Comparative *In-Vivo* Activities of Diminazene, Suramine, Quinapyramine and Homidium Bromide on *Trypanosoma evansi* infection in Mice

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Abstract:

Chemotherapy and chemoprophylaxis are the main methods of trypanosomal control. This study was done to compare the *in vivo* efficacy of four commonly used antitrypanosomal drugs. Twenty five Swiss Webstar mice (groups of fives) infected with locally isolated *Trypanosoma evansi* strains were used. Four groups were intraperitoneally injected by therapeutic doses of Diminazene aceturate (Berenil): 3.5 mg/kg, Suramine (Naganol): 10.0 mg/kg, Quinapyramine (Antrycide): 5.0 mg/kg and Homidium Bromide (Ethidium Bromide): 1.0 mg/kg. The fifth group of mice was used as a non-treatment control. Animals with heavy parasitic burden were cured by both Naganol and Berenil after 2 and 3 days of therapy, respectively. Unfortunately, Berenil caused death in 2/5 of experimental animals next day of therapy while Naganol showed no detectable toxic effects. Other drugs either failed to cure the infection or produced toxic effects in animals. In conclusion, Naganol is recommended for treatment of *Trypanosoma evansi* infection of mice.

Introduction:

Trypanosomiasis has continued to disrupt human life, animal husbandry and wild life in most parts of the world (Kuzoe 1993). The field control of animal trypanosomiasis has, over the years, relies on two broad strategies: using chemotherapeutic agents on infected animals and vector control. In general, however, the chemotherapeutic approach is used much more widely than vector control because it is easier to kill the trypanosomes than the flies (WHO 1998). Current methods of treatment of trypanosomes are still unsatisfactory because the number of available drugs is limited and the treatment is usually associated with severe side effects (Kaminsky and Brun 1998). The emergence of drug resistant trypanosomes implies failure of treatment or prevention, and if no other

active drugs are available, animals have to rely on their own immune defenses alone to combat the disease (Uilenberg 1998).

Chemotherapeutic drugs disrupt or block one or more of the vital processes which are essential to the parasite. Some compounds have specific effects on some enzyme system or block essential metabolic pathways. The exact way in which they work is often not known or only incompletely understood (Zhang *et al.* 1991). Chemotherapy, by stopping the multiplication of the trypanosomes, helps the immune system to overcome the infection (Osman *et al.* 1992). Chemotherapeutic drugs are toxic to the trypanosomes and often have a similar disruptive effect on the cells of the host (Jennings *et al.* 1977), and so should always be used with care and at the recommended dose level only (Homeida *et al.* 1981). It is estimated that in Africa 25-30 million doses of trypanocidal drugs are used annually in the treatment of animal trypanosomiasis, but the population of animals at risk indicated that ten times this figure were necessary (Ilemobade and Buys 1970).

Many investigators have reported therapeutic trials of *Trypanosoma evansi* with the use of different chemotherapeutic drugs (Homeida *et al.* 1980, Bacchi *et al.* 1998, Tuntasuvan *et al.* 2003). This study was done to compare *in vivo* action of the four commonly used chemotherapeutic drugs (Diminazene, Suramine, Quinapyramine and Homidium Bromide) on mice infected by locally isolated *T. evansi*.

Materials and Methods:

Trypansome: a cryopreserved strains of *T. evansi* originally isolated from naturally infected camels in Al-Ahsa Area, Saudi Arabia, that were propagated in laboratory bred rats, were extracted, purified and adjusted to yield 10^4 parasites (Al-Mohammed 2006) were used for infection of experimental animals.

Animals: Twenty-five female Swiss Webstar mice weighting 20-25gm of each were used for *in vivo* drug tests. Mice (groups of fives) were treated with four antitrypanosoma drugs at the appropriate concentrations and one group of animals was used as control.

Drugs: Drugs were dissolved and prepared as aliquots (according to manufacturer's instructions), to be injected intraperitoneally (I.P.) in the following concentrations: Diminazene aceturate (Berenil, Hoechst, Germany): 3.5 mg/kg, Suramine (Naganol, I.C.I., UK): 10.0 mg/kg, Quinapyramine (Antrycide, Bayer, Germany): 5.0 mg/kg and Homidium bromide (Ethidium bromide, May and Baker, UK): 1.0 mg/kg.

In vivo drug activity- each mouse was inoculated I.P. with 10^4 parasites and infection was allowed to develop for 14 days when treatment was initiated on day 15. Mice were checked daily (one day before therapy and thereafter for 17 days) for parasitaemia in blood collected from tail vein, (study period = 31 days). Animals were considered cured when no trypanosomes were detected during the 17 days of observation period (Bacchi *et al.* 1998).

Table (1)

State of parasitaemia in mice infected with *T. evansi* after treatment with four trypanocides.

days	Mean No of parasites in blood smear																	
Control	14	15	16	17	18	19	20	21	21	23	24	25	26	27	28	29	30	31
	183	147	79	33	0	12	52	93	137	214	0	0	25	59	214	157	104	98
	0	8	43	83	117	135	168	205	222	269	341	417	399	325	298	189	150	131
	121	200	254	290	305	317	261	214	138	94	55	34	20	0	0	13	75	115
	253	194	103	88	37	15	0	0	0	1	7	28	50	113	147	203	225	270
	55	76	100	106	127	201	232	163	105	97	38	4	0	2	36	85	133	190
Ethidium bromide	172	180	173	190	200	0	0	2	26	163	178	231	157	192	214	250	194	167
	9	12	45	230	300	325	398	413	215	176	50	0	0	150	97	45	3	0
	230	400	x															
	35	130	x															
	320	400	x															
Naganol	40	60	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	50	300	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	120	200	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	158	300	75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	311	350	95	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Berenil	150	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	0	300	210	97	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	130	200	x															
	200	300	x															
	400	350	130	70	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Antrycide	170	320	x															
	15	75	x															
	87	200	x															
	40	110	x															
	100	230	x															

X = animals died

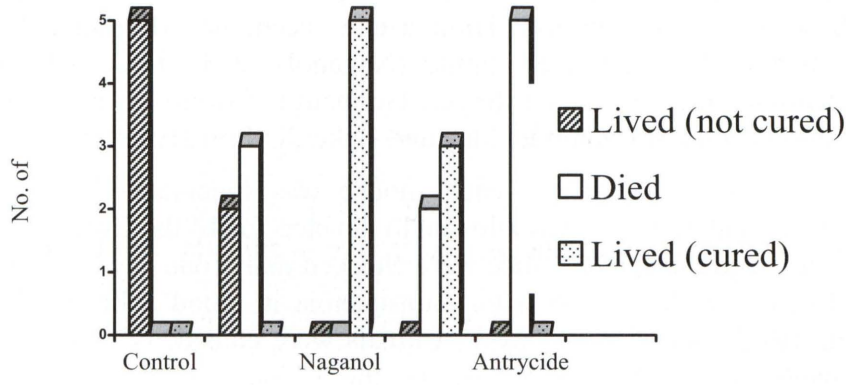

Figure (1) : Effect of four Trypanocides on *T. evansi* infected mice.

Results:

The effect of antitrypanosomal chemotherapy is shown in table (1) and figure (1). In the control group, parasitaemia showed the usual undulating course. In Ethidium bromide treated mice, three mice died next day of treatment (day 16). The other two mice showed parasetaemia not statistically different from control group with no effect of the drug on the parasites. In Naganol treated mice, the five animals showed dramatic response to the drug and clearance of parasetaemia occurred on the second day of chemotherapy. The mean number of parasites in blood smears on the day of chemotherapy was 242 that dropped on the next day to a mean of 42 followed by complete clearance of parasetaemia on the second day (day 17). In Berenil treated mice, two animals died next day of chemotherapy and the other three mice responded well to treatment. The mean number of parasetaemia on the day of therapy was 270 that dropped to a mean of 113.3 on the first day, 55.6 on second day and complete cure on third day of therapy (day 18). In Antrycide treated mice, all five mice died next day of I.P. chemotherapy.

Discussion:

Drug control of animal trypanosomiasis relies essentially on three drugs, namely: Ethidium bromide, Berenil ac eturate and Naganol. More recently Antrycide has been reintroduced because of the need to especially combat camel trypanosomiasis. The results obtained from this study clearly demonstrated that Naganol and Berenil were powerful antitrypanosomal compounds with a specific activity *in vivo* comparable to Ethedium bromide. Both drugs are curative 100% in model trypanosome infections and were effective in animals with heavy parasite burden (range of parasitaemia was 60-350 and 200-300 parasites, respectively). Importantly, the toxicity of Naganol was very low if compared with Berenil which caused death in 2/5 of experimental animals.

Berenil was reported by Tuntasuvan *et al.*, (2003) to cause mild to severe toxicity in horses and mules after injection, with minimal protective effect of the drug. Berenil was proved to be effective for the treatment of surra in cattle, buffalo, sheep, pigs and camels (Peregrine and Mamman 1993), but was reported to cause fatal reactions in camels, horses and dogs at doses which are considered to be normal and harmless in cattle (Sirivan *et al.* 1994).

Although showed toxicity and death of 3/5 of animals, Ethidium bromide had no therapeutic action on the remaining two animals of this study indicating that the strains of *T. evansi* isolated in Al-Ahsa might be resistant to that drug; a serious problem in the chemotherapy of Surra. Although the exact mechanism of drug resistance is insufficiently known, two mechanisms have been proposed by Uilenberg (1998); adaptation and selection theories. Impairment of the host immune system may lead to the rapid development of drug resistance by *T. evansi* under experimental conditions in mice (Osman *et al.* 1992). Reports of drug resistant *T. evansi* (or even multiresistant strains) are emerging from allover the world (Brun and Lun 1994, WHO 1998). Care must be taken in reporting drug resistance since the inaccessibility of the drug to tissue stages of trypanosomes or the insensitivity of some stages in the life-cycle of the trypanosome to the drug could be the reason (Jennings *et al.* 1977).

Antrycide, on the other hand, showed much toxicity to mice after I.P. administration and cause death of all five animals. Swiss Webstar mice did not tolerate a single-dose regimen of 0.5 mg/kg antrycide which appeared to be in the borderline for acute toxicity. Singh *et al.*, (1993) used antrycide in treating *T. evansi* in infected dogs with complete recovery of two dogs, while another dog died on the day therapy was initiated. These results agreed with Uilenberg (1998) who described toxicity problem of antrycide in cattle and horses. It is apparent that the toxicity of drugs differs in different species of animals. Much controversy results could be noticed about the efficiacy of the drug in both *in vivo* and *in vitro* studies. Kaminsky and Zweygarth (1989) reported that care must be taken when evaluating anti-trypanosomal drugs for in vitro potency because drugs might be inactive in the *in vitro* system but still be efficacious *in vivo*.

Because of its low toxicity margin and the inability to use it at higher dosage rates, antrycide has lost its popularity for use against *T. evansi.*

Collectively, the data presented indicate that suramine followed by berenil are the best trypanocidal drugs for *T. evansi*. Antrycide should be avoided due to its major toxic side effects.

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مقارنة فاعلية الدامنيزين، السورامين، القوابايرامين و بروميد الهوميدوم في معالجة فئران مصابة بسلالة *Trypanosoma evansi* المسبب لمرض الهيام (النوم)

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المخلص :

يعتبر استخدام أدوية مضادات الطفيليات للوقاية والعلاج أحد الطرق الرئيسية لمكافحة طفيل *Trypanosoma evansi* المسبب لمرض الهيام (النوم). أجريت هذه الدراسة لاختبار تأثير أربعة من الأدوية الشائعة في علاج هذا المرض بين الحيوانات حيث تم استخدام عدد ٢٥ من الفئران السويسرية (مقسمة إلى خمس مجموعات) والتي أمرضت سلفا بحقنها جميعا بجرعات متساوية من سلالة معزولة من طفيل *Trypanosoma evansi*.

حقنت ٤ مجموعات في التجويف البروتوني بالأدوية وهي كالتالي: المجموعة الأولى استورات الدامنزين (بيرنيل) بجرعة مقدارها ٣,٥ مليجرام للكيلوجرام، المجموعة الثانية سورامين (نجانول) بجرعة مقدارها ١٠ مليجرام للكيلوجرام الواحد، المجموعة الثالثة القوابايرامين (انتراسيد) بجرعة مقدارها ٥ مليجرام للكيلوجرام. أما المجموعة الرابعة فحقنت بمركب بروميد الهوميدوم (بروميد الايثديم) بجرعة مقدارها ١ مليجرام للكيلو جرام. وتركت المجموعة الخامسة بدون علاج كضابط للاختبار.

هذا وقد تم شفاء الفئران من الإصابة بواسطة مركب النجانول بعد يومين من بدء العلاج أما البيرنيل فتم الشفاء بعد ثلاثة أيام إلا أن البيرنيل تسبب في موت ٢ من ٥ من الفئران المعالجة في اليوم التالي لبدء العلاج بينما لم يتسبب النجانول في موت أي من الفئران. ولقد فشلت بقية الأدوية في شفاء المرض وربما تسببت في آثار سمية في الفئران.