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Full Length Research Paper

The Therapeutic Efficacy of Artesunate and Diminazene in the Treatment of Experimental *Trypanosoma brucei brucei* Infection in Rats

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ABSTRACT

Seventy healthy adult albino rats of both sexes weighing 150 – 200g were used to investigate the therapeutic efficacy of artesunate (Rekmal(R)) alone, diminaze neaceturate (Berenil®) alone and a combination of both drugs in the treatment of experimental *Tyrpanosoma brucei brucei* infection. The rats were separated into 7 groups (A-G) of 10 rats each. Groups A and B served as the uninfected untreated and infected untreated controls respectively, while groups C and D were infected and treated with 2.4mg/kg and 4.8mg/kg of artesunate (Rekmal(R)) respectively. Group E rats were infected and treated with 2.4mg/kg of artesunate and 3.5mg/kg of diminazene aceturate, while rats in group F were infected and treated with single dose, 7.0mg/kg of diminazen eaceturate. Infected group G rats were treated with 7.0mg/kg of diminazene aceturate and 4.8mg/kg of artesunate. Parasitaemia was established in all the infected groups after 4 days and treatment was instituted on day 12 post-infection. Artesunate at 2.4- 4.8mg/kg suppressed the level of parasitaemia but could not clear the parasites from the blood or prolong the life of the rats. Anaemia recorded was most severe in the infected untreated rats. There was relapse of infection in one rat each in groups F and G showing 90% treatment success.In conclusion, artesunate at 2.4 – 4.8mg/kg is less effective in treating *T.b. brucei* infection in rats. Diminazeneaceturate at 7.0mg/kg or its combination with artesunate at 4.8mg/kg achieved 90% success.

Keywords: Artesunate; diminazene aceturate; *Trypanosoma brucei brucei;* rats.

INTRODUCTION

Trypanosomosis affects man, domestic and wild animals (Radostits et al, 1994). Human trypanosomosis (sleeping sickness) is caused by *Trypanosomabrucei* gambiense and *T. brucei* rhodesiense, while African animal trypanosomosis is caused by *T. bruceibrucei*, *T. vivax*, *T. congolense*, *T. evansi* and host of others (Losos, 1986; Onyeyili and Egwu, 1995). Trypanosomes are commonly transmitted biologically by tsetse flies (*Glossina*spp) but Ikede and Losos (1972) reported few cases of mechanical and transplacental transmission.

*Trypanosoma b. brucei*is a tissue invasive species which causes orchitis in males, infertility in females, poor growth and milk production, emaciation, anaemia and death if the disease is not arrested early (Radostits et al., 1994).

Diminazene aceturate (Berenil(R)) is a drug of choice for the treatment of animal trypanosomosis due to its high therapeutic index and low incidence of drug resistance (Williamson, 1976; Aliu, 2007). But cases of relapse of infection have been reported in animals infected and treated with different doses of diminazeneaceturate (Losos,1986;Onyeyili and Onwualu, 1991, Aliu, 2007).

Artesunate (Rekmal(R)) is a strong anti-malarial agent recommended for treating chloroquine-resistant

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Plasmodium falciparum malaria. Some anti-malarial agents such as Chloroquine, Metakelfin® and Camoquine® have been reported to be less effective in treating trypanosomal infections in rats (Onyeyili et al., 1994; Egbe-Nwiyi et al, 2005; 2011). Trypanosomosis and plasmodosis are both protozoan parasites which affect man (Losos 1986; Sweetman 2002;Aliu, 2007).
The objective of this study was to investigate the therapeutic efficacy of artesunate (Rekmal$^{(R)}$) alone and its combination with diminazeneaceturate in the treatment of experimental *Trypanosoma brucei brucei* infection of rats.

MATERIALS AND METHODS

Experimental Animals:

Seventy healthy adult albino rats of both sexes weighing 150-200g obtained from the National Veterinary Research Institute (NVRI) VOM, Plateau state, Nigeria were used. They were maintained on a standard laboratory diet (Vital feeds LTD, Jos) and housed in clean plastic cages at room temperature (30-35^{o}C). Water was provided *ad libitum*. The rats were screened for haemoparasites before the commencement of the experiment (Jain, 1986).

Trypanosomes:

Trypanosomabruceibrucei, field isolates from slaughtered pig at Nsukka abattoir in 2010 were used. The parasites were maintained by serial passages in rats. One milliliter of phosphate buffered saline solution (pH 7.4) diluted infected blood from donor rats containing approximately $1x10^6$ trypanosomes was intraperitoneally injected into each rat in groups B-G.

Test Drugs:

Artesunate (Rekmal$^{(R)}$, Lincoln Pharmaceuticals LTD, India) and diminazeneaceturate (Berenil$^{(R)}$, Hoechst, Germany) were prepared for administration following manufacturers' specifications.

Experimental design:

Sixty of the seventy rats were experimentally infected with *T.b. brucei* ($1x10^6$ trypanosomes). The infected animals were divided into 6 equal groups (B-G) of 10 rats each and were treated as follows; Group B was untreated control, while groups C and D were treated with 2.4mg/kg and 4.8mg/kg of artesunate respectively. Rats in group E were treated with 2.4mg/kg of artesunate and 3.5mg/kg of diminazeneaceturate. Group F rats received 7.0mg/kg of diminazeneaceturate as a single dose while rats in group G received 7.0mg/kg of diminazeneaceturate and 4.8mg/kg of artesunate. Group A rats served as uninfected untreated control. Artesunate (Rekmal®) and diminazeneaceturate (Berenil®) were given intramuscularly. The infected rats were treated on day 12 post-infection (Pi) and artesunate treatment in groups C,D,E and G continued on days 13-17 Pi using half of the dose used at start on day 12 Pi (i.e. 1.2mg/kg in groups C and E and 2.4mg/kg in groups D and G). Tail blood samples were collected from rats pre- and post-parasite inoculations. Following treatments, the blood collected was used to determine parasitaemia and haematological changes. Packed cell volume (PCV) determined every 4 days by micro- haematocrit method was used to assess haematological response while parasitaemia was measured every 2 days by haemocytometry (Jain, 1986).

Statistical Analysis:

The data obtained were summarized as means ± standard deviations and compared by analysis of variance (ANOVA) (Chatfield, 1983).

RESULTS

The infected rats developed parasitaemia within 4-6 days after inoculation. The mean pre-patent periods in groups B, C, D, E, F and G were 4.8 ±1.0, 4.8±1.0, 4.6±1.0, 4.6±1.0, 4.0±0.0 and 4.8±1.0 days respectively. There were no significant difference ($P>0.05$) in pre- patent periods among the infected groups (B-G). The level of parasitaemia increased gradually from day 6 post-infection (Pi) in all the infected rats and reached peak on day 12 Pi when the infected rats were treated (Fig. 1).

The parasitaemia level decreased in groups C-G by day 2 post-treatment (PT) (day 14 Pi). Trypanosomes were cleared from the peripheral blood in groups E, F and G rats while the level of parasitaemia slightly decreased in groups C and D by day 4 post- treatment (PT) (day 16 Pi). Atesunate at 2.4mg/kg and 4.8mg/kg doses in groups C and D respectively slightly suppressed the level of parasitaemia from day 2-6 PT and the level gradually increased from days 8 and 10 PT respectively in groups C and D (Fig. 1).

There was relapse infection in one of the rats in group F (infected and treated with 7.0mg/kg of diminazeneaceturate) 24 days after treatment showing 90% treatment success. But the trypanosomes from that rat were cleared from the peripheral blood 34 days PT. Similarly, one of the rats in group G (infected and treated with 7.0mg/kg of diminazeneaceturate and 4.8mg/kg of artesunate) had relapse infection 52 days after treatment and subsequently disappeared from the peripheral blood 2 days later (day 54 PT) and reappeared on day 64 PT.

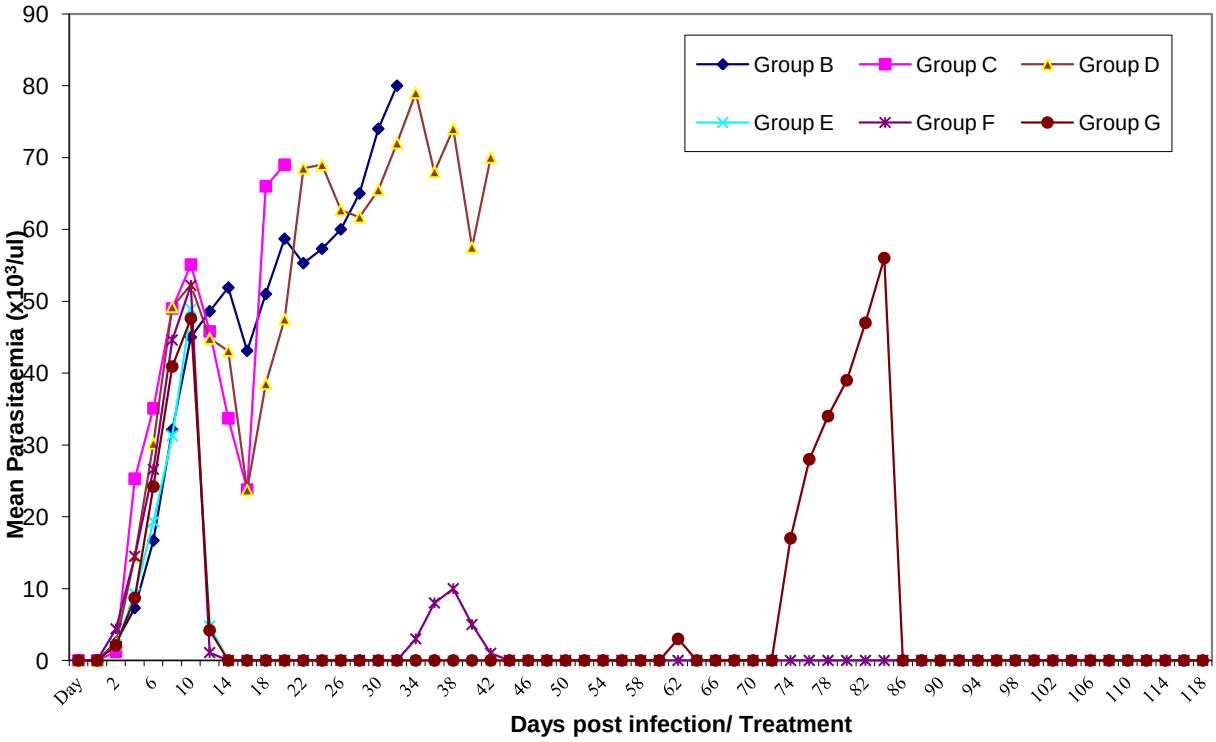

Fig.1:
Mean parasitaemia of *T. brucei brucei* infected untreated rats (Group B), infected and treated with 2.4mg/kg of artesunate (Group C), 4.8mg/kg of artesunate (Group D), 2.4mg/kg of artesunate and 3.5mg/kg of Berenil® (Group E), 7.0mg/kg of Berenil® (Group F) and 7.0mg/kg of Berenil® and 4.8mg/kg of artesunate (Group G).

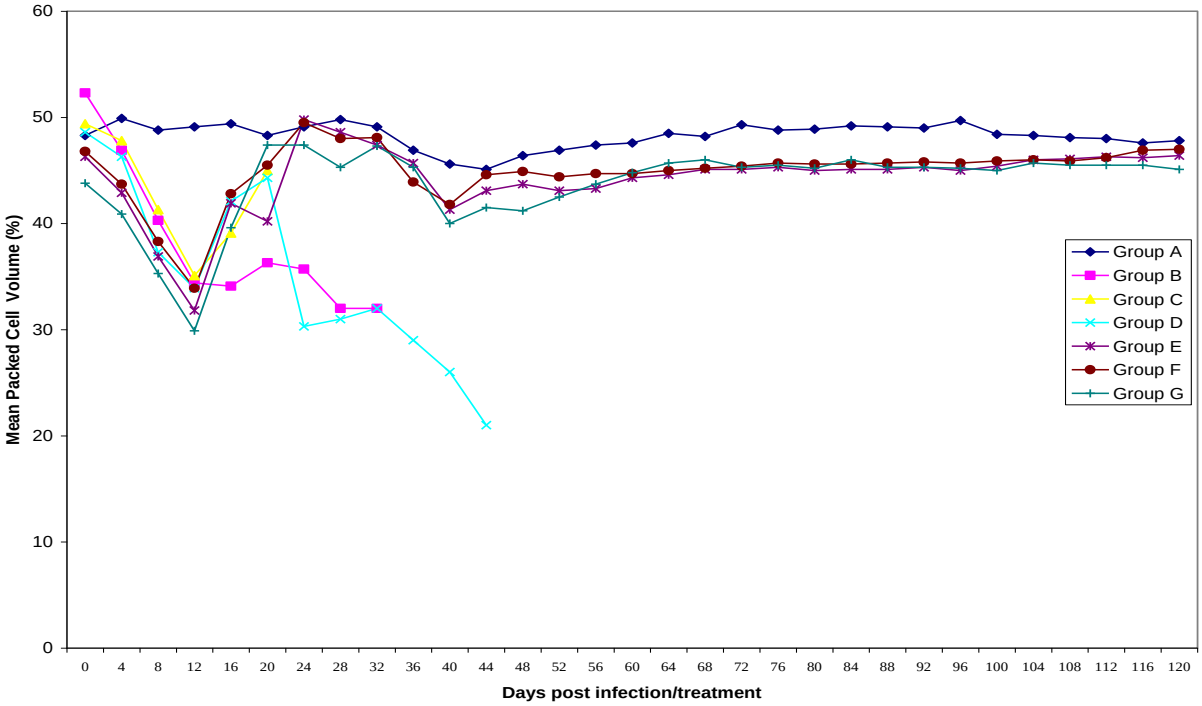

Fig.2:
Mean packed cell volume of uninfected untreated rats (Group A), infected untreated (Group B), infected and treated with 2.4mg/kg of artesunate (Group C), 4.8mg/kg of artesunate (Group D), 2.4mg/kg of artesunate and 3.5mg/kg of Berenil® (Group E), 7.0mg/kg of Berenil® (Group F) and 7.0mg/kg of Berenil® and 4.8mg/kg of artesunate (Group G)

The mean survival time in groups B, C, and D were

23.6± 5.6, 18.0± 3.7 and 26.0±10.3 days respectively. There was no significant difference (P>0.05) in the survival time in groups B, C, and D. The rat with relapse infection in group G died on day 71 PT.

The PCV decreased in all the infected groups by day 4 Pi and became remarkable from day 8-16 Pi (Fig. 2). On day 20 Pi (day 8 PT), the PCV level increased in groups C-G but that of group B continued to decrease until day 36 Pi (day 24 PT) when all the rats in that group died (Fig. 2). The PCV value in group D started decreasing from day 12 after treatment while those of groups E, F and G started appreciating. All the rats in groups C and D died on day 12 and 36 PT respectively (Fig. 2).

One of the rats in group F (infected and treated with 7.0mg/kg of diminazeneaceturate) had a relapse infection day 24 post-treatment with a PCV of 53%. The PCV declined to 39% on day 28 PT and rose again to 45% on day 32 PT. The parasites were cleared from the peripheral blood on day 36 PT with a PCV of 46% and the PCV stabilized and returned to pre-infection value from day 40 PT and no parasites were detected up to day 120 PT (when the experiment was terminated).

The rat with relapse infection in group G had a PCV of 42% on day 52 PT. The parasites disappeared from peripheral blood 2 days later (54 days PT) with a PCV of 44%. The same rat showed relapse parasitaemia 64 days PT with a PCV of 35% and the PCV came down to 30% on day 71 PT. The rat died on day 76 PT and no relapse occurred in any other rat as at 120 days PT. The PCV values in groups E-G returned to their pre-infection values (Fig. 2).

DISCUSSION

The results of the study showed that all the infected rats developed patent parasitaemia within 4-6 days post-infection. This is in agreement with the findings of Onyeyili and Onwualu (1991) and Egbe- Nwiyi et al (2005) in rats infected with *T. b. brucei* and treated with anti-malarial agents. The present study also demonstrated that Artesunate at 2.4-4.8mg/kg is less effective in the treatment of trypanosomosis. The drug temporarily suppressed the level of parasitaemia and 4.8mg/kg dose rate slightly increased the survival time (26.0±10.3 days) when compared with (23.6±5.6 days) that of the infected untreated.

The combination of the drug at 2.4mg/kg with diminazeneaceturate at 3.5mg/kg cleared the trypanosomes from the peripheral blood of the infected rats in group E but its combination at dose of 4.8mg/kg with 7.0mg/kg of diminazeneaceturate recorded 90% success as there was relapse infection in one of the rats. Relapse parasitaemia was also recorded in one of the rats in group F treated with single dose, 7.0mg/kg of diminazeneaceturate. The absence of relapse parasitaemia in group E treated with lower doses of artesunate (2.4mg/kg) and diminazeneaceturate (3.5mg/kg) combination may be attributed to synergism between the drugs. This type of result had earlier been reported in rats infected with *T. b. brucei* and treated with lower doses of diminazeneaceturate (3.5mg/kg) and Metakelfin$^{(R)}$ (10.0mg/kg) Egbe-Nwiyi et al (2005). The 90% treatment success witnessed in group F (treated with 7.0mg/kg of diminazeneaceturate alone) and G (treated with 7.0mg/kg of diminazeneaceturate and 4.8mg/kg of artesunate) showed effectiveness. The relapse parasitaemia in one rat each in groups F and G may not likely be due to under dosage considering the level of success but to hiding of the parasites in some drug inaccessible sides such as spleen, lymph nodes, brain and micro-circulation (Mamman et al 1994; Aliu, 2007). Cases of relapse have been reported earlier in rats infected with trypanosome species and treated with diminazeneacturate at 3.5-10.5mg/kg (Losos, 1986; Onyeyili and Onwualu, 1991; Egbe-Nwiyi et al 2006).

The none clearance of the parasites by artesunate alone agreed with the reports of other workers (Anika et al 1987; Onyeyili et al, 1994; Egbe-Nwiyi et al 2005; 2011) who observed the inability of some conventional anti-malarial agents to clear trypanosomes due to *T. bruceibrucei*from peripheral blood of rats. The inability of artesunate to perform such function may suggest that the drug lacks specific anti-parasitc activity directed towards trypanosomes.

Diminazeneaceturate belongs to the aromatic diamidines and the latter have strong specific anti-trypanosomal activity. This is achieved by interfering with trypanosomal DNA synthesis and aerobic glycolysis leading to the emergence of Kinetoplastic trypanosomes (Jones et al 1977; Riou and Bernard, 1980; Aliu, 2007). Maxie and Loses (1977) reported that diminazeneaceturate administration resulted in the release of trypanosomes from the micro-circulation into the general circulation. The workers suggested that the drug does not kill trypanosomes directly but makes them accessible to other defence mechanisms of the body, such as the macrophage system. It is pertinent to note that diminazeneaceturate has other effects on the parasites which include the inhibition of basic amino acid transport (Gutteridge, 1966), phospholipid synthesis (Gutteridge, 1969) and oxygen uptake (Hill and Hutner, 1968). Artesunate on the other hand, exerts its anti-malarial activity by cleavage of endo-peroxide bridge by iron producing free radicals (hyper- valent iron species, epoxides, aldehydes and dicabonyl compounds) which damage biological macro-molecules

causing oxidative stress in the cells of the parasite (Meshnick 1994).

The anaemia observed in all the infected groups was most severe in the infected and untreated rats. Anaemia is a normal finding in animal trypanosomosis (Anosa 1988; Murray and Dexter, 1998) and it usually results due to trypanosomal antigen-antibody complex, erythrophagocytosis, production of a range of enzymes that play a role in red cell damage and host of other factors (Mellors, 1985; Losos, 1986; Murray and Dexter, 1988). Lower doses of diminazeneaceturate (3.5mg/kg) and artesunate (2.4mg/kg) treatment caused full recovery of PCV while higher doses (7.0mg/kg and 4.8mg/kg) of the same drugs and 7.0mg/kg of diminazene aceturate as a single dose caused 90% recovery of PCV. The inability of diminazene aceturate at 7.0 mg/kg, to achieve 100% treatment success is not in agreement with the previous reports (Egbe-Nwiyi et al 2005;2011) in rats infected with *T. brucei brucei* and treated with 7.0mg/kg of diminazene aceturate. The relapse recorded in the two rats could not be attributed to strain of species of trypanosome used, as some strains of trypanosomes are genetically resistant or sensitive to diminazene aceturate (Mbwambo et al 1988). Considering the higher percentage of treatment success (90%) and 10% relapse in each group (F and G), it might be that the parasites in the two rats remained in drug inaccessible sites where they are not exposed to curative concentrations of the drug (Mamman et al 1994; Onyeyili and Egwu 1995) and this led to the progressive decrease in PCV values of the two rats until they died. The gradual recovery of PCV in the completely cured rats in groups E (10 rats), F (9 rats) and G (9 rats) is a manifestation that the drugs were in sufficient concentrations to have cleared the parasites from the peripheral blood. This observation is in consonance with the findings of previous workers (Poltera, 1980; Onyeyili and Egwu, 1995).

In conclusion, artesunate at 2.4-4.8mg/kg appeared to be less effective in the treatment of trypanosomosis due to *T. bruceibrucei* in rats and the drug seemed to potentiate the chemotherapeutic activity of diminazeneaceturate as there was no relapse when lower doses of diminazeneaceturate (3.5mg/kg) and Artesunate (2.4mg/kg) were combined. Relapse infection occurred in one rat each in groups F (infected and treated with 7.0mg/kg of diminazeneaceturate alone) and G (infected and treated with 7.0mg/kg of diminazeneaceturate and 4.8mg/kg of artesunate). There is need to evaluate the pathogenicity of the relapsed strain of the *T. b. brucei*and the efficacy of different combinations and regimens of artesunate and diminazeneaceturate in late stage trypanosomosis.

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