The efficacy of furazolidone against experimental infections with *Trypanosoma evansi* in camels and mice in Sudan : comparisons with quinapyramine and suramin

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Efficacité du furazolidone contre les infections expérimentales à *Trypanosoma evansi* chez les dromadaires et les souris au Soudan : comparaison avec la quinapyramine et la suramine — L'efficacité du furazolidone contre les infections expérimentales à *Trypanosoma evansi* a été étudiée chez les dromadaires et les souris. Les critères de mise en évidence de l'effet trypanocide du furazolidone comprenaient la recherche de *T. evansi* dans le sang et les tissus, ainsi que les changements pathologiques et cliniques dans quelques tissus spécialement choisis.

Chez les souris infectées, le furazolidone à la dose orale unique de 320 mg/kg de poids vif a entraîné l'élimination complète, pendant 4 semaines, du parasite circulant dans le sang. A cette dose, aucun effet toxique n'a été constaté sur les souris traitées. L'efficacité de la quinapyramine à dose unique sous-cutanée de 2 mg/kg de poids vif s'est révélée identique.

Chez les dromadaires infectés, le furazolidone administré à la dose de 10 mg/kg de poids vif pendant 5 jours n'a pas abouti à l'élimination des protozoaires du sang. Le traitement des mêmes animaux à la quinapyramine (injection sous-cutanée de 5 mg/kg de poids vif) et, 4 semaines plus tard, à la suramine (25 mg/kg de poids vif en intraveineuse) a entraîné l'élimination des parasites du sang pendant 21 jours au maximum, après quoi des rechutes sont apparues.

L'étude a montré qu'en dépit de l'effet trypanocide du furazolidone chez la souris, ce produit s'est révélé inefficace contre *T. evansi* chez le dromadaire du moins aux doses tolérables par cette espèce. *Mots clés :* Dromadaire - Infection expérimentale - *Trypanosoma evansi* - Souris - Trypanocide - Furazolidone - Quinapyramine - Suramine - Soudan.

INTRODUCTION

Infections with *Trypanosoma evansi* are prevalent in many tropical countries and cause tremendous losses in life and productivity of camels (for a review see 6). Little progress seems to have been made in the field of chemotherapy and prevention of trypanosomiasis due to *T. evansi* since the introduction of quinapyramine and suramin a few decades ago. Isometamidium chloride (samorin) has been shown to be either irregularly effective or even ineffective in the prophylaxis and treatment of camel trypanosomiasis (2, 3, 9, 10, 11).

In the present study, an attempt has been made to assess the therapeutic effectiveness of the nitrofuran drug, furazolidone, which is known to be effective against a variety of bacteria (*e.g. Campylobacter, Salmonella* and *E. coli*) and protozoa (*e.g. Giardia, Coccidia* and *Trichomonas*). Brief notes on the effect of this drug against *T. evansi* in mice have been published recently (1, 13), and these studies seem to indicate that this drug, at rather high doses (320 and 160 mg/kg, orally) is useful in treating mice experimentally infected with *T. evansi.* Lower doses were ineffective. These findings led us to evaluate this drug in the natural host of the parasite, the camel. The results obtained were compared with those obtained after conventional therapy with quinapyramine and suramin.

MATERIALS AND METHODS

Animals

Ten healthy camels (*Camelus dromedarius*) of both sexes, weighing 160-320 kg and ranging from 3-7 years old were purchased from the Khartoum area (known to be free from trypanosomiasis), and kept in a fly-proof house. They were given sorghum grains, hay and water *ad libitum.* The animals were numbered 1 through 10, and were divided into three groups : Group I (n^{os} 1 to 6), Group II (n^{os} 7 to 9) and Group III (n° 10).

Healthy white albino mice (locally bred) of both sexes, weighing 20-25 g were used. They were kept in clean plastic cages and allowed free access to drinking water and pelleted diet. They were divided at random into six groups designated I to VI.

T. evansi

A local strain was isolated from naturally infected camels in the Gadamballia region of the southeastern Sudan, an area where camel trypanosomiasis is prevalent. The parasite was maintained in albino white

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mice by syringe passage. Trypanosomes were separated from blood of infected mice showing a high parasitaemia using the method described before (2). Each experimental mouse received 500 000 parasites intraperitoneally while each experimental camel received 1 million parasites.

Experimental infection

(a) Camels

(I) Six camels (n^{os} 1 to 6) were infected with trypanosomes (Day 1) and then treated orally with furazolidone (12 mg/kg for 5 days) after one week.
(II) Three camels (n^{os} 7 to 9) were infected but not treated.
(III) One camel (n° 10) was kept as an uninfected for control.

(b) Mice

(I) 5 mice were kept as uninfected untreated controls.
(II) 5 mice were each infected with trypanosomes (Day 1).
(III) 5 mice were each infected as above (Day 1) and treated with an oral dose of furazolidone (320 mg/kg) on Day 8.
(IV) 5 mice were each infected as above (Day 1) and treated with an oral dose of furazolidone (320 mg/kg) on Day 22.
(V) 5 mice were given furazolidone at a dose of 320 mg/kg orally.
(VI) 5 mice were infected as above (Day 1) and treated subcutaneously with quinapyramine (2 mg/kg) on Day 8.

Treatment with furazolidone

Furazolidone of particle size 5 µm (« Furazolidon », Orphahell, The Netherlands) was administered orally to camels at a dose rate of 12 mg/kg for 5 days as recommended by the British Pharmacopoeia (Veterinary), 1977, for susceptible-infections in mammals. The drug was given to mice at a dose of 320 mg/kg as recommended by ZHANG (13). In both species, the drug was given 7 days after infection when parasitaemia was high.

Treatment with quinapyramine and suramin

After the experiment with furazolidone, the camels remained infected (see below) so it was decided to see if quinapyramine or suramin would be effective in reducing parasitaemia. Quinapyramine salt (supplied by May & Baker Laboratories) was given subcutaneously to camels (n^{os} 1 to 6)) at a dose rate of 5 mg/kg. For comparison, 5 mice were given the drug at a dose rate of 2 mg/kg subcutaneously. Suramin (« Naganol », Bayer, Germany) was administered to camels at a dose rate of 25 mg/kg (i/v) as recommended by the manufacturer. No sign of interactions between the administered drugs were noted in mice or camels.

Histopathological methods

Small pieces of heart, lung, liver, kidneys, spleen and lymph nodes of selected mice in each group were fixed in 10 p. 100 v/v formol-saline and cut at 5 µm thickness, and stained with haematoxylin and oesoin.

RESULTS

Effect of furazolidone on trypanosomiasis in mice

Some of the results of this experiment are shown in table I. In infected untreated mice (Group II), the trypanosomes appeared in blood within 12 h, disappeared in one mouse within 31 days, relapsed in 3 mice and never disappeared in one mouse. In mice treated with furazolidone one or three weeks after infection with *T. evansi,* the parasites disappeared from the blood three to seven days after treatment. The blood remained free from the trypanosomes for four weeks following which parasitaemia recurred. Furazolidone treatment in uninfected mice (Group V) did not cause obvious adverse effects, and the animals were killed at day 30.

Histopathological findings in mice

In infected untreated mice (Group II) there was some evidence of hepatic damage manifested by focal necrosis, haemosiderin deposition, and infiltration of the portal tract by mononuclear cells. There was also congestion and haemorrhage in the spleen, kidney and lungs. In Groups III, IV and VI only slight congestion was seen in the spleen and hearts of mice n^{os} 13, 17, 26 and 27. No obvious histopathological findings could be seen in mice in Groups I and V.

Effect of furazolidone on camel trypanosomiasis

As shown in table II, furazolidone (10 mg/kg liveweight for 5 days) was not effective in removing the parasite

TABLE I The effect of furazolidone (320 mg/kg orally) and quinapyramine (12 mg/kg) on mice experimentally infected with* **T. evansi** *(500 000 trypanosomes).*

	Mice n°	Onset of parasitaemia (h)	Trypanosome disappeared from blood (day)	Fate of mice at day
Group I (uninfected untreated controls)	1	—	—	30 (killed)
	2	—	—	30 (killed)
	3	—	—	30 (killed)
	4	—	—	30 (killed)
	5	—	—	30 (killed)
Group II (infected untreated)	6	12	31	40 (died)
	7	12	*	40 (killed)
	8	12	**	40 (killed)
	9	12	*	38 (died)
	10	8	*	40 (killed)
Group III (infected then treated after a week)	11	12	3 (after treatment)	30 (killed)
	12	8	5	30 (killed)
	13	8	6	30 (killed)
	14	12	3	30 (killed)
	15	12	7	30 (killed)
Group IV (infected then treated after 3 weeks)	16	6	3 (after treatment)	30 (killed)
	17	12	6	30 (killed)
	18	6	5	30 (killed)
	19	12	3	25 (died)
	20	8	7	21 (died)
Group V (uninfected given furazolidone only)	21	—	—	30 (killed)
	22	—	—	30 (killed)
	23	—	—	30 (killed)
	24	—	—	30 (killed)
	25	—	—	30 (killed)
Group VI (infected then treated with quinapyramine)	26	6	2	60 (killed)
	27	12	4	60 (killed)
	28	8	3	60 (killed)
	29	6	4	60 (killed)
	30	6	6	60 (killed)

* *Relapsing parasitaemia.* ** *Trypanosome never disappeared.*

from the blood stream. Monitoring the infection by the diagnostic methods mentioned before, parasitaemia remained for at least one month.

Effect of quinapyramine and suramin on trypanosomiasis

Following the failure of furazolidone in eliminating *T. evansi* infection in camels, an attempt was made to cure the infection with quinapyramine at the recommended dose of 5 mg/kg. Following the same procedure for diagnosis used before, it was found that the treatment was only effective in removing the parasite from the blood for periods ranging from 5 to 21 days. Relapses then occurred. Infected mice (Group VI) given the drug at a dose of 2 mg/kg were apparently cured for a period of two months, following which relapses occurred in four out of five mice. Another attempt was made to treat the experimental infection in camels with the therapeutic dose of suramin (25 mg/kg). This dose removed the parasite from the blood for up to 16 days. Three to five days after the recurrence of parasitaemia, 3 of the camels died and the rest were slaughtered.

TABLE II The effect of furazolidone (12 mg/kg for 5 days orally) on camels experimentally infected with **Trypanosoma evansi** *(10^6 parasites).*

	Camels n°	Onset of parasitaemia (h)	Trypanosome disappeared from blood (day)
Group I (infected treated)	1 2 3 4 5 6	12 24 8 12 12 —	Never disappeared
Group II (infected untreated)	7 8 9	12 6 8	Never disappeared
Group III (uninfected untreated control)	10	—	—

DISCUSSION

Several drugs have been tried against *T. evansi* infections in camels and other domestic and laboratory animals (2, 4, 5, 7, 11, 12). More recenlty furazolidone has been shown to possess a remarkable efficacy against *T. evansi* in mice comparable to that of quinapyramine (1). Therefore it was of interest to try the drug in infected camels. The dose which was effective in mice (320 mg/kg) was too high and probably toxic to camels. Lower doses were not attempted because it has been shown to be ineffective as trypanocidal drugs in mice (13). It was shown previously that camels are susceptible to furazolidone toxicity at doses which are apparently harmless to other species (ALI, unpublished observation). Therefore the dose recommended for mammals by the British Pharmacopoeia (Veterinary), 1977, was chosen. At this dose the drug failed to remove the parasite from blood making it unsuitable as a trypanocidal drug in camels when used at this dose. Treatment with the « classical » trypanocidal drug quinapyramine and suramin were effective only in removing the protozoan from the blood stream for up to 21 days. Repeated treatment with these drugs may, therefore, be necessary. With furazolidone, however, repeated treatment is not without some risk due to its known cumulative toxic properties.

The observation that mice were not affected either clinically or histopathologically by furazolidone at a dose rate of 320 mg/kg, while the same drug and diminazine aceturate and isometamidium chloride all affect camels adversely at doses which are apparently harmless in other animals (2, 8), seems to indicate that camels are particularly susceptible to the toxic effects of trypanocidal drugs. The reason for this particular susceptibility of camels is not known with certainity. It may possibly be due to a deficiency in the liver metabolizing capacity of camels, in particular the absence of some important drug metabolizing enzymes in this species, or to the biotransformation of these drugs into toxic metabolites. Experiments are in progress in this laboratory to study some drug metabolizing enzymes in the camel.

It is known that *T. evansi* is becoming more resistant to suramin especially when the drug is used regularly in the field (4). Quinapyramine is practically no longer commercially available. Recently it has been shown that isometamidium chloride eliminates *T. evansi* from experimentally infected camels for periods of only 3 weeks or less (2). Thus the research for effective and safe anti-trypanosomal drugs must continue. Considering the great economic significance of camels in tropical countries, the importance of the search for such drugs cannot be over-emphasized.

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The efficacy of furazolidone against experimental *Trypanosoma evansi* infection was studied in camels and mice. The criteria for assaying the anti-trypanosomal effect of furazolidone included

ALI (B. H.), HASSAN (T.), MALIK (K. H.). Eficacia de furazolidone contra las tripanosomosis con *T. evansi* en los dromedarios y los ratones en Sudán. Comparación con la quinapiramina y la suramina. *Rev. Elev. Méd. vét. Pays trop.*, 1986, **39** (2) : 197-201.

Se estudió la eficacia de furazolidone contra las tripanosomosis experimentales con *T. evansi* en los dromedarios y los ratones. Se evidenció la acción tripanocida de furazolidone por la bús-

examination of blood and tissue for *T. evansi* and the clinical and pathological changes seen in some selected tissues.
In infected mice furazolidone at a single oral dose of 320 mg/kg liveweight produced complete elimination of the parasite from the blood stream for four weeks. At this dose no toxic effects were seen in the treated mice. Quinapyramine at a single subcutaneous dose of 2 mg/kg liveweight produced similar effects.
In infected camels furazolidone at an oral dose of 10 mg/kg liveweight for five days did not remove the protozoan from the blood stream. Treatment of the same infected camels with quinapyramine (5 mg/kg liveweight subcutaneously), and four weeks later by suramin (25 mg/kg liveweight intravenously), removed the parasite from the blood for periods up to 21 days, following which relapses recurred.
The study indicated that despite the anti-trypanosomal effect of furazolidone in mice, it was found ineffective against *T. evansi* in camels when given at doses tolerated by this species. *Key words :* Dromedary - Experimental infection - *Trypanosoma evansi* - Mouse - Furazolidone - Quinapyramine - Suramin - Sudan.

queda de *T. evansi* en la sangre y los tejidos así como por modificaciones patológicas y clínicas en algunos tejidos elegidos.
En los ratones infectados, una sola dosis de 320 mg/kg de peso vivo por vía oral provocó la eliminación completa, durante 4 semanas, del protozoario en la sangre. Con esta dosis, no se observó ningún efecto tóxico en los ratones tratados. Se notó también la eficacia de una dosis única de 2 mg/kg de peso vivo de quinapiramina por vía subcutánea.
En los dromedarios infectados, la administración de 10 mg/kg de peso vivo durante 5 días no eliminó los tripanosomas. El tratamiento de los mismos animales con quinapiramina (inyección subcutánea de 5 mg/kg de peso vivo) y 4 semanas más tarde con suramina (25 mg/kg de peso vivo por vía intravenosa) provocó la eliminación de los tripanosomos durante 21 días al máximo, pero después se observaron recaídas.
El estudio mostró que a pesar del efecto tripanocido de furazolidone en el ratón, este producto fué ineficaz contra *T. evansi* en el dromedario, por lo menos con las dosis tolerables por dicha especie. *Palabras claves :* Dromedario - Ratón - Tripanosomosis - Infección experimental - *Trypanosoma evansi* - Tripanocido - Furazolidone - Quinapiramina - Suramina - Sudán.

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