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The chemotherapeutic efficacy of diminazene aceturate and lithium chloride against relapse infection of Trypanosoma brucei in rats

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Abstract

The chemotherapeutic efficacy of diminazene aceturate (Berenil®) and lithium chloride (LiCl) in relapse infection of trypanosomiasis was investigated in rats experimentally infected with Trypanosoma brucei brucei. The study showed that the combination of diminazene aceturate at (7 mg/kg) and LiCl (10 mg/kg) appeared more effective therapeutically than diminazene aceturate, or diminazene aceturate and LiCl and dexamethasone group, as more of the rats in the diminazene aceturate and LiCl treated-group remained aparasitaemic for longer days (60 days). Relapse parasitaemia occurred on days 10 and 12 in the diminazene aceturate (7 mg/kg) group, diminazene aceturate (7 mg/kg) and LiCl (10 mg/kg) plus dexamethasone (1 mg/kg) treated group respectively, while relapse parasitaemia did not occur in the diminazene aceturate and LiCl treated group until day 20. Histopathological examination of the brain did not show any signs of inflammatory reaction in the diminazene aceturate and LiCl treated group and dexamethasone treated group. However lesions associated with meningoencephalitis, such as cellular infiltration of mononuclear cells, perivascular cuffings and perivascular congestion and oedema were observed in the diminazene aceturate; diminazene aceturate and LiCl treated groups.

Introduction

Trypanosomiasis is a serious disease of various species of animals and man. It is an important parasitic disease in Africa where tremendous economic damages have been made in livestock industry. Drug resistance is one of the serious problems that complicate treatment of many infectious diseases and in the case of trypanosomiasis, has stimulated the search for chemotherapeutic control of the disease. Although much work has been carried out on the efficacy of many trypanocides in various species of animals (Wilson et al., 1958; Fairclough, 1963; Verma et al., 1973; Wilson et al., 1975), the effective drug control of the disease is still problematic. Poor distribution of trypanocidal drug (diminazene aceturate) to the infected and intracellular sites, diminished activity of the drug in animals with suppressed immune system, under-dosage and lodging of the parasite in the cryptic site (brain) are among the host related factors that influence the trypanocidal drug resistance (Hawking, 1963). This pharmacokinetic problem, i.e. poor distribution of drug may play important role in relapse phenomenon where re-infection occurs after chemotherapy. Relapse infection may be due to the fact that the parasites that localize in the cryptic site evade the trypanocidal effect of the drug (diminazene aceturate) mainly due o its large molecular size which prevents it from crossing the blood brain barrier. This limits its effectiveness, accounting for the numerous reported cases of relapse after chemotherapy with the drug. Therefore certain reversible breakdowns of the blood brain barrier might have therapeutic use in permitting greater entry of the drug into the brain. In this regard, hyperosmolar solutions like lithium chloride and sucrose have been used for the osmotic opening of the blood brain barrier (Allison et al., 1976; Spatz et al., 1976).

As a strategy to combat the pharmacokinetic problem, a study on the effect of increasing the permeability of blood brain barrier (BBB) to diminazene aceturate using hyperosmolar agents (lithium chloride and sucrose) was carried out in rats.

Materials and methods

Experimental animals: Twenty eight adult albino rats (Wistar Breed) of both sexes (males and females) and average weight (120g) were used in the experiment. They were obtained from the Nigeria Institute for Trypanosomiasis Research (NITR) Vom Plateau State, Nigeria. The rats were fed poultry chick mash (Bendel feed and flour Mill LTD Ewu. Nigeria) and water was provided ad-libitum. They were maintained in a tsetse fly proof house.
Drugs and Chemicals:

(i) Diminazene aceturate (Berenil, Hoechst Farbwerte AG, Frankfurt, Germany) was used at a dosage of 7 mg/kg body weight administered intramuscularly (i.m.).

(ii) Lithium chloride (LiCl) (BDH Chemicals LTD Poole, England) was used at a dosage of 10 mg/kg i.m.

Experimental groups:

Group Q1: Infected rats treated with diminazene aceturate alone.

Group Q2: Infected rats treated with diminazene aceturate (7 mg/kg) + lithium chloride (10 mg/kg), i.m.

Group Q3: Infected rats treated with diminazene aceturate (7 mg/kg) + lithium chloride (10 mg/kg) + dexamethasone (1 mg/kg) i.m.

Group Q4: Infected, untreated control.

Treatment was initiated in all the groups, except the control (Q4), fourteen days post inoculation when infection was well established on assessment of parasitaemia by "wet rapid matching" method of Lumsden and Herbert (1976) and Buffy coat technique (Murray et al., 1977). The dexamethasone added was to prevent inflammatory reactions usually associated with diminazene aceturate and lithium chloride therapy or extravasation of the parasites in the brain.

At the end of the experimental period, rats from diminazene aceturate, diminazene aceturate and lithium chloride, diminazene aceturate and lithium chloride plus dexamethasone and control groups were sacrificed and their brains were collected for histopathological studies. Clinical signs, packed cell volume (PCV), relapse parasitaemia and rectal temperature were monitored, in the course of the experiments.

Results

Group Q1: Infected rats treated with diminazene aceturate alone.

The infected rats became positive for trypanosomes 4 days after inoculation and showed the following clinical signs: rough hair coat, decreased packed cell volume (PCV), anorexia, emaciation and pyrexia. Diminazene aceturate therapy was initiated. Twenty four hours after the injection of berenil at 7 mg/kg i.m., the clinical signs were reversed until relapse infection occurred. The effects of diminazene aceturate treatment on T. b. brucei infection are shown in Figs. 1 and 2. Trypanosomes were not detectable in the blood of the infected rats 24 hours after diminazene aceturate treatment. The PCV increased steadily with the commencement of treatment. Relapse parasitaemia occurred after 10 days of aparasitaemia following treatment with diminazene aceturate, but the PCV declined progressively from day 19 post treatment. Although there was an observable fall in mean rectal temperature, there was no marked difference from that of control. Between the 10th and 40th day of treatment relapse parasitaemia occurred in 100% of the rats treated with diminazene aceturate.

The cerebral alterations in the diminazene aceturate treated rats after 40 days were those of cellular infiltration of a few mononuclear cells. In the cerebral cortex there was no major histopathological lesion except perivascular oedema.

Group Q2: Infected rats treated with diminazene aceturate and lithium chloride.

The clinical signs of infection like in Group Q1 disappeared 4 hours following treatment with diminazene aceturate (7 mg/kg) and lithium chloride (10 mg/kg). The effects of diminazene aceturate and lithium chloride treatment are shown in Figs. 1 and 2. Trypanosomes cleared from the blood stream of the infected rats after 24 hours of treatment. The PCV increased steadily with the commencement of treatment. Relapse parasitaemia occurred after 10 days of aparasitaemia following treatment with diminazene aceturate and lithium chloride, but the PCV declined progressively from day 19 post treatment. Although there was an observable fall in mean rectal temperature, there was no marked difference from that of control. Between the 10th and 40th day of treatment relapse parasitaemia occurred in 100% of the rats treated with diminazene aceturate and lithium chloride.
Chemotherapeutic efficacy of diminazene and lithium chloride


rapidly and steadily but declined after relapse infection occurred.

Relapse parasitaemia did not occur in this group until 20 days after treatment (Fig. 2). By 60th day of treatment relapse parasitaemia occurred in 3 out of the 7 rats representing 42.8 percent survival.

The histopathological changes observed in diminazene aceturate and lithium chloride treated group, after 60 days, included presence of scotchy macrophages and perivascular oedema.

Group O2: Infected rats treated with diminazene aceturate and lithium chloride plus dexamethasone

Figs. 1 and 2 show the effects of diminazene aceturate and lithium chloride plus dexamethasone in T. b. brucei infected rats. The clinical signs like rough hair coat, dullness, decreased packed cell volume, (PCV) and pyrexia observed prior to treatment with diminazene aceturate (7 mg/kg) and lithium chloride (10 mg/kg) plus dexamethasone (1 mg/kg) did not clear until 72 hours after treatment. Similarly trypanosomes did not clear from the blood stream of the rats until after 72 hours in rats treated with diminazene aceturate alone or combination of diminazene aceturate and LCI plus dexamethasone. T. b. brucei were cleared from the blood of rats treated with diminazene aceturate alone, and diminazene aceturate and LCI after 24 hours, whereas clearance of parasitaemia did not occur until after 72 hours in rats treated with diminazene aceturate and LCI plus dexamethasone. This could be associated with the anti-inflammatory effect of dexamethasone which could induce immunosuppression in the rats. This agrees with the work of Odika et al. (1987) which showed that dexamethasone has a catalytic effect on the course of infection in animals inoculated with this strain of trypanosom.

Relapse parasitaemia was observed in the infected and diminazene aceturate treated rats on day 10 following treatment. While it did not occur until day 12 in the diminazene aceturate and LCI plus dexamethasone treated-groups and day 20 in the diminazene aceturate and LCI group respectively (Fig. 2). By day 34, relapse had occurred in all the rats in the diminazene aceturate treated group, 4 out of 7 in the diminazene aceturate and LCI treated group and 6 out of 7 rats in the diminazene aceturate and LCI plus dexamethasone treated-rats. In fact 3 rats in the diminazene aceturate and LCI treated group remained aparasitaemic and without any clinical signs of infection for more than 60 days post treatment. Representing 42.8 percent survival.

The delay of relapse parasitaemia ob- served in the diminazene aceturate and LCI treated group may be due to the effective distribution of diminazene aceturate in the various organs and increased concentra tion of diminazene aceturate in the brain caused by LCI (Odika, 1993).

In all the treated groups the packed cell volume (PCV) of the infected rats improved in contrast to the PCV of the infected, untreated control which continued to fall until all the rats died (Fig. 5). The mean rectal temperature before and after treatment did not show any marked difference from that of control.

In the present study the histopathological changes such as perivascular cuffings, cellular infiltration of mononuclear cells, perivascular cuffings, perivascular congestion and oedema.

Discussion

Four days after the experimental rats were infected with T. b. brucei organisms they showed a gradual loss of condition which include rough hair coat, anaemia and lethargy which are similar to those reported earlier in acute cases of T. b. brucei infection in dogs and mice (Looss and Boede, 1972, Odika et al., 1987). When the infection was treated with diminazene aceturate alone, diminazene aceturate with LCI, and a combination of diminazene aceturate, LCI and Dexamethasone, the parasite cleared from the blood-stream (parasitaemia) in all the treated groups before relapse occurred as varying lengths of time depending on the drug or drug combina tion used for treatment. The treatment with diminazene aceturate and LCI seemed to be more effective when com pared to the groups treated with diminazene aceturate alone or combination of diminazene aceturate and LCI plus dexamethasone. T. b. brucei were cleared from the blood of rats treated with diminazene aceturate alone, and diminazene aceturate and LCI after 24 hours, whereas clearance of parasitaemia did not occur until after 72 hours in rats treated with diminazene aceturate and LCI plus dexamethasone. This could be associated with the anti-inflammatory effect of dexamethasone which could induce immunosuppression in the rats. This agrees with the work of Odika et al. (1987) which showed that dexamethasone has a catalytic effect on the course of infection in animals inoculated with this strain of trypanosom.
Azene aceturate and LiCl plus dexamethasone treated group appeared to be attributed to the dexamethasone which prevented inflammatory reaction due to either extravasation of the trypanosome or presence of diminazene aceturate or LiCl in the brain.

In conclusion the combination of diminazene aceturate (7 mg/kg) and LiCl (10 pg/kg) did not completely prevent relapse in T. b. brucei infected rats but improved the therapeutic efficacy of diminazene aceturate by significantly delaying the incidence and occurrence of relapse parasitaemia. The present study showed that the increase in brain concentration of diminazene aceturate by LiCl enhanced the chemotherapeutic efficacy of diminazene aceturate in cerebral trypanosomiasis, a phenomenon that will require further investigation.

Acknowledgement

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