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Report on the Use of the Trypanocidal Drug "TRYPAN"

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INTRODUCTION

It is well known that developing an effective trypanocidal drug, effective against all trypanosomes, is a difficult task. The same active substances have been used for the past 30 years. Immunity against diminazene and other trypanocidal substances have recently registered growing freqency in Western and Eastern Africa. For this reason I have concentred on developing a trypanocidal drug with prolonged effectiveness, which is still active after 90 days.

In the development stages of the drug, much time was spend trying to solve the problem of storing the drug in tropical climates. This problem was solved satisfactorily some months ago. Laboratory tests shown that the drug remains stable and unchanged for several months even when exposed to temperatures of more than 70℃. This means that it will be possible in future to transport and store the drug at high temperatures, providing certain conditions are met.

Physical chemistry

In the formulation of TRYPAN, it has been combined with diminazene-di-acceturate (diamidinophenyltriazene diaceturate tetrahydrate), Phenazone and Procaine. Diminazene is an aromatic diamidine dirived from Surfen C (Jensch 1958). The molecule is marketed as the diaceturate salt and consists of two amidinophenyl moieties linked by a triazene bridge, p,p-diamidinodiazo-aminobenzene diaceturate tetrahydrate.

The most diminazene-di-aceturate preparations are marketed in combination with the stabiliser and antipyretic molecule, phenyldimethyl pyrazolon (antipyrine) or Phenazone. In comparison with other product, TRYPAN is combined with Procaine hydrochloride. This combination has a synergistic and an additive effect in comparison with the other trypanocidal drugs.

TRYPAN has a painless, antipyretic and long-lasting effect. According to the results of the several tests, the TRYPAN has been judged as beeing the most effective Trypanocidal drug against infections with *Trypanosoma congolense, T. vivax, T. brucei* and *T. evansi, Babesia bigemina, B. canis* or other *B. aswell* as against *Theileria annulata*. Furthermore it has been confirmed that after a single injection of 15 ml, an animal is protected against a new reinfestation during a period of three months. TRYPAN can be used for curative and preventive treatment.

MATERIAL AND METHODS

The test results dated April 6, 1998, which we recently received from Tanzania, confirmed once again that TRYPAN is more effective against all trypanosomasis, *Babesia* and ECF parasites.

1) Nicholaus and Mwinuka (Frebruary 1998) in Ruvuma Region has demonstrated sensivityy of *T. congelense* "ADRI" to different doses of diminazene aceturate (TRYPAN) administered a four days post injection in mices.

Female Swiss white mice weightung 29-40g and 6-10 weeks old, randombred at Sokoine University of Agriculture, Morogo, were used for trypanosome maintenance and for the drug sensitive tests.

For the both drugs Isametadium and Diminazene, dilutions used were such that a mouse weighing 20g receive 0.20ml of the drug.

Initial treatment was carried out at five days post infection and the second treatment at nine days post infection.

RESULTS

Table 1: Sensitivity of *T. congolense* "ADRI" to different doses of diminazene aceturate administered a four days post infection in mice

	Drugs dose mg/kg.											
D.P.T.	0.00	7.0	0.5	14.5	21.0	28.0	35.0	42.0	49.0	56.0	63.0	72.0
0	5/5	5/5	5/5	5/5	4/4	5/5	5/5	5/5	4/4	5/5	4/4	4/4
3	5/5	3/5	2/5	1/5	1/5	0/4	0/5	0/5	0/5	0/4	0/5	4/4
35	5/5	5/5	5/5	5/5	5/5	4/4	5/5	5/5	4/4	5/5	3/4	3/4

D.P.T.: Days post treatment, one mouse died of drug toxicity - estimated CD_{80} 770 mg/kg.

Diminazene aceturate at 70mg/kg was schown to cure mly 25% of mice infected with *T. congolense* ADRI (Animal Disease Research Institute).

Table. 2

Days	Drugs dose (mg/kg)					
Treatment	0.00	16.0	17.0	18.0	19.0	20.0
0	5/5*	5/5	5/5	5/5	5/5	5/5
2	5/5	3/5	2/2	2/4	4/4	2/5
4	5/5	1/4	0/1	0/4	0/4	0/4
6	5/5	0/4	0/1	0/4	0/4	0/3
60	5/5	4/4	1/1	4/4	4/4	3/3

*Number of parasitaemic mice/Total number of mice tersted

Table 2: Shows that with spaced drug treatment regimen cure rates were 75%, 33% and 0% for BB-S, B-S, and b-B respectively and 80%, 100% for S-S, S-B and S-BB respectively. Mortalities unrelated to relapse parasitaemia accured in all treated with isometadium chloride fallowed by diminazene aceturate (TRYPAN) at 42 mg/kg.

DISCUSSION

The results presented here show that with spaced combinaion of diminazene aceturate and isometadium chloride, it is possible to cure infection *T. congolense* "ADRI" strain which express high levels of resistance to both diminazene aceturate and isometadium chloride. The best results (100%) were obtained with isometadium chloride at 10 mg/kg followed by diminazene aceturate 21 mg/kg.

Furthermore, this report confirm all other clinical tests carried out in Central Africa,

Benin and Ethiopia, thus further strengthening TRYPAN's advantages and its clinical superiority as a drug.

At the same time to work on the development of new substances or molecules, it would be interesting to try to combine the existing trypanocidal substances like Diminazene, Pentamidine, Isometadium, Metronidazole and perhaps through a new combination, to reduce the trypano-resistance. It is possible that the combination of two active substances increase the spectrum of the activity and the clinical application.

It has been a known fact for years that diminazene aceturate cures *T. evansi* in buffaloes, but also in camels. Several clinical tests have been carried out since 1960 as described in a report by Lead (1961). Raisinghani and Lodha confirm in their report that diminazene were successfully applied to treat camels in India with a dosage of 1.25 mg/kg body weight and 5 mg/kg body weight, curing infections caused by *T. evansi*. It is known that applying diminazene to camel can be toxic to the animal. This knowledge, however, is based upon high-dosed injection of 7.5 mg to 20 mg/kg body weight and more. The normal dose is at 3.5 mg/kg body weight, which has shown no side-effects. For this reason TRYPAN has been successfully applied to the treatment of *T. evansi* in camels in the United Arab Emirates (UAE).

Another problem we are also concerned with is the problem of trypano-resistance. Trypano-resistance has been documented over the last 30 years. A report by Njogu, Dolan, A.R., Wilson, B. and Sayer, P.D. (1985) have reported of trypano-resistance in Boran cattle, and in 1987 by Reiter, B. and Seitz, A. and also in the reports by Zhang, G.C. (1992) and by Balltz on the in vitro sensitivity of *T. evansi*, as well as in the report by Peregrine, A.S., Gray, M.A., and Molloo, S.K. Mwinuka, N.T. (1998) has also reported trypano-resistance in Tanzania. (ADRI).

The problem of the trypano-resistence particularly in Kenya is very important and is growing. The initial experiments in Kenya and Dubai, TRYPAN was schown to be highly active against all *Trypanosoma* particularly *T. congolense, T. vivax, T. brucei*, and *T. evansi*. Several experiments showed that TRYPAN can be used for curative and preventive treatment.

The development of trypanocidal drug resistance is among the most serious problems for the control of African animal trypanosomiasis. This is due to firstly the using the same of trypanocidal drugs. The problem of the trypano-resistance is also resulted of the using the same molecules over 30 years.

OTHER CLINICAL REPORTS ON TRYPAN

The following reports on TRYPAN have been received:

1) Ndokouif, F. and Ndoma ,F., The Animal Health Authorities in the Central African Republic (30.04.1994). The report has demonstraded (A) the preventive chemotherapeutical effect of isometodium and TRYPAN over 110 days; and (B) trypanocidal infections on the cattle treated with TRYPAN solution were not detected during the three last parasitological controls. However, the two first parasitological controls treated with isometadium had detected that an infection of *T. vivax* has resisted to the treatment. Most probably this is a chemoresistance. TRYPAN effectiveness against *Babesia* in comparison to the other diminazene is quite strong. Considering these excellent test results, the Animal Health Authorities concluded that the TRYPAN solution can be introduced in the Central African Republic .

TESTS

Materials and Methods:

Berenil and Veriben which belong to the family of the Diamidines, Trypamidium which belong to the family of the Phenatridines, and TRYPAN solution, a new product which is related to the Diamidines, were comparatively examined in therapeutical tests.

The tests were carried out on Zebu-Mbororos-Cattle, they are porters of several kinds of *Trypanosoma*. The animals were reared on a farm situated in humid bush savannah-area, where several kinds of hematophac flies breed enormously:

- *Glosina fusca*, - *Glosina fuscipes* and - Tabanide.

The obvious heaviness of the flies in the area was about 60 flies per day and trap. This means a certain risk in view of special sorts of *Trypanosoma*. A first visit had given the possibility to find the cattle which were bearers of *Trypanosoma* and *Babesia*.

In this case the signs were the followings :

- slimming, - punctured coat, - morbid increase of the superficial lymph node,
- anaemia, and - infestation of several kinds of tick.

A blood test of a capillary of an ear was catched into two-micro test tubes prepared with Heparin and afterwards sealed with Plasticin. The blood tests made in duplicate had served to dertermine the result of the centrifuged sediment and the sorts of the *Trypanosoma* by following the methode of Max Murray.

Distribution of blood had been made to prove the hemoparasites with GIEMSA-colour - method. The twenty cattle choosen as porters of *Trypanosoma* were devided into 4 groups: - Group Berenil, - Group Veriben, - Group Trypamidium and - Group Trypan solution.

Before treatment with the several trypanocides, the temperature of the animals was increased by measuring in the rectum. Parasitologic controls were carried out every four hours with treated animals having the aim to dertermine the time of lysis of the parasites and the effectiveness of the trypanocides.

The preventive-chemotherapeutical effect of the products has been controled every months during 110 days hematoscopies with the treated animals.

Results:

TRYPAN in liquid form has unlike isometadium proved to be more affective in the chemoprophylasis in the Central African Republic, longer than the prolonging effectiveness of both products, the diminazene-diaceturate (TRYPAN) at unique dose (3.5mg/kg) controlled all ordinary infections of *T. vivax*, *T. congolense* and *T. brucei*, and had been controlled with a healing rate of 60% within 8 hours and a healing rate of 100% after 12 hours. Its effectiveness against *Babesia* in comparison to other diminazene is quite strong; two parasites per test had been proved.

2) Clinical Report from The Pharnavet (National Veterinary Pharmacy) and ADIVET in Benin, (12.09.95): Experiments in this laboratory, have demonstrated the effectiviness of all trypanocidal products is similar after 2 and 3 days but the reinfestation come back after 7 to 10 days. TRYPAN unlike to the other products, the reinfestation come back later after 80 days with a rate of 25 %.

TESTS

Material and Methods:

This research was carried out with Zebu-Mbororos-Cattles on a farm during 110 days and the preventive-chemotherapeutical effect of the product has been controlled after 7 days, 10 days, 30 days and every months.

Seventy (70) cattle choosen as porters of *Trypanosoma* were devided in 5 groups :

Drugs	Trypanosomes.					
	T. vivax		*T. congolense*		*T. brucei*	
	Before	After	Before	After	Before	After
TRYPAN	8	0	1	0	0	0
TRYPAZEN	12	0	1	0	0	0
FATRYPAZEN	18	1	1	0	0	0
VERIBEN	12	0	5	0	6	0
BERENIL	9	0	2	0	0	0

TRYPAN, TRYPAZEN, VERIBEN, BEERENIL, are effective after 8/12 hours with a healing rate of 100% only FATRYBANYL the healing rate is 94.74% after 24 h. After 3 days the effectiveness all products is with a rate of 100 %.

Rate of the infection after 7 days :

TRYPAZEN: 8.33%, FATRYBANYL: 27.27%, VERIBEN: 18.75%, BERENIL: 11.11%, TRYPAN: Unlike the others, the reinfestation come back later after 60 and 80 days with a rate of 25%.

The Pharnavet concluded "Considering these excellent test results, the TRYPAN can be used for the program against the Trypanosomiasis to protect the cattle breeding for curative and preventive treatment in the condition to repeat in regular interval the treatment but not exceed 80 days".

3) The Laboratory for Veterinary Diagnostics from Bohicon / Benin, confirmed that with TRYPAN it was proved its preventive and curative chemotherapeutical effect and that its broad Trypanocidal activity permits with only one administration to eliminate all *T. vivax*, *T. congolense* with a rate of 100 % after 24h.

4) Akbar, S.J., Manawar, G., Ul-Haq, A., Khan, S.M.and Khan, M.A., (Dubai Camel Hospital, PO.BOX 9220, Dubai, 1998) have also demonstrated the efficacy of three trypanocidal drugs for the treatment and/or prophylaxis of trypanosomiasis in the camels.

TEST

Material and Methods:

A total of 16 animals of both sexes, aged about two years apparently healthy and free from trypanosomiasis were selected for the study. They were divided into four groups, each group comprised four camels. One group was kept as negative control while the remaining three groups were iniculted with *Trypanosoma evansi* subcuteously using a dose of 0.02 ml containing 5 million trypanosomes. Groups I, II and III were treated with Melarosamine, Diminazene and Quinapyramine. Group IV served as non-infected and non-treated control.

Results:

After five to six days of infection, animal showed signs of anorexia. Evaluation of buffy-coat and blood examination was performed in all three groups and were found positive for this parasite. The infected and non-infected camel died after 24 days, showing emaciation and weakness. Of three treated animal died after 34 days, while in one of the treated camel disease relapsed, showing signs of fever and anorexia. Cure rate of 66.66% for Diminazene (Trypan)) and Melararosamine (Cymelarsan) and 33.33% Quinapyramine (Triquin).

5) Fazil, M.A., Mombasa, Kenya (1998), has also demonstrated that 50 head of cattle average weight 250 kg were injected i.m. with TRYPAN SUSPENSION at rate of 20 ml per animal, started showing signs of the infection after 59 days.