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PHARMACOKINETICS OF LONG ACTING AMOXICILLINS IN BUFFALO CALVES IN PAKISTAN

Rasheed[1]M. A[*]., M. Ashraf, M. O. Omer[1], S. Khan[2]

[1]Department of Pharmacology and Toxicology; [2]Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan
[*]Corresponding author: dr_aadil@hotmail.com

ABSTRACT

In this present study pharmacokinetic parameters of two long acting preparations of amoxicillin were compared in buffalo calves. Study was performed on sixteen healthy buffalo calves. Calves were divided in two groups, A, B. Each group contains eight animals. In first phase, calves of group A were administered Farmox L.A and calves of group B were administered Clamoxyl L.A IM at rate of 15mg/kg bodyweight. In second phase, after a washout period of 2 weeks post administration, group A that received treatment with Farmox L.A were administered Clamoxyl L.A and vice versa at same dose rate. Blood sampling was done prior and after drug administration at different time intervals up to 48 hours. Plasma was taken apart and stored at -20 ºC till analysis. Amoxicillin concentration in plasma was found out by using HPLC. Pharmacokinetic parameters of both preparations were compared. It was found out, that there is no significant variation in pharmacokinetics of Clamoxyl L.A and Farmox L.A, but the area under curve of both drugs showed that the drug stay in body maximally up to 24 hours. So, it can be concluded that both the products are bioequivalent in their rate and extent of drug absorption and it should be repeated after 24 hours instead of 48 hours.

Key words: Pharmacokinetics, Long acting Amoxicillins, Buffalo Calves, HPLC.

INTRODUCTION

Amoxicillin is semi-synthetic penicillin. It inhibits bacterial cell wall synthesis by stopping the cross-linking of peptidoglycan strands. It is close analogue of ampicillin whose antibacterial spectrum is comparable to that of Chloramphenicol and Tetracyclines. It absorbed well when it is given orally and when through parenteral administration. It is also distributed well in liver, lungs and kidneys (Plumb 2002). It is acid stable and frequently used in dogs and cats orally (Watson 1992). While in ruminants and equines, it is commonly used by parenteral route (Adams 2001). Its sodium salt is water-soluble and, in solution form is not much stable. Its trihydrate salt is not water-soluble and is available in the market in the form of tablets, capsules and as injectables preparations. In veterinary clinical practice amoxicillin is being used through parenteral route as aqueous and oily suspension. Oil base preparations are when used, they maintain effective plasma level upto 48 hours (Adams 2001). Amoxicillin, in urine, is excreted in unchanged form. Its half-life of elimination is from 1.0 to 1.5 hours in various animal species. It has good efficacy against gram positive and negative bacteria such as *Streptococci, Staphylococci, Corynebacterium, E. coli*, *Salmonella, Pasteurella, and Shigella* etc. It has widespread use in veterinary practice for the treatment of infectious diseases of respiratory tract, alimentary tract, urogential tract, soft tissue infections, skin infections, post surgical infections and for secondary bacterial infections of viral diseases (Adams 2001). Clinical trials demonstrated that it was effective for the treatment of hemorrhagic septicemia and black quarter disease under local field conditions (Ramzan *et al* 2003). Amoxicillin has good efficacy against a wide range of important diseases such as metritis, mastitis, abscesses, shipping fever, Pylonephritis, foot rot, anthrax, etc (Laurence *et al* 2006). Pharmacokinetic studies are of utmost importance to design an appropriate dosage regimen but it may be different from one specie to another specie, and the local environmental conditions where the drug is being used (Toutain and Lees 2004). It is therefore important to characterize the pharmacokinetic parameters of various antimicrobial agents and to suggest the appropriate dosage regimen based on these parameters for the target species under local conditions (Nawaz 1994, and Nawaz *et al* 1998).

Various brands of same generic drugs are approved for the purpose of healthy competition of prices, but it should not be at the cost of quality of the drugs. It has been observed that if Good Manufacturing Practices are not properly followed. This will not only affect the quality of the product but also be a loss for the consumers. Drug may be effective for the purpose it is used for, but therapeutic failure may occur due to the substandard commercial preparation, which may aggravate the disease condition. The present project is designed to perform pharmacokinetics and bio-equivalence studies of various brands of the amoxicillin for the benefit of the patients.

MATERIALS AND METHODS

Animals: For this study sixteen healthy buffalo calves between the ages of 6 to 12 months were kept in the animal shed of University of Veterinary and Animal Sciences Lahore. Their health status was monitored by blood and physical examination. The calves were provided fodder and water at lib. Albendazole dewormer was given to all animals fifteen days prior to drug administration.

Drugs and Chemicals: Clamoxyl™ L.A (Reference Product) manufactured by Pfizer and Farmox™ L.A (Test Product) manufactured by Farvet Pakistan) were purchased from local market. Acetonitrile (ACN), Methanol and di-sodium hydrogen Phosphate ($Na_2 HPO_4$) were purchased from Merck. Amoxicillin standard was purchased from Sigma-Aldrich (USA).

Experimental Protocol: The study was cross over design. Calves were divided in two groups A, B having eight animals in every group. In first phase calves of group A were administered Farmox L.A (Test Product) intramuscular at rate of 15mg/kg bodyweight and calves of group B were administered Clamoxyl L.A (Reference Product) intramuscular at rate of 15mg/kg bodyweight. In second phase of study, after a washout period of 2 weeks post administration, group A that received treatment with the Farmox L.A (Test product) were administered Clamoxyl L.A (Reference Product) and vice versa at the same dose rate.

Collection of Blood Samples: Before drawing the blood, area above the juggler vein was cleaned and disinfected with swab of methylated spirit. Then 5ml blood was collected by direct pricking of vein in test tubes containing heparin prior and after drug administration at 0.166, 0.333, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0 and 48.0 hours. Centrifugation of blood samples were done for 10 minutes at 4000 rpm. Then the plasma was separated in small capped plastic bottles and placed at -20 ºC till analysis.

Standard Solutions: Amoxicillin Stock solution (1mg/ml) was prepared by adding 100mg of standard of amoxicillin in 100ml H_2O: ACN (95:5). From the stock solution working solutions were prepared by dilution with H_2O:ACN (95:5) to get concentrations of 0.05, 0.1, 0.5, 1, 10, 25, 50 and 100 µg per ml. All the solutions were kept away from light and stored at -20°C. Calibration standards were achieved by adding up known concentration of amoxicillin to plasma that is free from any drug. The final concentrations of 0.75, 1.5, 3.0, 6.0, 12.0 and 25 µg/ml were achieved. Plasma solutions was placed away from light at -20°C till it used.

Extraction Procedure: Protein extraction procedure was used for the extraction of amoxicillin from plasma. This was done by transferring 400 µl aliquot of each plasma sample to 1.5 ml polypropylene tube. Then, 800µl of cold methanol was added. After a vortex mixing, centrifugation was done (13500 rpm at 4°C for 15 min). A 100µl aliquot of supernatant was then shifted to HPLC injection vials. Then 20 µl was allowed to inject in chromatographic system. Quality controls runs were carried out in duplicate for each batch. To avoid inter-assay variation the analysis of plasma samples from single animal was performed on same day.

Instrument and Chromatographical Conditions: Analyses was performed on Shimadzu HPLC having LC-20AT. LC Solution software of HPLC was used to process the data. Mobile phase was the blend of di-sodiumhydrogen phosphate buffer (0.02mol/L), pH=3 and ACN (Acetonitrile) (95:5 v/v). The column was Discovery® HS C18 (5µmx25cmx4.6mm) having temperature 35ºC and flow rate 1.5ml/min. A peak of amoxicillin was observed at 8:02 minutes by UV absorbance at 230nm. Quantification of amoxicillin was obtained by plotting amoxicillin peak height ratios as a function of concentration.

RESULTS AND DISCUSSION

The concentration of amoxicillin in plasma was measured by HPLC. The results of standard curve are given in figure 1. Chromatograms of plasma from buffalo calf and spiked with 25 µg/ml were given in figure 2, The Chromatogram after the administration of commercial preparation of amoxicillins in buffalo calf having concentration of amoxicillin in plasma (5 µg/ml) was given in figure 3. Pharmacokinetic software (APO pharmacological analysis MW /PHARM version 3.02) was used to analyzed the data. One Compartmental open model was used, Area Under Curve (µg.h/ml), Peak Plasma Concentration (Cmax, µg/ml), Time to reach peak plasma concentration (Tmax, Hour), Volume of Distribution (Vd, Litre), Elimination Half Life (T1/2, Hours) , Mean Resident Time (MRT, Hours), Total Body Clearance (CI, Litre/hr). The Area under curve for both Clamoxyl L.A, and Farmox L.A, at 15mg/kg bodyweight is shown in figure 4. The difference is non significant. This shows that both the drugs have same concentration at different intervals of time. The pharmacokinetics parameters of Clamoxyl L.A, and Farmox L.A, are given in table 1. Time to reach peak plasma concentration (Tmax), The volume of distribution (Vd), Peak Plasma Concentration (Cmax) of both Clamoxyl L.A and Farmox L.A. showed non significant results. The half life of elimination of Clamoxyl L.A and Farmox L.A showed non significant difference, but differ from the elimination half-life calculated by *Kyung-Hwan Yoon et al* (2004) in healthy male volunteers after a single oral dose of amoxicillin/ clavulanic acid (Augmentin®) and by *Reyns*

et al (2007) in pigs after 20 mg/kg oral administration of amoxicillin. The difference in elimination half life may be due to the species difference and variation in dose rate. The Total Body Clearance (CI), and Mean Resident Time (MRT) of Clamoxyl L.A and Farmox L.A showed non significant difference.

Table 1: Comparison of One Compartmental Pharmacokinetic Parameters of Clamoxyl L.A and Farmox L.A (n=16) following intramuscular dosage at the rate of 15mg/kg to Sixteen Calves.

Parameters	Clamoxyl L.A		Farmox L.A		% Difference	P Value
	Mean	± SD	Mean	± SD		
Area Under Curve (AUC) (h.mg/l)	18.51	4.33	19.16	4.39	3.51	0.675
Clearance(CL) (l/h)	0.84	0.20	0.82	0.22	2.38	0.717
Volume of Distribution (VD /F)(l)	4.36	1.64	3.73	1.43	14.44	0.261
MRT(h)	5.83	1.05	5.31	1.09	8.91	0.183
Tmax (h)	2.73	1.12	3.25	1.33	19.04	0.248
Cmax (mg/l)	1.82	0.47	1.51	0.67	17.03	0.141

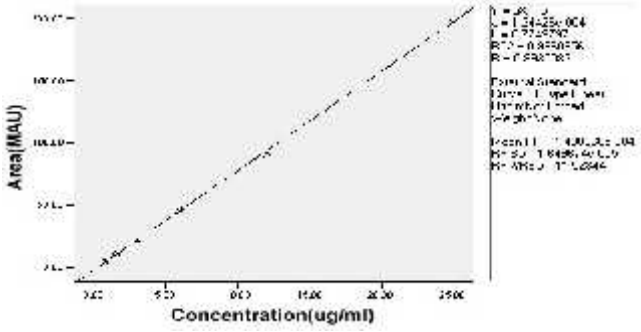

Figure 1: ***Calibration Standard Curve of Amoxicillin at different Concentrations (µg/ml) VS Area (MAU)***

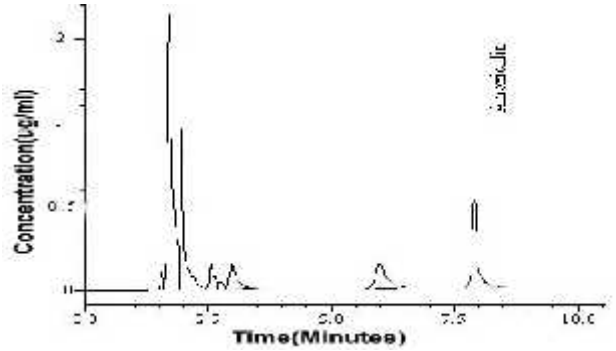

Figure 2: Chromatogram of spiked Amoxicillin (25µg/ml) in Buffalo Calf.

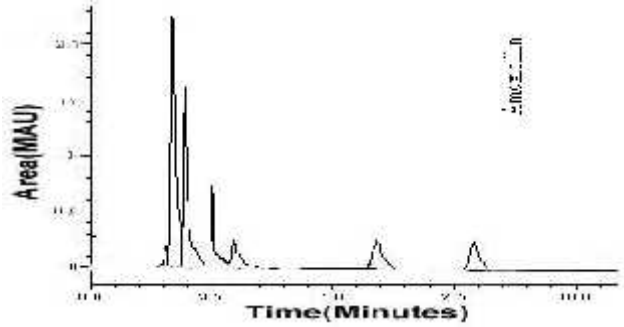

Figure 3: Chromatogram of plasma sample having Amoxicillin (Clamoxyl L.A, 5 µg/ml) in Buffalo Calf.

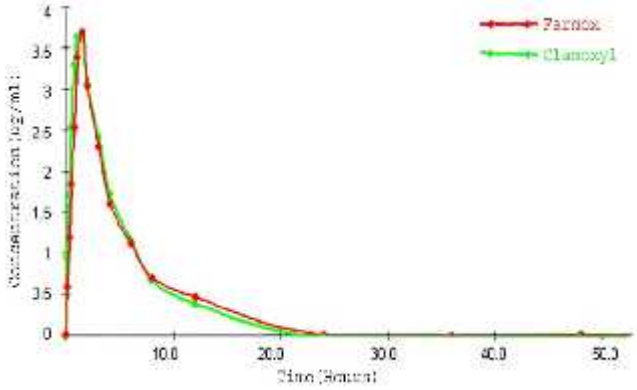

Figure 4: Graphical comparison of average plasma concentrations of Clamoxyl L.A and Farmox L.A (n=16) in buffalo calves.

Conclusion: After examining the data, there was no significant difference between the Pharmacokinetics of Clamoxyl L.A (Reference Product) and Farmox L.A (Test Product) after single intramuscular administration at rate of 15mg/kg body weight in sixteen buffalo calves. But the area under the curve of both drugs showed that the drug remain present in the body maximally up to 24 hours. So, it can be concluded that the both the products are bioequivalent in their rate and extent of drug absorption and it should be repeated after 24 hours instead of 48 hours.

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