Research Article

Fosfomycin in vivo Penetration in Swine Intestinal Cells

Pérez Gaudio DS1,2*, Martínez G1,2, Fernández Paggi MB1,2, Decundo JM1,2, Romanelli A1,2, Mozo J3, Dieguez SN1,4 and Soraci AL1,2

1Laboratorio de Toxicología, Centro de Investigación Veterinaria de Tandil, Departamento de Fisiopatología, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Tandil, Buenos Aires, Argentina
2Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
3Agencia Nacional de Promoción Científica y Tecnológica, Buenos Aires, Argentina
4Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC), La Plata, Buenos Aires, Argentina

*Corresponding author: denisa@vet.unicen.edu.ar

ABSTRACT

Enteric diseases have a high economic impact on animal production, being the interstitial and intracellular fluids, the main sites of infection (biophase) of the pathogens responsible for these disorders. Fosfomycin is an antibiotic widely used for the treatment and prevention of swine infections caused by resistant bacteria. For most of the important pathogens in swine production, fosfomycin MIC90 has been established in 0.25-4 μg/mL. Calcium fosfomycin concentrations in swine intestinal cells were previously determined by in vitro and ex vivo studies, although, still now, there are no in vivo studies showing the exposure of minimum inhibitory concentrations of fosfomycin in the enteric infectious site. According to this background, the aim of this research was to determine in vivo calcium fosfomycin concentrations on swine intestinal cells. Four clinically healthy post-weaning piglets 4-5 weeks old were used. Animals were sacrificed after 15 days of calcium fosfomycin consumption in the drinking water (30 mg/kg). After slaughtering, jejunum was removed. Intracellular concentrations of the antibiotic were analyzed by HPLC MS/MS and they ranged from 0.82 to 2.05 μg/mL. These concentrations exceed the MIC90 of Salmonella enterica (4 μg/mL).

Key words: Fosfomycin, in vivo, Enterocytes, Swine, MIC90

INTRODUCTION

In pig production, weaning is considered as a critical period for piglets. It is characterized by a decrease in food intake that leads to a status of undernutrition, affecting other aspects of animal physiology and metabolism (Dirkzwager et al., 2005). During this period animals are more susceptible to infectious diseases (Nabuurs et al., 1993). In this regard, enteric diseases have a high economic impact on animal production, being the interstitial and intracellular fluids, the main sites of infection (biophase) of the pathogens responsible for these disorders (Dosen et al., 2007; Ross, 2006). It should be noted that most of the enteric diseases are caused by intracellular organisms, such as Salmonella sp. (facultative intracellular) and Lawsonia intracellularis (obligate intracellular) (Pedersen et al., 2008; Pluske et al., 1996), although other microorganisms that are located at the extracellular level are also important, such as Escherichia coli, Brachyspira hyodisenteriae and Brachyspira pilosicoli.

Different antibiotics have been used for decades to reduce pathogen infections in pigs, leading to irrational use. For this reason, many bacteria have become resistant to the most frequently used antimicrobials (Dirkzwager et al., 2005; Mathew et al., 1998; Rood et al., 1985). Fosfomycin (cis-1,2-epoxyphosphonic acid), an intensive production widely used drug (Serrano, 2002), is among the most recently used antimicrobials. It is a broad-spectrum antibiotic, structurally unrelated to other classes of agents. Fosfomycin 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, 2010). It has a low molecular weight (138,059 Da) and its chemical structure is similar to that of phosphoenoxypropane. In animals, the fosfomycin-calcium salt formulation is used orally, whereas the more water-
soluble disodium salt is used intravenously and intramuscularly.

The use of fosfomycin in animals and humans has been proposed because of its low toxicity and potential efficacy (Gallego et al., 1974). It is also widely used in animal production due to its rapid effect, good tolerance and lack of side effects (Aramayona et al., 1997; Caraminiana et al., 2004). It is indicated for the treatment of a variety of porcine bacterial pathogens (Haemophilus parasuis, Streptococcus suis, Pasteurella multocida, Bordetella bronchiseptica, Staphylococcus hyicus, E. coli and Salmonella enterica) associated with stress and/or several viral diseases (Martineau, 1997). For most of the important pathogens in swine production, fosfomycin minimum inhibitory concentration which kills the 90% of the microorganisms (MIC90) has been established in 0.25-4 μg/mL (Fernández et al., 1995; Ibar et al., 2009; Sumano et al., 2007). In this regard, an aspect of importance is that fosfomycin exhibits a time dependent-killing, so it kills bacteria when its concentrations remain constantly above the MIC (Aliabadi and Lees, 1997; Toutain et al., 2002). Therefore, 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 et al., 1991; Toutain et al., 2002).

Another relevant point to consider is that antibiotics with high lipid solubility are able to penetrate cell membranes, however, fosfomycin is a hydrophilic drug and, therefore, transmembrane passive diffusion appears as a mechanism of lower cellular penetration (Hoger, 1985). In this regard, Soraci et al. (2012) and Pérez et al. (2012a), have demonstrated that disodium fosfomycin penetrates into pulmonary epithelial lining fluid and in respiratory cells (HEP-2 cells), respectively, and reaches concentrations that exceed the MIC90 for the most important pathogens in swine respiratory infections. These findings show that disodium fosfomycin is an alternative for the treatment of intracellular respiratory infections in pigs. Disodium fosfomycin penetration has also been demonstrated in swine white blood cells at concentrations higher than the MIC90 (Pérez Gaudio et al., 2018a). In previous studies, we have determined calcium fosfomycin pharmacokinetics and bioavailability in post weaning piglets after oral administration of a 30 mg/kg dose. According to our studies, F% of calcium FOS is low (20). Therefore, considering the large quantity of drug that remains in the intestine and that fosfomycin is also a good antibiotic to treat pathogens at the extracellular level, calcium fosfomycin would be appropriate for the treatment of some enteric infections in pigs (Pérez et al., 2012b). Previously, we have also studied the penetration of calcium fosfomycin into an in vitro model of intestinal cells (IPEC-J2 cells), where cultures were subjected to a concentration of 580 μg/ml (Pérez et al., 2013). On our work group we have also demonstrated ex vivo calcium fosfomycin penetration on swine intestinal explants (Pérez Gaudio et al., 2018b). Both in the IPEC-J2 cell assays and in the studies with intestinal explants, calcium fosfomycin reached intracellular concentrations higher than the MIC90 for pathogens responsible for swine enteric diseases. Although we have determined, by in vitro and ex vivo studies, calcium fosfomycin concentrations in swine intestinal cells which exceed the MIC90 for enteric pathogens, still now, there are no in vivo studies showing that this antibiotic reaches adequate concentrations in the enteric biophase where the most important pathogens operate. According to this background, the aim of this work was to determine in vivo calcium fosfomycin concentrations on swine intestinal cells.

### MATERIALS AND METHODS

#### Animals
To carry out this study, four clinically healthy post-weaning piglets 4-5 weeks old were used. Animals were weighed, identified and housed in pens in the weaning room, with free access to water and food.

#### Antibiotic
Calcium fosfomycin (98.9% of purity) was from Bedson Laboratory, Pilar, Buenos Aires, Argentina. For PO administration, calcium fosfomycin formulation (30 mg/kg), was weighed and diluted in drinking water, taking into account a water consumption per animal of 1 L daily.

#### Experimental design
After 15 days of antibiotic consumption in the drinking water, pigs were sacrificed. After slaughtering, small intestines (jejunum) were immediately removed and intestinal contents were washed and detached with physiological saline solution (PSS). Samples were transported to the Laboratory of Toxicology at 2-4°C. Jejunum samples were cut into fragments of 3 cm in length and then opened along the mesenteric border. Tissues were placed in 60-mm Petri dishes. To eliminate the mucus and the remaining intestinal contents, they were washed twice with PSS by shaking for 10 min. The mucosa was cut into circular pieces of 1.3 cm², with a weight of 0.1 g (Nietfeld et al., 1991; Zha et al., 1995). Samples were analyzed by HPLC-MS/MS, using our previously developed methodology as described in Pérez Gaudio et al. (2018b).

### RESULTS
A total of 16 samples of intestinal mucosa (four per animal) were analyzed. The average intracellular concentration of fosfomycin in intestinal cells after consumption of the antibiotic in drinking water for 15 days (at a rate of 30 mg/kg of b.w.) was 1.35 μg/mL, with an SD of 0.37. Figure 1 shows the obtained concentrations for each sample.

![In vivo intracellular concentrations of calcium fosfomycin in swine intestinal cells](image)

**Fig. 1:** Intracellular penetration of calcium fosfomycin in intestinal cells, after a 15-day continuous consumption of the antibiotic in drinking water.
DISCUSSION

In vivo calcium fosfomycin concentrations (between 0.82 and 2.05 μg/mL) were significantly lower than in our previous in vitro studies on IPEC-J2 cells (P<0.005) (Pérez et al., 2013). Among the factors that influence these lower concentrations it should be considered the processes that the drug suffers in vivo in the animal as fosfomycin degradation by the acid pH of pigs’ stomach, the great adsorption of this drug to the food (Pérez et al., 2012b), and dilution with digestive fluids at intestinal level, not only with the volume of liquid present (0.62 L) (Ruckebusch et al., 1981), but also with secretions that are dumped into the duodenum from the gallbladder and pancreas. In addition, the intestinal epithelium is renewed every 2-3 days and in the in vivo study, there was a continuous entry of the drug with many cells exposed to a low concentration of the antibiotic.

Conclusions

Concentrations found in vivo exceed the MIC₉₀ of intestinal pathogens such as E.coli (0.5 μg/mL), although they are lower than the MIC₉₀ of Salmonella enterica (4 μg/mL). Both pathogens are aerobic-anaerobic facultative, and most of their life cycle goes outside the cell. The MIC₉₀ of fosfomycin for L. intracellularis, obligate intracellular pathogen, has yet not been determined. However, calcium fosfomycin penetration on intestinal explants infected with this pathogen has been confirmed (Pérez Gaudio et al., 2018b).

REFERENCES


