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The effects of hyperosmolar agents lithium chloride and sucrose on the brain concentration of diminazene aceturate in rats

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The concentrations of diminazene aceturate in the brain of *Trypanosoma brucei brucei* infected and uninfected rats treated with diminazene aceturate (3.1 mg/kg, im) and either LiCl (2.5, 5.0 and 10 μg/kg) or sucrose (0.25, 0.5 and 1.0 g/kg) were determined. When diminazene aceturate was administered at a standard dose of 3.1 mg/kg (im), the addition of LiCl (10 μg/kg, im) increased significantly ($P<0.05$) the concentration of the drug in the brains of both trypanosome infected and normal infected rats. The addition of sucrose (1.0 g/kg, im) instead of LiCl failed to give any significant increase in diminazene aceturate levels in the brain. The diminazene aceturate levels were significantly ($P<0.05$) higher in the organs (brain, kidney, liver and spleen) of trypanosome infected compared to uninfected rats. The concentration of diminazene aceturate in the organs increased significantly ($P<0.05$) with increasing concentrations of LiCl.

Key words: Sucrose; Blood-brain-barrier; Diminazene aceturate; Lithium chloride; Trypanosomiasis

1. Introduction

The poor distribution of drugs to infected tissues like the brain is one of the host related factors associated with relapse trypanosomiasis infection (Jennings et al., 1979). This pharmacokinetic problem is observed in the treatment of cerebral trypanosomiasis with the existing drugs. Diminazene aceturate (Berenil®) is the effective routine drug for treating trypanosomiasis in Nigeria. Its large molecular size does not easily cross the blood brain barrier (BBB) with the result that in cerebral trypanosomiasis, the trypanosomes evade its trypanocidal action. This may be responsible for the relapse often seen in animal trypanosomiasis (Williamson 1970; Gutteridge and Coombs 1977; Jennings et al., 1977; Onyeyili and Anika 1989). Chukwu et al. (1990) reported that the brain is the cryptic site for the localization of Trypanosoma *brucei brucei* in animals suffering from cerebral trypanosomiasis, a location which is hardly reached by drugs due to poor distribution. In such infections, the parasites lodge in this site and evade the action of diminazene aceturate because

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of its inability to enter the cryptic site in therapeutic concentration. As a strategy to combat this failure to reach therapeutic levels, hyperosmolar compounds like lithium chloride (LiCl) and sucrose could be used in combination with trypanocides to achieve osmotic opening of the BBB with the resultant effective chemotherapy. Lithium chloride and sucrose have been used as hyperosmolar solutions in osmotic opening of the BBB (Spatz et al., 1976). The present study was therefore designed to:

(1) determine the effect of increasing the permeability of the BBB using the hyperosmolar agents, lithium chloride and sucrose, on the brain concentration of diminazene aceturate.

(2) determine which of the hyperosmolar agents is more effective in increasing the permeability of the BBB to diminazene aceturate.

2. Materials and methods

2.1. Experimental animals

Adult albino rats (Wistar breed) of both sexes weighing approximately 120 g each were used in the experiment. They were obtained from Nigerian Institute for Trypanosomiasis Research (NITR) Vom, Plateau State, Nigeria. The rats were fed chick mash (Bendel Feed and Flour Mill LTD. Ewu Nigeria) and provided with water ad libitum. The animals were evenly distributed in the various treatment groups of 5 rats each and maintained in cages at a room temperature of 27°C and 12 hours lighting period. Strict compliance with the principles of animal welfare was observed throughout the experimental period.

2.2. Trypanosome

Trypanosoma brucei brucei (Gboko CT/70/NITR) was obtained from NITR. The strain was maintained by serial passage in donor rats from where infective trypanosomes for experimental infection were obtained.

2.3. Drugs and chemicals

Drugs

The diminazene aceturate (Berenil® Hoechst Ferbwerk, A.G. Frankfurt, West Germany) was a yellow reconstitutable powder for injection and marketed as lactate gluconate mixture. One gramme of the drug contained 44.5% of the active ingredient. A 7% (w/v) solution was used for this investigation and the prepared solution was used within hours of preparation.

Chemicals

LiCl (BDH chemicals LTD Poole, England) used for the experiment was a white hygroscopic crystalline reconstitutable powder and was prepared as a 10% (w/v) solution.Sucrose (Cartivalues, England) was reconstituted into a 10% (w/v) solution. Hydrochloric acid (Cartivalues, England) was prepared to obtain 1N HCL. Other chemicals used were alpha-1-naphthylethlenediamine (BDH laboratory, Poole England) and ammonium sulphamate (BDH laboratory, Poole England).

2.4. Methods

Sixty-five rats obtained from the same source (NITR) were used for the experiment. The rats were divided into 13 groups of 5 rats each. The first 6 groups (A,B,C,D,E and F) were uninfected while the 7 remaining groups (T,U,V.W,X,Y and Z) were inoculated intraperitoneally with approximately 1.25×10^6 T. b brucei (in 1 ml of phosphate buffer glucose, PBS). Both infected and uninfected rats were treated with diminazene aceturate and LiCl; diminazene aceturate and sucrose; while only the infected rats were treated with diminazene aceturate alone as shown in Table 1.

Previous data suggest that peak levels of diminazene aceturate occur at 6 h post dose by intramuscular route (Onyeyili and Anika, 1989). Therefore 6 h after each treatment, the rats were sacrificed and the following organs collected; brain, spleen, liver and kidney. They were placed in petridishes containing PBS and stored in the freezer at $-21°C$ until the diminazene aceturate content of each was determined by a colorimetric method (Raether et al., 1972) in which 0.3 g of each organ was added to 2 ml of 10% trichloroacetic acid. After thorough homogenization and mixing, the sample was left to stand for 30 min, then centrifuged at $900 \times g$ for 15 min. 1 ml of clear supernatant was added to 1 ml of 1N HCL, heated in a waterbath for 30–60 min and allowed to cool under room temperature. It was then diazotized with 0.2 ml of 0.5% sodium nitrite solution. After 3 min 0.5 ml of 1% ammonium sulphate solution was added and the mixture was thoroughly shaken. After 3–5 min 1 ml of 0.4 alpha-naphthylethylenediamine solution was added and the developing colour

TABLE 1

Treatment methodology for infected and uninfected groups of rats treated with diminazene aceturate and LiCl; diminazene aceturate and sucrose; or diminazene aceturate alone

Groups	Uninfected	Groups	Infected
A	Diminazene aceturate (3.1 mg/kg) + LiCl (2.5 μg/kg) im	T	Diminazene aceturate (3.1 mg/kg) + LiCl (2.5 μg/kg) im
B	Diminazene aceturate (3.1 mg/kg) + LiCl (5 μg/kg) im	U	Diminazene aceturate (3.1 mg/kg) + LiCl (5 μg/kg) im
C	Diminazene aceturate (3.1 mg/kg) + LiCl (10 μg/kg) im	V	Diminazene aceturate (3.1 mg/kg) + LiCl (10 μg/kg) im
D	Diminazene aceturate (3.1 mg/kg) + Sucrose (0.25 g/kg) im	W	Diminazene aceturate (3.1 mg/kg) + Sucrose (0.25 g/kg) im
E	Diminazene aceturate (3.1 mg/kg) + Sucrose (0.5 g/kg) im	X	Diminazene aceturate (3.1 mg/kg) + Sucrose (0.5 g/kg) im
F	Diminazene aceturate (3.1 mg/kg) + Sucrose (1.0 g/kg) im	Y	Diminazene aceturate (3.1 mg/kg) + Sucrose (1.0 g/kg) im
		Z	Diminazene aceturate (3.1 mg/kg) im

im = given intramuscularly

was measured in a Coleman spectrophotometer at 540 nm. The minimum detectable level of this method is 0.005 μg/g of tissue.

3. Results

The mean concentration of diminazene aceturate in various organs after the administration of diminazene aceturate (3.1 mg/kg) and LiCl (2.5, 10 μg/kg), and diminazene aceturate (3.1 mg/kg) and sucrose (0.25, 0.5 and 1.0 g/kg), or diminazene aceturate alone (3.1 mg/kg), are in Tables 2–4. Peak concentrations of 0.032 ± 0.004 and 0.124 ± 0.001 μg/kg of diminazene aceturate were obtained in the brain of rats infected with *T. b. brucei* and treated with diminazene aceturate (3.1 mg/kg) and sucrose (1 g/kg), and diminazene aceturate (3.1 mg/kg) and LiCl 10 μg/kg), respectively.

The mean concentration of diminazene aceturate in the spleen, kidney and liver of infected and uninfected rats treated with diminazene aceturate and LiCl showed an increase over the values obtained from trypanosome infected and uninfected rats treated with diminazene aceturate and sucrose (Tables 3 and 4). Similarly the mean diminazene aceturate concentration in the spleen, liver and kidney of rats infected and treated with diminazene aceturate alone generally appeared to yield higher drug levels in these organs than co-administration with 2.5 μg/kg LiCl and 0.5 g/kg of sucrose (Tables 2–4). Generally, the concentrations of diminazene aceturate in the

TABLE 2

The concentrations of diminazene aceturate in various organs of infected rats treated with diminazene aceturate (3.1 mg/kg)

Organs	Conc. (μg/kg)
Brain	0.017 ± 0.010
Spleen	0.253 ± 0.020
Kidney	0.602 ± 0.190
Liver	0.481 ± 0.060

TABLE 3

The concentrations of diminazene aceturate (μg/kg) in various organs of *T. b. brucei* infected and uninfected rats, treated with diminazene aceturate (3.1 mg/kg) and LiCl (2.5, 5, 10 μg/kg)

Organs	2.5 μg/kg	5 μg/kg	10 μg/kg (LiCl)
A. Infected rats			
Brain	0.031 ± 0.001	0.080 ± 0.030	0.124 ± 0.010
Spleen	0.205 ± 0.030	1.599 ± 0.050	1.686 ± 0.210
Kidney	0.493 ± 0.080	1.872 ± 0.140	2.386 ± 0.070
Liver	0.446 ± 0.070	2.028 ± 0.250	2.092 ± 0.010
B. Uninfected rats			
Brain	0.023 ± 0.002	0.032 ± 0.002	0.070 ± 0.009
Spleen	0.162 ± 0.010	0.239 ± 0.030	0.345 ± 0.020
Kidney	0.392 ± 0.020	0.639 ± 0.150	0.860 ± 0.060
Liver	0.314 ± 0.006	0.563 ± 0.010	0.979 ± 0.010

TABLE 4

The concentrations of diminazene aceturate (μg/kg) in various organs of *T. b. brucei* infected and uninfected rats, treated with diminazene aceturate (3.1 mg/kg) and sucrose (0.25, 0.5, 1.0 g/kg)

Organs	0.25 g/kg	0.5 g/kg	1.0 g/kg (sucrose)
A. Infected rats			
Brain	0.023±0.002	0.026±0.002	0.032±0.004
Spleen	0.162±0.010	0.204±0.010	0.442±0.030
Kidney	0.329±0.020	0.441±0.020	0.547±0.050
Liver	0.314±0.060	0.314±0.006	0.541±0.150
B. Uninfected rats			
Brain	0.023±0.002	0.025±0.002	0.033±0.004
Spleen	0.147±0.010	0.160±0.010	0.179±0.030
Kidney	0.240±0.001	0.291±0.010	0.308±0.010
Liver	0.260±0.03	0.408±0.040	0.445±0.070

organs of rats (infected and uninfected) treated with diminazene aceturate and LiCl increased significantly ($P<0.05$) as the dose of LiCl increased, with the exception of values obtained for the spleen (5 and 10 μg/kg) and liver (5 and 10 μg/kg) of infected rats (Table 3) where the increases were not statistically significant.

4. Discussion

The results of the present study appeared to show that there was a significant increase in the concentration of diminazene aceturate in the organs of trypanosome infected rats treated with diminazene aceturate and LiCl (at 5 and 10 μg/kg) when compared with similar rats treated with diminazene aceturate alone or diminazene aceturate and sucrose (0.5 and 1 g/kg) (Fig. 1).The difference in the concentrations of diminazene aceturate in the brain observed between the LiCl treated and other groups are attributable to the osmotic opening of the BBB by LiCl. Lithium chloride, as a result of being a hyperosmotic agent, could cause shrinkage of cell layers at tight junctions in the choroid plexus resulting in the widening of the pores and consequent increased permeability of the BBB to diminazene aceturate (Rapoport, 1970, 1974). In general, the organ concentration of diminazene aceturate in rats treated with diminazene aceturate and LiCl increased with increasing doses of LiCl (Tables 3 and Fig. 1) except in the liver and spleen of infected rats treated with 5 and 10 μg/kg of LICL, indicating a pharmacological response. As mentioned, lithium chloride was observed to be more effective than sucrose (at the doses used) in increasing the entry of diminazene aceturate into the brain and various organs, a situation which indicates that LiCL was a more active hyperosmolar agent considering that sucrose (0.25–1 g/kg) was used at much higher doses than LiCl (25–10 μg/kg).

The present study showed that diminazene aceturate was readily distributed to various organs of the body within six hours of administration, which agrees with the findings of other investigators (Onyeyili 1982; Kellner et al., 1985). It was observed that within six hours of administering diminazene aceturate (3.1 mg/kg) and LiCl (10 μg/kg) the highest concentrations of diminazene aceturate were

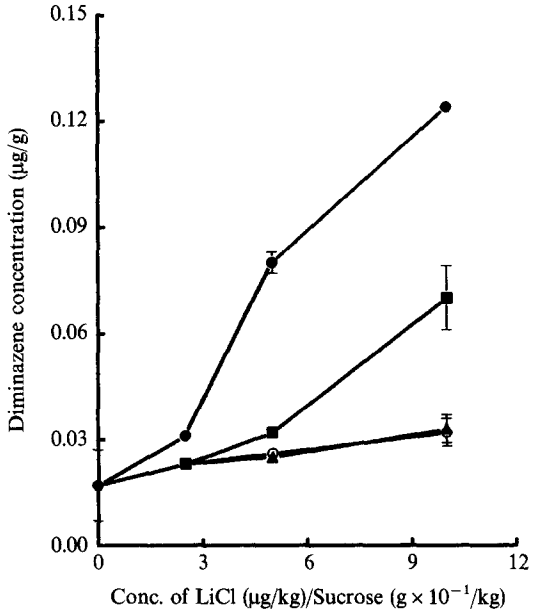

Fig. 1. The effect of LiCl or sucrose on the concentration of diminazene aceturate in the brain.

obtained in the kidney and liver of both infected and uninfected rats (Table 3). This suggests that diminazene aceturate accumulates mainly in the kidney and liver, which are the main organs of excretion and biotransformation, respectively. In general higher concentrations of diminazene aceturate were also observed in the liver, kidney and spleen of rats treated with diminazene aceturate and LiCl (at the highest dose of LiCl) compared with the rats treated with diminazene aceturate alone (infected rats) or diminazene and sucrose (Tables 2–4). However, the diminazene aceturate concentration in infected rats treated with diminazene aceturate alone generally yielded markedly higher drug levels in these organs than co-administration with 2.5 μg/kg of LiCl and 2.5 g/kg or 5 g/kg of sucrose. This phenomenon probably suggests that the least dose of LiCl that will significantly increase diminazene aceturate concentrations in other organs of infected rats is 5 μg/kg while that of sucrose (even though is not apparently significant for kidney and liver) is 1 g/kg.

In conclusion, diminazene aceturate was effectively distributed to various organs of the body within six hours of administration. The concentration of diminazene aceturate increased significantly ($P<0.05$) in the brain, liver, kidney and spleen of rats treated with LiCl and diminazene aceturate, particularly at 5 μg/kg and 10 μg/kg of LiCl. The increase appeared to be higher in T. b. brucei-infected rats than in uninfected rats probably due to increased body temperature during infection leading to increased drug permeability. From the study LiCl was more effective than sucrose (at the doses used) in increasing the permeability of the blood-brain-barrier to diminazene aceturate. A combination therapy of diminazene aceturate and LiCl may be useful for treating cerebral trypanosomiasis in animals even though care must be taken not to exceed the safe dose of LiCl to avoid toxic neurological reactions and death observed in rats treated with higher doses. In our subsequent

work it was observed that LiCl at the dose of 10 mg/kg, i.m co-administered with diminazene aceturate was safe in rats (Odika et al., 1995).

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