Efficacy of amprolium on laboratory rats infected with Trypanosoma brucei brucei

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African trypanosomiasis is fatal without proper treatment. The potential antiparasitic and immunomodulatory effect of Amprolium against Trypanosomabruceibrucei on Albino rats was investigated. Amprolium a structural analogue of thiamine (vitamin B1) has a thiamine inhibition mechanism. The study involved five groups of rats (with five rats each). Four groups were infected intraperitoneally with T. bruceibrucei. Groups III, IV, and V were treated by oral administration of Amprolium solution according to body weight. The other two groups serve as positive and negative controls. Various pathological and haematological data like Parasitaemia, Packed Cell Volume (PCV), Temperature and Weight changes were examined. Statistical analysis of ANOVA showed that the drug caused an insignificant decrease in parasitaemia and maintained a fairly significant body weight in infected animals in comparison to untreated ones. The effect was most prominent when Amprolium was administered simultaneously with T. bruceibrucei inoculation. The Packed Cell Volume however decreased throughout the experimental period (treatment not withstanding) in all the animals. As the experiment progressed a marked trypanocidal effect was observed as most of the blood trypanosomes were killed and a few very sluggish in movement. This observation was made in animals of Group IV and V which survived up to 15 days post-inoculation in comparison to the untreated. This data suggests that the anti-thiamine capability of Amprolium may be a promising therapy for T. brucei infection.

Key words: Amprolium, trypanosomiasis, Trypanosoma brucei brucei.

INTRODUCTION

Today, there are only a handful of active drugs available for treatment of Human and Animal African trypanosomiasis. The current line of treatment is problematic for many reasons: firstly, the drugs are expensive, harmfully toxic requiring extensive hospitalization (WHO, 1995). Also there arose the challenge of drug resistant on the part of the trypanosome. Treatment depends on the stage of infection. Treatment of the haemolymphatic stage is based on pentamidine and suramin. Melarsoprol, is the only treatment option available for late stage sleeping sickness (Bouteille et al., 2003). Although the most recent and effective drug against sleeping sickness, eflornithine is not widely available, it is difficult to administer, and costly for use under African health care conditions (Bouteille et al., 2003).

Trypanosomiasis is a fatal protozoan disease caused by an obligate haemolytic parasite – Trypanosome, which lives in the blood and tissue of the human or animal host (Stevens and Brisse, 2004). Several species of trypanosomes occur as parasites in a wide variety of animals and humans causing diseases in them. The African disease producing trypanosomes are parasites which spend part of their life cycle in the tissue of a mammalian host and part in the body of an insect vector Glossina sp. or the tsetse fly (Lynnes and David, 1993; Jerry, 2014). The tsetse fly transmitted trypanosomes cause sleeping sickness in humans and nagana (animal trypanosomiasis) in domestic animals in Africa. Depending on the species of trypanosome, the parasites
multiply largely in the blood of the mammalian host but may invade extravascular sites (lymphatics, connective tissue, central nervous system) (Michael and Turner, 1998; Roberts and Janovu, 2002). Trypanosomiasis of man and his livestock is an important public health and economic problem that has continued to devastate several parts of Sub-Saharan Africa as well as parts of South America (Sachs, 2010). The disease has been known for over a century and has had major bad effect on domestic animals as well as humans (Rocha et al., 2004). Every year it causes major losses on the economic, agricultural, and medical sectors of many countries most of which are in Africa (Lejon et al., 2002; Sachs, 2010). Trypanosomiasis has been reported in West and Central Africa (caused by Trypanosoma brucei gambiense), and in East and southern Africa (caused by Trypanosome brucei rhodesiense). Over a decade ago, there arose a dramatic resurgence of Gambian trypanosomiasis in Central Africa, especially in the Democratic Republic of Congo, Angola, Sudan (Pepin and Media, 2004; Moloo, 1993; Simarro et al., 2003). T. brucei rhodesiense on the other hand causes virulent diseases in animals known as Nagana (WHO, 2008; Jerry, 2014). Most cases of trypanosomiasis are chronic, but acute disease which may be fatal within a week, can also occur. Common clinical features of African trypanosomiasis occur in two distinct phases; the early stage, and the late stage (Taylor and Authie, 2004). In the early stage symptoms such as bouts of fever, headaches, joint pains, muscle aches, enlargement of lymph glands, Oedema and itching are observed (WHO, 2006). In the later stage more obvious signs and symptoms of the disease appear and they includes changes of behaviour, anaemia, confusion, mental disorders, loss of weight, insomnia, sensory disturbances and poor coordination, disturbance of sleep cycle, which gives the disease its name (Legros et al., 2002; CDC, 2010). The primary clinical signs observed in African Animal Trypanosomiasis (AAT) are anaemia. Followed by a pronounced decrease in packed cell volume (PVC), hemoglobin, red blood cell, and white blood cell levels, intermittent fever, loss of condition, lymphadenopathy (WHO, 2006). Milk yield may be decreased in dairy animals, cardiac lesions, diarrhea, keratitis, laceration, appetite loss and other clinical signs have also been reported (Seed and Hall, 1985).

Thus, there is an urgent need for novel, safe, rapidly-acting and inexpensive agents for the treatment of both human and animal African trypanosomiasis (WHO, 2004). Amprolium is a coccidiostat (antiprotozoal) used for the treatment and prevention of coccidiosis in calves, goats, sheep, chickens and turkeys. It is a thiamine (vitamin B1) antagonist, its pharmacological effect relies on competitive inhibition of thiamine uptake (Susan et al., 2006). Trypanosomes depends to a greater extent on the host’s supplies of carbohydrates, proteins, lipids, and some micronutrients. Thiamine is present in cells in the form of thiamine triphosphate (TTP), thiamine diphosphate (TDP), thiamine monophosphate (TMP) and free thiamine (Makarchikov et al., 2003). Some of the micronutrients which trypanosome may take up from exogenous sources are P-aminobenzoic acid, vitamins such as thiamine, folic acid, riboflavin, cobalamin, ascorbic acid and nicotinamide (Igbokwe, 1995). By blocking uptake it prevents carbohydrate synthesis (Sarett, 1960; Rindi et al., 1966). In this study, Amprolium which inhibits thiamine absorption in the system is hereby examined on its effect on experimental trypanosomiasis, using T. brucei rhodesiense as a case study. The thiamine requirement of T. brucei rhodesiense within the mammalian host is also evaluated.

MATERIALS AND METHODS

Study area

The research work was carried out at the Parasitology Division of the Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Nigeria. The experimental Animals (Albino Rats) were housed at the animal house of the Institute.

Animals and Parasites

Albino rats and Trypanosoma brucei. were obtained from the animal house and experimental room respectively of the Parasitology Division of NITR. The animals were kept in net cages with wood shavings on the floor of the cage to serve as bedding. Proper hygienic measures such as cleaning of the animal house, regular changing of the bedding, washing of the drinker bottles was maintained throughout the period of the experiment..

Diet (feeding) of experimental animals

The animals were fed adequately with rations of pelletized feed (grower marsh). Water was given to them in drinker bottles.

Preparation of Amprolium solution

Fresh oral solutions of Amprolium were prepared in plain bottles on daily basis. Adequate amount of clean water was used in the preparation and the animals were dosed according to their body weight.

Pre-experimental examination (screening) of experimental animals

Pre-experimental screening of the albino rats were done prior to the commencement of the experiment. The initial Packed Cell Volume, weight, and temperature of the rats were taken. These were done to provide data that would
Table 1. Grouping, infection, and treatment of experimental animals.

<table>
<thead>
<tr>
<th>EXPERIMENTAL GROUP</th>
<th>INFECTION</th>
<th>TREATMENT</th>
<th>NUMBER OF EXPERIMENTAL ANIMALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Not Infected</td>
<td>Not Treated (Negative control)</td>
<td>5</td>
</tr>
<tr>
<td>Group II</td>
<td>Infected</td>
<td>Not Treated (Positive control)</td>
<td>5</td>
</tr>
<tr>
<td>Group III</td>
<td>Infected</td>
<td>Pre-Inoculation treatment (Prophylaxis); Starts 7 days before Inoculation.</td>
<td>5</td>
</tr>
<tr>
<td>Group IV</td>
<td>Infected</td>
<td>Post-Inoculation treatment (Chemotherapy); Starts at patency.</td>
<td>5</td>
</tr>
<tr>
<td>Group V</td>
<td>Infected</td>
<td>Treatment starts immediately after inoculation.</td>
<td>5</td>
</tr>
</tbody>
</table>

The infection was done with Trypanosoma brucei brucei, and treatment with Amprolium solution.

Experimental design

A total of twenty five (25) Trypanosome-free Albino rats were used. The rats were kept in five (5) groups of five (5) rats each.

Experimental procedure

Baseline data of the experimental parameters were obtained on the first day of the experiment before inoculation of the trypanosomes into the animals. Data investigated throughout the experiment were the Packed Cell Volume (PCV), Parasitaemia, Body weight, and Body (Rectal) temperature of the animals. The animals were kept in five labeled cages of five rats each. After grouping, labeling and numbering, the animals were left to acclimatize for one week prior to infection and treatment.

On the first day of the experiment (day 1), animals in groups II, IV, and V were inoculated. Those of group III were only treated without inoculation, and were done so for seven (7) days after which they were then inoculated. Treatment started immediately for rats in group V and lasted for five (5) days. Animals in group IV however did not receive treatment until day 3 post-inoculation when parasitaemia came up (at patency). Group II animals were used as the positive control; they were inoculated on day one and were not treated at all. These (group II) served as a model to observe the full effect (signs and symptoms) of Trypanosoma brucei brucei infection on Laboratory rats. These symptoms served as a guideline in understanding the effectiveness of the test drug-Amprolium on trypanosomes. This was done by comparing the experimental data and clinical sings of the treated and untreated groups. Parasitemia, weight, and temperature of the experimental animals were taken on daily basis starting on day 2 post inoculation. The Packed Cell Volume (PCV) was taken at three (3) days interval throughout the experiment. Observational studies of each of the groups were conducted at different stages of the experiment.

Results

All groups were well and hearty pre-inoculation periods up till day 4 post – inoculation (when parasitaemia came up). The animals began showing signs of restlessness, loss of appetite, dullness, paleness and loss of condition. This unhealthy condition progressed in groups II, IV, V through day 5 to day 8 post inoculations. In group III none of the above signs was observed except that the animals became less active as the treatment progressed. However starting from day 10 of the experiment (3 days post-inoculation of Group III), the afore mentioned signs started to manifest and progressed slowly and steadily.

Infection of Albino rats with T. brucei brucei manifested after a pre-patent period of 3-4 days. All infected animals developed parasitaemia that was detected by wet blood film examination. Parasitaemia level varied in the different groups. An irregular rise and fall in parasitaemia was observed which in most of the cases led to the death of all infected animal. The parasite number increased as the experiment progressed. Pre-infection Packed Cell Volume (PCV) of the experimental animals ranged from 46.00% to 50.20%. The PCV of the untreated control (Group II) decreased gradually and consistently down to 35.5% prior to the death of the animals on day 9 post inoculation. Meanwhile PCV of Groups III, IV and V recorded similar pattern of steady decrease. However there was a gradual and consistent increase in the PCV of the uninfected control, and this lasted throughout the period of the experiment. An increase in the rectal temperature of all infected animals was observed at the onset of parasitaemia. A pre-patent temperature range of 35.80° to 37.20° was recorded on day 1 of the experiment before the infection of the animals. However in all the infected groups, there was a significant (P>0.05)
Table 2. Anova Table of the Parasitaemia ($X \times 10^6$) of T. brucei infected Albino rats treated with Amprolium.

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>427540.861</td>
<td>3</td>
<td>142513.62</td>
<td>1.152</td>
</tr>
<tr>
<td>Within Groups</td>
<td>7425272.354</td>
<td>60</td>
<td>123754.539</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7852813.215</td>
<td>63</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chart 1: Parasitaemia ($X \times 10^6$) of T. brucei infected Albino rats treated with Amprolium.

Table 3. Anova Table of the effect of Amprolium treatment on the Packed Cell Volume (PCV) % of T. brucei infected Albino rats

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>2064.075</td>
<td>4</td>
<td>516.019</td>
<td>1.717</td>
</tr>
<tr>
<td>Within Groups</td>
<td>7515.072</td>
<td>25</td>
<td>300.603</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9579.148</td>
<td>29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chart 2. PCV of T. brucei infected Albino rats treated with Amprolium.
Table 4. Anova Table of the effect of Amprolium treatment on rectal temperature (°C) of T. brucei infected Albino rats.

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>3251.563</td>
<td>4</td>
<td>812.891</td>
<td>4.58</td>
</tr>
<tr>
<td>Within Groups</td>
<td>13310.577</td>
<td>75</td>
<td>177.474</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16562.139</td>
<td>79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Chart 3. Effect of Amprolium treatedment on rectal temperature (0°C) of T. brucei infected Albino rats.

Table 5. Anova Table of the effect of Amprolium treatment on Body weight (g) of T. brucei infected Albino rats.

<table>
<thead>
<tr>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>24492.561</td>
<td>4</td>
<td>6123.14</td>
<td>9.479</td>
</tr>
<tr>
<td>Within Groups</td>
<td>48447.145</td>
<td>75</td>
<td>645.962</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>72939.706</td>
<td>79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

decrease in temperature prior to the death of each of the animals. The uninfected control (Group I) had a progressive weight gain throughout the period of the experiment. The weight changes in the untreated control (Group II) seemed less prominent with the weight values fluctuating within a small margin (47.56g to 54.25g). Group III, IV, and V had a fluctuating weight gain and loss throughout the experiment.

**DISCUSSION**

**General signs and symptoms**

The results of this study indicate that Amprolium was able to produce useful effects in reducing the effects of T. brucei brucei on Albino rats, and prolonging the life span of the infected animals. The pre-patent period recorded for the trypanosome to come up was in line with the parasite's known incubation period of 5-10 days (Mare, 2000). The animals manifested a chronic to acute form of trypanosomiasis which was characterized by continuous presence of trypanosome in the blood. T. brucei infected rats died of the infection when parasitaemia build up to unprecedented levels. This high level of parasitaemia of course overwhelmed the host. Clinical and pathological manifestation of anemia, emaciation, fever, reduced food and water intake, weight loss and severe illness that led to death as observed were typical of T. brucei brucei infection (Taylor and Authie, 2004).

However with Amprolium treatment of the animals, a marked reduction of these symptoms was observed which was very prominent when treatment with Amprolium commenced simultaneously with trypanosome inoculation. This resulted in an increase in the survival time among the treated infected animals. It may be said
that anti-thiamine action of Amprolium led to obstruction of the parasites metabolism and reproduction, thus contributing to a reduction of blood parasites and the harmful pathogenic effects of T. brucei brucei. Amprolium acts as a thiamine analogue, successfully inhibiting the absorption of thiamine in the animals’ system. Thiamine on the other hand is known to be very useful in glucose metabolism. All organisms use thiamine in their biochemistry (Igbokwe, 1995). An observational suggestion by Baker et al., (1999) revealed an up-regulation of glycolysis in T. brucei brucei infected mice, which is likely a result of parasites in the blood streams. Previous research has shown that T. brucei depends on glycolysis for its supply of ATP (Baker et al., 1999). The reason for the increase in survival time among the infected animals is however not very clear but is thought to be related to the anti-thiamine activity of Amprolium on the animals, which however interferes with the availability of blood glucose to the trypanosomes.

Parasitaemia

The initial increase and subsequent fluctuations in the level of parasitaemia further points to unconducive breeding and survival environment for the parasites. The results obtained in the study showed that Amprolium have the ability to disrupt the glucose synthesis of the trypanosomes. This is indicated by the rise and fall of the parasite in the blood, death (non motility) of most of the trypanosomes and less activeness (slow movements) of a few of them. This goes in agreement with the work of Ira Singer (1961), who deduced that the thiamine concentration in the blood of T. brucei infected rats were increased by threefold which could be related to the rise in parasitaemia. We can therefore say that the significant reduction in the level of parasitaemia (at some points) among the long surviving animals, compared with the untreated control goes to show the relationship between parasitaemia and survival time. It is obvious therefore that the drug may be producing its effects by reducing parasite load in infected animals.

Packed Cell Volume (PVC)

One of the most prominent features of Animal trypanosomiasis is anemia (CDC, 2010). African trypanosomiasis is accompanied by a severe drop in Packed Cell Volume and Red blood cell counts due to erythrocyte destruction (Akol et al., 1986). Also (Jenkins et al., 1980) reported that T. brucei brucei is a tissue parasite which induces anemia in infected rabbits, as in other susceptible animals such as cattle, dogs, rats and mice. Throughout the period of this experiment, the mean PCV values of all infected animals progressively decreased till death. This is in accordance with the work of Igbokwe and Mohammed (1992). Treatment with Amprolium appears to have an insignificant effect in maintaining the PCV of the experimental animals. This was indicated from the steady and progressive decrease in the PCV of all the animals (treated or not) as the experiment progressed. This study thus revealed that in T. brucei brucei infection, anemia cannot be controlled by a thiamine analogue.

Temperature and weight

Starting from the onset of parasitaemia, the temperatures of the infected animals increased significantly. Fever as one of the known signs of Animal trypanosomiasis was thus evident. The mean temperature of all the infected animals whether treated or not was high as the infection progressed. Treatment with Amprolium do however had some maintenance or sustenance effects on the weight of the animals. They seemed to have weight increase
(though slightly) through a greater part of the experiment. This wasn’t in accordance with the typical signs of weight loss and emaciation associated with African trypanosomiasis (Mann et al., 2009). This weight maintenance ability of the treatment however was not sustained as the animals started losing weight two days to their death. We may say that it was as a result of the overwhelming effects of the parasites towards the terminal stage of the infection, which was likely to cause tissue and organs damages. This knowledge is made more confirmatory from the work of Nwaorgu and Iwuala (1981) who reported histopathologic changes after T. brucei infection manifesting as lesions in various tissues and organs, anemia, emaciation, and eventually death.

The result of this experiment has further highlighted the pathological effects of T. brucei brucei on mammalian host (Albino rats). More importantly, the trypanocidal capability of Amprolium has undoubtedly been tested and the drug has been found to be effective by prolonging the survival time of the trypanosome infected rats; though not necessarily curing the infection. Also the drugs ability to cause massive mortality of the blood parasites is a quality worthy of note. The mechanism by which Amprolium exhibits this trypanocidal action was not determined. However the drug is more likely to be producing its effects through its anti-thiamine action as explained earlier. The initial increase and fluctuation in the level of parasitaemia could also be attributed to the Variant surface glycoprotein (VSG) coat of the parasite which later makes them become elusive to host immune system and chemotherapy (Vickermann, 1985).

CONCLUSION

The study concludes that conclude therefore that Amprolium can significantly reduce parasitaemia load, weight loss and emaciation in T. brucei brucei infected Albino rats. The rise and fall in parasitaemia and weight charts are a clear indication to such facts. The outcome of the study has also indicated that T. brucei brucei can cause severe infection in animals and subsequent death unless effectively treated. Meanwhile the best effective use of Amprolium was obtained when the drug was given simultaneously with the trypanosome inoculation. This trypanocidal ability of Amprolium led to a reduction of blood parasites thus prolonging the survival time of the infected animals.

REFERENCES


