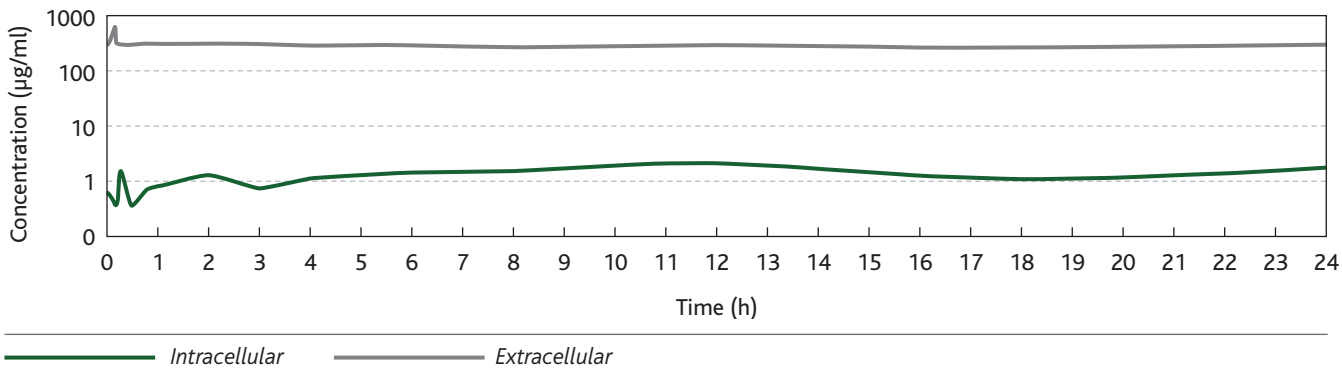


Figure 4 – *Disodium fosfomycin kinetics in HEp-2 cells (130µg/mL)*



12 hours Tmax (see Figure 3). Figure 4 shows the intracellular and extracellular concentrations of fosfomycin, when cells were incubated with 130µg/mL of disodium fosfomycin, from zero to 24 hours. There were statistically significant differences ($p<0.05$) between HEp-2 cells dosed with 280µg/mL and HEp-2 cells incubated with 130µg/mL (see Figure 5). In regard to cellular penetration of the antibiotic in HEp-2 cells incubated with calcium fosfomycin, the Tmax was eight hours and the concentrations ranged between 0.46 and 1.11µg/mL (see Figure 6). Figure 7 shows the intracellular and extracellular kinetics of calcium fosfomycin in HEp-2 cells (130µg/mL). There were no significant differences ($p>0.05$) between the two formulations at the same concentration (disodium fosfomycin vs. calcium fosfomycin, 130µg/mL), compared to the Cmax (1.60 vs. 1.11µg/mL, respectively) and Tmax (12 hours vs. eight hours, respectively) (see Figure 8).

Fosfomycin area under the curve (AUC), Cmax and Tmax in weaning piglets were determined by Soraci *et al.*, (2011). Fosfomycin AUC_{0-t}, after an intramuscular (IM) dose of disodium fosfomycin (15mg/kg), was 99.00µg-h/mL. AUC_{0-t} after an oral dose of calcium fosfomycin was 39.6µg-h/mL. The ratio of fosfomycin AUC in HEp-2 cells compared with AUC in serum (AUCHEp-2/AUCserum) was 0.48; 0.27 and 0.49, for the 280µg/mL disodium fosfomycin dose, 130 disodium fosfomycin dose and 130 calcium fosfomycin dose, respectively. The Cmax were 43.00µg/mL for the disodium salt which was intramuscularly administered, 3.60µg/mL for the calcium salt which was orally administered, 2.79µg/mL for the 280µg/mL disodium fosfomycin HEp-2 cells dose, 1.60µg/mL for the 130µg/mL disodium fosfomycin HEp-2 cells dose, and 1.11µg/mL for the 130µg/mL calcium fosfomycin HEp-2 cells dose. These concentrations were achieved at different times. Tmax were: 0.75 hours (fosfomycin disodium salt in serum), three hours (fosfomycin calcium salt in serum), 12 hours (280 and 130µg/mL disodium fosfomycin doses in HEp-2 cells) and eight hours (130µg/mL calcium fosfomycin dose in HEp-2 cells). Table 1 shows some pharmacokinetics parameters of fosfomycin in serum (after a single IM dose of 15mg/kg. BW. and after a single oral dose of 30mg/kg b.w.) and in HEp-2 cells (after incubation with disodium fosfomycin; 280 and 130µg/mL, and with calcium fosfomycin, 130µg/mL).

DISCUSSION

For many clinically relevant pulmonary pathologies in pigs, respiratory cells represent a major site of infection by intracellular pathogens (biophase) (Schentag, 1990; Toutain *et al.*, 2002; Ross, 2006; Došen *et al.*, 2007). We decided to use HEp-2 cells as an *in vitro* model to estimate intracellular fosfomycin concentrations in respiratory cells. Although the cell line derives from human tissues, it is considered a representative model due to its respiratory origin and it has been widely used for the study of human and animal respiratory pathogens (Roblin *et al.*, 1992; Reddy and Kummar, 2000, Reddy and Hayworth, 2002).

The capacity of certain antibiotics to penetrate cell membranes represents an important pre-requisite for their *in vivo* efficacy against intracellular bacteria (Mandell, 1973; Johnson *et al.*, 1980; Yourtee and Root, 1982; Jacobs and Wilson, 1983; Höger *et al.*, 1985). In the present study, we found that the concentrations of fosfomycin were significantly lower in HEp-2 cells than in serum, at all sampling times. At all doses and formulations assayed, fosfomycin was taken up by HEp-2 cells. Antibiotics with high lipid solubility are able to penetrate cell membranes (Mandell, 1973; Johnson *et al.*, 1980). However, fosfomycin is known to be a hydrophilic drug and, therefore, passive transmembrane diffusion is an improbable explanation for its uptake (Höger *et al.*, 1985). Apparently, passive transport through the bacterial cell membrane is not a likely mechanism. Nevertheless, intracellular penetration of a small quantity of the drug by this mechanism cannot be rejected. Milagre *et al.*, (2011) demonstrated that fosfomycin is also taken up by a proteic transport system. Kahan *et al.*, (1974) have shown the existence of two different active transport mechanisms for fosfomycin. In some bacteria, the drug is incorporated via the sn-glycero-3-phosphate transport system.

Table 1 – *Fosfomycin pharmacokinetics parameters in serum and HEp-2 cells*

Parameters	Serum		HEp-2 cells		
	Disodium Fosfomycin	Calcium Fosfomycin	Disodium Fosfomycin		Calcium Fosfomycin
	IM, 15mg/kg BW	PO, 30mg/kg BW	280µg/mL	130µg/mL	130µg/mL
AUC_{0-t} µg.h/mL	99.00	39.60	47.70	27.70	19.60
C_{max} µg/mL	43.00	3.60	2.79	1.60	1.11
T_{max} (h)	0.75	3.00	12.00	12.00	8.00

Figure 5 – *Disodium fosfomycin intracellular kinetics in HEp-2 cells (280µg/mL vs. 130µg/mL)*

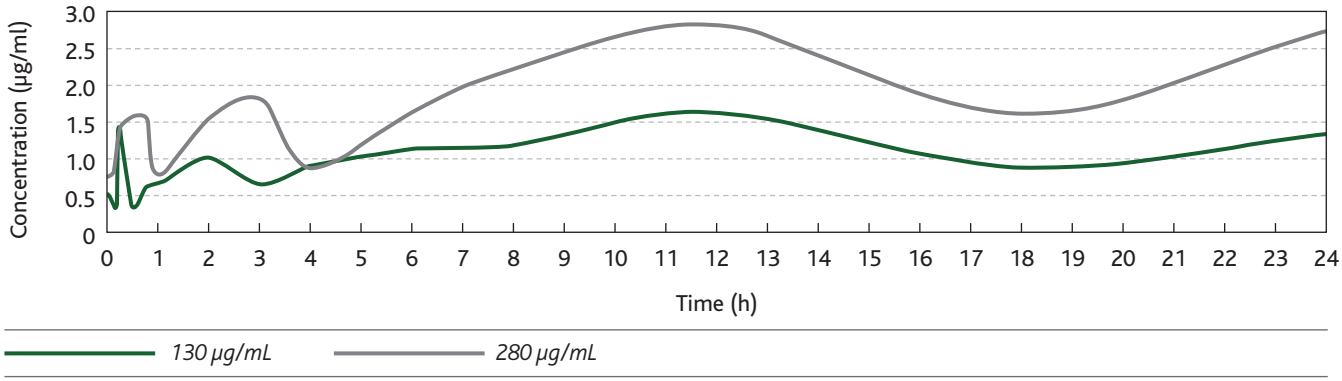


Figure 6 – *Calcium fosfomycin intracellular kinetics in HEp-2 cells (130µg/mL)*

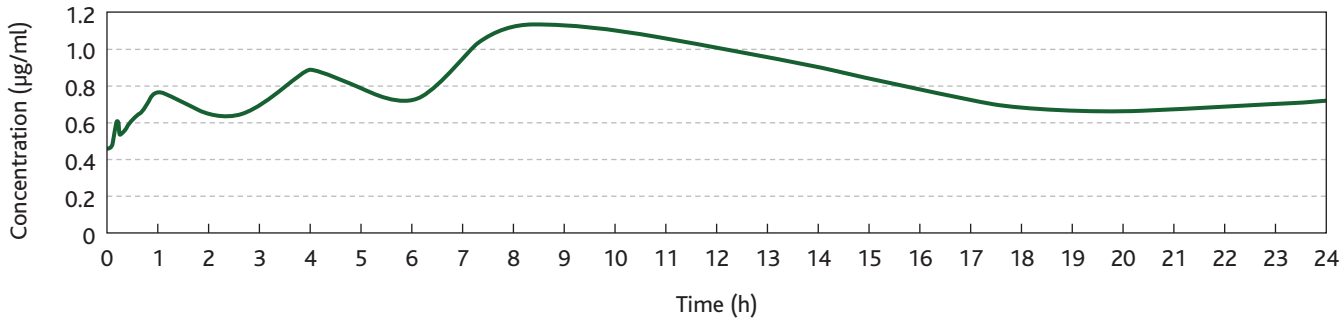


Figure 7 – *Calcium fosfomycin kinetics in HEp-2 cells (130µg/mL)*

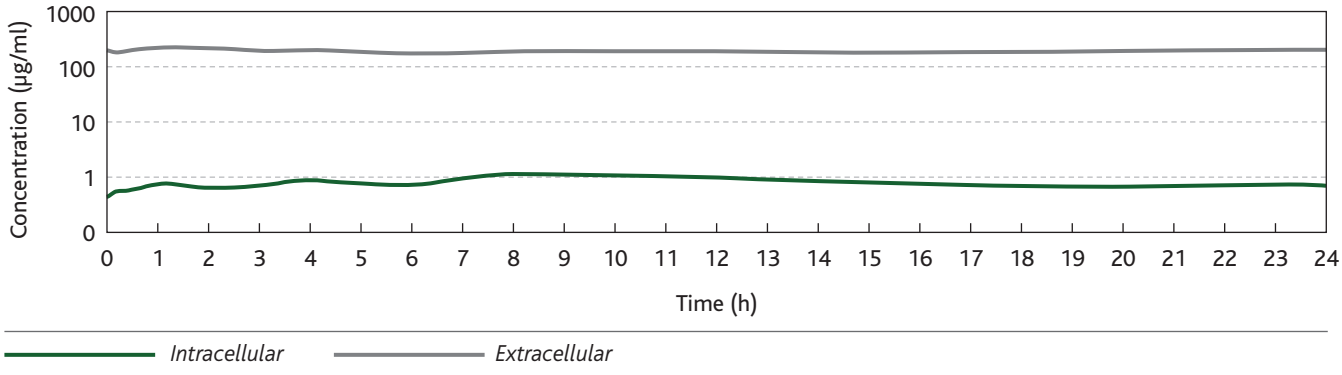
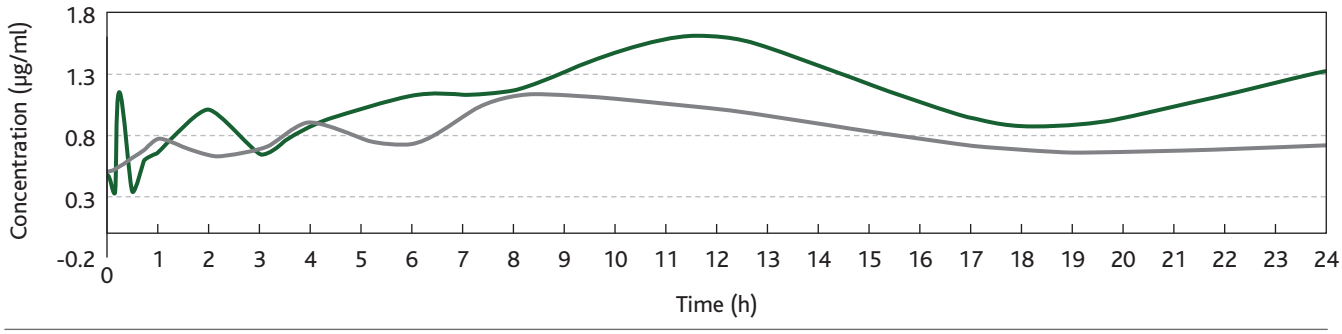


Figure 8 – *Intracellular kinetics in HEp-2 cells: disodium fosfomycin (130µg/mL) vs. calcium fosfomycin (130µg/mL)*



Under certain conditions, the glucosa-6-phosphate system, which can be externally induced, may also be used. Höger *et al.,* (1985) suggested the presence of a similar active transport mechanism for fosfomycin in human polymorphonuclear cells, as described for bacteria. We hypothesised that a similar mechanism takes place in animal cells.

Bacteria may survive within cells and remain unaffected by those antimicrobial agents which are unable to reach the intracellular space. There are several antibiotics with known intracellular accumulation and efficacy against intracellular pathogens. However, their clinical application might be limited by their adverse effects on important neutrophil functions. Such inhibitory actions of antibiotics on host defense mechanisms might be critical in immune-compromised hosts (Woodruff *et al.,* 1977). This undesirable effect is not present after fosfomycin administration (Gobernado, 2003). On the contrary, Morikawa *et al.,* (1996) and Honda *et al.,* (1998) have shown it has immune-modulatory effects on lymphocytes. Krause *et al.,* (2001) have demonstrated an increased power of destruction of micro-organisms when neutrophils are incubated in the presence of fosfomycin.

Besides all this, an antibiotic taken up intracellularly can only be clinically useful if it retains its bactericidal activity. Many infectious diseases are caused by facultative organisms that are able to survive within cells. The intracellular location of these micro-organisms protects them from the host defense mechanisms and from antibiotics with poor penetration into phagocytic cells (Briones *et al.,* 2008). In swine production, the intracellular and interstitial fluids are the biophase of *Mycoplasma hyopneumoniae, Bordetella bronchisceptica, Salmonella choleraesuis, Pasteurella multocida* and Streptococcus spp. – facultative intracellular organisms that cause respiratory disease. These bacteria would be killed as soon as the antibiotic reaches a bactericidal concentration within the cell (Höger *et al.,* 1985). Therefore, as described, the choice of an antibiotic is dependent on its direct antimicrobial activity. Generally, this property is detected *in vitro* by determination of the MIC (Herbert, 2002). A fosfomycin MIC90 of 0.25-0.5µg/mL (Fernández *et al.,* 1995) has been determined for the most important pathogens in swine production. We found that the intracellular penetration was around 0.25-1.23% of the incubation dose and concentrations were always higher than the MIC90 for the most important pathogens in swine production.

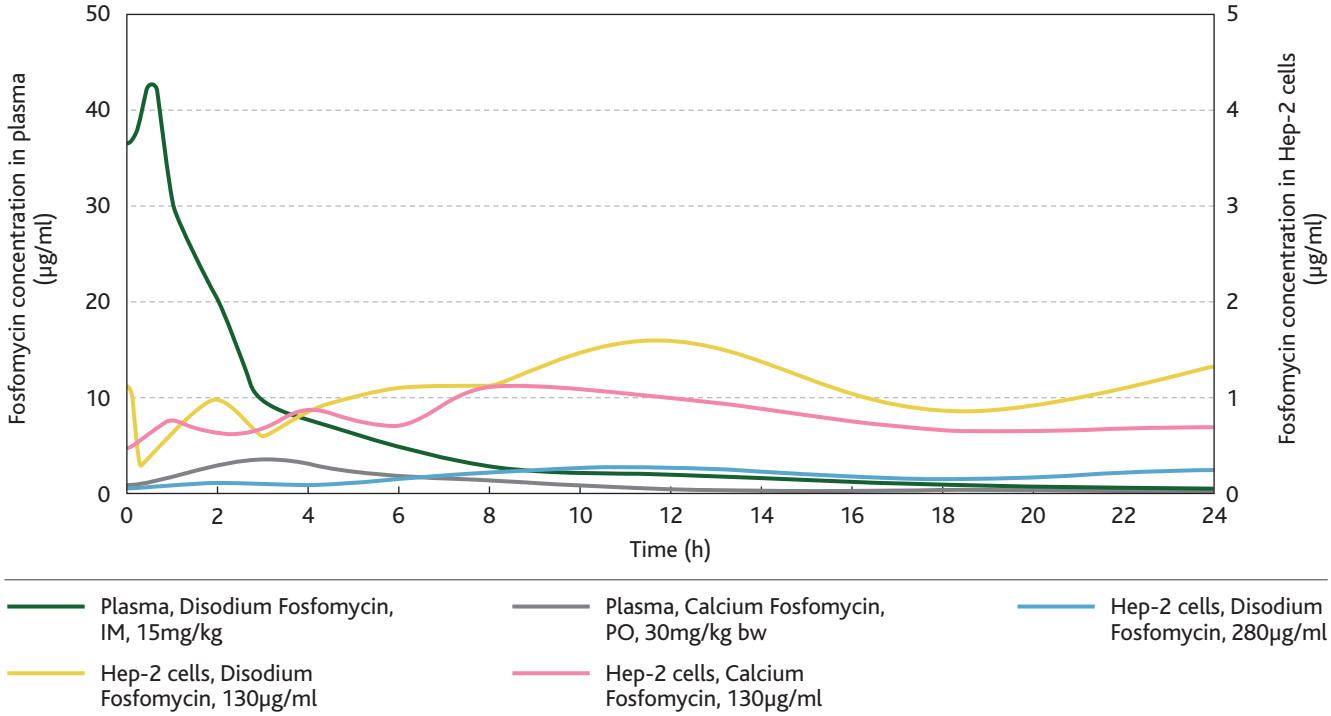
The efficacy of antimicrobial agents against pulmonary infections depends on their local concentrations in the lung (Kiem and Schentag, 2008). Studies of Pharmacokinetic/Pharmacodynamic (PK/PD) of several antibiotics have demonstrated the importance of measuring the concentrations of antibiotics at the infection sites, taking into account that the distribution of the drug may vary according to the tissues (Nix et al, 1991; Schentag and Ballow, 1991; Toutain et al, 2002). The penetration of drugs into various tissues is best described by the use of the AUC , which responds to variations in concentration with time (Schentag and Ballow, 1991; Kiem and Schentag, 2008). The comparative AUCHEp-2 vs. AUCserum was considered significant ($p<0.05$). The AUCHEp-2/AUCserum ratio for the 280µg/mL disodium fosfomycin dose was 44% higher than for the 130µg/mL disodium fosfomycin dose. This is due to the differences between fosfomycin incubation doses. However, when comparing the AUCHEp-2/AUCserum ratios of both fosfomycin formulations at the same concentration, we found that calcium fosfomycin exceeds 45% of disodium fosfomycin. In addition, it even exceeds the AUCHEp-2/AUCserum obtained with the 280µg/mL dose. Since there were no significant differences ($p>0.05$) between the two formulations at the same concentration, we consider that, although orally administered fosfomycin calcium (PO) has a low bioavailability, the amount of drug that reaches the cells can efficiently penetrate them. Furthermore, the Tmax is reached four hours earlier with calcium fosfomycin than with disodium fosfomycin (eight hours vs. 12 hours).

When the time period exceeding the MIC for the relevant pathogen (t>MIC) is maximised (%T>MIC), optimal bacterial killing by fosfomycin will be achieved (Sumano *et al.,* 2007; Gutierrez, 2008; Popovic *et al.,* 2009). In addition, an effective bacterial killing can be expected when the MIC for the pathogen is covered for at least 60-70% of the dosing interval (McKellar *et al.,* 2004). Streptococcus spp. is considered an important secondary agent in respiratory diseases of pigs (Gardner and Hird, 1990; Galina *et al.,* 1994; Done and Paton, 1995; Christensen *et al.,* 1999; Thanawongnuwech *et al.,* 2000; Carr, 2001; Cloutier *et al.,* 2003, Došen *et al.,* 2007). Fosfomycin MIC90 for this pathogen has been determined at 0.25µg/ml (Fernandez *et al.,* 1995; Sumano *et al.,* 2007). In the present study, fosfomycin concentrations in HEp-2 cells were above the MIC90 for Streptococcus spp up to 24 hours and at all doses and formulations assayed (see Figure 9).

CONCLUSIONS

Fosfomycin MIC90 is 0.25-0.5 µg/mL for the most important pathogens in swine production. As demonstrated in this study, concentrations achieved at the cellular level are remarkably higher. These findings make fosfomycin an excellent alternative for the treatment of intracellular infections in pigs. To further corroborate our *in vitro* studies, additional experiments should be carried out in pigs infected with respiratory pathogens.

Figure 9 – *Mean concentrations of fosfomycin in plasma and HEp-2 cells*



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