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Pharmacokinetic Characteristics and Withdrawal Times of Amoxycillin in Ducks

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ABSTRACT

The pharmacokinetic characteristics of amoxycillin (AMX) were observed on healthy ducks. Each animal was administered intravenously (i.v.), intramuscularly (i.m.), subcutaneously (s.c.) and orally (p.o.) at a single dose level of 20 mg/kg body weight. The amoxycillin in plasma was detected up to 5 hours (h) after i.v. and i.m. administration but it was found up to 10 h and 6 h after s.c. and p.o. administration, respectively. However, the drug could be absorbed at the peak plasma level within 30 min after i.m and s.c. administration while it was detected at 60 min after p.o. administration. The pharmacokinetic parameters after i.v. administration were as follows; the elimination half-life ($t_{1/2}\beta$) = 42.0 ± 3.5 min, the elimination rate constant (K_{el}) = 2.87 ± 0.31 h^{-1}, the apparent volume of distribution ($V_{d(area)}$) = 0.75 ± 0.21 l/Kg, the total body clearance (Cl_B) = 1.59 ± 0.52 l/Kg/h and bioavailability following i.m., s.c. and p.o. administration were 93.33 ± 5.23 %, 91.11 ± 7.41 % and 34.67 ± 5.06 %, respectively. Finally these available data could be used for establishing the dosage regimen as well as to allow the preslaughter withdrawal times and maximum residue limits for ducks.

Key words: pharmacokinetic, withdrawal time, antibiotic, amoxycillin, duck species

INTRODUCTION

Amoxycillin (AMX), broad spectrum antibiotic of the amino-penicillin group that is widely used for treating urinary, respiratory, skin and gastrointestinal bacterial infections in animals however, resistant strains of Gram-negative and Gram-positive bacteria have been clinical problems almost from the inception of β-lactam antibiotic use in humans and Veterinary practices. On the other hand, AMX is broad spectrum beta-lactam with a chemical structure and antibacterial activity similar to ampicillin (APC), but the advantages of AMX over APC are a more rapid bactericidal effect and a more complete absorption after oral administration. Moreover, the systemic availability of AMX is about twice that of APC that occur after the same oral dose of APC in domestic animals. The revival of interest in AMX and APC had led to many investigations elucidating the disposition of these drugs in various animal species. Nevertheless, There is no report of therapeutic regimes for broad spectrum penicillins in ducks, though the pharmacokinetics and clinical use of amoxycillin and other antibiotics have been widely studied in other avian species (Clark, 1986; Dorrestein *et al*., 1987; Lawrence, 1988; Limpoka, 1992; Poapolathep et al., 1998 and 2000).

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The objective of the present investigation was initiated to determine the basic pharmacokinetic parameters of amoxycillin trihydrate obtained following intravenous, intramuscular, subcutaneous and oral administration because the kinetic behaviors reports of antimicrobial drugs are scarce in ducks, as well as to compare some considerable different of pharmacokinetic values of the other broad spectrum penicillins in ducks (Poapolathep *et al*., 2001a and 2001b) in order to resolve the problems of using antimicrobial drugs in duck species, thereafter, the therapeutic regimes of AMX should be concerned for duck.

MATERIALS AND METHODS

Drug

Amoxycillin trihydrate formulation, (lot.no. AM 8860699) that was diluted with sterile water before administration at an identical dose of 20 mg/kg body weight for each duck. The standard drug preparation was used by amoxycillin (Sigma, lot 108H0647), calculated potency free base /mg.

Animals

The experiments were performed on 120 healthy ducks with an average weight of 1.82 ± 0.28 kg, and ducks were separated to four groups (30 ducks per each group). The animals were fed with commercial standard diet that free from any chemotherapeutics three times per day and water *ad libitum*. Throughout the study they were housed in the animal cages at Division of Experimental animal, Faculty of Veterinary Medicine, Kasetsart University.

Experimental design

The experiment was examined for AMX in which the determination of fundamental pharmacokinetic parameters was studied. The animals were taken for intravenous, intramuscular, subcutaneous and administration. On each occasion 2.5 ml blood samples were collected randomly, using heparinized syringes, following a single dose of AMX from brachial vein at 0, 15, 30 min and 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24 h after administration. Blood samples were separated by a centrifugation (1000 x g) for 15 min to collect the plasma, which stored at –20°C until analysis. All of plasma samples were analyzed for AMX after storage within 1 month.

Method of analysis

The concentration of AMX was analyzed by a microbiological diffusion method (Anhalt 1985, Limpoka 1992), using *Micrococcus luteus* ATCC 9341 as test organisms which was purchased from Scientific and Technology institute of Thailand. Standard dose-response curves were obtained using buffered AMX solutions. The sensitivity of detection of AMX was 0.025 μg/ml of standard preparation.

For AMX plasma determination, briefly, the bacteria were kept in trypticase soy broth (oxoid) and thawed just before use. Antibiotic medium (Muller Hinton medium, pH 6.0) was adjusted by 0.1 NHCl, it was steriled for 20 minutes at 121°C. The motten agar was inoculated with *Micrococcus luteus* ATCC 9341 in broth. The medium was poured into 10 × 15 cm glass plates. Each glass plate contained 32 ml of inoculated edium hardened for 30 minutes in the refrigerator being punched out. Then 10 mm wells were punched out from agar allowing 8 holes per plate and filled 2 holes of each glass plate with the standard AMX solutions at 0.025 and 0.1 μg/ml. The remaining 6 holes were examined by plasma samples. The samples were allowed to diffuse for 45 min at room temperature prior to incubation. Finally, the glass plates were incubated for 24 h at 37°C, thereafter, the inhibition zones of standard preparations and samples were measured using caliper vernia and the concentrations were recorded from plots of log concentration plus zone diameter of plasma.

Calculation of pharmacokinetic parameters

The pharmacokinetic characteristics of

plasma concentration time profile for AMX i.v. dosing which evaluated by modified standard technique. These data were calculated for each animal by two-compartment pharmacokinetic model based on the criteria of improvement in the sum square by plot of residuals (Baggot, 1977 and Limpoka, 1992). The following pharmacokinetic parameters were obtained according to the equation previously described by Baggot (1977), Limpoka (1992) and Craigmill *et al*. (1994).

The term of Cp^0 is the extrapolated plasma concentration time curve at zero-time of the first part of the curve was also determined. A was determined by the residual method of the plasma concentration vs time curve (O' Flaherty, 1981), and B was extraolated from the elimination phase (β–slope). The α and β are hybrid rate constants describing the initial and terminal decline in plasma concentration and are composed of the microrate constants ($K_{12,}$ K_{21}) of the model (Gibaldi and Perier 1982). The $t_{1/2}\alpha$ (distribution half-life), $t_{1/2}\beta$ (elimination half-life), AUC (area under the curve), $V_{d(area)}$ (apparent volume of distribution during the post-distribution phase), V_c (volume of central compartment), F (bioavailability) and Cl_B (total body clearance) were determined using the following equations.

$t_{1/2}\alpha = \ln 2/\alpha$

$t_{1/2}\beta = \ln 2/\beta$

$K_{21} = A(\beta) + B(\alpha)/ A+B$

$K_{el} = (\alpha)(\beta)/ K_{21}$

$K_{12} = \alpha+\beta- K_{21} - K_{el}$

$V_c = Dose/C_p^o$

$V_{d(area)} = Dose/(AUC)(\beta)$

$AUC = (A/\alpha) + (B/\beta)$

$F = AUC_{other} / AUC_{i.v.}$

$Cl_B = (K_{el})(V_c)$

These equations were used to obtain pharmacokinetic parameters for two-compartment model.

Statistic analysis

The mean ± SD of pharmacokinetic parameters were calculated. The significant differences of the mean plasma concentration among amoxycillin, ampicillin and penicillin-G in ducks after oral administration were performed by the Student's *t* – test or Welch's *t*-test.

RESULTS

The mean ± SD pharmacokinetic parameters of AMX were determined by a two-compartment pharmacokinetic model after i.v. administration in ducks were shown in Table 1. The mean plasma concentration-time curve of AMX was depicted using best-fit lines in Figure 1.

Mean plasma concentrations of AMX considerably different at various routes and times (Table 2). AMX plasma concentration following i.m. and s.c. administration increased rapidly at 30 min but it peaked at 1 h after p.o. administration. Differences in peak plasma concentrations after p.o. administration were found among amoxycillin, ampicillin and penicillin-G (Poapolathep *et al*., 2001a and 2001b). It showed that AMX peak plasma concentration was the highest, however, the peak plasma level of ampicillin was higher than penicillin-G after p.o. administration. (Table 3 and Figure 2). Hence, these levels were also higher than the therapeutic level (Brander, 1991; Limpoka, 1992).

DISCUSSION

The fate, disposition and bioavailability of amoxycillin were studied following a single intravenous, intramuscular, subcutaneous and oral administration to ducks. The obtained pharmacokinetic data were compared with those after i.v. administration and i.m., s.c., and p.o. fraction absorbed at the dose level 20 mg/kg body weight. In this present study, the comparison of the peak plasma concentration among amoxycillin, ampicillin and penicillin-G (Poapolathep *et al*., 2001a and 2001b) after oral administration in ducks

Table 1 Pharmacokinetic data (mean ± SD) for amoxycillin (AMX) determined following intravenous administration at a single dose of 20 mg/kg in ducks.

Pharmacokinetic parameters (units)	AMX
Cp^o (μg/ml)	36.0 ± 4.75
A (μg/ml)	24.5 ± 3.50
α (h^{-1})	11.0 ± 3.54
B (μg/ml)	24.5 ± 4.80
β (h^{-1})	1.65 ± 0.14
$t_{1/2}\alpha$ (min)	6.3 ± 1.40
$t_{1/2}\beta$ (min)	42.0 ± 3.50
K_{12} (h^{-1})	3.45 ± 1.10
K_{21} (h^{-1})	6.33 ± 1.39
K_{el} (h^{-1})	2.87 ± 0.31
V_c (l/Kg)	0.56 ± 0.09
$V_{d(area)}$ (l/Kg)	0.75 ± 0.21
Cl_B (l/Kg/h)	1.59 ± 0.52
$Bioavailability_{i.m.}$ (%)	93.33 ± 5.23
$Bioavailability_{s.c.}$ (%)	91.11 ± 7.41
$Bioavailability_{p.o.}$ (%)	34.67 ± 5.06

Note : Pharmacokinetic parameters of amoxycillin were determined by a two-compartment pharmacokinetic model.

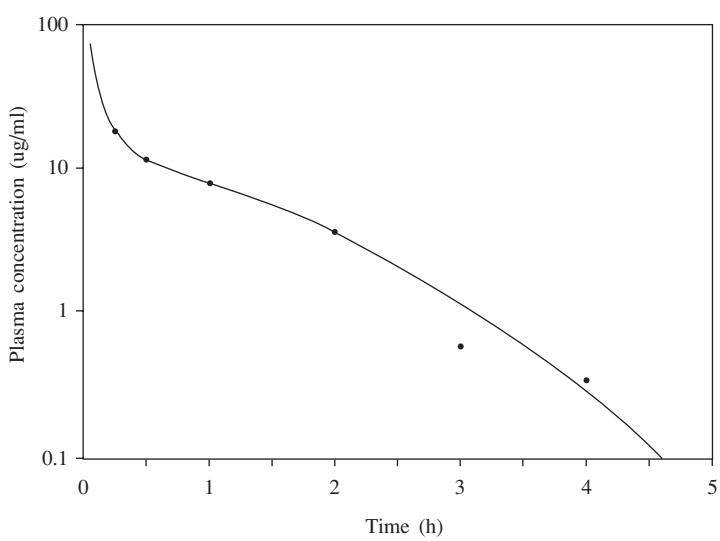

Figure 1 Semilogarithmic plot of mean amoxycillin (AMX) plasma concentration-time profile following a single i.v. administration of 20 mg/kg b.w. in ducks.

Table 2 Mean ± SD plasma concentrations in ducks administered with a single dose of amoxycillin trihydrate i.v., i.m., s.c. and p.o. at a dose of 20 mg/kg b.w.

Hours after administration	Plasma concentrations (ug/ml)			
	i.v.	i.m.	s.c.	p.o.
15 (min)	17.98 ± 2.01	8.49 ± 1.05	6.72 ± 1.38	1.20 ± 0.56
30 (min)	11.42 ± 1.80	14.32 ± 1.69	10.64 ± 2.02	1.70 ± 0.59
1	7.80 ± 1.12	8.50 ± 0.61	9.50 ± 1.10	4.29 ± 1.71
2	3.74 ± 1.95	6.47 ± 0.82	7.09 ± 1.21	1.35 ± 0.46
3	0.59 ± 0.16	2.33 ± 0.82	4.22 ± 0.49	0.79 ± 0.18
4	0.35 ± 0.17	0.23 ± 0.08	3.27 ± 0.74	0.46 ± 0.11
5	0.06 ± 0.02	0.09 ± 0.04	1.49 ± 0.33	0.25 ± 0.08
6	NM	NM	0.68 ± 0.15	0.13 ± 0.05
8	NM	NM	0.13 ± 0.04	NM
10	NM	NM	0.05 ± 0.02	NM

Note : NM = concentrations below measurable levels.

Table 3 Comparative (mean ± SD) plasma concentration-time profiles of penicillin-G (Pen-G), ampicillin (APC) and amoxycillin (AMX) following single oral administration of 20 mg/kg b.w. in ducks.

Hours after administration	Plasma concentrations (Mean ± SD)		
	Amoxycillin	Ampicillin[a]	Penicillin-G[b]
15 (min)	1.20 ± 0.56	0.95 ± 0.33*	1.38 ± 0.21*
30 (min)	1.70 ± 0.59	1.40 ± 0.25*	1.61 ± 0.20
1	4.29 ± 1.71	2.52 ± 0.54**	1.11 ± 0.23**
2	1.35 ± 0.46	1.30 ± 0.24	0.22 ± 0.07**
3	0.79 ± 0.18	0.66 ± 0.24*	0.14 ± 0.05**
4	0.46 ± 0.11	0.21 ± 0.08**	0.09 ± 0.05**
5	0.25 ± 0.08	0.07 ± 0.02**	NM
6	0.13 ± 0.05	NM	NM

Note : NM = concentrations below measurable levels.

[a, b] = data from Poapolathep *et al*., 2001a and 2001b, respectively.

*, $p<0.05$; ** $p<0.01$: Significant different from AMX.

were crucial to develop a fundamental data on the disposition of these drugs. Now, the further study on the pharmacokinetic of the other antibiotics in ducks is in progress to test if different species respond to drugs differently, especially in chicken. Because Veterinarians currently suggest the similar dose rate in which depended on chicken, in order to resolve the problems of using antimicrobial drugs in ducks. Therefore the therapeutic regimes of antibiotic should be examined in ducks.

In present study, the mean ± SD bioavailability of amoxycillin in ducks was found to be93.33 ± 5.23 % after i.m. administration, therefore, it is likely that the dose of amoxycillin

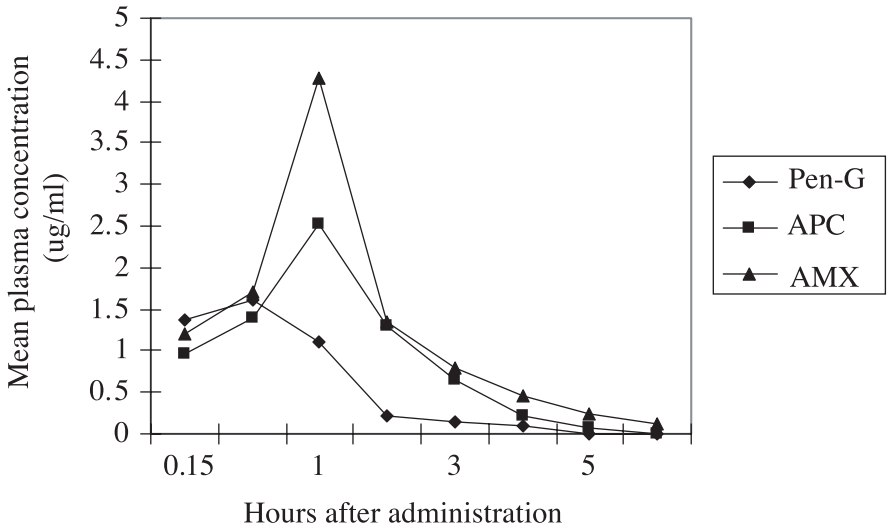

Figure 2 Comparative mean plasma concentration-time profiles of penicillin-G (Pen-G), ampicillin (APC) and amoxycillin (AMX) following single oral (p.o.) administration of 20 mg/kg b.w. in ducks.

was almost completely absorbed. The i.m. bioavailability value was also higher than from s.c. (91.11 ± 7.41 %) and p.o. (34.67 ± 5.06 %) bioavailability. Moreover, the drug was detected in plasma up to 10 h after s.c. administration while it was observed up to 5 h after i.v. and i.m. administration and up to 6 h was found after p.o. administration. In addition, the maximum plasma concentrations (C_{max}) were approximately 14.32 ± 1.69 μg/ml at 15 min after i.m. administration and 10.64 ± 2.02 μg/ml at 30 min and 4.29 ± 1.71μg/ml at 1 h after s.c. and p.o. administration, respectively. These results indicated that amoxycillin is the best absorbed after i.m. administration. Besides, the pharmacokinetic parameters could be determined after i.v. administration in order to compare with the other species and broad spectrum penicillins in ducks. The results of mean pharmacokinetic parameters after i.v. administration were as follows: the elimination half-life ($t_{1/2}\beta$) = 42.0 ± 3.50 min; the total body clearance (Cl_B) = 1.59 ± 0.52 l/kg/h; the elimination rate constants (K_{el}) = 2.87 ± 0.31 h^{-1}; the apparent volume of distribution ($V_{d(area)}$) = 0.75 ± 0.21 l/kg however, the pharmacokinetic parameters after i.v. administration were in line well with the estimates in other studies of antibiotic in animals (Limpoka, 1992). Following p.o. administration, the peak plasma concentration differed among amoxycillin, ampicillin and penicillin-G. The significant differences ($p<0.05$) were found that, the peak plasma concentration of penicillin-G (30 min) was reached more rapidly than ampicillin and amoxycillin at 1 h after oral administration but the peak plasma values of amoxycillin was the highest (4.29 ± 1.71 μg/ml) and higher than both of the ampicillin (2.52 ± 0.54 μg/ml) and penicillin-G (1.61 ± 0.20 μg/ml). It indicated that amoxycillin is more efficiently absorbed ($p<0.01$) than ampicillin and penicillin-G after oral administration. Furthermore, the amoxycillin was detected in plasma up to 6 h but ampicillin and penicillin-G were observed in plasma up to 5 h and 4 h after p.o. administration, respectively. Therefore, these data were also related to estimates of broad spectrum penicillin in the other species (Limpoka, 1992, 1997).

In conclusion, following i.m., s.c. and p.o. administration, drug plasma concentration-time profiles appeared to follow the pattern similar to that expected for intravenous administration. The biphasic nature of plasma concentration-time curve suggested that a two-compartment pharmacokinetic model would provide an accurate description of pharmacokinetic behaviors. At present, we needed to further elucidate in oral dosing because these drugs always are used in animals by oral administration. However, amoxycillin, ampicillin and penicillin-G in plasma after oral administration were higher than the therapeutic level (Brander 1991; Limpoka, 1992). Finally, for amoxycillin treatment in ducks, an identical dose rate of 20 mg/kg b.w. would be suggested for each routes. Although, the peak plasma concentration of amoxycillin was the most effective and maintained in the plasma longer than ampicillin and penicillin-G., the metabolites of amoxycillin sholud be confirmed by HPLC. For the elimination half-life value of amoxycillin, it revealed that 98% of ingested drug was excreted from the blood circulation within 4.30 h in ducks. However, the tissue residues should be further investigated in order to get insight into the tissue uptake as well as to account the withdrawal times of AMX in ducks.

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