

Haematological Status of *T. brucei*-infected Rats Treated with Ibuprofen

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ABSTRACT

Available drugs in the management of African *Trypanosomiasis* are toxic, regimented and costly, generally non affordable by the poor and hence the need to continue research work in seeking inexpensive, readily available and less cumbersome approaches to the management and treatment of the disease. Ibuprofen belongs to a class of drugs called non-steroidal anti-inflammatory drugs (NSAIDs). It was chosen not only because it chelates iron, but also because it is cheap, readily affordable and available. In this study, *Trypanosoma brucei*-infected rats were treated with ibuprofen at three days before infection (prophylaxis), three days post infection (early stage) and ten days post infection (late stage). Three sets of controls were set up against the experiment: positive, negative and normal control. Haematological indices of the serum of each group of rats were obtained for day 6, 12 and 18. It was observed that the PCV and Hb levels were significantly lower in the late stage-rats compared to the normal control. Ibuprofen was able to extend the life span of the rats for 2, 4 and 7 days in late, early and prophylaxis stages respectively. Thus, ibuprofen could be a useful, cheap and readily available drug in the management of African sleeping sickness for residents of disease endemic areas.

keywords: *Trypanosomiasis*, Ibuprofen, Iron chelator and *T. brucei brucei*

INTRODUCTION

African Trypanosomiasis or sleeping sickness is a great scourge to man and domestic animals in Africa (Cox, 2004; Abenga and Lawal, 2005; Chretien and Smoak, 2005). Available drugs are toxic, regimented and costly, generally non affordable by the poor (Kioy and Mattock, 2005; Moore, 2005), which makes it imperative that researchers must continue to seek inexpensive, readily available and less cumbersome approaches to the management and treatment of the disease (Jannin and Cattand, 2004; Moore, 2005). Ibuprofen belongs to a class of drugs called non-steroidal anti-inflammatory drugs (NSAIDs) (Chen *et al*, 2005). Other members of this class include Naproxen (Aleve), Indomethacin (Indocid), 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 lower levels of prostaglandins. As a consequence, inflammation, pain and fever are reduced. The FDA approved ibuprofen in 1974.

Kennedy *et al*, 1990 established the fact that ibuprofen was able to attenuate 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

the phosgene.

The aim of the study was to use ibuprofen to chelate iron from the vicinity of trypanosomes' ribonucleotide reductase, thereby reducing or preventing the proliferation of the parasites. Ibuprofen has been shown to operate by an alternate mechanism of action, that is, to act as an iron chelator (Kennedy *et al*, 1990).

MATERIALS AND METHODS

Masterials

Trypanosoma brucei brucei strain was obtained from Veterinary and Livestock Studies Department, Nigerian Institute for Trypanosome Research (VLS-NITR), Vom, Jos, Plateau State, Nigeria. The parasite was injected intraperitoneally into rats and maintained by repeated passaging into other rats.

Ninety Wistar rats (*rattus norvergicus*) with an average weight of 260 ± 20 g were used for the experiment. The rats were obtained from the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria and were acclimatized for 14 days before the commencement of the experiment. The animals were maintained on standard animal feed (Bendel animal pellets) and clean water *ad libitum*.

Ibuprofen B.P. 400mg: 2-[4(2-methylpropyl) phenyl] propanoic acid, with Chemical Formula: $C_{13}H_{18}O_2$ was obtained from Ayo Pharmacy, Ilorin, kwara State. It is a product of Dr. Meyers, Meyer vitabiotics, Jersey. All haematological indices were monitored at the Unilorin teaching

span for 4 and 2 days, respectively. The haematological parameters for days 6, 12 and 18 are shown on **table 1-3**. The data's show varying but general decline in the Hb, PCV and WBC levels from days 6 to 18 and from treated to untreated.

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,

Table 1: Haematological indices for day 6

S/N	Rat Group-ings	Hb (g/dl)	PCV (%)	RBC ($\times 10^{12/l}$)	MCV (fl)	MCH (pg)	MCHC (g/dl)	WBC ($\times 10^9/l$)	Plate-lets ($\times 10^9/l$)	Neurophis (%)	Lympho-cytes(%)	Eosin
1	Normal control	12.35	42.0	6.95	61.0	18.5	30.0	17.25	449.5	14.5	87.0	06
2	Positive Control	13.65	45.5	7.29	62.5	18.5	30.0	29.70	639.5	20.5	79.5	07
3	Negative Control	11.90	39.9	5.78	69.5	18.5	26.5	11.90	417.0	20.0	82.5	08
4	Prophylatic Stage	12.45	43.0	7.34	63.0	20.0	28.0	20.30	38.8	15.7	82.6	09
5	Early Stage	12.65	39.9	6.69	59.0	19.0	32.0	11.15	619.5	45.0	49.5	08
6	Late Sage	11.80	39.9	6.81	58.0	17.5	30.0	8.90	571.0	10.5	84.5	03

DISCUSSION

Ibuprofen is noted for its antipyretic, anti-inflammatory and analgesic properties (Omudhome *et al.*, 2005; Omudhome, 2006). It is also an established fact that ibuprofen was able to

high fever will be subsided by ibuprofen's antipyretic properties while at the same time having all inflammatory responses looked into and taken care of by its anti-inflammatory properties. In addition to all these, even a patient diagnosed to

Table 2: Haematological indices for day 12

S/N	Rat Groupings	Hb (g/dl)	PCV (%)	RBC ($\times 10^{12/l}$)	MCV (fl)	MCH (pg)	MCH C (g/dl)	WBC ($\times 10^9/l$)	Platelets ($\times 10^9/l$)	Neurophis (%)	Lymphocytes (%)
1	Normal control	12.20	43.0	7.16	60.0	17.0	28	19.80	791	14	86.0
2	Positive Control	11.75	39.0	6.31	66.5	19.5	30	13.40	485	11	85.0
3	Negative Control	11.95	38.5	6.18	62.0	19.5	31.5	9.55	502	13	87.0
4	Prophylatic Stage	12.50	42.0	7.19	64.6	18.0	29.0	22.30	678	12	85.7
5	Early Stage	11.55	38.5	6.155	63.0	19.5	30.5	7.80	542	29.5	69.5
6	Late Sage	11.45	36.0	6.148	62.0	19.5	31.5	8.75	656	06	94.0

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).

The reduction in parasitaemia and life extension as observed in early and prophylactic treatment (16 and 19 days respectively) compared with the untreated but infected rats (12 days), as observed in Figure 1 shows that ibuprofen has the potentials of being used in the clinical management of trypanosomiasis.

Trypanosomiasis is usually accompanied with such symptoms as pains, high temperature, inflammatory responses and a host of others. 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

be at the late stage of the infection could hope for life extension, even if it is just for a day.

How does ibuprofen achieve this? Its major mechanism of action has to do with inhibiting an enzyme called cyclo-oxygenase (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 catalyzes the synthesis of prostaglandins (cyclo-oxygenase), resulting in lower amounts of prostaglandins. As a consequence, inflammation, pain and fever are drastically reduced.

The haematological parameters in Tables 1-3 did not exactly give a definite pattern but generally they show that the Hb, PCV and WBC are significantly lower in late stages of infection compared to the early stages of infection. These show

Ibuprofen B.P. 400mg: 2-[4(2-methylpropyl) phenyl] propanoic acid, with Chemical Formula: $C_{13}H_{18}O_2$ was obtained from Ayo Pharmacy, Ilorin, kwara State. It is a product of Dr. Meyers, Meyer vitabiotics, Jersey. All haematological indices were monitored at the Unilorin teaching hospital laboratory.

Inneculation of parasite

The tail of an infected rat was cleaned and the tip of the tail was cut with a clean pair of scissors. Then the blood was extruded into about 0.5ml of 0.9% normal saline. The extruded blood and saline was swirled to mix and drawn into a 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 in the solution. 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.

Experimental design

Six groups of 15 rats each were used.

Group 1(A): 15 rats Normal rats, neither treated nor infected was kept as control for all the groups. Distilled water was administered to them daily till the end of the experiment to serve as placebo.

Group 2(B): 15 rats were treated not infected (positive control)

Group 3(C): 15 rats were not treated but infected (negative control)

Group 4(D): 15 rats were used for prophylactic stage parasitaemia count.

Group 5(E): 15 rats were used for early stage parasitaemia count.

Group 6(F): 15 rats were used for late stage parasitaemia count

Ibuprofen (40mg/kg body weight) were adminis-

tered intraperitoneally daily to infected rats and parasitaemia were recorded daily until days 6, 9, 12, 15 and 18 when three rats taken from each group are sacrificed for enzyme analysis and monitoring of the blood's haematological indices: (PCV, RBC, WBC, HB, MCV, MCH, MCHC, Platelet, Neutrophils Lymphocytes and Eosin) in all the six groups.

On days 6, 9, 12, 15 and 18, 3 rats from each group were anaesthetized using cotton wool soaked in chloroform. Blood samples were collected by cardiac puncture using needle and syringe from where they were transferred into EDTA-containing tubes. This is to prevent the blood from clotting.

Determination of haematological indices Wintrobe's microhaematocrit, improved Neubauer haematocytometer and Sahli's methods

On days 6, 9, 12, 15 and 18, blood was collected from the rats. Haemoglobin (Hb), Packed cell volume (PCV), Red blood cell (RBC), White blood cell (WBC) were determined using Wintrobe's microhaematocrit (John and Wintrobe, 2008 and John *et al*; 2009), improved Neubauer haematocytometer (Sukcharoen *et al*; 1994) and Sahli's methods (Hermann Sahli, 1994) respectively.

Differential count with Wright's Stain

The differential WBC counts were obtained by making a differential count with Wright's stain and percentage count taken for lymphocyte, eosinophils and neutrophils.

RESULTS

As shown in figure 1 the infected rats administered with ibuprofen (40mg/kg body weight) intraperitoneally demonstrated that prophylactic treatment extended the life span by 7 days while early and late stage treatments extended the life

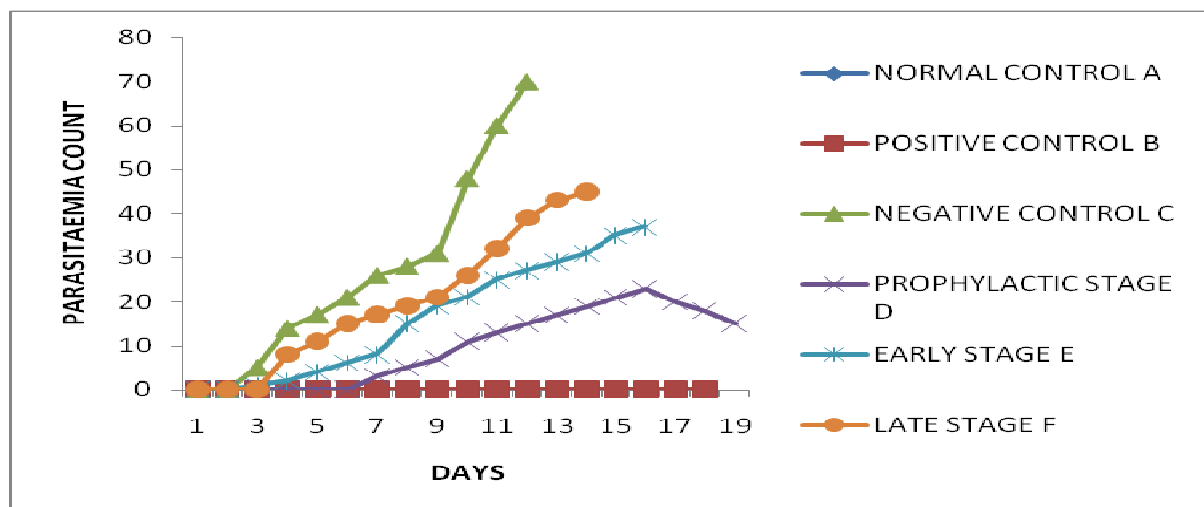


Fig. 1: The Parasitaemia of ibuprofen-treated *T. brucei* infected rats

nificantly lower in late stages of infection compared to the early stages of infection. These show and confirm the gradual decline of the patients towards anaemia which usually characterize the late stages of infection. The same trend was also observed when Hb, PCV and WBC levels were compared between infected-treated rats and infected-untreated rats.

This observation may be related to the decline in iron level as the Hb and PCV reduces with infection. The notable reduction in parasite level in the blood of the host may not be unconnected with the treatment of the rats with ibuprofen and 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.

Ibuprofen is readily available, cheap and non toxic, even though it has not succeeded in totally curing African Trypanosomiasis at this dose we suggest that ibuprofen at varying doses (below its

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Table 3: Haematological indices for day 18

S/N	Rat Grouping	Hb (g/dl)	PCV (%)	RBC(x 10 ¹² /L)	MC V(fl)	MCH (pg)	MCHC (g/dl)	WBC (x10 ⁹ /L)	Platelets (x10 ⁹ /L)	Neutrophil (%)	Lymphocytes(%)
1	Normal control	11.3	38.0	6.30	69.0	19	30.0	12.1	451	10	90
2	Positive Control	12.4	38.0	6.35	60.0	20	33.0	219	679	07	93
3	Negative Control	11.1	36.5	5.61	65.5	20	30.5	9.4	646	14	86
4	Prophylatic Stage	11.6	37.0	6.35	58.0	18	32.0	11.7	678	33	67
5	Early Stage	11.1	35.0	6.29	56.0	18	32.0	18.0	527	05	95
6	Late Sage	10.3	37.0	5.87	63.0	18	28.0	13.7	556	14	86

LD50) could be used in the management of the disease in an endemic area.

Conclusion.

At the end of this research work, we concluded that ibuprofen could be a useful, cheap and readily available drug in the management of African sleeping sickness for residents of disease endemic areas.

We therefore recommend the use of ibuprofen in the management of the disease, trypanosomiasis which has become a scourge to man and his domestic animals. The drug is readily available and affordable to the less privileged.

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