

Full Length Research Paper

Plasma kinetics of intravenously administered lactose-in-saline in healthy and *Trypanosoma congolense* infected rams

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The effect of *Trypanosoma congolense* infection on plasma kinetics of intravenously administered lactose was investigated in apparently healthy and *T. congolense* infected rams. Four rams infected with *T. congolense* and 4 uninfected rams were each infused intravenously with repeated doses of 0.5 g/kg body weight lactose in saline, thrice daily at 4 h interval. Blood was collected at specified intervals and analyzed for residual lactose. Plasma kinetic parameters such as biologic half-life ($t_{1/2}$), elimination rate constant (K_e), total clearance (C_T) and volume of distribution (V_d) were calculated from the data obtained. The mean values for the $t_{1/2}$, K_e , V_d and C_T for the infected group were 5.328 ± 1.045 , $1.682 \pm 0.289 \text{ h}^{-1}$, $508.75 \pm 41.987 \text{ ml/kg}$ and $68.378 \pm 17.571 \text{ ml/kg/h}$, respectively. The mean values for the uninfected group were 5.336 ± 0.753 , $2.408 \pm 0.817 \text{ h}^{-1}$, $517.00 \pm 196.592 \text{ ml/kg}$ and $53.223 \pm 14.888 \text{ ml/kg/h}$ respectively. Infection with *T. congolense* significantly affected only the elimination rate constant K_e .

Key words: *Trypanosoma congolense*, trypanosomosis, lactose.

INTRODUCTION

Chemotherapy and vector control strategies are the principal methods used in the management of African trypanosomosis. However, these methods have shortcomings which include repertoire of compounds, parasite resistance to drugs, toxicity due to long period of treatment and lack of complete eradication of the vector. The phenomenon of antigenic variation has rendered the prospect of vaccination hopeless. Hence, there is need to seek for new chemotherapeutic and chemoprophylactic agents against trypanosomosis (Atawodi et al., 2002).

Since anemia is said to be the major cause of death during trypanosomosis infection it is suggested that if it can be prevented, this may increase the body's ability to fight off the infection.

Lactose is a major type of sugar found in milk and milk

products, including human milk. Lactose is not found naturally in any other food aside from dairy products (Walstra and Jenness, 2007). Lactose and other β -galactoside compounds have been used to inhibit erythrophagocytosis of desialylated RBC's by rat peritoneal macrophages (Kolb and Kolb-Bachofen, 1978; Nagamura and Kolb, 1979; Kolb et al., 1980; Schlepper-Schafer and Kolb, 1980) the rapid sequestration of homologous desialylated erythrocytes from the blood stream of rabbits has been considerably inhibited by intravenously administered lactose (Muller et al., 1981).

More recently, infusion of lactose has been suggested to be of importance as a base to a trypanocidal agent because it can inhibit phagocytosis and sequestration of the desialylated red cells (Umar et al., 2008). Also the kinetics of intravenously infused lactose in cattle infected with *T. vivax* was followed by Umar et al. (1998). This work presents the effect of *T. congolense* infection on plasma kinetics of intravenously administered lactose, in healthy and *T. congolense* infected "yankassa" rams.

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MATERIALS AND METHODS

Parasites

T. congolense (NITRE-53624609) was obtained from the National Institute of Trypanosomiasis Research (NITR), Jos, Nigeria.

Experimental animals

8 rams ("Yankassa" breed) between the ages of 12 - 18 months were purchased from a tse-tse fly free area. The animals were dewormed with Albendazole and vaccinated against black leg, foot rot, pneumonia, systemic mastitis, heart water, wound infections, ecto and endo parasites with Terroxy LA (Habel Yuanzheng Pharm Liability Co. Ltd. 10, Shizheng Road, Shijiazhuang Hebei China.), Aflamec and Diazintol (Alfasan International B.V 3340 AB Woerden Holland.). The animals were housed and fed with hay, wheat offal, groundnut hump, corn, guinea corn, cowpea chaff. Clean water was made available to the animals in clean drinkers.

Infection and lactose infusion

The animals were divided into 2 groups with 4 animals in each group. The group i animals were the uninfected rams which were given lactose in saline at a dose rate of 0.5 g lactose kg⁻¹ body weight 3 times at 4 h interval, via jugular venepuncture from day 3 to 20 post-infection. The group ii animals were the lactose treated infected animals which were infected via jugular venepuncture with blood containing 106 trypanosomes harvested from a donor ram at peak parasitemia and were given lactose in saline at a dose of 0.5 g lactose kg⁻¹ body weight 3 times at 4 h interval, via jugular venepuncture from day 3 to 20 post-infection (p.i).

Sampling and analyses

On the first day of lactose infusion, 5 ml of blood was collected via the jugular vein first at 15 min interval, then at 30 min interval followed by hourly intervals for 4 h. After the second and third infusions, blood was collected at hourly intervals into tubes with EDTA for the determination of plasma lactose concentrations by the method of Wahba (1965) after removal of interfering substances from the samples by the method described by Horowitz et al., (1951) on subsequent days 5ml of blood was collected from each animal before the first lactose infusion for that day commenced. Parasitemia was estimated by the microhaematocrit method of Herbert and Lumsden (1976).

Calculation of kinetic parameters

The plasma lactose concentration for each animal was plotted against the sampling times on semi logarithmic graph papers. The biological half-life ($t_{1/2}$), elimination rate constant (K_e), total clearance (C_T) and apparent volume of distribution (V_d) of lactose in the animals were calculated from the semi-log plots as described by Grahame-Smith and Aronson (1992).

The $t_{1/2}$ was defined as the time taken for half the concentration of lactose in plasma at a given time to disappear; while the K_e was the amount of lactose eliminated from the body via all routes per unit time (h). Total clearance was defined as the volume of fluid from which the lactose was eliminated per hour, while V_d was the total volume of plasma in which the administered lactose needed to be dissolved to give a uniform concentration. The $t_{1/2}$ was read off directly from the plot and K_e was the slope of the graph. The V_d and C_T were calculated using the following equations.

$$V_d = D/C_0$$

$$C_T = V_d \times \ln 2/t_{1/2}$$

Where D = amount of lactose administered to the animal and C_0 = cognitive lactose concentration in plasma this is the theoretical peak lactose concentrations in the plasma when the administered dose is evenly distributed in the body, it is obtained as the intercept of the plot on the concentration axis.

Data analysis

All statistical analyses were done using student's t-test.

RESULTS

The profile of mean plasma lactose concentrations in the uninfected and lactose treated infected groups are shown in Figure 1. The profile for the lactose treated infected group showed that after the first and second infusions, the level of lactose reached a peak and fell down to pre-infusion level. However, after the third infusion, the level of lactose did not return to pre-infusion level. Also, subsequent infusions showed that lactose level did not return to pre-infusion level throughout the duration of the experiment Figure 2.

The profile of mean plasma lactose concentrations in the uninfected group showed that the level of lactose reached a peaked after the first, second and third infusions and returned to pre-infusion level (Figure 1). The level of lactose was maintained at near pre-infusion level after subsequent infusions throughout the duration of the experiment (Figure 3).

Figure 4 presents profiles of mean parasitemia of the infected groups. Parasites were first detected in the blood stream of infected treated group on day 6 p.i and the parasitemia rose to a peak on day 9 p.i through to day 11 p.i after which the parasitemia declined and parasites were not detected in the blood by day 15 p.i. The parasites came up on day 16 p.i, thereby starting the second parasitemic wave. In the infected control group, parasites were first detected on day 7 p.i. Parasitemia rose to a peak on day 11 p.i and declined through to day 14 p.i, the second parasitemic wave started on day 15 p.i. At periods of peak parasitemia, there was no significant difference in the parasitemias of the 2 groups.

Plasma kinetic parameters of lactose in the infected and uninfected groups (Table 1) were calculated from semi-logarithmic plots. In the uninfected group, the mean biologic half-life ($t_{1/2}$), was calculated as 5.33 ± 0.39 h as compared to 5.32 ± 0.53 h in the infected treated group, which showed no significant difference ($P > 0.05$). The mean volume of distribution (V_d), total clearance (C_T) and elimination rate constant (K_e) were calculated as 28.69 ± 1.09 mg/kg, 162.92 ± 14.95 mg/kg/h and 2.682 ± 0.289 h⁻¹, respectively for the infected group while the values for the uninfected group were 21.66 ± 2.84 ml/kg, 153.73 ± 10.85 mg/kg/h and 2.408 ± 0.817 h⁻¹, respectively.

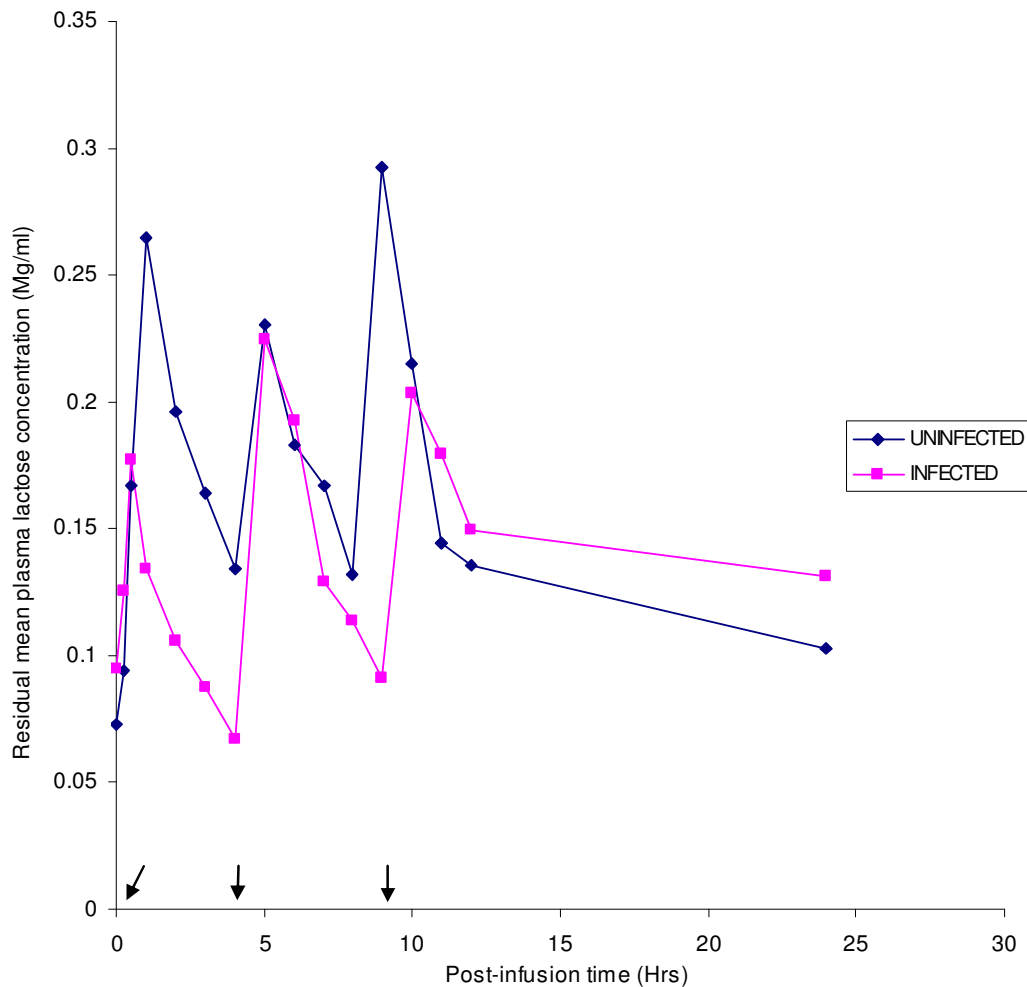


Figure 1. Profile of mean plasma lactose in uninfected and infected group after three four hourly doses of lactose. ↓ Indicate points where lactose infusion was done.

Table 1. Calculated Plasma kinetic Parameters for Lactose in Infected and Non-Infected Rams given Triple Intravenous Dose of 0.5 g Lactose per kg body weight.

Animal No.	$t_{1/2}$ (h)	V_d (ml kg ⁻¹)	C_T (ml kg ⁻¹ hr ⁻¹)	K_e (h ⁻¹)
Infected				
A	5.496	454.00	57.256	1.702
J	5.775	527.00	91.616	2.080
M	6.531	495.00	52.539	1.422
P	5.298	551.00	72.088	1.525
Mean±S.D	5.775 ± 0.541	506.750 ± 41.987	68.378 ± 17.571	1.682 ± 0.289^a
Uninfected				
H	5.360	518.00	75.134	1.791
K	4.502	414.00	63.741	2.064
N	5.124	332.00	44.909	3.610
O	6.317	392.00	43.011	2.168
Mean±S.D	5.326 ± 0.753	414.00 ± 77.510	56.699 ± 15.447	2.408 ± 0.817^b

Values are mean ± S.D for replicate determinations. Values with different superscripts across the group are significantly different ($P < 0.05$).

$t_{1/2}$ = Biological half-life; K_e = Elimination rate constant for lactose; V_d = Apparent volume of distribution for lactose; C_T = Total Clearance.

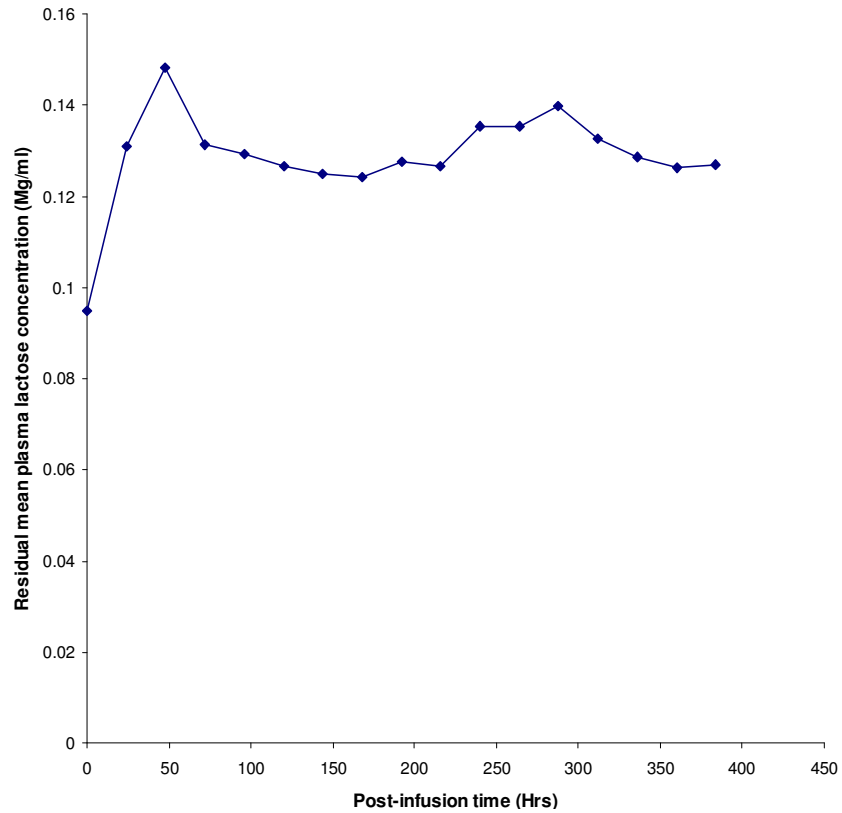


Figure 2. Profile of mean plasma lactose concentration in the lactose treated infected group at 24 h intervals.

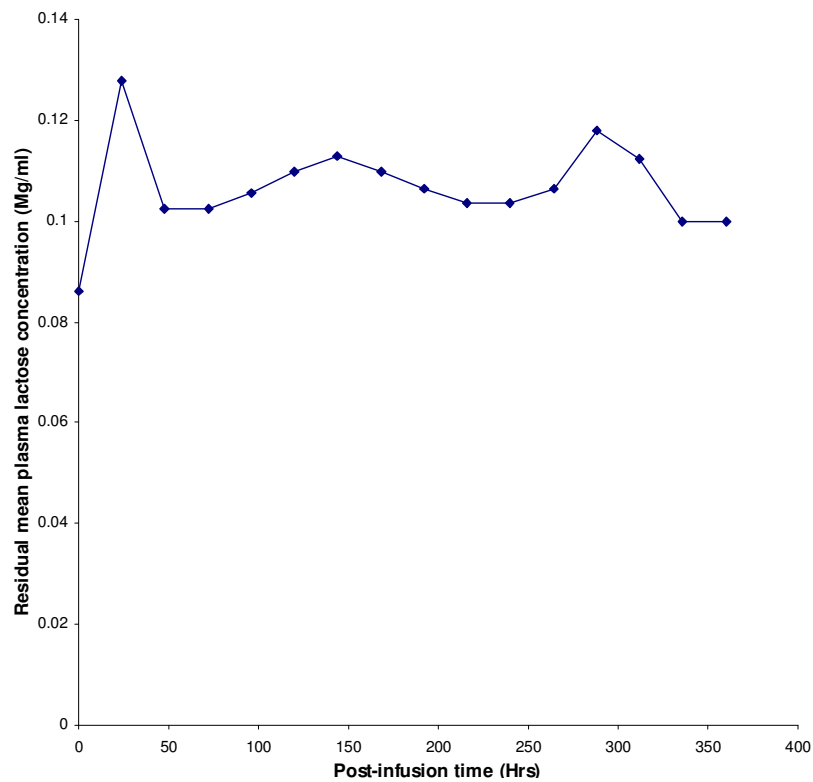


Figure 3. Profile of mean plasma lactose concentration in the uninfected group at 24 h intervals.

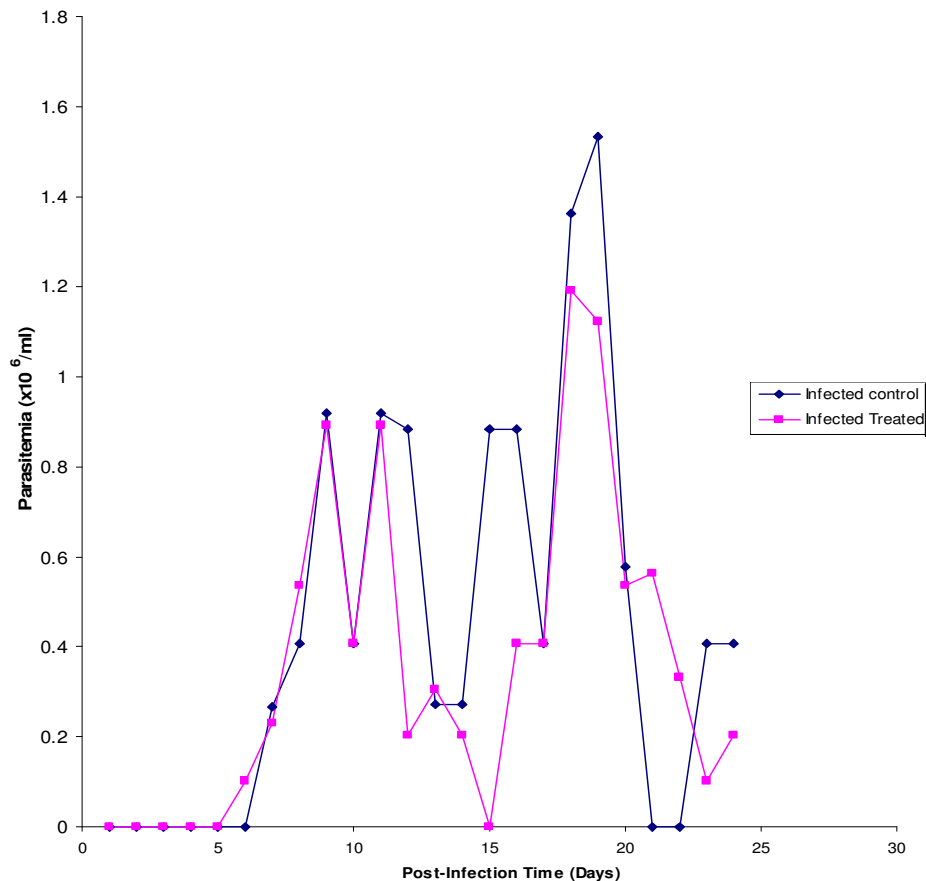


Figure 4. Profiles of parasitemia in infected control and infected treated groups.

DISCUSSION

During lactose infusion in the uninfected and infected groups, a sharp increase in the concentration of plasma lactose was observed which indicates rapid distribution of administered lactose in the vascular system. This was followed by an initial rapid fall which indicates the period of distribution of the administered lactose into the extracellular spaces. The non-accumulation of lactose upon repeated dosing in uninfected rams was perhaps due to the rapid elimination of lactose as indicated by the K_e .

The mean $t_{1/2}$ and K_e indicated that lactose was rapidly eliminated and for lactose to be used effectively as a base to a trypanocidal agent, repeated doses must be given at fairly frequent interval (Muller et al., 1981) to maintain therapeutically effective levels of lactose in plasma. Umar et al. (1998b) reported a significant difference in the mean $t_{1/2}$ plasma lactose of the infected and uninfected groups. However, this report shows that there was no statistical difference in the mean $t_{1/2}$ of the infected and uninfected groups. This may be because the infection was not established in the infected animals before lactose infusion commenced in the present work: contrary to the procedure adopted in the previous experi-

ment (Umar et al., 1998a) which showed that lactose infusion started after parasitemia was established. Our aim for doing the contrary was to see what effect administration of lactose before the onset of parasitemia would have on the course of the infection. Hence, the mononuclear phagocytic system had not been fully activated in this work before commencement of lactose infusion. In *T. congolense* infected rams, after the third infusion, there was accumulation of lactose in the blood such that plasma concentrations of lactose never fell back to pre-infusion levels. This may be a direct consequence of the infection. This finding agrees with previous reports (Umar et al., 1998a, b). Macrophages are released into circulation during the course of the infection. These macrophages have on their surfaces beta-D-galactoside specific lectins (Kelm et al., 1986; Kelm and Schauer, 1986; Kuster and Schauer, 1981; Muller et al., 1983) which could bind the desialylated erythrocytes as well as lactose (Kelm and Schauer, 1986; Kolb et al., 1980; Kolb and Kolb-Bachofen, 1978). The lactose bound to macrophageal lectins is unavailable for elimination and hence remains in circulation longer.

In conclusion, *T. congolense* infection in rams did not cause significant changes in the $t_{1/2}$, V_d and C_T since lactose therapy began before the onset of parasitemia

was established.

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