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Effect of *Salmonella thyphymurium* Infection on the Pharmacokinetics of Ceftriaxone in Sokoto Red Goats

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ABSTRACT: This study was designed to investigate the effect of *Salmonella thyphimurium* infection on the plasma kinetics of ceftriaxone in Sokoto red goats. In a randomised two-way study, 10 healthy male goats were divided into two groups of five each, and either received a single intramuscular (IM) injection of 1g ceftriaxone only or 1g Ceftriaxone plus inoculation with *S. thyphimurium*. Non compartmental pharmacokinetic parameters were measured in plasma samples by microbiological assay using *Escherichia. coli* as the test organism. The pharmacokinetic parameters of Ceftriaxone alone were as follows: absorption half life 0.509±0.004hr, elimination half-life 0.580±0.012hr, maximum plasma concentration (C_{max}) 45.56±0.191µg/ml, Time to attain C_{max} 0.7±0.123hr, area under the curve(AUC) 144.06±1.711µg-hr/ml, area under mean curve (AUMC) 313.46±6.156µg-hr*hr/ml, volum of distribution (Vd) 485.28±15.725ml/kg, Cl 578.751±6.880ml/hr/kg, MRT 2.18±0.020hr. These were significantly influenced by *S. thyphimurium* infection; with the absorption half life as 0.318±0.084hr, elimination half-life 0.866±0.113hr, Cmax 41±2.915µg/ml, Tmax 0.5hr, AUC 90.14±2.952µg-hr/ml, AUMC 175.84±6.948µg-hr*hr/ml, Vd 1175.36±181.30ml/kg, clearance 928.549±30.675ml/hr/kg, MRT 1.94±0.060hr . These findings suggest that *S. thyphimurium* infection in Sokoto red goat significantly influences the pharmacokinetics of Ceftriaxone. Further studies are required to buttress these findings and to establish the mechanism of interaction.

Keywords: pharmacokinetics, *Salmonella thyphimurium*, ceftriaxone, Sokoto red goats.

INTRODUCTION

Ceftriaxone is a third generation cephalosporin that is widely used in bacterial infections. It has a good activity against several strains of *Salmonella* and its long half life and single daily dosing proffers it with a positive advantage. Several studies have documented pharmacokinetics of ceftriaxone in healthy situation; these parameters may differ in situation of infection due to fever, inflammation and transient dysfunction of the liver and kidneys during the infection (Gopal *et al*., 1994, Etuk and Onyeyili, 2006). *S. thyphimurium* infection was shown to alter the distribution of chloramphenicol (Etuk and Onyeyili, 2006) and a fall in peak plasma levels was observed in *Pasteurella haemolytica* infected calves (Burrows *et al*., 1986), and *Escherichia coli* infected veal calves (Groothuis *et al*., 1979). However, the peak plasma concentration of amoxicillin remained unchanged in *S. thyphimurium* infected pigs (Agerso *et al*., 2000). Pharmacokinetics of ceftriaxone was affected by pulmonary infection **(**Wang *et al.,* 2005**),** and achieved peak abscess levels of about 40% of the peak serum levels (Dezfulian *et al.,* 1993). Ceftriaxone has been shown to displace phenytoin in serum in vitro and invivo (Dasgupta *et al.,* 1991), and decreased the efficacy of morphine in rats (Rawls *et al.,* 2007). A polyherbal drug (Fibrosin) decreased the body clearance of ceftriaxone (Sar *et al.,* 2006) while the clearance was significantly increased during hemofiltration and hemodialysis (Matzke *et al.,* 2000). Ceftriaxone is extensively used in many bacterial infections, but to the best of our knowledge no data is available for the effect of *S. thyphimurium* infection on the plasma kinetics of ceftriaxone in Sokoto red goats, hence the justification for this study. Therefore, the purpose of this study is to investigate whether *S. thyphimurium* infection has any effect on the pharmacokinetics of ceftriaxone in Sokoto Red Goats.

MATERIALS AND METHODS

Sample collection

(a) Drug: Generic Ceftriaxone injection, Panpharma Laboratories, France.

(b) Animals: Ten apparently healthy male Sokoto red goats aged six to twelve months and weighing 10-13kg were purchased from Dange market in Dange-Shuni Local Government Area of Sokoto State, Nigeria. The goats were housed separately in groups of five after a computer generated randomisation and conditioned in pens with concrete floors in the large animal unit of Usmanu Danfodiyo University Sokoto. They were fed on wheat bran, bean offal, cowpea hay, while water was provided ad libitum. Before the commencement of the experiment, the goats were confirmed to be in healthy condition. The animals were acclimatised for a period of two weeks, and evaluated once every 24 hours after purchase for respiratory and pulse rates, rectal temperature and colour of conjunctivae and buccal mucous membrane.

Drug Administration : The first group received 1g of ceftriaxone only; the second group received 1g of ceftriaxone and inoculation with *S. thyphimurium*. All drugs were administered via the intramuscular route. Venous blood samples collected via the jugular vein (5ml) were drawn pre-dose and at 0 , 0.25, 0.5 , 1 , 1.5 , 2 , 2.5 , 3 , 4 , 6 , 8,12, 24 and 48 hours. The samples were collected in heparinized centrifuge tubes. All the samples were centrifuged and plasma separated and frozen until running the analysis.

Determination of the regression equation for the microbiological assay: Pooled plasma from healthy Sokoto red goats was used as diluents to constitute 2.5, 5, 10, 15,20,25,30,35,40,45 and 50ug/ml Ceftriaxone solution. Using the agar well diffusion technique, the inhibition of the growth of *Escherichia coli* was then identified and measured using a vernier calliper. A regression equation Y=ax+C and correlation co-efficient was then generated from the concentration response curve using Graphpad instat software (Zuluaga *et al*., 2009, Singh *et al*., 2008, Issa *et al*., 2007a).

Infection of sokoto red goats with S*almonella typhimurium:* The method of Otesile *et al* (1990) was used. Stock culture of *S. typhimurium* initially obtained from clinical isolates was used. Each of the five goats in the second group received orally a 10ml suspension containing $2x10^9$ organism/ml. Blood was collected aseptically from the goats jugular veins for haematological tests. Clinical features indicating infection were observed and noted in the infected goats as per the pre-validated models.

Pharmacokinetic analysis: Pharmacokinetic parameters were calculated by inputting the concentration time data obtained into the pharmacokinetic software PK Solution 2.0 and curve stripping was performed using the least-square technique.

Statistical analysis: The mean, standard error of mean, spearmanôs correlation, and p-values were calculated using Graphpad instat, statistical software.

RESULTS

Table 1 shows mean zones of inhibition, standard error of mean, and spearmanôs correlation coefficient of various Ceftriaxone concentrations when tested against several microorganisms to be selected for the microbiological assay.

This study reveals that *E. coli* is most sensitive to Ceftriaxone of all the organisms tested and has the best correlation when concentration is plotted against respective zones of inhibition and was selected as the assay ($R= 0.9935$, $p<0.0001$).

Table 2 shows plasma Ceftriaxone concentrations against time following a single intramuscular administration in healthy and *S. thyphimurium* infected Sokoto red goats, while Table 3 shows summary of pharmacokinetic parameters of Ceftriaxone in healthy and infected Sokoto red goats.

S. thyphimurium infection in the goats resulted in a rise in rectal temperature, significant reduction in feed intake, passage of frequent watery and offensive faeces, and lethargy, and the haematological examinations revealed a

significant rise in white blood cell count. Infecting the Sokoto red goats with *S. thyphimurium* caused a fall in the mean maximum plasma concentration (41±2.915µg/ml) at about 0.5 hours after drug administration, when compared with the value of mean maximum plasma concentration (45.56±0.191µg/ml) obtained in the healthy Sokoto red goats at about 0.7 hours after drug administration (P=0.1572) (Table 3).

Table 1: Mean zones of inhibition, standard error of mean, and spearmanôs correlation coefficient of various ceftriaxone concentrations

Concentration of Ceftraixone (µg/ml)	Zone of Inhibition of growth of *Klebsiella* (mm)	Zone of Inhibition of growth of *E.coli* (mm)	Zone of Inhibition of growth of *Proteus*(mm)
2.5	20.4±1.01	24.0±0.13	23.6±1.74
5	24.2±1.20	27.0±0.12	26.5±1.02
10	23.0±1.10	28.5±1.56	26.6±1.09
15	25.4±0.99	31.0±0.11	28.0±0.93
20	24.7±1.12	31.5±0.16	28.1±1.02
25	24.8±1.00	32.0±0.11	28.3±2.06
30	24.8±1.00	33.0±0.09	28.2±1.05
35	26.5±0.63	33.5±0.08	29.2±0.84
40	27.0±1.53	34.2±0.11	28.7±1.00
45	26.5±0.96	34.98±0.08	29.4±0.67
50	27.4±0.74	36.25±0.11	30.3±0.47
R^2	0.5344	*0.9935	0.5612

All values of ZI are mean ±SEM of n=6 observations. R=Spearmanôs Correlation coefficient. *=Selected R= 0.9935, p<0.0001

The plasma concentration of ceftriaxone in both healthy and infected Sokoto red goats showed a steady decline afterwards until minimum measurable plasma concentrations of 1.73±0.00 and 1.2±0.10µg/ml were obtained at about 6 hours after drug administration respectively (Table 2).

The pharmacokinetics behavior of Ceftriaxone in both healthy and infected Sokoto red goats after intramuscular injection could be best described by a one compartment open model with a first order kinetic (Figure 1 and 2).

There was a statistically significant effect on the area under the curve (144.06±1.711 and 90.14±2.952 µghr/ml), volume of distribution (485.28±15.725 and 1175.36±181.30 ml/kg), clearance (578.751±6.880 and 928.549±30.675 ml/hr/kg), mean residence time (2.18±0.020 and 1.94±0.060hr) (P<0.05) respectively when pharmacokinetic parameters of the healthy goats were compared with the infected goats (Table 3).

Table 2: Plasma concentrations of Ceftriaxone following a single intramuscular administration in healthy and *S. thyphimurium* infected Sokoto red goats

Time (hr)	Health Goats Conc. (µg/ml)	Infected Goats Conc. (µg/ml)
0.25	32±0.51	19±2.91
0.5	45±0.45	41±2.91
1.0	45±0.17	32±1.22
1.5	37±0.38	25±0.00
2.0	28.5±0.33	19±1.00
2.5	27±0.44	15±1.58
3.0	25±0.95	15±1.58
4.0	19±0.95	5±1.58
6.0	1.73±0.00	1.2±0.10

This table shows the mean concentrations±SEM against time for all animals in the group (n=5).

Table 3: Summary of pharmacokinetic parameters of Ceftriaxone in healthy and infected Sokoto red goats

PK parameters	Healthy goats (mean±sem)	Infected goats (mean±sem)	P-value
Absorption Half life (hr)	0.509±0.004	0.318±0.084	P=0.053
Elimination Half life (hr)	0.580±0.012	0.866±0.113	P=0.0664
C_{max} (µg/ml)	45.56±0.191	41±2.915	P=0.1572
T_{max} (hr)	0.7±0.123	0.5±0	
AUC (µghr/ml)	144.06±1.711	90.14±2.952	P<0.0001
AUMC (µghr*hr/ml)	313.46±6.156	175.84±6.948	P<0.0001
Vd (ml/kg)	485.28±15.725	1175.36±181.30	P=0.0053
Cl (ml/hr/kg)	578.751±6.880	928.549±30.675	P<0.0001
MRT (hr)	2.18±0.020	1.94±0.060	P=0.0162

Key: C_{max} = maximium plasma concentration; T_{max} = time to attain the C_{max}; AUC = area under the curve (area under the concentration-time curve); AUMC = area under the mean curve; Vd = volume of distribution; Cl = clearance; MRT = mean resident time.

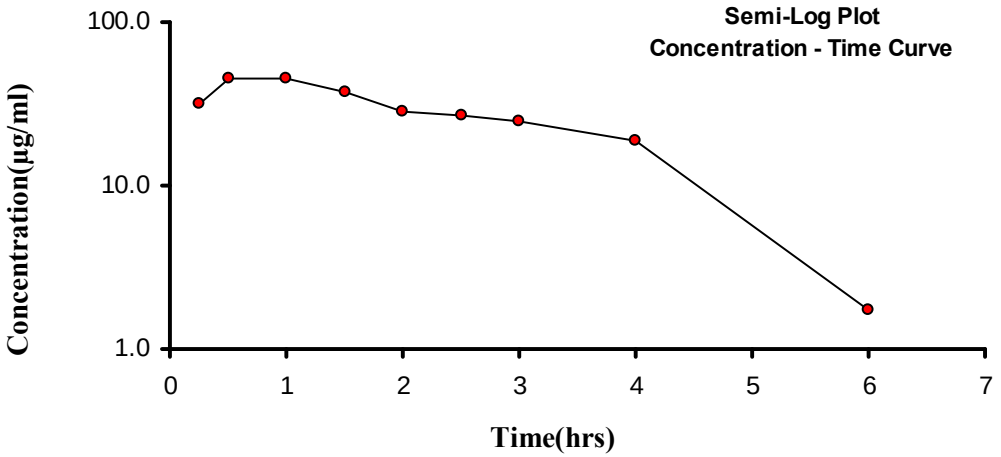

Figure 1: Semi-Log Plot of concentration-time curve for ceftriaxone in healthy Sokoto red Goats

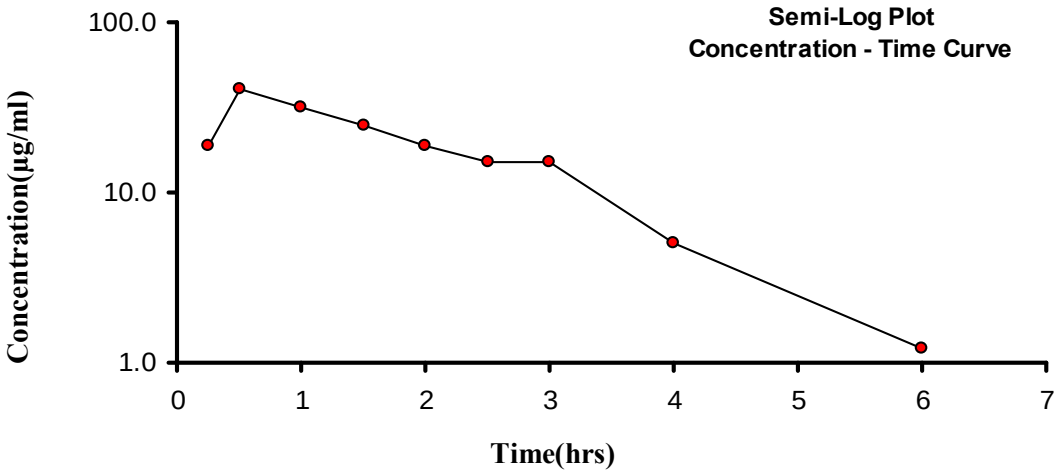

Figure 2: Semi-Log of concentration-time curve of ceftriaxone following a single intramuscular administration in *S. thyphimurium* infected Sokoto red goats.

DISCUSSION

The effect of *S. thyphimurium* infection on the pharmacokinetics of ceftriaxone in Sokoto red goats was examined in this study with either the administration of 1g ceftriaxone only or 1g ceftriaxone plus inoculation with *S. thyphimurium*. *S. thyphimurium* infection was shown to influence the ceftriaxone plasma kinetic behavior (Table 3). The effect of the interaction on the maximum plasma concentrations (C_{max}), absorption and elimination half life were not statistically significant ($P>0.05$); whereas there was a statistically significant effect on the area under the curve (AUC), volume of distribution (Vd), clearance (Cl), mean residence time (MRT) ($P<0.05$) when pharmacokinetic parameters of the healthy goats were compared with the infected (Table 3). In a similar study Etuk and Onyeyili (2006) concluded that *S typhimurium* infection did alter the distribution of chloramphenicol, increase the loss of the drug and reduced its mean residence time in the body of the Sokoto red goats. Agerso *et al*., (2000) reported that the peak plasma concentration of amoxicillin remained unchanged in *S. typhimurium* infected pigs. A decrease in the peak plasma levels of chloramphenicol was observed in *Pasteurella haemolytica* infected calves (Burrows *et al*., 1986). In a similar study, a low chloramphenicol plasma level was observed in *E. coli* infected veal calves Groothuis *et al*., (1979). The decrease in plasma concentration observed in the infected goats may be due to fever and inflammation induced by *S. typhimurium* infection, which may be due to the decrease in the distribution phase intercept in the infected animals (Etuk and Onyeyili, 2006). Fever and inflammation are cardinal features in bacterial infection, which may inturn causes an increase in heart rate and cardiac output, increasing blood flow to the liver and kidneys, all these could lead to increase in the rate at which the drug is delivered to both organs which are important sites of drug excretion (Etuk and Onyeyili, 2006). The above may somewhat explain the increase in the total body clearance in infected goats.

CONCLUSION

This study revealed that *S. thyphimurium* infection significantly ($p<0.05$) influenced the pharmacokinetic parameters of ceftriaxone in Sokoto red goats. The results of our investigations as presented in tables 2 and 3 may imply that a dose adjustment may be necessary when administering ceftriaxone in *S. thyphimurium* infected Sokoto red goats. Further studies are however required to buttress these findings and establish the mechanism of interaction.

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