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Disposition Kinetics of Amoxicillin in Healthy, Hepatopathic and Nephropathic Conditions in Chicken after Single Oral Administration

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Abstract

Fifteen broiler chickens (COBB 400) of 42 days of age weighing 1.8 to 2.0 kg were equally divided into 3 groups, each consisting of 5 birds. Hepatopathy was induced by oral administration of paracetamol while nephropathy was induced by intravenous administration of uranyl nitrate. Kinetic study was investigated in healthy, hepatopathic and nephropathic birds following single oral administration of amoxicillin at 40 mg kg^{-1}. Blood samples were collected at different time schedule. Plasma concentrations of amoxicillin in healthy, hepatopathic and nephropathic birds were 41.90 ± 5.59, 9.93 ± 0.76 and 38.75 ± 6.08 µg ml^{-1}, respectively at 1 hr; 15.34 ± 1.99, 18.57 ± 1.66 and 67.40 ± 2.62 µg ml^{-1}, respectively at 4 hr and 2.03 ± 0.28, 15.54 ± 0.82 and 30.63 ± 1.58 µg ml^{-1}, respectively at 24 hr. Maximum plasma concentration was detected at 1 hr in healthy birds (41.90 ± 5.59 µg ml^{-1}), at 8 hr in hepatopathic birds (23.51 ± 1.64 µg ml^{-1}) and at 4 hr in nephropathic birds (67.40 ± 2.62 µg ml^{-1}). The drug could not be detected in plasma beyond 24 hr in healthy, 72 hr in both hepatopathic and nephropathic birds. The concentration of amoxicillin was significantly ($P < 0.01$) higher in most of the samples of hepatopathic and nephropathic birds compared to healthy birds. Significant higher values ($P < 0.01$) of $t_{1/2}$ K, AUC, and MRT and lower values of K and Cl_B in the hepatopathic and nephropathic birds in comparison to healthy birds were observed.

Key words: Chicken, Disposition kinetics, Amoxicillin, Hepatopathic, Nephropathic.

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Introduction

Amoxicillin is penicillinase susceptible semi-synthetic penicillin. The drug has shown potent antimicrobial activity against respiratory tract infection caused by *Streptococcus pneumoniae*, *Streptococcus pyogenes*, urinary tract infections caused by *E. coli*. *Pneumococci* and *H. influenzae* are generally susceptible to amoxicillin. Amoxicillin-clavulanate is the most active agent against the broad spectrum of aerobic and anaerobic bacteria. It is rapidly and completely absorbed from the G.I. tract after oral administration in chicken.[1] Amoxicillin has been used to treat urinary tract infections as well as streptococcal infection of upper respiratory tract and skin. The kinetic behavior of many drugs altered following administration by different routes in induced diseased models of goats.[2-4] Semi-synthetic amino penicillin like amoxicillin is used in poultry to combat the disease caused by susceptible microorganisms. Although literature in respect to pharmacokinetics in healthy birds[1,5] are available, modification of pharmacokinetics during diseased condition is scarcely available. With this idea the present research work was carried out to study the oral disposition kinetics of amoxicillin in healthy birds, its modification in hepatopathic and nephropathic condition.

Materials and Methods

Experimental birds. The birds were healthy, 42 days old broiler chickens (COBB 400) of 1.8 to 2.0 kg body wt and of both sexes. The birds were examined clinically to evaluate health status. The animal room was cleaned and fumigated with potassium permanganate and commercial formaldehyde solution (1:10) for 48 hours. The cages, feeding troughs and watering troughs were cleaned with detergent and disinfected with potassium permanganate solution (5 %), 24 hr before starting the experiment. They were housed in cages and were maintained on broiler finisher ration and provided water *ad libitum.*

Drugs and chemicals. Amoxicillin in powder form (purity > 85 %) was provided kindly by M/s Wockhardt Veterinary Private Limited, Bombay, India. All the chemicals of analytical grade used in the experiment were purchased from E. Merck (India) and Sigma Chemical Company (USA).

Experimental design. Fifteen healthy birds weighing between1.8 and 2 kg were selected and divided into three groups (Group I, II, and III) of which group I was considered as control, while group II birds were made hepatopathic following oral administration of paracetamol (500 mg kg^{-1}) for 7 consecutive days[6]. Likewise birds of group III were made nephropathic following intravenous administration of uranyl nitrate (2 mg kg^{-1}) for 4 consecutive days.[6] Different biochemical estimations were carried out to assess the intensity of liver and kidney damage.[6-9] The dose level of paracetamol (500 mg kg^{-1}) and uranyl nitrate (2 mg kg^{-1}) for inducing hepatopathy and nephropathy were determined from the pilot studies[6]. A single dose of amoxicillin at 40 mg kg^{-1} was administered orally to each bird of the healthy (Group I), hepatopathic (Group II), and nephropathic birds (Group III). Amoxicillin was administered after 7 days of paracetamol administration in group II chicken and after 4 days of uranyl nitrate administration in group III chicken. The research protocol was approved by the Institutional Animal Ethics Committee.

Collection of blood. A vein flow catheter (22G) was introduced into the left wing vein of the bird and fixed with adhesive tape. Blood samples were collected through the vein flow catheter of the birds from all the three groups at 0 (pre-drug control), 0.16, 0.33, 0.5, 1, 2, 4, 8, 12, 18, 24, 48, 72 and 96 hr post administration, in heparinized test tubes, and centrifuged at 3000 rpm for 30

minutes to separate the plasma for the estimation of the drug.

Preparation of calibration curve. A stock solution (100 µg ml^{-1}) of amoxicillin in distilled water was prepared. The drug solution varying from 0.5 to 10 µg in 5 ml aliquot was mixed with 0.5 ml of plasma, 0.5 ml of 70 % perchloric acid was added and distilled water was added to make a 5 ml aliquot. Optical densities of the drug molecule of different concentrations were read at 235 nm, by a double beam UV-VIS spectrophotometer. The concentrations were then plotted against optical densities on a graph paper to obtain a standard curve. The recovery percentage of amoxicillin from the plasma was above 83 and the detection limit for amoxicillin by UV-VIS spectrophotometer was 1 µg ml^{-1}.

Estimation of the drug concentration. Plasma (0.5 ml) was mixed with 4 ml of distilled water and 0.5 ml of 70 % perchloric acid was added to precipitate plasma protein. The mixture was shaken for 5 min. and then centrifuged for 40 min. at 2500 rpm. The supernatant was collected and read at 235 nm by a double beam UV-VIS spectrophotometer. Concentrations of the drug at different time intervals were obtained from the standard curve prepared previously and expressed as µg $ml^{-1}$of the plasma.

Pharmacokinetic parameters. Some pharmacokinetic variables were determined using a computerized curve fitting software program "PHARMKIT" supplied by the Department of Pharmacology, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry, India. The other kinetic parameters were estimated following the standard formula.[10]

Statistical analysis. The pharmacokinetic parameters for each bird were determined and the mean values and standard error (SE) were calculated. Mean values, SE, analysis of variance, and independent sample t test of the tabulated data were calculated, where applicable, using the SPSS statistical software program.

Results

Mean value of plasma half life of BSP and plasma AST activity following consecutive daily oral administration of paracetamol at 500 mg kg^{-1} in birds have been presented in Table 3. The $t_{1/2}$ value for BSP clearance and plasma AST activity gradually increased along with time and maximum increase was recorded after consecutive daily oral administration of paracetamol for 7 days. Likewise, plasma urea nitrogen and creatinine level gradually increased following daily intravenous administration of uranyl nitrate at 2 mg kg^{-1} for 4 days (Table 3).

A semilogarithmic plot of the mean plasma concentrations of amoxicillin against time, in healthy, hepatopathic, and nephropathic birds after a single-dose oral administration at 40 mg kg^{-1} has been presented in Fig. 1. Maximum plasma concentration of amoxicillin (41.90 ± 5.59 µg ml^{-1}) was recorded at 1 hr (Table 1), which then followed by a gradual decline and a minimum drug concentration of 2.03 ± 0.28 µg ml^{-1} was recorded at 24 hr in healthy birds. On the other hand, C_{max} (maximum plasma concentration) of amoxicillin was recorded at 8 hr (23.51 ± 1.64 µg ml^{-1}) and C_{min} (minimum plasma concentration) of the drug was recorded at 72 hr (5.58 ± 0.34 µg ml^{-1}) in hepatopathic birds (Table 1). C_{max} of plasma amoxicillin was recorded at 4 hr (67.40 ± 2.62 µg ml^{-1}) and C_{min} of amoxicillin was recorded at 72 hr (5.34 ± 0.28 µg ml^{-1}) in nephropathic birds (Table 1). Amoxicillin could not be detected in plasma beyond 24 hr in healthy, and 72 hr in hepatopathic and nephropathic birds. The concentration of amoxicillin was significantly ($P < 0.01$) higher in most of the samples of hepatopathic and nephropathic birds compared to healthy birds.

The disposition kinetic parameters of amoxicillin in healthy, hepatopathic and

nephropathic birds following a single-dose oral administration at 40 mg kg^{-1} have been described in Table 2. Absorption rate constant (K_{abs}) of amoxicillin in healthy, hepatopathic and nephropathic chicken were 3.24 ± 0.14, 0.23 ± 0.01 and 0.90 ± 0.17 hr^{-1}, respectively. Absorption half life ($t_{1/2}$ K_{abs}) values were 0.21 ± 0.01, 3.03 ± 0.15 and 0.91 ± 0.21 hr in healthy, hepatopathic and nephropathic birds, respectively. Elimination rate constant (K) were respectively 0.11 ± 0.01, 0.02 ± 0.002 and 0.03 ± 0.002 hr^{-1} in healthy, hepatopathic and nephropathic chicken while elimination half life ($t_{1/2}$ K) values were 6.04 ± 0.13, 32.30 ± 1.35 and 19.62 ± 0.33 hr in healthy, hepatopathic and nephropathic birds, respectively. Area under curve (AUC) of amoxicillin was highest in nephropathic followed by hepatopathic and minimum was in healthy birds. On the other hand, volume of distribution (Vd_{area}) was highest in hepatopathic followed by healthy and minimum was recorded in nephropathic chickens (Table 2). Maximum retention time (MRT) values were 8.86 ± 0.19, 47.54 ± 1.64 and 27.25 ± 0.18 hr in healthy, hepatopathic and nephropathic birds, respectively.

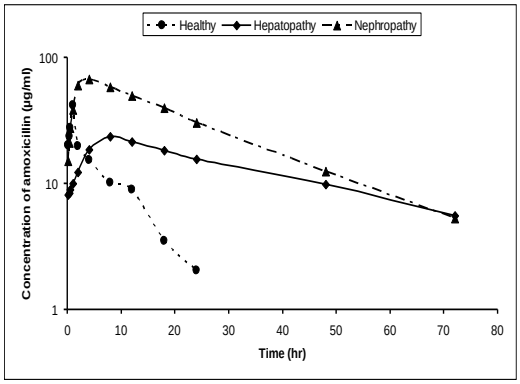

Fig 1. Semilogarithmic plot of the mean plasma concentration of amoxicillin against time in healthy, hepatopathic and nephropathic birds after a single dose oral administration of amoxicillin at 40 mg kg^{-1}

Table 1. Mean plasma concentration (µg ml^{-1}) of amoxicillin in healthy, hepatopathic and nephropathic birds after single dose oral administration at 40 mg kg^{-1}.(Mean of 5 replicates)

Time	Group I	Group II	Group III
0.16	20.20 ± 2.65	8.16 ± 0.64	15.12 ± 5.46
0.33	23.58 ± 3.07	8.42 ± 0.66	21.06 ± 5.93
0.5	27.61 ± 3.76	8.88 ± 0.66	27.75 ± 6.45
1	41.90 ± 5.59	9.93 ± 0.76	38.75 ± 6.08
2	19.75 ± 2.74	12.38 ± 0.99	59.74 ± 4.02
4	15.34 ± 1.99	18.57 ± 1.66	67.40 ± 2.62
8	10.17 ± 1.31	23.51 ± 1.64	58.45 ± 2.73
12	8.92 ± 1.07	21.40 ± 1.48	49.80 ± 2.52
18	3.48 ± 0.38	18.35 ± 1.13	39.53 ± 1.94
24	2.03 ± 0.28	15.54 ± 0.82	30.63 ± 1.58
48	BDL	9.90 ± 0.65	12.46 ± 0.71
72	BDL	5.58 ± 0.34	5.34 ± 0.28

BDL: Below detection limit.

Table 2. Mean kinetic parameters of amoxicillin following single dose oral administration at 40 mg kg^{-1} in healthy, hepatopathic and nephropathic birds. (Mean of 5 replicates with SE)

Kinetic parameters	Group I	Group II	Group III
K_{abs} (hr^{-1})	$3.24^{a} \pm 0.14$	$0.23^{b} \pm 0.01$	$0.90^{c} \pm 0.17$
K (hr^{-1})	$0.11^{a} \pm 0.01$	$0.02^{b} \pm 0.002$	$0.03^{c} \pm 0.002$
$t_{1/2}$ K_{abs} (hr)	$0.21^{a} \pm 0.01$	$3.03^{b} \pm 0.15$	$0.91^{c} \pm 0.21$
$t_{1/2}$ K (hr)	$6.04^{a} \pm 0.13$	$32.30^{b} \pm 1.35$	$19.62^{c} \pm 0.33$
AUC (µg hr ml^{-1})	$235.93^{a} \pm 29.78$	$935.98^{b} \pm 66.62$	$1878.71^{c} \pm 101.02$
MRT (hr)	$8.86^{a} \pm 0.19$	$47.54^{b} \pm 1.64$	$27.25^{c} \pm 0.18$
Cl_B (L Kg hr^{-1})	$0.10^{a} \pm 0.01$	$0.02^{b} \pm 0.002$	$0.01^{c} \pm 0.002$
Vd_{area} (L Kg^{-1})	$0.87^{a} \pm 0.12$	$0.91^{b} \pm 0.04$	$0.34^{c} \pm 0.01$
C_{max} (µg ml^{-1})	$41.90^{a} \pm 5.59$	$23.51^{b} \pm 1.64$	$67.40^{c} \pm 2.62$
C_{min} (µg ml^{-1})	$2.03^{a} \pm 0.28$	$5.58^{b} \pm 0.34$	$5.34^{b} \pm 0.28$

Values of dissimilar superscript in horizontal line vary significantly ($P < 0.05$).

K_{abs}: Absorption rate constant; **K:** Elimination rate constant; **$t_{1/2}$ K_{abs}:** Absorption half life; **$t_{1/2}$ K:** Elimination half life; **AUC:** Area under curve; **MRT:** Mean residence time; **Cl_B:** Total body clearance; **Vd_{area}:** Apparent volume of distribution; **C_{max}:** Maximum drug concentration achieved; **C_{min}:** Minimum drug concentration achieved.

Table 3. Composite table showing biochemical values in chicken before induction of hepatopathy or nephropathy (0 day) and after induction of hepatopathy (7 day) and nephropathy (4 day)

Parameters	Groups			
	Days	G1 (Control)	G2 (Hepatopathy)	G3 (Nephropathy)
AST (µg pyruvic acid/mg protein/hr)	0	$2.28^{ax} \pm 0.18$	$2.46^{ax} \pm 0.19$	NC
	7	$2.31^{ax} \pm 0.16$	$4.26^{by} \pm 0.14$	NC
BSP clearance, $t_{1/2}$ (hr)	0	$0.058^{ax} \pm 0.004$	$0.050^{ax} \pm 0.004$	NC
	7	$0.057^{ax} \pm 0.003$	$0.100^{by} \pm 0.003$	NC
BUN (mg dl^{-1})	0	$5.93^{ax} \pm 0.29$	NC	$5.18^{ax} \pm 0.24$
	4	$5.88^{ax} \pm 0.31$	NC	$11.66^{by} \pm 0.39$
Creatinine (mg dl^{-1})	0	$1.38^{ax} \pm 0.14$	NC	$1.46^{ax} \pm 0.13$
	4	$1.41^{ax} \pm 0.13$	NC	$6.22^{by} \pm 0.22$

NC: Not calculated.

Dissimilar superscript a, b, showed significant difference ($P < 0.05$) between groups and superscript x, y, showed significant difference between days in a particular parameter.

Discussion

Plasma concentration of amoxicillin at 0.16 hr in healthy birds was 20.20 µg ml^{-1}, which is much higher than that of nephropathic and hepatopathic chicken in sequence. This coupled with absorption rate constant (K_{abs}) suggests that antibiotic is poorly absorbed in hepatopathic and nephropathic condition. This is possible since it has been reported that in renal failure condition, absorption of drug through gastrointestinal tract is poor and inconsistent in human being. Beside any insult of important organs also inhibit the gastrointestinal transit period[12] and consequently the drug is poorly absorbed but the absorption period continues for longer time. This might be the plausible reasons of significantly higher absorption half life ($t_{1/2}K_{abs}$) in both hepatopathic and nephropathic condition compared to healthy birds. This also corroborated the finding of maximum plasma concentration of drug at 1 hr pd in healthy chicken, and at 8 hr and 4 hr pd in hepatopathic and nephropathic condition, respectively. Attainment of maximum plasma concentration of amoxicillin at 1 hr pd in healthy chicken also corroborated the finding of Anadon *et al.*(1996),[1] in chicken despite the differences in doses (40 mg kg^{-1} in present experiment and 10 mg kg^{-1} by Anadon *et al*. (1996)[1] Elimination rate constant (K) in healthy chicken of the present experiment was 0.11 hr^{-1}, while Anadon *et al.* (1996),[1] reported much lower (0.078 hr^{-1}), the differences might be attributed due to the administration of different doses of amoxicillin. Elimination rate (K) of amoxicillin in hepatopathic and nephropathic chickens was significantly lower than that of healthy chicken; conversely the elimination half life ($t_{1/2}K$) was significantly greater than that of the values of healthy birds. Kidney failure modulated the drug metabolism.[11]

Sooud *et al.* (2004),[5] and Anadon *et al.* (1996),[1] used the same dose of Amoxicillin in chicken and reported the elimination half life were 9.16 and 1.13hr, while in this experiment it was found to be 6.04 hr in healthy birds. Area under curve (AUC) value of healthy chicken was much smaller compared to those reported by Sooud *et al*. (2004),[5] and Anadon *et al.* (1996)[1]. AUC values of hepatopathic and nephropathic chickens have considerably increased suggesting that pathologic condition of liver and kidney altered the values. Area under curve values of healthy birds was 0.87 L Kg^{-1}, while that reported by Anadon *et al.* (1996)[1] was 0.054 L Kg^{-1}. The differences of kinetic values that have been observed in healthy birds and reported values of Sooud *et al.* (2004),[5] and Anadon *et al.* (1996)[1] might be due to difference of doses of amoxicillin administered through oral route. Hepatopathic condition slightly increased, while nephropathic condition considerably decreased the Vd_{area} values of plasma amoxicillin in this experiment. As expected total body clearance value indicates that in hepatopathic and nephropathic condition, amoxicillin is very slowly excreted from the body.

In conclusion, oral absorption of amoxicillin was slow and the process continued for long time. Pathologic condition of liver and kidney altered the kinetic values of oral amoxicillin in chicken and therefore under the condition, the dose of amoxicillin administration by oral route needs to be carefully adjusted.

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