International Journal of Pharmacology

ISSN 1811-7775

science alert

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Asian Network for Scientific Information

International Journal of Pharmacology 2 (1): 28-32, 2005
ISSN 1811-7775

The Effect of *Salmonella* infection on the Plasma Kinetics of Chloramphenicol in the Sokoto Red Goats

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Abstract: This study was to determine the effect of *salmonella typhimurium* infection on the plasma kinetic profile of chloramphenicol in the locally breed goats of Sokoto state, Nortwestern Nigeria. Chloramphenicol administered intravenously at the dose of 25 mg kg^{-1} body weight to healthy and *salmonella typhimurium* infected goats; assayed chemically and pharmacokinetic parameters assessed by use of two compartment open model has shown significant changes in the kinetic profile of the drug after the infection. The mean plasma concentrations of the drug were lower in the inoculated goats. The volume of distribution ($V^d\beta$), total body clearance (Cl) and elimination constant (β) were significantly higher while the distribution half life ($t½\alpha$), elimination half life ($t½\beta$) and the Area Under the Curve (AUC) were significantly reduced after the infection. *Salmonella typhimurium* infection has altered the distribution of chloramphenicol, increased the loss of the drug and reduced its mean residence time in the body of the goats.

Key words: *Salmonella typhimurium,* chloramphenicol, pharmacokinetic, Sokoto red goats, plasma

INTRODUCTION

Chloramphenicol is a potent curative antimicrobial drug that has gained popularity in the treatment of typhoid fever in man. Its effect is usually bacteriostatic but, at high concentrations it may be bacteriocidal. Significant blood levels were observed following oral chloramphenicol administration to horses and monogastric animals[1]. In horses less than 50% of the orally administered dose (50 mg kg^{-1}) was absorbed and produced therapeutic serum, synovial and peritoneal fluids concentrations which persisted for only a short period. There was a decrease in the amount absorbed with repetitive oral dosing[2]. There appear to be a considerable species variation in the plasma half-life of chloramphenicol in horses (1-2 h), cattle (2-3 h) and pigs (1-3 h)[3,4]. Though there have been pharmacokinetic studies of chloramphenicol in animals, we are not aware of any in red Sokoto goats, especially those infected with *Salmonella typhimurium* organisms.

The objective of the present study was to compare the kinetic profile of chloramphenicol in healthy and *Salmonella typhimurium* infected goats following a single intravenous administration of the drug.

MATERIALS AND METHODS

Animals and treatment: Five goats of both sexes weighing between 15 to 20 kg were used. The animals were purchased from the Sokoto cattle market and were kept under constant observation for a fortnight before the commencement of the experiments in order to acclimatise them to the new environment. The animals were also examined clinically for the possibility of any disease condition during this period. They were housed in clean goat pens with concrete floors and were maintained on hay and concentrate. Water was made available *ad libitum*. The same goats were used for both aspects of the study with an interval of one month between the healthy and diseased conditions.

The *Salmonella typhimurium* used was obtained from the Microbiology Laboratory of the College of Health Sciences, Usmanu Danfodiyo University, Sokoto. The goats were inoculated with *Salmonella typhimurium*, intragastrically using a stomach tube, rectal temperatures were recorded intermittently and supplementary blood samples collected for differential white blood cell count. Chloramphenicol was administered to the infected goats after infection was established. Chloramphenicol was injected intravenously at the dose of 25 mg kg^{-1} body weight through the left jugular vein

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and the blood samples were collected from the right jugular vein.

Blood samples (6 mL) were collected by jugular venipuncture into the test tubes containing disodium EDTA as anticoagulant. Plasma was separated immediately after centrifugation at 1,500 rpm for 15 min and stored at -20°C until analysed. The timings of blood sample collection include 0, 0.08, 0.25, 0.5, 1.0, 2.0, 3.0, 6.0, 9.0, 12.0, 24.0, 48.0 and 72.0 h.

Inoculation of *Salmonella typhimurium:* The method of Agerso *et al.*[5] was used for the culture of the organism. *Salmonella typhimurium* used was prepared from a stock culture obtained from Microbiology Laboratory, College of Health Science, Usmanu Danfodiyo University. The organism was then sub-cultured on calf-blood agar for 24 h at 37°C. One colony was thereafter obtained and inoculated into a 10 mL aliquot of veal infusion broth (VB) and incubated at 37°C for 16 h without shaking. From the broth culture, 100 µL aliquots were inoculated into twelve 100 mL vials of prewarmed veal infusion broth and incubated for 8 h at 37°C without shaking. The twelve broth cultures were thereafter pooled and centrifuged at 200xg for 10 min at room temperature. The pellet obtained was resuspended in normal saline (0.9% NaCl) solution and viable counts of the organisms in the suspension determined. The suspension was further diluted using a standard dilution technique and each goat received 20 mL of this suspension in 100 mL of 10% $NaHCO_3$ containing $6x10^{10}$ colony forming units of the organisms intragastrically with the help of a stomach tube.

Chloramphenicol determination from the plasma: The method of Kakemi *et al.*[6] as modified by Hughes and Diamond[7] and Watson[8] was used for the determination of chloramphenicol in the plasma. The optical density of the resulting colour was measured at 430 nm using a spectrophotometer. Bilirubin interference with chloramphenicol assay was investigated using zero-hour and peak plasma concentration samples[9].

Calculation of pharmacokinetic constants: The pharmacokinetic parameters and constants were determined using standard methods[10]. The following parameters and constants were obtained: the half-life ($t_{½}$), concentration at zero-time (Cp°), distribution rate constant (α), elimination rate constant (β), zero-time intercepts (A and B). These constants were evaluated using graphic techniques, in which least square regression lines were calculated for the terminal (elimination) and the feathered (distribution) portions of the log plasma drug concentration time curves as outlined by Baggot[10].

The results were expressed as mean±SD Tests for significance between mean parameters in respect of healthy and *Salmonella typhimurium* infected goats were performed using students t-test for paired comparisons and the null hypothesis was rejected at $p<0.05$.

RESULTS

Inoculation with *Salmonella typhimurium* elicited a significant febrile response within 6 h in the goats. The inoculated goats became anorectic with significant increase in white blood cell count (about 75%) during the following twenty-four hours.

The inoculation with *S. typhimurium* to goats resulted in decreased peak mean plasma concentration of chloramphenicol (20.28±1.37 µg mL^{-1}) at 0.08 hours following drug treatment when compared to the concentration obtained at the same time period in the healthy goats (25.21±1.28 µg mL^{-1}) (Table 1).

The plasma levels of the drug thereafter showed a gradual decline in both the healthy and infected animals until a minimum levels of 0.48±0.07 and 0.64 ± 0.2 µg mL^{-1} were obtained at the 6 h post-treatment, respectively. The disposition kinetics of chloramphenicol after intravenous administration to *S. typhimurium* infected and healthy goats could best be described by a two-compartment model with first-order input and output (Fig. 1 and 2).

Table 2 shows the mean pharmacokinetic parameters. The character of the bi-exponential curve might best be described by:

$Cp^t = 28.1e^{-6.18}t + 6.2e^{-0.27t}$ (in healthy goats)
$Cp^t = 19.2e^{-6.30t} + 6.8e^{-0.50t}$ (in *S. typhimurium* infected goats)

where, Cp^t is the initial concentration in plasma (µg mL^{-1}) and (t) is the time (h). The plasma concentration of the drug at zero hour was calculated to be 34.30±4.52 and 26.0±2.44 µg mL^{-1} in the healthy and infected goats respectively and were found to be significantly different ($p<0.05$). The volume of distribution ($V^d\beta$), distribution rate constant (α) and elimination rate constant (β) were 3.01±0.24 L kg^{-1}, 6.18±0.21 h^{-1} and 0.27±0.06 h^{-1}, respectively in the healthy goats. For the infected goats, the calculated values were, 3.39±0.20 L kg^{-1}, 6.30±0.46 h^{-1} and 0.50±0.08 h^{-1}, respectively for the volume of distribution ($V^d\beta$), distribution rate constant (α) and elimination rate constant, respectively.

While the distribution half-life ($t_{½}\alpha$) was slightly smaller (0.113±0.05 h) in the infected goats than in the healthy goats (0.162±0.04 h); elimination half-life ($t_{½}\beta$) significantly, decreased. The half-life of elimination of

Table 1: Mean plasma chloramphenicol concentrations of healthy and *Salmonella* infected goats following a single intravenous dose of the drug (25 mg kg^{-1})

	Concentrations (μg mL^{-1})	
Time (h)*	Healthy goats**	*Salmonella* infected goats**
0.00	0.00±0.00	0.00±0.00
0.08	25.21±1.20	20.28±1.37
0.25	13.35±1.00	11.55±1.02
0.50	6.09±0.72	7.39±0.85
1.0	4.23±0.09	5.06±0.89
2.0	3.23±0.31	3.12±0.77
3.0	1.44±0.08	1.53±0.42
6.0	0.48±0.07	0.64±0.20
9.0	0.00±0.00	0.00±0.00

*Chloramphenicol was injected 15 min after collection of control sample
**Mean±SD based on 5 observations

Table 2: Pharmacokinetic parameters for chloramphenicol in healthy and *Salmonella typhimurium* infected goats following a single intravenous dose (25 mg kg^{-1}) of the dmg

Parameters	Healthy goats (n = 5)[a]	*Salmonella typhimurium* infected goats (n = 5)[a]
A (mg mL^{-1})	28.100±2.61	19.200±2.69[b]
B (mg mL^{-1})	6.200±0.78	6.800.±0.54
C_{po} (mg mL^{-1})	34.300±4.52	26.000±2.44[b]
AUC (h/mg/mL)	27.890±3.15	16.680±1.52[b]
CL (L/kg/h)	0.896±0.09	1.498±0.05[b]
V^{d}_{β} (L kg^{-1})	3.010±0.24	3.390±0.20[b]
$T_{½}(\beta)$ (h)	3.100±0.55	1.450±0.61[b]
$T_{½}(\alpha)$ (h)	0.162±0.04	0.113±0.05
K_{21} (h^{-1})	1.370±0.50	2.020±0.43
K_{12} (h^{-1})	4.040±0.91	3.220±0.61[b]
K_{el} (h^{-1})	1.230±0.33	1.560±0.21
Vc (L kg^{-1})	0.729±0.04	0.964±0.06[b]
α (h^{-1})	6.180±0.21	6.300±0.46
β (h^{-1})	0.270±0.06	0.500±0.08[b]

[a]: Data represents mean±SD
[b]: Data for *Salmonella* infected goats significantly different from those of healthy goats at $p<0.05$ using student's t-test

3.10±0.55 h in the healthy goats and 1.45±0.61 h in the infected animals (Table 2) were obtained.

The total body clearance (Cl) of 0.896±0.09 L/kg/h obtained in the healthy goats was lower than the total body clearance of 1.49±0.05 L/kg/h in the *Salmonella* infected goats.

DISCUSSION

The results presented in this study indicate that peak concentrations of 25.21±1.20 and 20.28±1.37 μg mL^{-1} were attained in healthy and *S. typhimurium* infected goats, respectively at 0.08 h (5 min) after dosing. These peak levels were lower than those obtained in healthy (80 μg mL^{-1}) and *Pasteurella haemolytica* infected (54 μg mL^{-1}) calves injected intravenously with chloramphenicol at 25 mg kg^{-1} body weight[11]. The peak concentration of 19.48 μg mL^{-1} in healthy dogs obtained by Watson[8] after administration of 20 mg kg^{-1} dose intravenously was lower than that of healthy goats as recorded in the present study. This is however, similar to

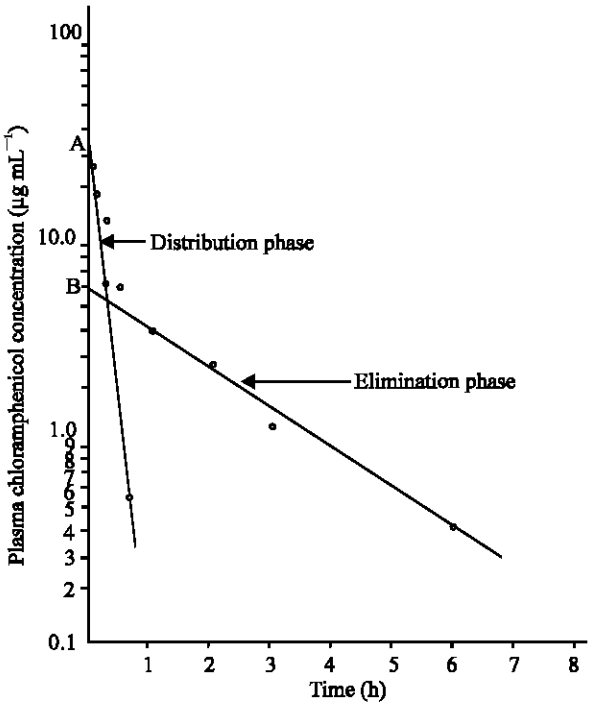

Fig. 1: Semilogrithmic plot of chloramphenicol disappearance in plasma versus time after administration of a single dose of chloramphenicol (25 mg kg^{-1}) intravenously to healthy goats

the peak concentration obtained in the *S. typhimurium* infected goats.

The lower plasma concentration of chloramphenicol observed in the infected goats may be ascribed to inflammation induced by *S. Typhimurium* infection. Inflammation is one of the most common and visible responses of vertebrate body to infections[12]. Inflammation is known to cause a loss of functional integrity of the tissue musculature thus allowing easy passage of water, electrolytes and macromolecules. Lower plasma concentration of chloramphenicol following infection in veal calves has been reported by Groothuis *et al.*[13] and Babale[14] in milking goats. Similar decline in plasma levels has been reported for gentamycin sulphate in febrile dogs[15]. However, Agerso *et al.*[5] reported that the peak plasma concentration of amoxicillin remained unchanged in *Salmonella typhiimurium* infected pigs in-spite of the observed significant increase in absorption of the drug from injection site. Significantly higher levels of amikacin in the plasma of febrile goats were observed by Agrawal *et al.*[16] after intramuscular administration of the drug. The contrast in the last two reported studies to the present one may be attributed to the differences in the type of drug and the route of

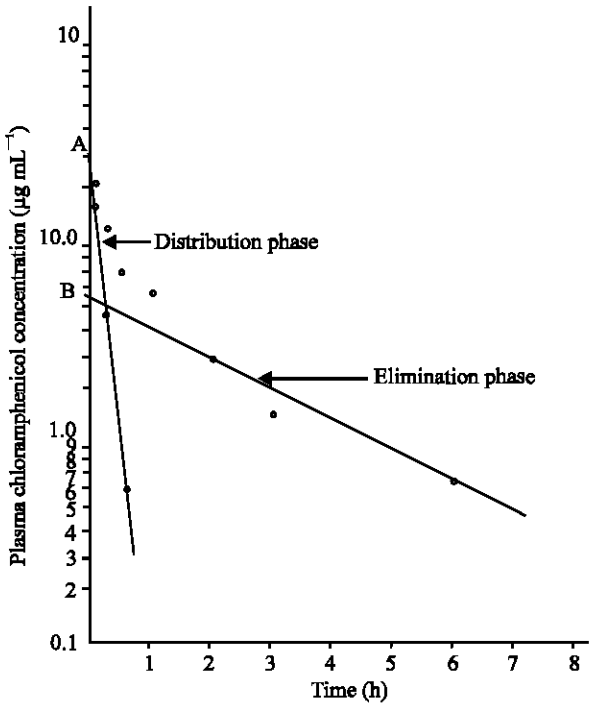

Fig. 1: Semilogrithmic plot of chloramphenicol disappearance in plasma versus time after administration of a single dose of chloramphenicol (25 mg kg^{-1}) intravenously to salmonella infected goats

administration used. Variations among species, breeds, age and analytical methods are known to have contributed to the differences in kinetic parameter reported by various workers[17].

The result of the pharmacokinetic studies (Fig. 1 and 2) indicated that chloramphenicol is eliminated from the plasma of goats in a biphasic process whether they were infected with *S. typhimurium* or not. A similar depletion pattern was observed in other healthy species of animals[8,11,18].

The kinetic disposition of chloramphenicol in goats was altered significantly by the presence of bacterial infection due to *Salmonella typhimurium*. The plasma concentration of chloramphenicol in the infected goats at zero time (Cp°) was significantly lower than that in healthy animals. This decrease may be attributable to a decrease in [A], the distribution phase intercept. The decrease in A parameter could most likely be due to the lowered plasma chloramphenicol concentration in the infected animals due to fever and inflammation as observed in this study. The infected animals showed increased rectal temperature and white blood cells count. The lower plasma concentration of the drug could be related to the significant ($p<0.05$) increase in V_c, the volume of central compartment observed in the present study (Table 2). Circulatory changes following fever could be accounted for by the increase in volume of central compartment[13].

The possible explanation to decreased elimination half-life and increased total body clearance in *S. typhimurium* infected goats when compared to healthy goats may be circulatory changes, such as increased heart rate and cardiac output and cutaneous peripheral vaconstriction. Fever resulting from bacterial infection could increase the blood flow to the liver and kidney. This will in turn increase the rate at which the drug is delivered to both organs which are important sites of the drug excretion from the blood plasma. Fever and inflammation which accompany bacterial infections could cause vasodilation and increased cell membrane permeability[19], with the loss of plasma components into the extravascular spaces. This in turn will decrease the effective drug plasma concentration. This may be responsible for the increased chloramphenicol volume of distribution noticed in the infected goats compared to the healthy animals and the lower distribution half-life in the infected (0.113±0.05 h) versus the healthy (0.162±0.04 h) goats. Significant alterations in pharmacokinetic parameters and dosage regimen of minocycline and oxytetracycline[20], sulphadiamidine[21] and cefazolin[22] have been reported in febrile animals. This study has shown that, *Salmonella* infection in goats can reduce the availability and mean residence time of the drug in plasma.

REFERENCES

1. Sisodia, C.S., V.S. Gupta, R.E. Dunlop and D.M. Radostits, 1973. Chloramphenicol concentration in blood and milk of cow following parental administration. Can. Vet. J., 9: 217-220.
2. Gromwall, R., M.P. Brown, A.M. Merritt and H.W. Stone, 1986. Body fluids concentration and pharmacokinetics of chloramphenicol given to mares intravenously or by repeated gavage. Am. J. Vet. Res., 47: 2591-2595.
3. Davis, E.L., J.D. Baggot and T.E. Powers, 1972. Pharmacokinetics of chloramphenicol in domesticated animals. Am. J. Vet. Res., 33: 2259-2265.
4. Pilloud, M., 1973. Pharmacokinetics, plasma protein binding and dosage of chloramphenicol in cattle and horses. Res. Vet. Sci., 15: 231-238.
5. Agerso, H., C. Friis and J.P. Nielsen, 2000. Pharmacokinetics and tissue distribution of amoxicillin in healthy and *Salmonella typhimurium*-inoculated pigs. Am. J. Vet. Res., 61: 992-996.

6. Kakemi, K., T. Arita and S. Ohashi, 1962. Absorption and excretion of drugs IV. Determination of chloramphenicol in blood. J. Pharm. Soc. Japan, 82: 342-347.
7. Hughes, D.W. and L.K. Diamond, 1964. Chloramphenicol in blood, simple chemical estimations in patients receiving multiple antibiotics. Antibiot. Sci., 114: 296-297.
8. Watson, A.D.J., 1979. Plasma chloramphenicol concentration in cats after parental administration of chloramphenicol sodium succinate. J. Vet. Pharmacol. Ther., 2: 123-127.
9. Mason, O.E., S.L. Kaplan, C.J. Baker and D.A. Ritter, 1979. Modification of the coloremetric assay for chloramphenicol in the presence of bilirubin. Antimicrob. Agents Chemother, 15: 544-546.
10. Baggot, J.D., 1977. Principles of Drug Disposition in Domestic Animals: The Basis of Veterinary Clinical Pharmacology. 1st Edn., W.B. Saunders Company. Philadelphia.
11. Burrows, G.E., P.B. Barto and B.R. Weeks, 1986. Chloramphenicol, Lincomycin and oxytetracycline disposition in calves with experimental pneumonic pasteurellosis. J. Vet. Pharmacol. Therap., 9: 213-222.
12. Ward, P.A., 1970. Neutrophils chemotactic factors and related clinical disorders. Arthritis Rheum., 13: 181.
13. Groothuis, D.G., H.V. Gogh and A.S. van Miert, 1979. The effect of *Echrichia coli* endotoxin induced fever on the blood levels of antimicrobial drugs after intravenous and intramuscular administration in veal calves. Trend in Vet. Pharmacol. Toxicity, 6: 77-86.
14. Babale, B.K., 1985. Effects of fever on plasma levels and pharmacokinetics of chloramphenicol in goats. In: Desertification submitted to Tuskegee Institute, Alabama, USA.
15. Pennington. J.E., D.C. Dale, H.Y. Reynolds and J.D. Maclowery, 1975. Gentamycin sulfate pharmacokinetics: Lower levels of gentamycin blood during fever. J. Infect. Dis., 132: 270-275.
16. Agrawal, A.K., SD Singh and C. Jayaehandran, 2001. Effect of fever on pharmacokinetics and dosage regimen of intramuscularly administered amikacin in goats. J. Vet. Sci., 2: 91-96.
17. Jayachandran, C., M.K. Singh and N.C. Banerjee, 1990. Pharmacokinetics and distribution of ampicillin in plasma, milk and uterine fluid of female buffaloes. Vet. Res. Commun., 14: 47-51.
18. Anadon, A., P. Bringas, M.R. Martinez-Larranage and M.J. Diaz, 1994. Bioavailability, pharmacokinetics and residues of chloramphenicol in the chicken. J. Vet. Pharmacol. Therap., 17: 66-69.
19. Trenholme, G.M., R.L. Williams, K.H. Riechmann, H. Frishcher and P.E. Carson, 1976. Quidine disposition during malaria and during induced fever. Clin. Pharmacol. Ther., 19: 459-467.
20. Huber, W.G., 1988. Tetracyclines. Veterinary Pharmacology and Therapeutics. (Eds.), Booth, N.H. and L.E. McDonald, (AMSC, Iowa, USA, Iowa State University Press), pp: 813-821.
21. Sande, G.H. and J.S. Mandel, 1980. Committee on the use of antibiotics in animal husbandry and veterinary medicine. J. Environ. Contamination, 22: 15-20.
22. Stowe, M., D. Terhune and P. Wilmore, 1980. A survey of use and misuse of tetracyclines. USA. Ani. health Meeting, 81: 331-338.