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Pharmacokinetics and Withdrawal Times of Ampicillin in Ducks

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ABSTRACT

Pharmacokinetics of ampicillin (APC) was investigated in ducks after drug administration at a single dose rate of 20 mg/kg body weight by intravenous (i.v.), intramuscular (i.m.), subcutaneous (s.c.) and oral (p.o.) routes. A two-compartment pharmacokinetic model was developed to describe the fate and disposition of this drug. Mean peak plasma concentrations of APC was 10.52 ± 2.06, 12.21 ± 0.66, 5.62 ± 0.95 and 1.61 ± 0.20 microgram/ml after i.v., i.m., s.c. and p.o. administration respectively. Ampicillin was shown to have an elimination half-life ($t_{1/2\beta}$) of 97 ± 9.55 min, while the elimination rate constant (K_{el}), the apparent volume of distribution ($V_{d(area)}$), and the total body clearance (Cl_B) were 2.09 ± 0.07 h^{-1}, 1.79 ± 0.41 L/Kg and 1.21 ± 0.19 L/Kg/h respectively. In addition, the bioavailability following different routes were 91.11 ± 7.2 % for i.m., 62.22 ± 8.03 % for s.c. and 17.78 ± 2.08 % for p.o. administration. The results suggested that when treating ducks, the pharmacokinetic behaviors of amipicillin should be considered in order to optimize the therapeutic dose, and to allow the preslaughter withdrawal time and maximum residue limits of ampicillin for duck.

Key words: pharmacokinetic, withdrawal time, antibiotic, ampicillin and duck species

INTRODUCTION

Ampicillin (APC) is broad spectrum penicillin derivative that is a one widely used for treating urinary, respiratory, skin and gastrointestinal bacterial infections in animals. It is 4-8 times more active against gram-negative bacteria and 50 times more resistant to gastric pH than penicillin-G, but is sensitive to Beta-lactamase (Campoli-Richards and Brogden, 1987). On the other hand, several factors affect the fate of drugs in animals (Gibson and Skett, 1994). These include species differences (Walker, 1980). Moreover, at present time there are insufficient pharmacokinetic data for clinical use of ampicillin in ducks. (Limpoka, 1992). The revival of interest in APC has led to many investigations elucidating the disposition of the drug in various animal species (Groothuis *et al*., 1978; Traver and Brown *et al*., 1991; Tufenkji *et al*., 1991).

The objective of the present study was initiated to investigate the fundamental pharmacokinetic data APC on ducks following intravenous (i.v.), intramuscular (i.m.), subcutaneous (s.c.) and oral (p.o.) administration, and then to determine some substantial different of some pharmacokinetic values because there are no reports of the fate and disposition data for broad spectrum penicillins in ducks, although the

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pharmacokinetics and clinical use of APC have been widely studied in other avian species (Clark, 1986; Dorrestein *et al*., 1987; Lawrence, 1988; Limpoka, 1992). In addition, Veterinarians usually currently suggest the similar dose rate of APC for chicken to use in ducks. In order to know the dosage regimen in ducks the pharmacokinetics of APC in ducks has been studied.

MATERIALS AND METHODS

Drugs

Ampicillin formulation, (lot.no.CL 5880699) was diluted to solution with sterile water before administration at an identical dose of 20 mg/kg body weight to each duck. The standard preparation was used by APC (Merck, lot K226-15278 916), calculated potency free base /mg.

Animals

The experiments were carried out on 120 healthy ducks with an average weight of 1.33 ±0.31 kg and were devided into four groups (30 ducks per each group). The animals were fed commercial standard diet that free from any chemotherapeutics three times per day and had access to water *ad libitum*. Throughout the study they were kept in the animal cages at Division of Experimental animal, Faculty of Veterinary Medicine, Kasetsart University.

Experimantal design

The experiment was performed in 120 healthy ducks for APC, in which the determination of fundamental pharmacokinetic parameters were carried out. The animals were separated in four groups for intravenous (i.v.), intramuscular (i.m.), subcutaneous (s.c.) and oral (p.o.) administration. On each occasion 2.5 mL blood samples were taken randomly using heparinized syringes following a single dose of APC from brachial vein just before and at 0.15, 0.30, 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24 h after administration. Plasma was separated by centrifugation (1000 x g) for 15 min and stored at –20°C until analysis. All of plasma samples were analyzed for APC after storage within 1 month.

Method of analysis

The concentration of APC was determined by the microbiological diffusion method (Anhalt 1985, Limpoka 1997), using *Micrococcus luteus* ATCC 9341 as test organisms that purchased from Scientific and Technology Institute of Thailand. Standard dose-response curves were obtained using buffered APC solution. The sensitivity of detection of APC was 0.05 ug/ml of standard preparation.

The bacteria were kept in trypticase soy broth (oxoid) and thaw just before use. For the assay of APC, Antibiotic medium (Muller Hinton medium) pH 6.0 was adjusted by 0.1 N. HCl. It was steriled 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, which were kept at 4°C. Each glass plate contained 32 mL of inoculated medium hardened for 30 minutes in the refrigerator before punching out of the holes. After drying the plates for 1 h at 37°C, 10 mm diameter agar wells were punched out from the agar plate allowing 8 holes per plate. Then, filled 2 holes of each glass plate with the standard APC solution at 0.025 and 0.1 ug/ml and the remaining 6 holes with the assay plasma. This standard was used 2 glass plates per plasma sample and allowed the plasma to diffuse for 45 minutes at room temperature prior to incubation. Finally, the glass plates were incubated at 37°C 24 h, 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 the data on plasma concentration time profile for APC i.v. dosing which evaluated by semilogarithm

technique (Baggot, 1977; Limpoka, 1992) using a semilog paper and fitting curves by table curve 2D program. 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. 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. B was calculated from elimination phase (β-slope). A was calculated by residual method (O' Flaherty, 1981). The a and b 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 1982a). $t_{1/2\alpha}$ (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), Bioavailability and Cl_b (Total body clearance) were determined by the following equations.

The following equations were used to obtain these pharmacokinetic parameters for two-compartment pharmacokinetic model.

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

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

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

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

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

$V_c = Dose/C_p^o$

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

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

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

Statistic analysis

The mean ± SD of pharmacokinetic parameters were calculated. Statistical analysis of data was performed using Microsoft Excel version 7.0, Window 97.

RESULTS

The mean ± SD pharmacokinetic parameters and bioavailabilities of APC were determined by the two-compartment pharmacokinetic model after i.v. administration on ducks that shows in Table 1. The mean plasma of APC concentration time profile following i.v. administration was depicted using best-fit lines in Figure 1.

Mean plasma concentration-time profile of APC in ducks was considerably different. Differences in plasma pharmacokinetic behaviors after different routes of administration were observed for APC with respect to $t_{1/2}\alpha$, $t_{1/2}\beta$, $K_{el,}$ $V_{d(area),}$ Cl_B and bioavailability following i.m., s.c. and p.o administration. Nevertheless, the peak plasma concentration of APC was found within 15 min following i.m. administration while it was detected at the peak level within 30 min and 60 min after s.c. and p.o. administration respectively. Hence, these levels are higher than the therapeutic level (Limpoka, 1992). The data of mean ± SD plasma concentration at different times after administration were shown in Table 2.

DISCUSSION

In present reports, the pharmacokinetic behaviors of ampicillin (APC) were determined on clinically healthy ducks. Each animal was administered as an intravenously (i.v.), intramuscularly (i.m.), subcutaneously (s.c.) and orally (p.o.) at a single dose rate of 20 mg/kg body weight. A two-compartment pharmacokinetic model was developed to describe the fate and disposition of ampicillin. Ampicillin was shown to have an elimination half-life ($t_{1/2b}$) of 97 ± 9.55 min, while the elimination rate constant (K_{el}), the apparent volume of distribution ($V_{d(area)}$), and the total body clearance (Cl_B) were 2.09 ± 0.07 h^{-1}, 1.79 ± 0.41 L/Kg and 1.21 ± 0.19 L/Kg/h, respectively. In addition, the bioavailabilities after i.m. administration was found at 91.11 ± 7.20 %, higher than those of the s.c.

Table 1 Pharmacokinetic data (mean ± SD) of ampicillin (APC) by intravenous administration at a single dose of 20 mg/kg in ducks.

Pharmacokinetic parameters (units)	APC
Cp° (ug/ml)	38.0 ± 6.66
A (ug/ml)	30.0 ± 3.21
α (h^{-1})	6.3 ± 0.49
B (ug/ml)	6.4 ± 2.33
β (h^{-1})	0.51 ± 0.09
$t_{1/2\alpha}$ (min)	11.00 ± 1.11
$t_{1/2\beta}$ (min)	97.0 ± 9.55
K_{12} (h^{-1})	3.19 ± 0.24
K_{21} (h^{-1})	1.52 ± 0.37
K_{el} (h^{-1})	2.09 ± 0.07
$V_{c'}$ (L/Kg)	0.53 ± 0.08
$V_{d(area)}$ (L/Kg)	1.79 ± 0.41
Cl_B (L/Kg/h)	1.21 ± 0.19
$Bioavailability_{i.m.}$ (%)	91.11 ± 7.2
$Bioavailability_{s.c.}$ (%)	62.22 ± 8.03
$Bioavailability_{p.o.}$ (%)	17.78 ± 2.08

Note : Pharmacokinetic parameters of ampicillin were determined by the two-compartment pharmacokinetic model.

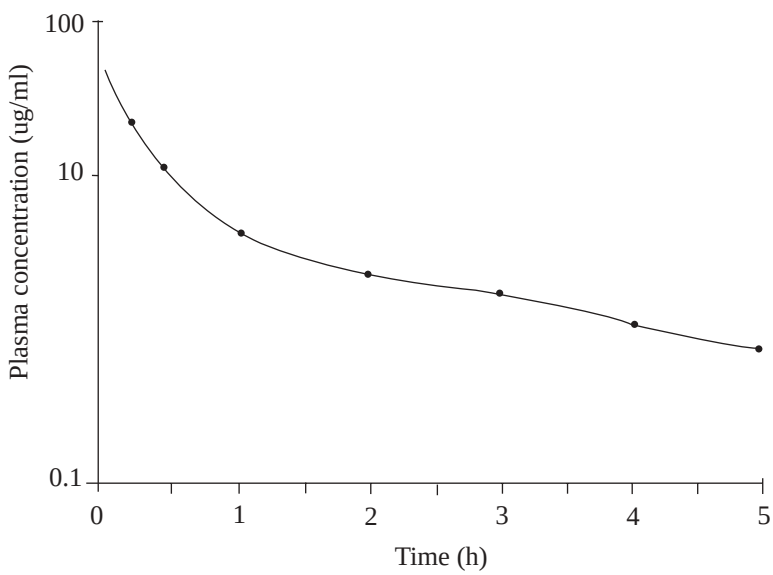

Figure 1 Semilogarithmic plot of mean ampicillin (APC) plasma concentration-time profile following single i.v. administration of 20 mg/kg b.w. in ducks.

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

Hours after administration	Plasma concentrations (Mean ± SD)			
	Intravenously	Intramuscularly	Subcutaneoasly	Orally
0.15	19.68 ± 2.21	16.58 ± 3.70	6.50 ± 2.37	0.95 ± 0.33
0.3	10.51 ± 1.00	11.89 ± 3.04	9.07 ± 2.00	1.40 ± 0.25
1	4.43 ± 1.06	6.38 ± 3.98	5.04 ± 1.10	2.52 ± 0.54
2	2.35 ± 0.91	3.98 ± 1.11	2.11 ± 0.77	1.30 ± 0.24
3	1.78 ± 0.47	1.05 ± 0.16	1.76 ± 0.62	0.66 ± 0.24
4	1.16 ± 0.26	0.83 ± 0.20	0.83 ± 0.19	0.21 ± 0.08
5	0.82 ± 0.12	0.25 ± 0.13	0.09 ± 0.03	0.07 ± 0.02
6	0.19 ± 0.18	0.07 ± 0.02	NM	NM

Note : NM = concentrations below measurable levels.

(62.22 ± 8.03 %) and p.o. (17.78 ± 2.08 %). It revealed that ampicillin was not completely absorbed after oral administration. On the other hand, the drug was the longest detected in plasma up to 6 h after i.v. and i.m. administration while it was observed up to 5 h after s.c. and p.o. administration. In addition, the maximum plasma concentrations (C_{max}) were approximately observed 19.68 ± 2.21 ug/ml after i.v and 16.58 ± 3.70 ug/ml after i.m. administration within 15 min but it was 9.07 ± 2.00 ug/ml and 2.52 ± 0.54 ug/ml within 30 min and 1 h after s.c and p.o. administration respectively. However, ampicillin in plasma after i.v., im., s.c. and p.o. administration were higher than the therapeutic level (Brander *et al*., 1991; Limpoka, 1992). Moreover, drug plasma concentration-time profiles following i.m., s.c. and p.o. administration were also 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 exactly description of pharmacokinetic behavior.

In conclusion for ampicillin treatment, a dose level of 20 mg/kg b.w. would be recommended to ducks by i.v., i.m. or s.c. but not by p.o. The metabolites of ampicillin suggested to be confirmed by HPLC.

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