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Effect of administration of Ibuprofen on the levels of parasitaemia, albumin and total protein concentration in rats infected with *Trypanosoma brucei brucei*

A. F. Sulaiman*, M. A. Akanji and M. T. Yakubu

Department of Biochemistry, University of Ilorin, Ilorin, Nigeria

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ABSTRACT: *Trypanosoma brucei brucei*-infected rats were administered intraperitoneally with ibuprofen at the dose of 0.02mg/kg body weight, thrice daily before infection (prophylaxis), three and ten days post infections (early and late stage respectively). Two sets of control groups were set up in parallel with the test groups viz: positive and negative controls. Parasitaemia was monitored on daily basis. The animals were sacrificed using diethyl ether, albumin (Grant et al., 1987) and the protein concentrations (Tietz, 1995) were determined in the liver, kidney, brain and serum using standard methods.

Albumin and total protein concentrations were significantly ($P < 0.05$) reduced in the tissues of the rats in the late stage compared to the positive control and treated groups while the concentrations of the biomolecules were significantly elevated ($P > 0.05$) in the serum of the late stage group compared to those of the positive control and treated groups. It was concluded that Ibuprofen could be a good candidate for the management of African sleeping sickness. Ibuprofen significantly ($P < 0.05$) reduces blood parasitaemia compare to the controls and extended the life span of the rats by 2, 4 and 7 days in the late, early and prophylactic stages respectively.

Keywords: Albumin, Trypanosomiasis, Total protein, Ibuprofen, *Trypanosoma brucei brucei*

Introduction

Trypanosomiasis refers to several diseases, in vertebrates, caused by parasitic protozoan trypanosomes. Two decades ago, more than sixty-six (66) million women, men and children in thirty-six (36) countries of sub-saharan Africa suffer from human African trypanosomiasis (WHO 1998). The other form of trypanosomiasis, called Chagas disease causes 21,000 deaths per year in Latin America (WHO 1998).

Recently however, only 9878 cases of the disease were reported in 2009, compared with almost 38 000 cases in 1998 and more than 17 600 in 2004. The number of new cases of human African trypanosomiasis reported to WHO has dropped to below 10 000 for the first time in 50 years (WHO 2011).

*Author to whom correspondence should be addressed.
E-mail: faoziyat20022002@yahoo.com
Tel: +234 8032127200

Ibuprofen belongs to a class of drugs called non-steroidal anti-inflammatory drugs (NSAIDs) (Chen *et al.*, 2005). The most common being Ibuprofen: (2-[4(2-methylpropyl) phenyl] propanoic acid). Other members of this class include Naproxen (Aleve), Indomethacin (Indocin), Nabumetone (Relafen) and several others. These drugs are used for the management of mild to moderate pain, fever, and inflammation. Prostaglandins are chemicals that are made by the body and are responsible for causing pain, fever and inflammation. Ibuprofen blocks the enzyme that makes prostaglandins, cyclooxygenase, (Hippisley-Cox and Coupland, 2005) resulting in reduction of prostaglandins. As a consequence, inflammation, pain and fever are reduced.

Kennedy *et al.*, 1990 suggested that ibuprofen attenuates lung injury from phosgene and that it does this by acting as an iron chelator, possibly preventing iron mediated oxidant generation and lipid peroxidation caused by phosgene. This research was therefore aimed at determining the concentrations of Albumin and Total protein concentrations in the tissues and serum of prophylactic, early and late stages ibuprofen-administered rats.

Materials and Methods

Trypanosoma brucei, *brucei* was obtained from Veterinary and Livestock Studies Department, Nigerian Institute for Trypanosome Research (VLS-NITR), Vom, Jos, Plateau State, Nigeria. **Ibuprofen** was a product of Dr. Meyers, Meyer Vitabiotics, Jersey.

Experimental animals

Sixty male Wistar rats weighing 200.89 ± 20 g were obtained from the small animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. They were acclimatized for 14 days before the commencement of the experiment and were fed with rat pellets (Bendel Feeds and Flour Mills Ltd; Ewu, Edo State) and clean water was provided ad libitum.

Assay kits and other reagents used were of analytical grade and were prepared using distilled water.

Inoculation of parasites

The tail of a *Trypanosoma*-infected rat was cleaned with distilled water soaked-cotton wool and the tip of the tail was cut with a clean pair of scissors. The blood was then extruded into 0.5ml of 0.9% normal saline. The extruded blood and saline were swirled thoroughly and the mixture drawn into 1 ml syringe. A drop of the solution was placed on a microscope slide and observed under the light microscope to ascertain the presence of the parasite. Inoculation into the peritoneal cavity of an uninfected rat was carried out when parasite suspension contained 3 or 4 *trypanosomes* per view at x100 magnification. The parasite was injected intraperitoneally into rats and maintained by repeated passaging into other rats. Parasite count was determined by counting the number of parasites per view under light microscope at x100 magnification.

Animal grouping and drug administration

The prophylactic treatment began 72 hours before the rats were infected, early stage treatment began the day parasite was sighted in the blood (3rd day post infection) while late stage treatment was administered 7 days post infection. The administered drug concentration was 0.02mg/Kg rat body weight.

Preparation of serum and tissue homogenates

Rats were anesthetized using cotton wool soaked in diethyl ether. Blood samples were collected using needle and syringe on cutting the jugular vein, the blood were then transferred into sample bottles containing EDTA. The blood was allowed to clot for 10 min at room temperature and then centrifuged using Laboratory centrifuge at 503 Xg for 5 mins. The serum was then separated from the clotted blood with a clean Pasteur pipette and stored frozen for 24 hours (Ogbu and Okechukwu, 2001). The tissues (liver, Kidney and Brain) were excised, cleansed of blood and superficial connective tissues and transferred into ice-cold 0.25 M sucrose solution.

1g of the liver, Kidney and Brain was cut with a sterile blade from the total weight of the liver and homogenized in ice cold 0.25 sucrose solution (1:5 w/v) using Teflon homogenizer. The homogenized tissue was diluted accordingly with 0.25M sucrose solution and then centrifuged at 56 Xg for 10 mins. The tissues' supernatants were freezed prior to analysis.

Determination of protein and albumin

Albumin and protein concentrations were determined using the procedure as described by Grant *et al.* (1987) and (Tietz, 1995) respectively.

Experimental Design

The experiment was divided into three phases of 3, 4 and 3 groups, with each group containing 5 rats each. 50 rats were used altogether for these experiments; Phase one has 3 groups (15 rats), Phase two has 4 groups (20 rats) and the third phase has 3 groups (15 rats) experimental groups each.

In the first phase, the first group, Group A (Positive control) contained rats that were neither treated nor infected, they serve as the positive control for the groups in this phase. Distilled water was administered daily to this group of rats. The second group, Group B (Negative control) were infected but not treated; while Group C were infected, treated with 0.02mg/kg rat body weight of Ibuprofen daily and parasitaemia were recorded daily until the death of the rats in each group.

The second phase: the first group contained rats that were neither treated nor infected, (positive control). Group two rats were used for prophylactic stage parasitaemia count while groups three and four rats were used for early and late stages parasitaemia count respectively.

Infected rats were treated at prophylactic, early and late stages of the infection with 0.02mg/kg rat body weight of Ibuprofen daily. Parasitaemia was monitored daily as the infection progressed.

The third phase, a positive and negative control groups were set up with a third infected group to which 0.02mg/kg rat body weight of Ibuprofen was administered intraperitoneally daily until day 10 when all the rats from each group were sacrificed and the blood and tissues were collected for the determination of total protein and albumin concentration.

Statistical Analysis

Data were expressed as mean $\pm$ standard error of mean (SEM) of five replicates. The data were subjected to statistical analysis using Analysis of variance (ANOVA) and complemented with Duncan Multiple Range Test (DMRT). Data were considered significant at $P < 0.05$.

Results and Discussion

The reduction in parasitaemia and life span extension as shown in Fig. 1 in the treated group (15 days) compared with the untreated but infected rats (8 days), showed that ibuprofen has the potentials of being used in the clinical management of trypanosomiasis. No parasite was seen in the blood of rats in the positive control group, as they were not infected.

Table 1: Total Protein Concentration (g/L) in *T. brucei* Infected Rats Treated with Ibuprofen

Treatment Groups	SERUM	KIDNEY	LIVER	BRAIN
Positive Control	22.96 $\pm$ 3.23 ^a	28.86 $\pm$ 2.22 ^a	23.27 $\pm$ 3.40 ^a	13.63 $\pm$ 2.11 ^a
Negative Control	71.39 $\pm$ 4.01 ^c	58.06 $\pm$ 3.23 ^c	43.63 $\pm$ 2.12 ^c	19.34 $\pm$ 1.21 ^b
Treated Group	38.54 $\pm$ 3.92 ^b	42.96 $\pm$ 2.31 ^b	39.60 $\pm$ 1.43 ^b	12.49 $\pm$ 2.32 ^a

Total protein concentrations as observed in *T. brucei* infected rats administered with (0.02mg/kg rat body weight) of Ibuprofen daily. Each value is an average count from 5 rats. Different superscripts denote concentrations significantly different from the positive control down the group.

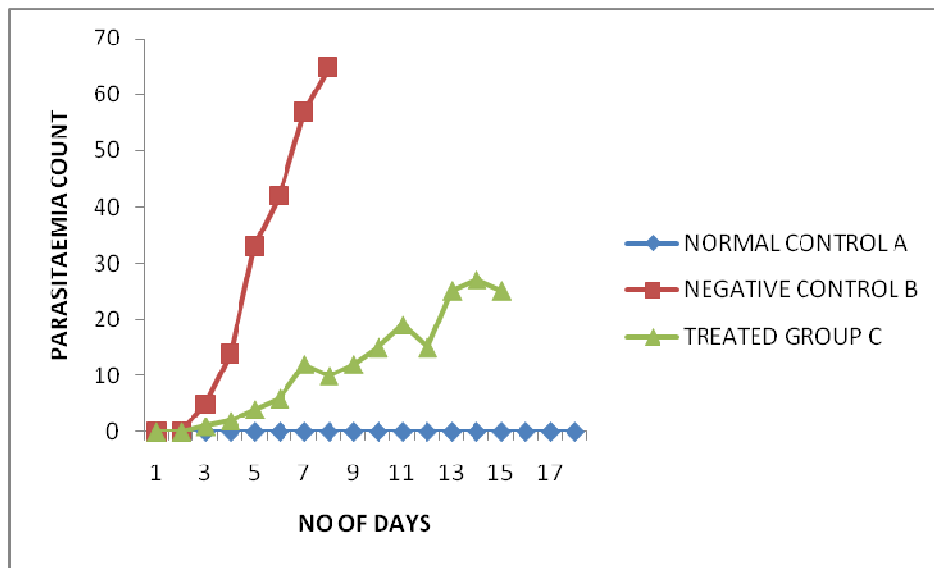


Figure 1: Treatment of *T. brucei* infected rats with Ibuprofen.
Each point is an average count from 5 rats.

The trend of life extension depicted in Figure 2 shows that ibuprofen if administered three days post infection, will not only lower the blood parasitemia level, but also extend life span of the rats by 7 days when compared with the late stage groups where treatment started 7 days post infection.

Ibuprofen is noted for its antipyretic, anti-inflammatory and analgesic properties (Chen et al., 2005). It has also been suggested that ibuprofen was able to attenuate lung injury from phosgene and that it does so by acting as an iron chelator, possibly preventing iron mediated oxidant generation and lipid peroxidation caused by phosgene (Kennedy et al., 1990).

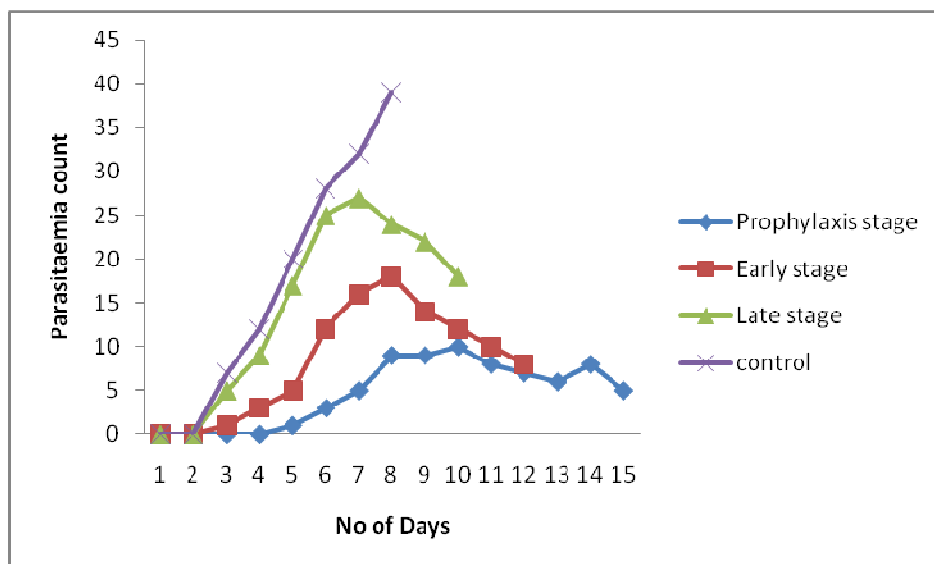


Figure 2: Life extension as observed in the Treatment of *T. brucei* infected rats with Ibuprofen. Each point is an average count from 5 rats.

Parasitaemia has been shown to correlate with severity of infection (Ekanem and Yusuf, 2008). The notable reduction in parasite level in the blood of the host may also not be unconnected with the fact that the drug may have

chelated or removed enough iron from the vicinity of trypanosomes' ribonucleotide reductase to reduce the proliferation of the parasites in hosts.

There was a significant ($p < 0.05$) increase in the total protein concentration (Table 1) in the serum and tissues of the negative control group compared to that of the positive control and treated groups, however, the total protein concentration in the brain of positive and treated groups were not significantly different.

The increase in serum total protein may have been due to increased release of tissue specific enzymes and other intracellular proteins secondary to parasite-induced cell membrane disruption (Pentreath and Kennedy, 2004; Orhue et al., 2005).

Release of erythrocytes-derived enzymes and proteins may be another possible source of plasma protein. Since haemolysis has been shown to be a common feature of *T. brucei* infection in mammals (Anosa and Kaneko, 1983; Anosa, 1988; Igbokwe and Mohammed, 1992; Egbe-Nwiyi and Antia, 1993; Igbokwe et al., 1994, 1998; Omotainse and Anosa, 1995). It is also likely that the increase in total protein may have been due to increased mass of parasite proteins as a result of increased parasitaemia or possibly increase in parasite derived intracellular enzymes and proteins as the parasites are lysed by the host immune system (Awobode, 2006).

Table 2: Albumin concentration (g/l) in *T. brucei* infected rats treated with Ibuprofen

Treatment Groups	Serum	Kidney	Liver	Brain
Positive Control	42.96±6.80 ^a	28.86±0.95 ^a	23.27±1.87 ^a	04.63±1.48 ^a
Negative Control	57.39±7.62 ^b	58.06±4.27 ^b	43.63±2.97 ^b	18.34±3.17 ^b
Treated Control	47.44±2.72 ^a	42.96±1.64 ^c	29.60±0.47 ^c	09.49±3.70 ^c

Albumin protein concentrations as observed in *T. brucei* infected rats administered with 0.02mg/kg rat body weight of Ibuprofen daily. Each value is an average count from 5 rats. Different superscripts denote concentrations significantly different from the positive control down the group.

Table 2 show similar trend as observed in the total protein concentration as there was a significant increase in the total albumin in the serum of infected rats (negative control) relative to their liver and brain. A significant reduction of the total albumin was observed in the kidney, liver and brain of Ibuprofen-treated rats, relative to the untreated (negative control) and positive control groups.

These changes in serum proteins and albumin accompanying infection with trypanosomes have been reported and reviewed in several studies (Frommel et al., 1988; January et al., 1991; Igbokwe and Mohammed, 1992; Ogunsanmi et al., 1994; Orhue et al., 2005; Ekanem and Yusuf, 2008).

The main biological functions of albumin are to maintain the water balance in serum and plasma and to transport and store a wide variety of ligands as fatty acids, calcium, bilirubin and hormones such as thyroxine. Albumin also provides an endogenous source of amino acids.

Hyperalbuminaemia has little diagnostic relevance except perhaps in dehydration while Hypoalbuminaemia is associated with such conditions as: analbuminaemia, impaired albumin synthesis in the liver, malnutrition, liver disease or malabsorption, generalized shock, burns or dermatitis; kidney disease and intestinal disease (Grant et al., 1987). Ibuprofen's major mechanism of action has to do with inhibition of cyclooxygenase (Young and Passmore, 1990). Prostaglandins are chemicals that are made by the body and are responsible for causing pain, fever and inflammation. Ibuprofen blocks the enzyme that makes prostaglandins (cyclooxygenase), resulting in lower levels of prostaglandins. As a consequence, inflammation, pain and fever are drastically reduced.

Pains, high temperature, inflammatory responses and a host of others are some of the symptoms associated with the disease, Trypanosomosis. A typical patient in a trypanosoma endemic area administered with Ibuprofen will most likely be cured or relieved of at least four of his symptoms or problems, depending on the various stages and extent of infection.

The analgesic properties in ibuprofen will most likely give instant relieve from joint pains and general body aches. Intermittent high body temperature and probably, high fever will be subdued by ibuprofen's antipyretic properties and all inflammatory responses subdued by its anti-inflammatory properties.

Earlier studies showed that trypanosomes release extra-cellular factors (Ekanem, 1989), which are believed to be toxic to the host. The death of treated animals even at low parasitaemia (Prophylactic treatment) could have been the

result of the toxic activities of these extra-cellular factors. More so when the extra-cellular factors have been reported to have pathological effects on host rats (Ekanem *et al.*, 1996).

Conclusion

The results of this study suggest that Ibuprofen could be useful in the clinical management of African sleeping sickness since it was able to reduce parasitaemia level in the infected and treated rats. The biochemical parameters measured were also significantly reduced when compared with infected but untreated rats. We also speculate that ibuprofen may have caused the depletion of parasitaemia in the infected and treated rats owing to their ability to form complex with heme iron, which is a by product of red blood cell lysis resulting from *trypanosoma* infection-induced erythrocyte membrane damage.

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