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Original Research Article

Biochemical Response of Red Sokoto Bucks Experimentally Infected With *Trypanosoma congolense and Trypanosoma brucei* Treatment and Relapse

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Abstract: Biochemical responses of Red Sokoto bucks experimentally infected with Trypanosomes were studied. Twenty four bucks were divided into 4 groups of 6 animals each (I, II, III, and IV). Group 1 served as the uninfected control group, Group II and III were inoculated with 2 ml of 1 x10^6 *Trypanosoma brucei* and *T. congolense* respectively while Group IV were inoculated with 2 ml of 1x10^6 parasites each of *T. congolense* and *T. brucei*. The bucks in all the infected groups were treated on day 14 post patency with Isometamidium Chloride (2%) at the dose rate of 0.5 mg/kg IM. Serum enzymes (Aspartate amino transferase, Alanine amino transferase, Alanine amino phosphate) were all elevated. Urea and potassium levels were increased, while the levels of Na^+, Cl^- and HCO_3 were decreased. The elevated biochemical parameters were more pronounced during the infection and relapse phases in the entire infected group. This study concludes that during trypanosome infection phases, serum enzyme; urea and potassium were elevated while Na^+, Cl^- and HCO_3 ions were decreased.

Keywords: Biochemical Responses, Isometamidium Chloride, Red Sokoto Bucks, Trypanosomosis.

INTRODUCTION

Trypanosomosis is a complex debilitating and often fatal disease caused by infection with one or more of the pathogenic tsetse transmitted protozoan parasites of the genus *Trypanosoma* [1-4]. The most important species responsible for the disease complex commonly known as nagana" in livestock includes *Trypanosoma brucei brucei, T. congolense* and *T. vivax* [1]. The somatic migration and phenomenon of antigenic variation Coetzer, *et al.* [19] of Trypanosome organisms present difficulty in the diagnosis of Trypanosomosis in animals, easier and quicker methods of field diagnosis of the parasite such as those with specificity like a molecular technique (PCR), direct (parasitological) and indirect (serological) Diagnostic methods with varying degree of sensitivity and specificity [5] are needed.

This work was designed to study the biochemical response of Red sokoto bucks experimentally infected with *Trypanosome brucei* and *Trypanosoma congolense*, to treatment with Isometamidium Chloride. The extent of protection conferred by the drug on the animals was also evaluated from the pattern of biochemical responses before and after treatment.

MATERIALS AND METHODS

Experimental Goats

Twenty-four Red Sokoto bucks aged 8 months to 1 year weighing 12.10±1.11 kg were procured from Kafur in Katsina State. The bucks were stabilized for four weeks during which 20% oxytetracyline (Tetranor®) at 200mg/kg, parasiticidal drug (1% ivermectin at 10mg/50kg) and oral multivitamins (Vitalite®) were administered. The animals were confined in arthropod-proof pens in the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. The bucks were provided with water and feed *ad-libitum*. The bucks were randomly picked, tagged and Grouped into 4 (I, II, III and IV) of 6 goats each. Group I goats served as the uninfected untreated control Group, Group II and III goats were intravenously inoculated with *T. brucei* and *T. congolense* (1x10^6 parasites each) respectively, while Group IV goats were inoculated with a mixed infection of *T. brucei* and T. *congolense* (1x 10^6 parasites each). The number of trypanosomes per inoculum was estimated as described by Herbert and Lumsden [6].

***Trypanosoma* parasite and Preparation of Inoculum**

Trypanosoma congolense and *Trypanosoma brucei* used in this study were obtained from the National Institute for Trypanosomiasis and Ochocerciasis Research (NITOR), Vom, Nigeria. The parasites were initially multiplied in albino rats by inoculating each of the stabilates into albino rats and monitored for parasitaemia using the thin blood smear method and viewed with the light microscope. Two milliliters of infected blood containing about 2 x 10^4 trypanosomes were harvested from the albino rats via the corneal artery using capillary tubes and 1.0 ml each intravenously inoculated into two donor goats to further multiply the parasites and obtain sufficient inoculum for the experimental infection. The number of trypanosomes injected into the savannah brown goats was estimated as described by Herbert and Lumsden [6]. The two donor goats showed patent parasitaemia days 7 and 13 for *T. brucei* and *T. congolense* respectively.

Monitoring of parasitemia and other physical parameters

Parasitemia was estimated daily using wet mounts of the jugular blood prior to patency of parasitaemia, while parasite clearance following treatment was determined daily for four weeks using the haematocrit centrifuge technique [7].

Rectal temperature of each animal in each group was taken using a digital thermometer two times daily at 8 am and 6 pm and daily average temperatures were obtained from the data.

The goats were weighed on a weekly basis throughout the experimental period using a bathroom scale and mean weekly weight recorded.

Biochemical profile

For serum biochemical profiles, 5 mls of blood were collected from each of the goats via jugular venepuncture into plain labeled tubes. The blood was allowed to clot at room temperature and centrifuged at 1000 g for 5 minutes. The supernatant fluid (serum) was collected and stored at -20°C to assay for serum total protein (TP), liver enzymes such as aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), α-glutamyl transferase using Rietman-Frankel AST method as described by Cheesebrough [8].

The serum samples were again analyzed for sodium (Na+) and potassium (K+) using flame emission spectrometry [8]; chloride (Cl-) by the method of Schales and Schales [9] as outlined by Coles [10]; bicarbonate (HCO3-) by titrimetric method (Cheesebrough,1991) modified from Van Slyke (1956). Blood Urea Nitrogen was determined by the method of Jandrassik *et.al. (*1958) cited by Cheesebrough [8]; Glucose oxidase-peroxidase method [8].

The samples for serum biochemical profiles were taken weekly for three weeks before the inoculation of the parasite and then 2 days prior to experimental infection and thereafter thrice a week for two weeks post infection followed by another two weeks post treatment to evaluate the changes in the acute phase of the infection based on this, collections were made every other day for three weeks for base line data.

Infected animals (Groups II to IV) were administered a single dose of 0.5 mg/kg body weight of isometamidium chloride (2%) intramuscularly while Group I served as the uninfected untreated control.

Statistical analysis

The data generated were expressed as mean ± SEM. Analysis of variance (ANOVA) with Turkey"s Mutiple comparison post-hoc tests using Graph Pad Prism version 4.0 for windows (from Graph Pad software, San Diego, California, USA) was used to compare the level of significance between the test Groups. Values of $P< 0.05$ were considered significant.

RESULT

Table-1: Mean ±SEM of serum biochemical indices (mMols) of Red Sokoto Bucks infected with *Trypanosoma brucei, T. congolense* a mixed infection of *T. brucei* and *T. congolense* and treated with isometamidium.

Serum Biochemical Indices (mMols)	Control	*T. brucei*	*T. congolense*	*T. brucei*+*T. congolense*
Aspartate amino transferase				
Pre-infection	83.06±15.92	83.17±10.98	83.06±15.92	84.17±13.96
Post-infection	83.17±10.98	105.5±5.82	89.76±8.56	126.1±7.21
Post-therapy	88.92±5.88	89.75±8.56	63.33±12.54	71.83±12.43
Relapse	88.92±5.88	94.75±6.10	126.1±7.21	173.6±9.49
Alanine aminotransferase				
Pre-infection	20.6±2.97	21.08±3.22	22.17±2.24	20.83±1.34
Post-infection	20.8±1.82	49.67±18.44	65.58±10.71	83.25±16.98
Post-therapy	20.17±1.72	18.75±2.40	16.92±4.38	49.67±18.44
Relapse	20.17±1.72	39.67±17.44	65.58±10.71***	83.25±16.98***
Alkaline phosphatase				
Pre-infection	20.6±2.97	20.08±3.2	22.17±2.24	20.83±1.34
Post-infection	18.9±2.38	93.25±27.63	48.75±5.29	62.42±7.20
Post-therapy	20.08±3.2	33.92±12.09	105.8±17.78	273.3±91.46
Relapse	20.83±1.34	83.5±4.73	105.8±17.78	273.3±91.46***
Urea				
Pre-infection	6.17±2.37	5.99±1.15	5.88±1.28	5.02±0.25
Post-infection	5.79±0.29	6.8±0.79	6.8±0.79	6.9±0.95
Post-therapy	5.99±1.15	4.69±1.06	6.77±1.04	3.91±0.22
Relapse	5.99±1.15	4.69±1.06	6.77±1.04	5.88±1.28

*** = P<0.001

Table-2: Mean ±SEM of serum electrolytes (mMols) of Red Sokoto bucks infected with *Trypanosoma brucei, T. congolense*, a mixed infection of *T. brucei* and *T. congolense* and treated with isometamidium chloride.

Serum electrolytes (mMols)	**Uninfected control**	***T. brucei* infected Group**	***T.congolense* infected Group**	***T. brucei* +*T. congolense* infected Group**
Sodium ion (Na^+)				
Pre-infection	140.7±1.10	140.5±1.08	140.4±0.49	140.3±0.36
Post-infection	140.9±1.18	38.9±1.25**	139±1.27**	138.5±0.25**
Post-therapy	139.8±1.87	39.5±0.29**	138.5±0.41**	139.8±1.87**
Relapse	138.9±1.25	139.5±0.29	138.5±0.41	118.9±22.4**
Potassium ion (K^+)				
Pre-infection	4.52±0.49	4.86±0.62	4.96±0.43	4.96±0.43
Post-infection	4.22±0.08	4.48±0.14**	4.48±0.14**	4.75±0.11**
Post-therapy	4.13±0.11	4.58±0.35**	4.86±0.62**	4.36±0.23**
Relapse	4.13±0.11	4.58±0.35	4.22±0.08	3.26±0.26**
Chloride ion (Cl^-)				
Pre-infection	86.7±10.96	98.67±1.65	86.6±15.26	100.3±0.76
Post-infection	86.6±15.26	6.6±15.26**	86.6±15.26**	88.3±2.16**
Post-therapy	88.3±2.16	100.7±0.80**	100.3±0.76**	101.6±1.04**
Relapse	88.3±2.16	100.7±0.80	98.67±1.65	99.3±2.16
Bicarbonate ion (HCO_3)				
Pre-infection	24.5±0.85	24.42±0.65	24.42±0.56	24.42±0.55
Post-infection	24±0.93	3.33±0.71**	22.58±0.55**	19.55±3.22**
Post-therapy	24.67±0.63	26±1.16**	24±0.42**	22.7±0.60**
Relapse	24.67±0.63	26±1.16	23±0.52	22.58±0.55

** = P<0.001

DISCUSSION

The observed rise in AST (Aspartate aminotransferase) activity can be attributed partly to cellular damage caused by the trypanosomes lysis, while the increase in ALT (Alanine Aminotransferase) activity probably and have results from host destruction of trypanosomes [11]. Compared with the previous report of [12] in which ALP (Alanine Amino Phosphate), AST and LDH enzymes displayed increase during trypanosomosis.

The increase in level of K^+ in all the infected animals in this study agree with similar reports by Ikejani [13] and Zwemer and Culbertson [14] in *T. congolense* and *T. brucei* infected rats. RBC destruction and damaged tissues are said to be the sources of extra increase in K^+.However, the increase in K^+ corresponds with the fall in bicarbonate level which is the normal occurrence physiologically in mammals in case of metabolic acidosis due to increase in K^+ level [15].

Furthermore, the observed fall in the levels of Na+ and Cl- agrees with the known physiological observations that increase in plasma K^+ level is always accompanied with subsequent fall in plasma levels of Na+ ions due to some metabolic dysfunctions [16].

The Na+ decrease suggests its removal possibly by leaving the plasma partly intracellularly among other serum components. This movement is supported by the significant increase in K^+ in the plasma since any movement of Na+ intracellularly is accompanied by displacement of K^+ from intracellular compartment into the plasma. Also K^+ movement out of the cell may lead to the retention of Na+ intracellularly hence the observed fall in K^+ extracellular concentration.

Also the observed decrease in levels of Cl^- in all the infected animals in this work agrees with similar reports by Sackey [15] in *T. brucei* and *T. congolense* infected Savannah Brown goat but in contrast to high levels reported by Fiennes *et al.* [17]. However it is a general belief that a corresponding fall in Cl^- level always follows a fall in Na+ level which was observed in this study due to movement in the same gradient with Na+.

Interestingly, the decrease in HCO_3^- levels in this study is in contrast to the reported increase in its level in *T. brucei* infected WAD sheep by Ogunsanmi *et al.* [18]. HCO_3^- is physiologically said to move in opposite direction (gradient) to K^+ions (Breazile, 1971). As such any movement of K^+ions extracellular and hence increase in plasma level leads automatically to movement of HCO_3^- ions intracellularly and as such fall in the plasma level, a phenomenon observed in this study. Also the fall in plasma Na+ level together with the Cl^- especially during the acute phase of the infection expecially after 4 and 12days post infection and increase in K^+ suggest a level of metabolic acidosis especially in the cases of diarrhea which was a very prominent symptom in the infected goats in this study.

CONCLUSION

Depressed haematological and biochemical parameters were more observed during infective stages and the reappearance of parasite in all infected Groups. Also, the use of isometamidium chloride in mixed *T. brucei* and *T. congolense* infection infection did not give the desired therapeutic efficacy in Red Sokoto bucks. Therefore, further study is advocated on the efficacy of the drug in Goats.

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