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# Studies on Trypanocidal Drug Sensitivity Against *Trypanosoma evansi* in Experimentally Infected Mice

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**Abstract:** Trypanocidal drug sensitivity studies were conducted to assess the efficacy of Diminazene diaceturate (Diminasan®) and Bis (aminoethylthio) 4-melaminophenylarsine dihydrochloride (Cymelarsan®) against *T. evansi* of Colombia strain in experimentally infected mice. Study was undertaken from November 2008 to April 2009. Diminazene diaceturate at dose range of 3.5, 7.0, 14.0 and 28.0 mg $kg^{-1}$ body weight failed completely to cure *Trypanosoma evansi*. All the mice died due to sever parasitaemia. From the present study the minimum dose necessary to achieve 100 % cure in at least 3 mice for diminazene diaceturate is >28.0 mg $kg^{-1}$ body weight. However, mice treated with Cymelarsan® at higher doses of 0.5, 1.0 and 2.0 mg $kg^{-1}$ body weight becomes effective treatment for *Trypanosoma evansi* Colombia strain in mice with no relapse for at least two months. The result obtained from this study will be important for designing similar study of *in vitro* assay to detect drug resistance against the available isolates of *T. evansi* from camel rearing areas of Ethiopia.

## INTRODUCTION

In developing countries like Ethiopia, livestock is an important part of the agricultural sector used for draught power, source of fertilizer, means of transportation and others. African animal trypanosomosis constrains agricultural production in areas of Africa that hold the continent's greatest potential for expanded agricultural production. Trypanosomosis contribute to the direct economic losses on livestock production. In tsetse infected areas of the country the most prevalent trypanosomosis species is *T. congolense* followed by *T. brucei brucei and T. vivax*[1]. Among the non tsetse transmitted animal trypanosomosis, *T. evansi* (the causative agent of surra) and *T. equiperdum* (the causative agent of dourine) are also common. In Ethiopia, surra is present in almost all areas where camels are found[2].

Surra is a disease of vertebrate animals caused by *Trypanosoma evansi*. The natural course of the disease is characterized by fever and anemia followed by emaciation, lethargy, oedema, cachexia and enlargement of the lymph nodes and spleen. Neurological symptoms occur late in the disease. Abortion may occur in late pregnancy. Acutely affected animals die within weeks or months but chronic infections may continue for several years[3, 4]. The clinical signs of the disease caused by *T. evansi* vary according to the trypanosome strain, the virulence of the particular isolate, species of the host,

unspecific factors affecting the animal such as other infection and stress and the local epidemiological situations[5, 6]. *Trypanosoma evansi* is a widely distributed hemoflagelate parasite of the kingdom Protozoa, phylum Sarcomastigophora, order Kinetoplastidae, family Trypanosomatidae and genus Trypanosoma. *Trypanosoma evansi* is of considerable veterinary importance that affects a variety of larger mammals including horses, mules, buffalos, cattle and deer. In Africa, Asia, the Middle East and South America, *T. evansi*, the causative agent of surra is important, especially in draft and transport animals and is exclusively transmitted mechanically by biting flies such as *Tabanus* and *Stomoxys* spp[7].

With the wide spread of drug resistant parasites into all trypanosomosis-endemic areas, development of new antitrypanosomal compounds and drugs to circumvent resistance is urgently needed. However, it appears unlikely that new compounds will be introduced in the near future because of lack of interest by the pharmaceutical industry in investing in research and development of antitrypanosomal drugs[8]. Hence, the need to intensify efforts at maintaining the efficacy of existing trypanocides is crucial. In Ethiopia escalating costs and other problems of initiating and maintaining tsetse control campaigns lead the livestock sector to be completely reliant on the use of trypanocidal drug to both prevention and control of the disease. However, almost all trypanocidal drugs were gradually losing their efficacy due to drug resistance[1]. Hence, there is an urgent need for assessment of efficacy of the drug in which representative number of trypanosomes isolate are examined for distribution and degree of efficacy, so as to work out the best possible therapeutic strategies and/or alternative control measures. As prerequisite for such undertaking, it is necessary to first obtain information on *in vivo* trypanocidal drug sensitivity patterns of the parasites in a mice model.

Therefore, the objective of the present study was to asses the trypanocidal drug sensitivity Diminazene diaceturate and Cymelarsan against *T. evansi* of Colombia strain in experimentally infected mice.

## MATERIALS AND METHODS

**Study area:** The present study was undertaken from November 2008 to April 2009 in the molecular Parasitology laboratory of Ethio-Belgium VLIR UOS funded dourine project which is found in the compound of Addis Ababa University Faculty of Veterinary Medicine, Debre Zeit. They were housed in a conducive environment in the laboratory.

**Experimental animals:** Swiss white mice 6 weeks of age, weighing 20 g, obtained from the breeding colony of the National Veterinary Institute (NVI) and maintained under standard commercial pelleted ration and water *ad libitum* in the laboratory of the VLIR-UOS funded Ethio-Belgium collaborative project Faculty of Veterinary Medicine, Debre Zeit.

**Experimental design:** Five donor mice were infected with the original stabilates from the ITM. The mice were infected with the stabilates after defrosting at room temperature and mixed with PSG using intra peritoneal route 0.2 mL. Then with in five to seven days time peak parasitemia was evident. It was successfully possible to serially passage the *Trypanosoma evansi* Colombia strain to the next mice. Each time cryostabilates were prepared and kept in liquid nitrogen.

Two drug sensitivity studies were conducted on *T. evansi* Colombia strain using diminazene diaceturate and cymelarsan. In each of the study 25 mice were divided randomly into five experimental groups (Group I-V) of five mice each. The first four groups (I-IV) formed the infected groups treated with different doses of trypanocidal drugs. The fifth group (Group V) served as untreated infected control. Each mouse in group I-IV was weighing on a digital balance prior to administration of trypanocidal drug for calculation of dosage and the average weight was taken to have a common dosage. *Trypanosoma evansi* Colombia strain was kindly obtained from Institute of Tropical Medicine (ITM) Antwerp, Belgium through the VLIR-UOS funded Ethio-Belgium Collaborative Project. The mice were infected with 1-10 trypanosomes per preparation which is estimated $10^3$-$10^4$ trypanosomes per mL[9] by inta peritoneal (i.p) inoculation with 0.2 mL blood from donor mice taken at peak parasitaemia.

**Treatment and monitoring:** Diminazene diaceturate (Diminasan, Batch DG/20337 Kuipersweg 9, 3449 JA Woerden, Holland) and Bis (aminoethylthio) 4-melaminophenylarsine dihydrochloride (Cymelarsan®, Lot B 09108A, MERIAL-17, rue Bourgelat 69002 Lyon-France) were used for treatment of infected mice in the treatment group. Mice in the treatment group were treated 24 h post-infection. Diminazene diaceturate was administered i.p at range doses of 3.5, 7.0, 14.0 and 28.0 mg $kg^{-1}$ body weight. Similarly, cymelarsan was given i.p at range of doses of 0.25 mg, 0.5, 1.0 and 2.0 mg $kg^{-1}$ body weight (Table 1). The control groups

Table 1: Treatment groups for *T. evansi* sensitivity studies in mice using diminazene diaceturate and cymelarsan

| Treatment groups | No. of mice per group | Drugs used (mg $kg^{-1}$) | |
|---|---|---|---|
| | | Diminazene diaceturate | Cymelarsan |
| I | 5 | 3.5 | 0.25 |
| II | 5 | 7.0 | 0.5 |
| III | 5 | 14.0 | 1.0 |
| IV | 5 | 28.0 | 2.0 |
| V | 5 | 0[a] | 0[a] |

[a] Distilled water used instead of trypanocidal drug

received the same amount of sterile distilled water via. i.p route. Injection solution was prepared by dissolving the required quantity of each compound in sterile distilled water before use according to respective manufacturer instruction. The required dose of drug was administered in 0.2 mL of solution for all treatment groups[10]. Mice were monitored every alternate day up to 60 days for the presence of trypanosomes by microscopic examination of wet smears of tail blood. Presence of trypanosomes in the blood smear was considered as indication of drug resistance.

## RESULTS AND DISCUSSION

The results of the present drug sensitivity test in mice indicated that diminazene diaceturate administered i.p at range of doses of 3.5, 7.0, 14.0 and 28 mg $kg^{-1}$ body weight failed completely to cure mice infected with *Trypanosoma evansi* of Colombia strain (Table 2).

In case of cymelarsan mice infected and treated with at a dose rate of 0.25 mg $kg^{-1}$ body weight only one mouse found to show relapse (2±1.87 days) and the remaining four mice died within 24 hours of treatment (Table 2). However, cymelarsan with higher doses 0.5, 1.0 and 2.0 mg $kg^{-1}$ body weight completely cured the infection in mice with no relapse at least for more than one month. Hence, the Minimum Curative Dose (MCD) of diminazene diaceturate and cymelarsan required to clear *Trypanosoma evansi* from experimentally infected mice was >28 and 0.5 mg $kg^{-1}$, respectively.

For both drugs there was a clear relationship between the time of relapse and the dose of the drug used. Mice treated at lower doses relapsed after a shorter time than mice treated with higher doses. All mice in the control groups showed high level of parasitaemia and died between days 1 and 4 after infection.

Lack of much interest by the pharmaceutical industry to venture into development of new antitrypanosomal drugs has been a major stimulus to an intensification of research into the few existing drugs. Those indicated for animal trypanosomiasis include: Isometamidium, Homidium and Diminazene, used primarily against *Trypanosoma congolense, T. vivax* and *T. brucei* and quinapyramine, mainly indicated for use against *T. evansi* infections.

The results from the drug sensitivity study disclosed that Cymelarsan® at higher doses of 0.5, 1.0 and 2.0 mg $kg^{-1}$ body weight becomes effective treatment for *Trypanosoma evansi* Colombia strain in mice with no relapse for at least two months. Cymelarsan® at doses of 0.25 mg $kg^{-1}$ body weight failed to completely cure the infection. A breakthrough has been made in the recent past, in the field of chemotherapy of *T. evansi* infections by the introduction of a new arsenic compound, melarsenoxide cysteamine (Cymelarsan®). Lack of efficacy of Cymelarsan® to clear parasitemia in mice and other domestic animals at the standard dose of 0.25 mg $kg^{-1}$ for *T. evansi* in camels has previously been reported. For instance Cymelarsan® was ineffective in buffaloes treated at doses ranging from 0.25-3 mg $kg^{-1}$[11] in goats treated at a dose of 0.3 mg $kg^{-1}$[12] in mice treated at doses of 0.25 mg and 0.5 mg $kg^{-1}$[13] and in cattle treated at a dose of 0.5 mg $kg^{-1}$[14]. This ineffectiveness of Cymelarsan® at a dose rate of 0.25 mg $kg^{-1}$ body weight could be attributed to the fact that the recommended dose should be used for the treatment of camels only. However, higher doses are required to treat *T. evansi* in other domestic animals and mice[12].

The water soluble trivalent arsenical agent melarsamine hydrochloride or MelCy (trade name, Cymelarsan®) has been effective against *T. brucei brucei* and *T. evansi* in camels, water buffaloes, goats and pigs. Its effectiveness against *T. equiperdum* in horses has also been shown[11]. Cymelarsan®; is, melamine-phenylarsine made by the conjugation of one equivalent of melarsen oxide and two equivalents of cysteamine[15]. Payne *et al*.[14], recommended a dose rate of 0.75 mg $kg^{-1}$ body weight administered intramuscularly for the treatment of *T. evansi* infection in Holstein Friesian cattle after experimental analysis of Cymelarsan®.

Diminazene diaceturate at dose range of 3.5, 7.0, 14.0, and 28.0 mg $kg^{-1}$ body weight failed completely to cure *Trypanosoma* evansi. All the mice died due to sever parasitaemia. From the present study the minimum dose necessary to achieve 100 % cure in at least 3 mice for diminazene diaceturate is greater than 28.0 mg $kg^{-1}$ body weight.

Table 2: Results of diminazene diaceturate and cymelarsan against *Trypanosoma evansi* Colombia strain in mice in Debre Zeit Ethiopia

| Drug | Doses (mg $kg^{-1}$) | No. mice treated/relapsed | Mean relapse interval in days±SD |
|---|---|---|---|
| Diminazene diaceturate | 3.5 | 5/5 | 1±0.5 |
| | 7.0 | 5/5 | 0.96±0.074 |
| | 14.0 | 5/5 | 2.85±0.44 |
| | 28.0 | 5/5 | 4±1.97 |
| Cymelarsan | 0.25 | 5/1 | 2±1.87 |
| | 0.50 | 5/0 | Cured (No. relapse) |
| | 1.0 | 5/0 | Cured (No. relapse) |
| | 2.0 | 5/0 | Cured (No. relapse) |
| Control | Distilled water | 5/5[a] | Died on days of 1-4 post infection |

No. Number; [a]Treated/Died

The classical treatment for surra is based on the drugs Suramin, Diminazene aceturate and Quinapyramine sulphate. Suramin has been used extensively for treatment of both animal and human trypanosomosis and ochocercosis. It is administered by intravenous injection over prolonged duration and is active against disease stages preceding central nervous system involvement.

Drug resistance is challenge to the use of Suramin. Suramin resistant *T. evansi* strains have been reported from various places such as the Sudan[16]. Resistance to Quinapyramine methyl sulphate develops quickly and treatment with the drug is normally followed by relapse. Its use in cattle is now not recommended as drug resistance develops rapidly and can be associated with cross resistance to all the other trypanocidal drugs in use[17]. Resistance to *T. evansi* to Suramin and diminazene could be induced in mice in the laboratory, indicating that inappropriate use of drugs may lead to a resistance in the field[18]. However, the water soluble arsenical agent, melarsamine hydrochloride or MelCy (trade name, Cymelarsan) has been shown to be very effective against *T. brucei brucei* and *T. evansi* in camels, water buffaloes, goats and pigs[11]. The drug was also found to be effective against Diminazene aceturate resistant *T. brucei brucei* and *T. evansi*.

## CONCLUSION

Chemotherapy for trypanosomiasis in domestic livestock depends on only a few compounds, of which several are chemically closely related. However, extensive use of the limited number of commercially available trypanocidal drugs resulted in the appearance of trypanosome strains resistant to these drugs and the discovery of new drugs has been painfully slow. To combat the problems associated with trypanocides, further research into the existing drugs is a prerequisite for their optimal usage in the overall effort of improving animal health and productivity through control of trypanosomiasis.

## RECOMMENDATION

Therefore, in light with the above conclusions the following recommendations can be forwarded:

*In vitro* assays should be performed to detect drug resistance against the available isolates of *T. evansi* from camel rearing areas of Ethiopia where surra is endemic and major problem.

Since, the available drugs treat *T. evansi* are limited, therefore, research is required on the possibility of use of drug combination therapies.

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