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Research Article

PHARMACOKINETICS OF AMOXICILLIN IN BROILER CHICKEN FOLLOWING A SINGLE ORAL ADMINISTRATION

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Amoxicillin was given to six nos. of chickens at a dose of 20 mg/Kg of body weight orally. Plasma concentration was monitored serially for 24 h after oral administration. Concentrations of amoxicillin were measured using high-performance liquid chromatography. K_a value and $t_{½}$ K_a were 1.38±0.17 (hr^{1}) and 0.51±0.07 (hr). On the other hand, elimination rate constant K and elimination half-life $t_{½}$ K were found to be 0.18±0.01 (hr^{-1}) and 3.95±0.14 (hr). The AUC and Vd_{area} values were 257.88±28.01 (µg hrL^{-1}) and 0.41±0.07 (L Kg^{-1}), while Cl_B value was 0.07±0.01 ($LKg^{-1}hr^{-1}$). The C_{max} value was 38.87±2.94 (µgml^{-1}) at T_{max} 1.50±0.09 (hr). The MRT value was 6.02±1.73 (hr). The results indicated that amoxicillin persist in poultry bird for a shorter period following moderate absorption from GI tract.

Key Words: Amoxicillin, Broiler poultry, HPLC, Oral kinetic

Amoxicillin is one of the amino-penicillin β-lactam antibiotics widely used in veterinary medicine due to its broad spectrum antimicrobial activity (Neu and Winshell, 1971; Palmer *et al.*, 1976 and Brander, 1977), good absorption, penetration into tissues and rapid bactericidal activity (Yeoman, 1977). Amoxicillin is effective against gram-positive organisms (like *Clostridium, Staphylococcus, Streptococcus* and *Corynebacterium)* and gram-negative organisms (for example, *BordeteUa bronchiseptica*, *Escherichia colt*, *Proteus mirabilis* and species of *Pasteurella*, *Salmonella* and *Haemophtlus)* affecting avian production. Therefore, amoxicillin is seen to be a valuable antibiotic in terms of

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therapeutic effect for treating bacterial infections in poultry. Keeping in view, the present study was conducted to determine the oral pharmacokinetic profile and oral bioavailability of amoxicillin in broiler chickens, and to relate the findings to a theoretically effective therapeutic regimen.

MATERIALS AND METHODS

Drugs and chemicals: Amoxicillin trihydrate was purchased from market Zovex® oral powder 50% W/W (Dey's Medical Stores Ltd.). Amoxicillin trihydrate analytical grade (>98% purity) was also obtained from M/S Alembic Chemicals Ltd, Vadodara as a gift and was used as standard to run HPLC. All other chemicals used in this study were obtained from E. Merck (India), Sigma Chemicals Co. (USA).

Experimental birds: Vaccinated and dewormed 40 days old six broiler male chickens (Hubbard X Hubbard) were purchased from a commercial poultry farm. The birds were placed in individual cage in the animal house of this laboratory one week before medication with optimum lighting facility. The birds were fed with feed as per Bureau of Indian Standard (BIS), 1992. Fresh water and feed were supplied *ad libitum*. Prior to start of this experiment, the animal room was cleaned and fumigated with potassium permanganate and commercial formaldehyde solution (1:10). All the feeders and watering troughs were properly cleaned and disinfected with potassium permanganate solution (5%) 24 hr before start of the experiment.

Experimental design and mode of administration: Chickens were given single oral doses of amoxicillin at 20 mg/ Kg body weight between 8 AM to 9 AM. Food was withheld for twelve hour before oral dosing until six hour after dosing. Blood samples were collected from the left brachial vein of each chicken and collected in heparinized tubes through a cannula at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 hours after drug administration. Plasma was separated after centrifugation and was stored frozen (-20ºC) until analyzed. The experimental procedure was approved by Institutional Animal Ethical Committee (IAEC).

Analytical Method: Analysis of amoxicillin in plasma was done by the HPLC method of Vree *et al.* (1978) with some modifications. An Agilent 1100 series high-performance liquid chromatography fitted with Diode Array Detector attached with a computer was used for analysis of amoxicillin.

Chromatographic separation was achieved on a 5µ Thermo 100A C_{18} 250x4.6 mm column condition by a mobile phase, mixture of 20 mM potassium dihydrogen orthophosphate buffer (pH4.5) and acetonitrile (70:30) with a flow rate of 0.5 mL min^{-1}at 230 nm wave length. Standard samples (20 µL) were injected into liquid chromatograph using a 25 µL loop with Hamilton syringe.

Extraction: To 0.5 mL of plasma, 2 mL of 0.33M perchloric acid was added and mixed

by vortexing for 20 sec. It was centrifuged at 15,000 rpm for 10 min by Beckman Coulter Centrifuge. This process was repeated thrice and the supernatant was transferred to another tube and evaporated to dryness on rotary evaporator (Eyela Digital Water bath). The residue was then reconstituted in mobile phase (1000 µL) and after syringe filter, 20 µL was injected into the column.

Pharmacokinetic parameters: Pharmacokinetic parameters were determined from computerized curve fitting programme 'PHARMKIT' supplied by the Department of Pharmacology, Institute of Postgraduate Medical Education and Research, Pondicherry, India. Pharmacokinetic parameters were determined for each bird individually. Semilogarithmic plot of mean plasma concentrations of amoxicillin against time with computerized best-fit line in broiler birds following single dose oral administration at 20 mg/Kg body wt.

RESULTS

The results of *in vitro* recovery experiment of amoxicillin from plasma ranged from 81 to 86%. The limit of detection of amoxicillin was 0.01 ppm. The chromatogram showed the well resolved peak of amoxicillin (technical grade) from plasma (Fig. 1). Therefore the method is satisfactory and subsequently used for extraction, clean up and estimation of amoxicillin from plasma of broiler poultry.

Data analysis: Plasma concentration-time data (Table 1) were fitted to a two-compartment open model for kinetic analysis. The therotical zerotime plasma concentration (C^0P µgmL^{-1}), absorption rate constant (K_a) expressed as hr^{-1}, absorption half –life (t½ K_a) expressed as hr, elimination rate constant(K) expressed as

Table 1. Plasma amoxicillin concentration (µg mL^{-1}) after single dose oral admin istration at 20 mg/Kg body weight in plasma of broiler poultry (Mean values of six replicates with S.E.)

Time (hr)	Concentration (µg mL^{-1})
0.25	19.67±1.74
0.5	24.90±1.88
1	38.87±2.94
2	30.93±4.47
4	23.70±3.29
6	17.86±2.78
8	12.13±1.35
12	5.43±0.38
24	0.67±0.05

Table 2. Pharmacokinetic parameters of amoxicillin after single dose oral administration at 20 mg/Kg body weight of broiler poultry (Mean values of six replicates with S.E.)

Parameters	Pharmacokinetic value
$K_a(hr^{-1})$	1.38±0.17
t½ K_a (hr)	0.51±0.07
$K(hr^{-1})$	0.18±0.01
t½K(hr)	3.95±0.14
AUC(µg hr L^{-1})	257.88±28.01
Vd_{area}(L Kg^{-1})	0.41±0.07
Cl_B(L $Kg^{-1}hr^{-1}$)	0.07±0.01
C_{max}(µg mL^{-1})	38.87±2.94
T_{max}(hr)	1.50±0.09
MRT(hr)	6.02±1.73

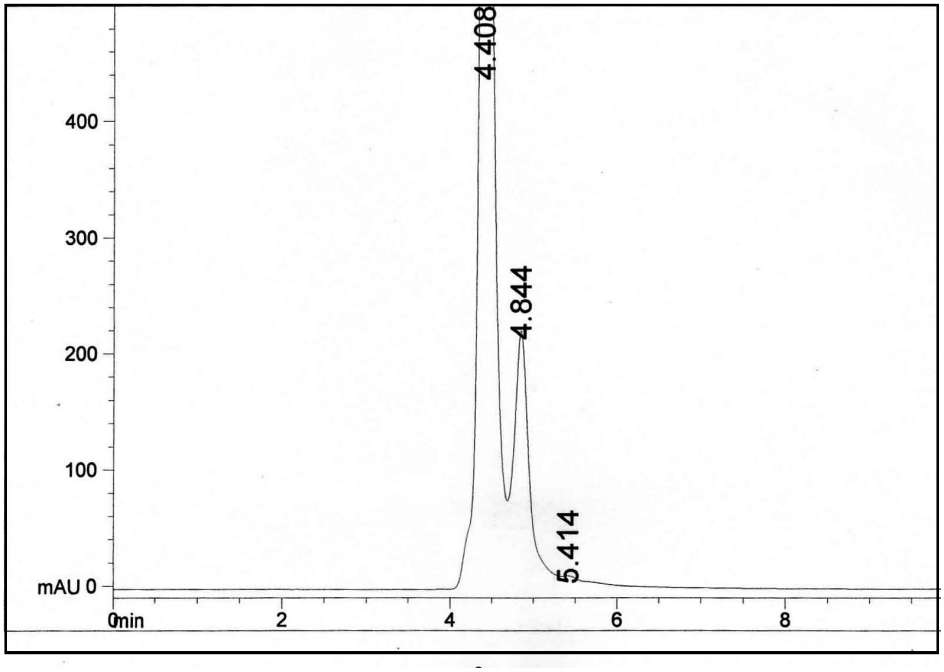

Fig. 1. Chromatogram of amoxicillin in plasma

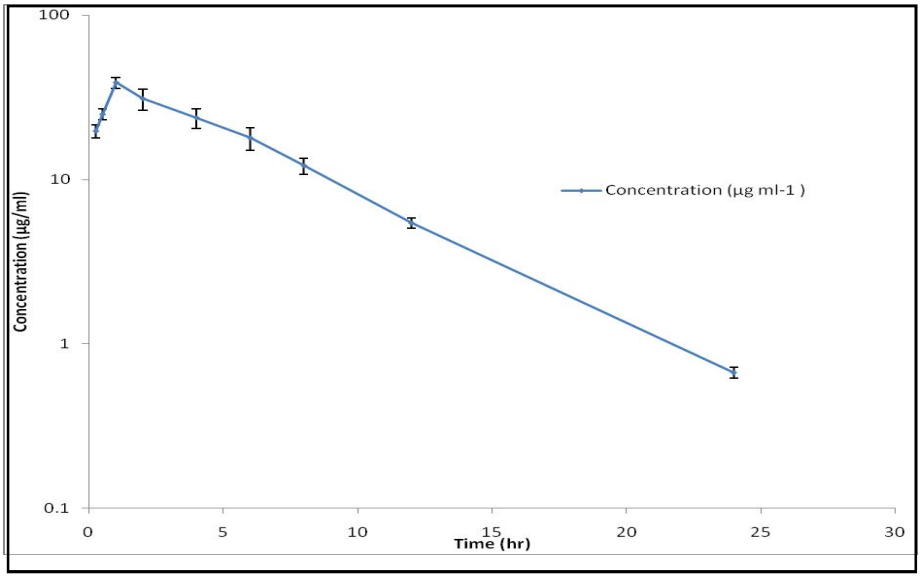

Fig. 2. Semilogharithmic plot of mean plasma concentration of amoxicillin after single dose oral administration at 20 mg/Kg bw of broiler poultry

hr^{-1}, elimination half life (t½ K) expressed as hr, apparent volume of drug distribution (Vd_{area}) based on total area under plasma drug concentration versus time curve (area method) expressed as L Kg^{-1}, total area under the plasma drug concentration (AUC) versus time curve from 't_0' to 't' after administration expressed as µg hr mL^{-1}, total body clearance (Cl_B) of a drug representing the sum of all clearance processes in the body expressed as L Kg^{-1} hr^1 were calculated by standard equations (Baggot, 1977).

Mean pharmacokinetic parameter of amoxicillin after single dose oral administration at 20 mg Kg^{-1}bw in broiler poultry has been presented in Table 2. It is transpired from the table that K_a value and t½ K_a were 1.38±0.17 (hr^{-1}) and 0.51±0.07 (hr). On the other hand, elimination rate constant K and elimination half-life t½ K were found to be 0.18±0.01 (hr^{-1}) and 3.95±0.14 (hr). The AUC and Vd_{area} values were 257.88±28.01 (µg hrL^{-1}) and 0.41±0.07(L Kg^{-1}), while Cl_B value was 0.07±0.01(L $Kg^{-1}hr^{-1}$). The C_{max} value was 38.87±2.94(µgmL^{-1}) at T_{max} 1.50±0.09 (hr). The MRT value was 6.02±1.73 (hr).

DISCUSSION

The values of AUC and Vd_{area} indicate moderate distribution throughout the body. On the other hand, value of Cl_B and MRT suggest shorter persistence of amoxicillin in broiler poultry which is further substantiated by t½ k of 3.95±0.14 (hr).

Therefore it may be concluded that Amoxicillin persist in poultry bird for a shorter period following moderate absorption from GI tract. Anadon *et al.* (1996) stated that after single oral administration, amoxicillin was rapidly absorbed, with T_{max} of 1.00±0.06 hr. Maximum plasma concentration was 160.40±4.67 μgmL^{-1} and mean amoxicillin concentrations > 15 μgmL^{-1} persisted for 24 hr. In pigeons, Dorrestein *et al.*(1986) observed that blood levels after oral administration of amoxicillin were comparable to the tissue level. Reyns *et al.* (2007) stated that elimination half-life of amoxicillin was 0.73 in pig and the mean maximal plasma concentrations of amoxicillin was 3.14 mg/L reached after 1.19 hr. In birds, the anatomy and digestive physiology are different from ruminant and other species. Therefore, the disposition parameters may vary widely among the species.

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