



## RESEARCH ARTICLE

### Pharmacokinetics of a Long-Acting Formulation of Azithromycin in Pigs

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#### ABSTRACT

The pharmacokinetics (PK) of a long acting 20% solution of azithromycin (AZ-LA) in pigs after a single peri-caudal injection at 10 and 20 mg/kg body weight was determined, using an aqueous formulation of the drug administered IV as reference. HPLC was used as analytical method.  $C_{max}$  was 1.4 and 2.5  $\mu\text{g/mL}$ , and  $T_{max}$  was 3.1 and 3.4 h, respectively for 10 and 20 mg/kg. Both preparations had 99.5% purity. Values for MRT were longer in the 10 mg/kg dose group (18.2 h) as compared to the 20 mg/kg group (16.5 h). Yet  $T_{1/2\beta}$  was comparable between both dose levels (8.5 h vs. 7.2 h, for 10 and 20 mg/kg, respectively). No linear increments linked to dose were found in variables such as relative bioavailability,  $C_{max}$  and apparent volume of distribution. Values for relative bioavailability (Fr) of the higher dose as compared to the lower were 86.81% and absolute bioavailability (F) was 110.5 for the lower dose and 115.9% for the higher. Best PK/PD ratios for clinical efficacy of azithromycin describe this antibacterial drug as time-dependent. Considering pig's serum concentrations of the AZ-LA preparation here studied after its peri-caudal injection and based on a theoretical minimal therapeutic concentration in plasma of 0.1  $\mu\text{g/mL}$  based on bacteriological testing, a dose-interval of up to 48 h can be achieved. The suitability of this preparation to treat pig respiratory diseases is addressed.

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#### INTRODUCTION

By having a 14 carbon ring, azithromycin (AZ), is regarded as an azalide antimicrobial drug, whose chemical structure differs from most macrolide antibacterial analogs. It can also be distinguished due to its higher potency against Gram-negative bacteria, without losing efficacy vs Gram-positive microorganisms. Additionally, azithromycin possesses a longer half-life and a notable volume of distribution. This latter feature accounts for its diffusion into cells, including phagocytes, and explains why this antibacterial drug is a preferred choice to treat diseases caused by various intracellular pathogens (Parnham *et al.*, 2014), and lung diseases with hypersecretion of mucus (Balloy *et al.*, 2014). However, there is no azithromycin formulation for veterinary medicine. Yet some of its exclusive pharmacokinetic features seen in other domestic species, makes it potentially suitable for veterinary medicine, such as a long serum elimination half-life ( $T_{1/2\beta}$ ): 13.97 h in cows and 61.29 h in sheep (Cárceles *et al.*, 2005b); 25.7 h in foals (Villarino and

Martin-Jiménez, 2013) and 32.5 h in goats (Cárceles *et al.*, 2005a) after oral administration of the drug. No information is available for the pharmacokinetics (PK) of AZ in pigs.

Based on pharmacokinetics/pharmacodynamics (PK/PD) ratios, AZ is used as a time-dependent antimicrobial in human medicine. This implies that serum and/or tissue concentrations should meet or exceed the minimal inhibitory concentration (MIC) (Treyaprasert *et al.*, 2007) of the infective microorganism for the majority of the dosing interval (Yousef and Jaffe, 2010). Considering the long persistence of AZ in the body in humans, as well as its well established *in vivo* post-antibiotic effect (Parnham *et al.*, 2014), a once a day oral dosing of the drug is customary in human medicine. In contrast, this may not be the case in pigs. Azithromycin has a bitter and disagreeable taste and it is unlikely to be suitable for in-feed medication in this species, unless a proper pharmaceutical preparation is accomplished. Similarly, a daily injection of this antibacterial agent, to treat a given disease in pigs, at a commercial level, is

unlikely, because such a procedure is time-consuming and costly; it imposes a great work load to producers and stress to affected pigs. Hence, a long acting formulation may be highly desirable. Definition of the PK of one such experimental preparation is here presented.

## MATERIALS AND METHODS

This trial was implemented at an experimental pig farm PRRSV-negative and free from *Actinobacillus pleuropneumoniae*. Mean pen temperature values were 19.5°C and thirty two healthy crossbred, castrated male pigs (Landrace-Yorkshire) with a mean weight of 10.4±0.6 kg were included. They were not medicated 15 days prior to this trial and four groups were randomly formed and housed in 8 separate rooms. Pig diet was provided *ad libitum* (55.5% N-free elements, 16.0% crude protein, 3.0% fat, and 6.5% crude fiber). One group was dosed with 10 mg/kg of a 20% long acting azithromycin (AZ-LA) preparation (AZ-LA<sub>10</sub>) (Azivex®, Laboratorios Karizoo SA de CV, Mexico), based on thioglycerol, citric acid and glicerol formal and the next group received a dose of 20 mg/kg (AZ-LA<sub>20</sub>). Injection site was in the lax tissue surrounding the tail (peri-caudal area) in a subcutaneous manner, delivering no more than 0.5 mL/injection site using 3 mL syringes and No.18 gauge needles. The other two groups AZ-IV<sub>10</sub> and AZ-IV<sub>20</sub>, received 10 and 20 mg/kg of an aqueous solution of azithromycin (Marozit®, a 10% solution of Laboratorios Liomont, SA de CV, México).

Left and right jugular indwelling catheters, 30-cm long (Becton, Dickinson and Company, Mexico) were implanted under surgical anesthesia. The loose ends were bandage-fixed and filled with 0.9% saline solution containing 100 IU of heparin for free-blood sampling or drug-dosing. Blood samples were obtained at 0 h (before injection) and at 0.50, 1, 2, 3, 4, 6, 8, 10, 12, 18, 24, 36, 48 and 60 h post-injection. Serum was collected after centrifugation and stored at -20°C until quantitative analysis was performed by chromatography, using a Jasco XLC HPLC system with a fluorometric detector and a diode array detector. Linearity for the standard curve was achieved between 0.1 to 8 mg/L ( $r > 0.995$ ). The intra-assay and inter-assay precision and recovery percentages for AZ were ≤5, ≤6 and 92%, respectively. The detection limit was 0.025 µg/mL and the quantification one, 0.05 µg/mL.

The serum concentration data of AZ, based on individual concentration vs time curves, were processed through non-compartmental analysis using WinNonlin (Version 3.2 Pharsight, Mountain View, CA, USA) software, obtaining:  $AUC_{0-\infty}$  = area under the concentration-time curve from zero up to  $\infty$  with extrapolation of the terminal phase;  $AUMC$  = area under the first moment of the concentration-time curve;  $MRT_{0-last}$  = mean residence time;  $\beta$  = elimination hybrid rate constant;  $T_{1/2\beta}$  = elimination half-life;  $T_{1/2ab}$  = absorption half-life;  $Vd_{AUC}$  = apparent volume of distribution of AUC;  $C_{S_0}$  = maximum serum concentration at zero time;  $C_{max}$  = maximum plasma concentration;  $T_{max}$  = time of maximum serum concentration;  $Cl_B$  = total body clearance;  $F$  = absolute bioavailability and  $Fr$  = relative bioavailability between doses.

Statistical differences were analyzed by means of Kruskal-Wallis and Dunn-tests, setting a significant error

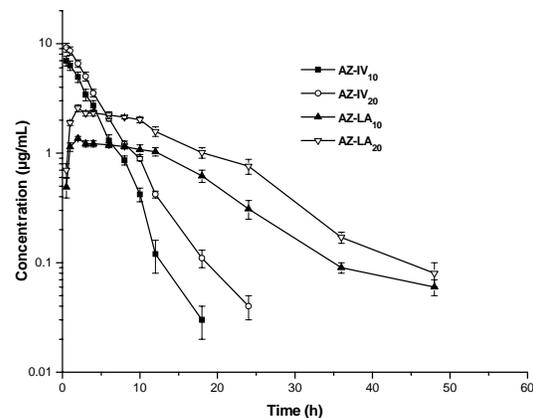
value not greater than  $P < 0.05$  (Statistical Package for the Social Sciences (SPSS).

After 15 days from injection, all animals from groups AZ-LA<sub>10</sub> and AZ-LA<sub>20</sub> were slaughtered and the peri-caudal area dissected, macroscopically inspected for lesions and routine histopathological analysis performed.

Determination of minimum inhibitory concentrations (MIC) of azithromycin was achieved as layout by Andrews (2001) for 20 field strains of both *Actinobacillus pleuropneumoniae* and *Escherichia coli* and using as control bacteria *Actinobacillus* sp49523 and *Escherichia coli* 25922 (NCTC 12241), respectively.

## RESULTS

Mean±SD plasma concentrations vs time profiles of AZ-LA 20% solution in pigs after a single SC peri-caudal injection of either 10 or 20 mg/kg, are presented in Figure 1. Also, this figure shows the serum levels of azithromycin after the 10 and 20 mg/kg IV injection of this drug. Table 1 shows values for pharmacokinetic parameters as well as their statistical comparison. As expected, no normal distribution for the four groups was the constant.



**Fig. 1:** Mean plasma concentrations of azithromycin in pigs after the SC-pericaudal injection of a long-acting preparation of azithromycin, either at a dose of 10 mg/kg (AZ-LA<sub>10</sub>) or 20 mg/kg (AZ-LA<sub>20</sub>). Intravenous profiles of the same dose are also presented (AZ-IV<sub>10</sub> and AZ-IV<sub>20</sub>).

Azithromycin showed a single open compartment kinetic behavior after its IV administration. Serum concentration at zero time ( $C_{S_0}$ ) was 9.11±0.3 µg/mL for group AZ-IV<sub>10</sub> and 11.38±0.5 µg/mL for group AZ-IV<sub>20</sub>. Terminal phase half-lives ( $T_{1/2\beta}$ ) were 2.8±0.4 h and 3.0±0.4 h, respectively for these two former groups. Correspondingly, apparent volume of distribution area under the curve ( $Vd_{AUC}$ ) values was 3.1±0.9 L/kg and 1.1±0.6 L/kg.

Maximum serum concentration ( $C_{max}$ ) of the long acting preparation were 1.4±0.2 µg/mL and 2.5±0.4 µg/mL for groups AZ-LA<sub>10</sub> and AZ-LA<sub>20</sub>, respectively. Terminal phase half-lives were in the same order 8.5±0.8 h and 7.2±1.0 h. Also, for the peri-caudal administration of azithromycin as LA preparation, MRT was only slightly larger for AZ-LA<sub>10</sub> as compared to the larger dose of 20 mg/kg (18.2 vs 16.5 h, respectively). This difference

**Table 1:** Pharmacokinetic variables (mean±SD) for a long-acting injectable preparation of azithromycin (AZ-LA) in pigs after the SC-pericaudal injection of either 10 mg/kg or 20 mg/kg. Intravenous injection of an aqueous preparation of azithromycin also at 10 and 20 mg/kg served as reference

Parameters	Group AZ-LA <sub>10</sub> (10 mg/kg) <sup>#</sup>	Group AZ-LA <sub>20</sub> (20 mg/kg) <sup>#</sup>	Group AZ-A <sub>IV</sub>	Group AZ-B <sub>IV</sub>
AUC <sub>0-∞</sub> (µg h/mL)	27.3±3.2 <sup>a</sup>	47.4±4.2 <sup>b</sup>	23.8±3.3 <sup>a</sup>	40.9±4.7 <sup>c</sup>
AUMC (µg h <sup>2</sup> /mL)	498.3±12.9 <sup>a</sup>	783.9±14.3 <sup>b</sup>	101.3±8.2 <sup>c</sup>	153.7±8.8 <sup>d</sup>
MRT (h)	18.2±0.5 <sup>a</sup>	16.5±0.5 <sup>b</sup>	4.3±0.3 <sup>c</sup>	3.8±0.4 <sup>c</sup>
β (µg <sup>-1</sup> )	0.1±0.02 <sup>a</sup>	0.08±0.02 <sup>a</sup>	0.013±0.006 <sup>b</sup>	0.024±0.007 <sup>b</sup>
T <sub>1/2β</sub> (h)	8.5±0.8 <sup>a</sup>	7.2±1.1 <sup>a</sup>	2.8±0.4 <sup>b</sup>	3.0±0.4 <sup>b</sup>
T <sub>1/2ab</sub> (h)	0.72±0.03 <sup>a</sup>	0.70±0.04 <sup>a</sup>	-	-
Vd <sub>AUC</sub> (L/kg)	-	-	3.1±0.9 <sup>a</sup>	1.1±0.6 <sup>b</sup>
C <sub>s0</sub> (µg/mL)	-	-	9.11±0.3 <sup>a</sup>	11.38±0.5 <sup>b</sup>
C <sub>max</sub> (µg/mL)	1.4±0.2 <sup>a</sup>	2.5±0.4 <sup>b</sup>	-	-
T <sub>max</sub> (h)	3.1±0.3 <sup>a</sup>	3.4±0.3 <sup>a</sup>	-	-
Cl <sub>B</sub> (mL/kg/h)	-	-	40	26
F (%) <sup>*</sup>	111	116	-	-
Fr (%)	115	87	-	-

Values within a row with no common super script differ significantly (P<0.05) Kruskal-Wallis/Dunn. <sup>\*</sup>Mean of means, <sup>#</sup>Injected peri-caudally, n = 8; AUC<sub>0-∞</sub> = area under the concentration-time curve from zero up to ∞ with extrapolation of the terminal phase; AUMC = area under the first moment of the concentration-time curve; MRT = mean residence time; β = elimination hybrid rate constant; T<sub>1/2β</sub> = elimination half-life; T<sub>1/2ab</sub> = absorption half-life; Vd<sub>AUC</sub> = apparent volume of distribution of AUC; C<sub>s0</sub> = maximum serum concentration at zero time; C<sub>max</sub> = maximum plasma concentration; T<sub>max</sub> = time to reach maximum serum concentration; Cl<sub>B</sub> = total body clearance; F = absolute bioavailability; Fr = relative bioavailability of the 10 vs the 20 mg/kg dose and viceversa.

**Table 2:** MIC's (µg/mL) for 20 field of *Actinobacillus pleuropneumoniae* and *Escherichia coli* and using as control bacteria *Actinobacillus* 49523 (NCTC 10229) and *Escherichia coli* 25922 (NCTC 12241)

MIC value (µg/mL)	<i>Actinobacillus pleuropneumoniae</i>	<i>Escherichia coli</i>
≤ 0.01	1	0
0.025	2	0
0.05	5	1
0.1	8	1
0.125	2	6
0.25	1	5
0.5	0	2
1	1	2
≥ 4	0	3
<i>Actinobacillus</i> 49523	2.0 µg/mL	
<i>Escherichia coli</i> 25922	4.0 µg/mL	

was statistically significant (P>0.05). Relative bioavailability (Fr) of the AZ-LA<sub>20</sub> group, as compared to AZ-LA<sub>10</sub> group, was 86.8 % and Fr of AZ-LA<sub>10</sub> as compared to AZ-LA<sub>20</sub> was 115.2 %, based on the following formula that considers the dose administered:

$$Fr = \text{AUC}_{20 \text{ mg/kg}} / 20 \text{ mg/kg} \div \text{AUC}_{10 \text{ mg/kg}} / 10 \text{ mg/kg} \times 100$$

Absolute bioavailability (F) was calculated to be 110.5 and 115.9%, respectively for the 10 and 20 mg/kg groups. Maximum serum concentration at a dose of 20 mg/kg was 78.6% higher than this value at a dose of 10 mg/kg and resulted to be statistically different (P<0.05). All other variables assessed, including T<sub>1/2β</sub> and T<sub>1/2ab</sub>, were not statistically different.

Setting minimal therapeutic concentration in plasma of 0.1 µg/mL, based on data derived from MIC values obtained (see Table 2), a dose-interval of 35 to 48 h can be achieved. The time period with serum concentrations of AZ at or above a useful level, was statistically indistinguishable between 20 and 10 mg/kg (P<0.001), as most bacteriological susceptibility patterns of *Actinobacillus pleuropneumoniae* and *E. coli* were at or below the referred values. All pigs remained clinically healthy throughout the study and no signs of visible pain were detected. Tissue swelling at peri-caudal area was not observed. Histopathological analysis of injection sites taken post-mortem did not show either macroscopic signs of an inflammatory response nor scar tissue.

## DISCUSSION

In humans, at least 10 metabolites of AZ have been described. They all possess antibacterial activity (Yousef and Jaffe, 2010). Yet, it has been reported that there is equivalence between HPLC and bioassay methods to quantify AZ in blood samples (Rivulgo *et al.*, 2013). This study disregarded the possibility that some different metabolites of azithromycin to the ones so far described in formal literature are present in pigs, and that they show antibacterial activity. Thus, based on the assumption that metabolites of azithromycin in pigs show no antimicrobial activity, PK data derived from HPLC of the parent compound only, as carried out in this study, will represent the kinetic behavior of the active fraction of this drug (Riedel *et al.*, 1992), and can be safely taken to derive PK/PD ratios. Furthermore, pharmacokinetics of AZ has been established for humans (Sampson *et al.*, 2014); cows (Lucas *et al.*, 2010); goats (Cárceles *et al.*, 2005a); sheep (Cárceles *et al.*, 2005b); foals (Villarino and Martínez-Jiménez, 2013) and dogs (Zur *et al.*, 2014), and in all these studies PK of the parent compound was regarded as the relevant one. Also, in all cases the IV and IM routes were assessed. Yet in any study a long-acting preparation was evaluated in pigs. In this context PK values obtained in this paper cannot be directly compared.

In this study, T<sub>1/2β</sub> for the peri-caudal administration of azithromycin were not essentially different between the two doses tested (P>0.05) and both values are similar to the T<sub>1/2β</sub> reported for cows, when injected with a dose of 10 mg/kg of a 30% ethanol-propylenglycol solution (7.2 to 8.5 in pigs and 13.97±11.1 in cows) (Lucas *et al.*, 2010). In contrast, values here obtained are considerably shorter than values reported for goats (Cárceles *et al.*, 2005a) (45.26±6.8 h for goats). This may be due to the fact that the preparation used for goats was entirely dissolved in PEG 200 (polyethylen glycol) with only 2% of alcohol butyl-aminobenzoate, while the experimental preparation used in this trial contained 200 mg/mL of azithromycin in thioglycerol, citric acid and glicerol formal.

Absolute bioavailability of AZ-LA<sub>10</sub> and AZ-LA<sub>20</sub> was 111% and 116%, respectively. It is worth pointing out that bioavailability values above 100% are not startling for pharmaceutical designs which display long action

and/or flip-flop kinetics (Yañez, *et al.*, 2011). This phenomenon could be interpreted as a flaw in sampling, quantifying the drug and/or processing the data, but can also account for a recycling phenomenon due to the distinctly lipid solubility nature of AZ, as well as for the attenuation of the first order clearance of the drug achieved by the combination of the vehicles previously mentioned (Sampson *et al.*, 2014).

It is important to stand out that pharmacokinetic variables did not show a linear increment as a result of doubling the dose from 10 to 20 mg/kg. This phenomenon is not entirely odd when injecting drug preparations with vehicles that are not merely aqueous (Yañez *et al.*, 2011). Furthermore Fr obtained showed, for the pericaudal injection site chosen in this trial, that a dose of 10 mg/kg has greater bioavailability as compared to the 20 mg/kg dose. This may be in part due to the fact that maximum absorption capacity of the injection site has been surpassed by the larger dose. Complexities of the possible interaction of vehicles, active principle and the special structure of the site in pigs, could explain this phenomenon (Craven *et al.*, 2002), but such an endeavor is beyond the scope of this paper. However, MRT values obtained for both doses and a dosing interval based on 0.1 µg/mL, allow the conclusion that either a 10 or 20 mg/kg dose every 48 h may suffice as a rational dose scheme in pigs for the preparation of azithromycin in study

It has been accepted that following administration, AZ is extensively distributed extra-plasmatically (Sampson *et al.*, 2014). This prominent pharmacokinetic characteristic of AZ explains, at least in part, why tissue concentrations remain high sometime after serum concentrations have declined to undetectable levels. Consequently, a low therapeutic concentration can be set in blood to establish a dosing interval. In this study it is possible to set 0.1 µg/mL, derived from the pilot study here performed and complies well with the minimum plasma therapeutic concentration adopted from human studies (Harahap *et al.*, 2012). This value allows a 48 h dosing interval. Further studies are warranted to assess whether or not lung concentrations of azithromycin meet the referred expectations.

There is no possibility that an additional useful slope beyond the 48 h sampling period was overlooked, because concentrations at 60 h were below the set effective serum concentration (0.1 µg/mL). Hence, it is necessary to test other vehicles, such as polymeric matrixes, before an ideal pharmaceutical design for AZ in pigs is achieved, and it is mandatory to determined drug safety of this pharmaceutical design in pigs, as well as its lung tissue kinetics. Additionally, drug residue analysis of AZ and metabolites should be performed when the ideal formulation is chosen. Hence, at this stage is not possible to recommend the clinical use of the AZ preparation here analyzed. However, the biggest challenge for the drug development of azithromycin in food-producing species is the international awareness to control antibacterial drug resistance in the world, limiting the use of many antimicrobial drugs for veterinary medicine (Anonymous, 2011), a view that is beyond the scope of this trial, and has many contending perspectives.

**Conflict of interest:** The authors declare that there were no competing interests and that the conceptual design, the conduct, the interpretation of results and all scientific

aspects of the study were not influenced by any pharmaceutical company.

**Author's contribution:** LG and HS conceived and designed the experiment. IM, LG and HS executed the experiment at the farm. JM-C and LO analyzed the sera while IM analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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