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The Comparative Study of the Tissue Kinetics of Chloramphenicol in Healthy and *Salmonella* Infected Goats

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Abstract: A comparative pharmacokinetic study of chloramphenicol (25 mg kg^{-1} intravenous) by colorimetric assay method in tissues/organs of healthy goats and *Salmonella typhimurium* infected goats has revealed that, the drug was present in high concentrations in various tissues at 0.08 h (5 min) post-drug administration except in the brain of healthy goats and skeletal muscle of both healthy and infected goats. The average concentrations of the drug in the liver, kidney, spleen, bone marrow, lungs, heart and brain were higher after the infection. The residence period of the drug was higher in the kidney and the skeletal muscle of the healthy goats (240 h; 10 days). There were however decrease in the half-lives, elimination rate constants of the drug in the liver, kidney, spleen, brain, heart and skeletal muscle after the infection. The present study indicated that, a single dose of intravenous chloramphenicol injection can persist up to 10 days and a shorter period (5 days) in the edible tissues of the healthy and *Salmonella* infected goats, respectively. The presence of chloramphenicol in some edible tissues for up to 10 days is of public health significance.

Key words: Chloramphenicol, *Salmonella typhimurium,* plasma, tissues, concentrations, goats

INTRODUCTION

The distribution of any drug following administration between the plasma and the various tissues in the body varies from time to time and from specie to specie. The correlation coefficients between plasma and the various body tissues obtained with sulphamethazine and sulphathiazole in several species of animals was greater than expected and in many cases were equivalent to 1,000[1]. Results of this nature are highly stimulatory to the desire to examine other classes of drugs of equal importance to both veterinary and human medicine. It was suggestive from the previous studies that, the plasma levels of chloramphenicol may have predictive value regarding tissue levels for the drug[2].

However it will be important to ascertain the tissue residue profile of this drug in animals especially those infected with *Salmonella* organisms. *Salmonella* infection is known to influence pathophysiologic changes which may influence the drug distribution to the tissues and organs[1]. Despite the widespread use of chloramphenicol in human and veterinary practice, no relevant data are available on how long the drug persists in edible tissues of red Sokoto goats. This is necessary in order to determine the withdrawal period of the drug especially in *Salmonella* infected goats treated with the agent to prevent public health hazard following consumption of the edible tissue by man. The objective of this study was to compare the tissue residue profile of chloramphenicol in healthy and *Salmonella typhimurium* infected red Sokoto goats treated intravenously with the drug.

MATERIALS AND METHODS

Fifty four adult red Sokoto goats of mixed sexes, weighing between 15.0 to 25 kg with a mean of 18.3 kg were used for the study. The animals were certified clinically healthy at the onset of the experiment. The goats were purchased from Sokoto Cattle Market, Sokoto. They were dewormed on arrival with Levamisole hydrochloride (Vamisole, Batch No. TL.VO405, Tuyil Pharm. Ind. Ltd., Nig.) orally at the rate of 7.5 mg kg^{-1}. They were housed in goat pens belonging to the Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto and were fed with hay and concentrates. Water was provided *ad libitum.* The goats were screened for the presence of *Salmonella* or other bacterias prior to the commencement of the study using

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blood obtained from jugular vein and placed in two culture bottles, one containing Robertson cooked meat broth and the other containing Brain heart infusion broth which were incubated to detect the presence of bacterial organisms.

The animals were randomly separated into two groups of 24 goats each. One of the groups was left as healthy goats, while the goats in the second group were infected with 20 mL of *Salmonella typhimurium* suspension in normal saline containing $6x10^{10}$ Colony Forming Units (CFU) mixed with 100 mL of 10% $NaHCO_3$ and administered intragastrically using a stomach tube based on the method of Agerso *et al.*[3].

Chloramphenicol was administered intravenously at the rate of 25 mg kg^{-1}. The treatment was made 24 h post infection when fever was well established in the infected group on assessment of the rectal temperature using clinical thermometer.

Sample collection and determination of chloramphenicol concentration: Ten grams of tissue samples (liver, skeletal muscle, brain, lungs, kidney, spleen and bone marrow) were taken post-mortem from the animals at 0.08, 0.25 0.5, 1, 3, 9, 12, 24, 48, 120, 240 360 and 480 h following the drug administration. Two goats from each of the groups were sacrificed at each period of sample collection. Two goats were initially sacrificed for the preparation of control tissues and tissue standards. The tissue samples were stored at –20°C until analysed. The experimental work area and all utensils were cleaned thoroughly after each slaughter to prevent cross contamination between sacrifice periods.

Chloramphenicol contents of the tissues were determined colorimetrically using the method developed by Kakemi *et al.*[4] as modified by Hughes and Diamond[5] and Watson[6].

Linear regression analysis was performed by standard procedures using average tissue concentrations and the slope of the elimination curve for chloramphenicol in each tissue determined. The half-lives and the elimination rate constants in various tissues were calculated[7].

RESULTS

Peak concentrations of the drug were established in the various tissues at 0.08 h (5 min) post-drug administration except in the brain of healthy goats and skeletal muscle of both healthy and *Salmonella* infected goats. Concentrations of 68.96 and 79.03 μg g^{-1} were obtained in the liver of healthy and *Salmonella* infected goats, respectively 0.08 h post administration (Table 1).

Table 1: Chloramphenicol concentrations (μg g^{-1}) in the liver of healthy and *Salmonella* infected goats

Time after injection (h)	Healthy goats	*Salmonella typhimurium* infected goats
0.08	68.96	79.03
0.25	35.08	40.81
0.50	18.34	19.93
1.00	14.30	14.63
3.00	10.98	12.01
6.00	8.42	10.03
12.00	7.06	7.11
24.00	6.32	4.22
48.00	2.09	1.36
120.00	0.38	0.72

Table 2: Chloramphenicol concentrations (μg g^{-1}) in the kidney of healthy and *Salmonella* infected goats

Time after injection (h)	Healthy goats	*Salmonella typhimurium* infected goats
0.08	53.01	69.01
0.25	26.22	33.93
0.50	13.18	17.12
1.00	10.23	13.02
3.00	7.03	10.88
6.00	5.16	8.21
12.00	4.33	7.31
24.00	4.02	5.01
48.00	3.61	2.19
120.00	0.83	0.91
240.00	0.10	0.00

Table 3: Chloramphenicol concentrations (μg g^{-1}) in the heart of healthy and *Salmonella* infected goats

Time after injection (h)	Healthy goats	*Salmonella typhimurium* infected goats
0.08	34.61	41.83
0.25	18.03	19.92
0.50	8.94	10.02
1.00	6.82	7.52
3.00	4.03	5.72
6.00	3.22	3.21
12.00	1.04	1.93
24.00	0.46	0.42
48.00	0.10	0.00

The concentrations in the liver showed a continuous decrease and at 120 h (5 days) post injection the concentrations were 0.38 and 0.72 μg g^{-1} in healthy and infected goats, respectively.

The highest concentrations of the drug obtained in the kidney of the healthy and *Salmonella* infected goats were at 0.08 h post administration (Table 2). The average concentrations obtained were 53.01 and 69.01 μg g^{-1} in healthy and *Salmonella* infected animals, respectively. The concentration decreased to 0.1 μg g^{-1} in healthy goats and was below detectable level in *Salmonella* infected goats 240 h post drug administration.

The chloramphenicol concentrations present in the heart muscle at 0.08 h after injection was 34.61 and 41.83 μg g^{-1} in healthy and *Salmonella* infected goats. These concentrations decreased continuously and at 48 h post drug administration the drug concentration was 0.1 μg g^{-1} in healthy animals while it was below detectable

Table 4: Chloramphenicol concentrations (μg g^{-1}) in the lungs of healthy and *Salmonella* infected goats

Time after injection (h)	Healthy goats	*Salmonella typhimurium* infected goats**
0.08	39.00	49.86
0.25	19.31	25.03
0.50	8.78	13.01
1.00	7.01	9.66
3.00	5.21	6.23
6.00	3.04	4.08
12.00	1.33	2.92
24.00	0.98	1.01
48.00	0.14	0.62
120.00	0.00	0.26

Table 5: Chloramphenicol* concentrations (μg g^{-1}) in the bone marrow of healthy and *Salmonella* infected goats

Time after injection (h)	Healthy goats	*Salmonella typhimurium* infected goats**
0.08	56.20	70.30
0.25	26.11	34.47
0.50	14.03	18.01
1.00	11.33	15.21
3.00	8.12	12.11
6.00	6.01	7.33
12.00	3.98	5.92
24.00	1.32	3.14
48.00	0.89	1.10
120.00	0.12	0.10

Table 6: Chloramphenicol concentrations (μg g^{-1}) in the spleen of healthy and *Salmonella* infected goats

Time after injection (h)	Healthy goats	*Salmonella typhimurium* infected goats**
0.08	41.33	53.11
0.25	23.11	26.03
0.50	11.03	14.11
1.00	9.61	10.23
3.00	6.22	7.52
6.00	4.08	5.04
12.00	2.63	3.98
24.00	0.86	1.29
48.00	0.52	0.63
120.00	0.11	0.00

levels in the infected goats (Table 3). The lungs showed peak concentrations of 39.00 and 49.86 μg g^{-1} in healthy and *Salmonella* infected goats. Detectable concentrations lasted for 48 h post drug treatment in healthy goats and 120 h in *Salmonella* infected goats (Table 4).

The drug was also observed to be distributed readily to the bone marrow. High concentrations of 56.20 and 70.30 μg g^{-1} were obtained 0.08 h post drug administration in healthy and *Salmonella* infected goats, respectively (Table 5). At 120 h post drug administration detectable levels were still obtained in the bone marrow of healthy (0.12 μg g^{-1}) and *Salmonella* infected (0.10 μg g^{-1}) goats. Concentrations of the drug occurred for 120 h after drug administration in the spleen of healthy goats while in the infected goats it occurred only for 48 h post drug administration. The highest concentrations of 41.33 and 53.11 μg g^{-1} were obtained at 0.08 h post drug treatment. Following continuous decrease the

Table 7: Chloramphenicol concentrations (μg g^{-1}) in the brain of healthy and *Salmonella* infected goats

Time after injection (h)	Healthy goats	*Salmonella typhimurium* infected goats**
0.08	0.10	24.51
0.25	0.36	13.02
0.50	0.63	7.22
1.00	0.41	6.01
3.00	0.32	4.88
6.00	0.28	2.92
12.00	0.18	1.01
24.00	0.10	0.62
48.00	0.00	0.21

Table 8: Chloramphenicol concentrations (μg g^{-1}) in the skeletal muscle of healthy and *Salmonella* infected goats

Time after injection (h)	Healthy goats	*Salmonella typhimurium* infected goats**
0.08	0.93	7.01
0.25	1.26	12.93
0.50	2.98	15.01
1.00	4.62	13.11
3.00	3.98	11.03
6.00	3.90	9.22
12.00	3.64	6.15
24.00	2.98	3.19
48.00	1.07	2.01
120.00	0.54	0.83
240.00	0.12	0.00

Table 9: The half-lives and elimination rate constants in various tissues in healthy and *Salmonella typhimurium* infected goats

	Healthy goats		*Salmonella typhimurium* infected goats**	
Tissues	Half-Lives (h)	Elimination Constant (h^{-1})	Half-lives (h)	Elimination Constant (h^{-1})
Liver	1.375	0.504	1.079	0.642
Kidney	3.554	0.195	1.265	0.548
Brain	21.000	0.033	1.129	0.614
Heart	1.950	0.355	1.589	0.436
Lungs	0.785	0.883	1.733	0.400
Bone marrow	1.529	0.453	1.181	0.587
Spleen	0.846	0.819	0.723	0.958
Skeletal muscle	23.896	0.029	3.039	0.228

level of the drug in the spleen also declined (Table 6). The concentrations in these tissues were markedly lower than those of the liver, kidney, bone marrow, lungs and spleen in both healthy and infected animals. The brain at 0.08 h has concentration of 0.1 μg g^{-1} and this increased to 0.63 μg g^{-1} at 0.50 h. Thereafter, there was continuous decrease and at 12 h post drug administration the concentration came down to 0.18 μg g^{-1}. At 0.08 h the brain of infected goats had a drug concentration of 24.51 μg g^{-1}. Detectable levels of chloramphenicol occurred in the brain of infected goats for 48 h (Table 7). In the case of skeletal muscle, concentrations of 0.93 and 7.01 were obtained at 0.08 h respectively in healthy and *Salmonella* infected animals, thereafter the concentrations increased and at 1.0 and 0.5 h, respectively, peak concentration of 4.62 and 15.01 μg g^{-1} were obtained in healthy and *Salmonella* infected

animals. Detectable levels of the drug were obtained in healthy animals at 240 h (0.12 μg g^{-1}) and in *Salmonella* infected goats at 120 h (0.83 μg g^{-1}) (Table 8).

The half-life of the drug in the brain was 21.0 h in healthy goats while in *Salmonella* infected goats it was 1.129 h. In healthy goats the half-life in the skeletal muscle was 23.896 h while it was 3.039 h in *Salmonella* infected goats. The half-lives of the drug in tissues of healthy animals appear to be higher than those of the infected animals (Table 9).

DISCUSSION

The results presented in this experiment showed that chloramphenicol is readily distributed to various organs and tissues of the body. Five minutes (0.08 h) post administration very high concentrations occurred in the excretory organs (liver and kidney) in all the groups. Reasonable concentrations have been reported earlier to occur in the liver and kidney of chickens treated orally[8]. The high concentrations obtained in the liver and kidney are expected. The kidney is the primary organ of elimination and the ability of the drug to penetrate the liver is high since the liver is the main organ of bio-transformation. Furthermore, the compound is not slowly and incompletely metabolised.

Low chloramphenicol concentrations were found in the brain and skeletal muscle of the healthy goats (Table 7 and 8). However, extensive chloramphenicol concentrations were obtained in these tissues following *Salmonella* infection. Chloramphenicol is known to have the ability to cross physiological barriers with concentrations occurring in such areas as the brain, being one of the few antibiotics to penetrate into the brain and cerebrospinal fluid[9]. The higher concentrations observed in the *Salmonella* infected animals may be as a result of inflammation caused by the *Salmonella* infections. It therefore means that penetration of the compound into the brain and skeletal muscle is markedly enhanced when the meninges of the brain and basal lamina and reticular fibers coating of the muscles are inflamed. Fever that often accompanies *Salmonella* infections may be another contributory factor.

The organs of the body, which are moderately supplied with blood such as the heart and lungs, also contain high chloramphenicol concentrations in both the healthy and *Salmonella* infected animals. This may be an indication of rapid distribution of the drug to tissues. The concentration of the drug in the heart and lungs obtained in *Salmonella* infected goats were higher than those of healthy goats. The higher drug concentrations in the tissues of diseased animals may have occurred due to increased uptake of the compound by these organs. The increased uptake may have resulted from fever which induced increased heart rate resulting in increased circulation of blood to organs and tissues[10].

Chloramphenicol concentrations present in the bone marrow and spleen were high in both healthy and *Salmonella* infected goats. Bone marrow is responsible mainly for the production of red blood cells and some leucocytes while the spleen is involved in haemopoiesis and immunological processes[11]. The presence of high concentrations of the drug in these two organs is of interest since the drug is known to induce aplastic anaemia in man and animals[9,12-14]. The *Salmonella* infected animals showed higher drug levels in these tissues than the healthy animals. This may have resulted from increased blood supply to these tissues due to increased heart rate resulting from fever induced by *Salmonella* infection. The drug has longer half-lives in the tissues of healthy animals when compared to infected animals. This may be due to higher elimination rate constants of the drug in the tissues of *Salmonella* infected goats compared to the healthy animals.

There is an apparent difference in the levels of residues in healthy and *Salmonella* infected goats. This tends to suggest that the disease process altered the response of the tissues to the drug. The present findings indicate that chloramphenicol persists in the tissues of the goat for up to 240 h (10 days) after intravenous injection in healthy goats and less than that in *Salmonella* infected goats. This should be given due consideration in the estimation of the withdrawal period for the drug.

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