

Trypanosomiasis in Zebu cattle. Reappearance of *Trypanosoma congolense* in brain tissue after treatment with Berenil

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RÉSUMÉ

Trypanosomoses chez le Zébu. Réapparition de *T. congolense* à partir du tissu cérébral.

Une souche de *Trypanosoma congolense* a pu être isolée du cerveau d'un zébu ouest-africain, traité à l'acétate de diminazène (Bérenil) à une dose de 8 mg/kg.

L'hypothèse d'une résistance médicamenteuse a pu être écartée, et le sous-dosage est expérimentalement exclu. Il a été également montré que la généralisation de la parasitémie chez les souris injectées par voie intracérébrale est possible.

Le cerveau peut être considéré comme un réservoir possible pour *T. congolense*. Après traitement au Bérenil, la réinvasion du système vasculaire des zébus par ce parasite en provenance du cerveau n'est donc pas exclue.

INTRODUCTION

The most significant pathogens involved in Nagana of cattle in West Africa, and especially in Upper Volta, are *Trypanosoma vivax*, *T. congolense*, and *T. brucei* (4). *T. brucei* invades and infects many parts of the body, parasitizing the blood, intercellular fluids, connective and parenchymatous tissues as well as the fluids of the body cavities. At first, it was believed that *T. congolense* and *T. vivax* only parasitized the vascular system, but recently LUCKINS and GRAY (9) were able to demonstrate *T. congolense* in extravascular sites.

Nagana of cattle is usually treated by administering trypanocidal drugs as a preventive measure, or in a curative manner. The most effective drug used in the curative treatment of

trypanosomiasis in West Africa is Diminazene aceturate (Berenil-Hoechst, FRG). It affords an uncompensating treatment for the various trypanosomal species responsible for the disease in cattle. However, Berenil and other compounds can readily induce drug-resistance in trypanosomes, as WILLIAMSON, (14), and others have shown.

JENNINGS et al. (6) have described a relapsing *T. brucei* infection in mice which occurred after treatment with Berenil and was not ascribable to drug-resistance or under-dosage of the medication. Because the blood-brain-barrier prevents Berenil from reaching the cerebrospinal fluid (CSF) in sufficient concentration to be effectively, it can be assumed that the reappearance of the parasite is due to its persistent infection of the brain tissue.

We treated Zebu cattle, which exhibited signs of *T. brucei*, *T. congolense* and *T. vivax* infection, with Berenil and found that reoccurring infections were not due to drug-resistant trypanosomes or under-dosage of the medication but some other mechanism. We recorded various times that animals became aparasitaemic after Berenil treatment and relapsed two to four weeks later. In this paper, we describe a study of four Zebu cattle wherein specific emphasis was placed on discovering the sites of the parasites during the aparasitaemic blood phase.

MATERIAL AND METHODS

Experimental animals

Cattle (No 1-4) selected for the study were male West African Zebus, 2 years old, and weighed about 200 kg. The sera from all the animals were positive for trypanosomiasis as tested by enzyme-linked immunosorbent assay (ELISA), a readily understandable fact attributable to trypanosomal infections of these animals because they came from tsetse-fly infested areas.

The rats used in the study were adult Wistar albino rats, 250-350 g, male and female.

SPF-Swiss strain mice, 16-20 g, were purchased from the Pasteur Institute, Abidjan.

The goats (Landrace) were purchased at the local market. They were checked and found to be parasitologically and serologically negative for trypanosomiasis.

Chemotherapy

The cattle were injected (i. m.) with 8 mg Diminazene aceturate (Berenil) per kg body weight, more than twice the recommended curative dosage for *T. congolense* and *T. vivax* of 3.5 mg per kg. The rats received 20 or 30 mg Berenil per kg of body weight. Both species tolerated the drug without noticeable side effects.

Origin of the test trypanosomes

All strains of trypanosomes used in the experiment were isolated from naturally infected animals and passaged *in vitro*. Because of logistic difficulties, they were not stabilates and may have consisted of a mixture of subspecies. The four Zebus used as subjects were confirmed to have a *T. congolense* infection prior to the experiment.

The *T. vivax* strain was isolated from a cow exhibiting massive parasitaemia at the Bobo-Dioulasso slaughter house and passaged twice in goats.

T. brucei came from a rabbit which was infected under laboratory control with *G. palpalis gambiense* flies. It was passaged twice in mice.

Using the « matching » method according to HERBERT and LUMSDEN (5), the jugular vein blood of cattle and tail blood of the rats and mice was examined for trypanosomes.

The parasites were identified by Giemsa and May-Grünwald staining of the jugular blood smears or after injection into rodents.

Experimental design

Since it had been confirmed that the four Zebus were previously infected with *T. congolense*, each animal received an intravenous infection of 10^6 *T. vivax* parasites in a respective dilution of heparinized blood in phosphate-saline-glucose (PSG), pH 8, and two weeks later 10^6 *T. brucei*. The stage of trypanosomiasis was monitored twice a week. 16 days after the last infection (*T. brucei*) cattle were treated with 8 mg/kg of Berenil (i. m.) and 11 days after the Berenil treatment when no trypanosomes could be demonstrated in the peripheral blood, the four animals were sacrificed. Bottles of whole blood as well as whole brain, spleen, liver, kidney and prescapular lymph gland were removed by sterile procedure. The organs were minced in Petri dishes and passed through a kitchen sieve and diluted to a 40 p. 100 suspension in sterile PSG. The fresh 40 p. 100 organ suspensions were immediately inoculated into recipient animals as shown in table I.

Bovine tissues examined	Rats		Goats	
	Volume injected intraperitoneally	Number of inoc. animals	Volume injected intravenously	Number of inoc. animals
Blood	3 ml	2	10 ml	1
Brain	1 ml	2	10 ml	1
Spleen	1 ml	2		
Liver	1 ml	2		
Kidney	1 ml	2		
Lymphgland	1 ml	2		

TABLE 1. — Inoculation scheme for blood and organs per head of infected cattle

RESULTS

Transmission of infection after chemotherapy

The organ suspensions taken from spleen, liver, kidney and the lymph glands of the four Zebus did not produce trypanosomiasis in the recipient rats within an observation period of 30 days.

Blood taken from animal no. 1 produced a *T. brucei* infection in one rat, the infection becoming apparent on the 6th day after inoculation.

However, two rats inoculated with the brain suspension of animal no. 2 developed a *T. congolense* parasitaemia 11 and 14 days, respectively after treatment.

Inoculation of blood samples from Zebus No. 2, 3, and 4, and brain suspension of 1, 3 and 4 failed to transmit the infection.

None of the inoculated goats showed signs of infection.

Experiments with isolated trypanosomes

Due to the fact that trypanosomes could be demonstrated in tissues of cattle after Berenil therapy, it was necessary to investigate the possibility of drug-resistance, or some other mechanism.

Two groups of 3 rats each were infected with 10^3 *T. brucei* in PSG, intraperitoneally, and examined daily for trypanosomes.

Group I was treated with a single intraperitoneal dose of 20 mg/kg Berenil, the recommended dosage for rats, 4 days after infection and a parasitaemia of 10^5 - 10^6 per ml blood.

Group I remained aparasitaemic up to the end of the experiment on day 42.

Rats of group II were treated with 20 mg/kg Berenil when massive parasitaemia had occurred on day 13 (10^8 *T. brucei* per ml blood), and showed a low grade parasitaemia following the Berenil which subsided only after a second dosage of 30 mg/kg on day 4. No trypanosomes could be found throughout the next 26 days.

Three groups of 3 rats each were infected with 10^3 *T. congolense* as previously described, and group I was treated with 20 mg/kg Berenil 4 days later when parasitaemia level reached 5×10^5 /ml blood. Group I remained aparasitaemic until the end of the experiment on day 60.

Rats of group II were treated on day 9 on which a level of 10^6 parasites/ml blood was demonstrated. Two rats exhibited no parasitaemia, and in one animal the trypanosomes remained depressed and disappeared only after an additional dosage of 30 mg/kg.

Among the rats of group III a peak parasitaemia was reached on day 15 (10^7 /ml), but no additional trypanosomes could be observed throughout the 60 days after treatment with 20 mg/kg Berenil.

A group of 5 mice was intracerebrally infected with 10^3 *T. congolense* parasites in PSG, pH 8.0 as described by KOPROWSKI (8). The parasites injected into the brain led to general infection of the mice that finally died from the disease. *T. congolense* could be demonstrated in the tail blood of all infected mice within 10 days.

DISCUSSION

It was possible to isolate a strain of *T. congolense* from two rats which had re-

ceived brain tissue suspensions taken from Zebu cattle treated with Berenil. We have repeatedly observed that relapsing trypanosomal infections occur in cattle within 2-4 weeks after treatment with 7 mg/kg Berenil. After treatment, the blood of these animals was parasitologically negative as studied by the wet film technique and haematocrit centrifuge technique (15).

MCLENNAN (12) has described an aparasitaemic interval following treatment of *T. vivax* in cattle with Berenil. Even after administering 100 ml of blood to recipient animals, it was not possible to demonstrate the existence of subpatent parasitaemia. The author has postulated that following the treatment with Berenil, it is feasible to believe that *T. vivax* survives in the treated animals other than in the blood stream.

LUCKINS and GRAY (9) were the first to describe an extravascular site of *T. congolense*, a finding that indicates that even for the economically significant trypanosome strains many pathogenic mechanisms have not been clarified. The same authors (10) found *T. congolense* in lymph nodes of a calf and a sheep, another indication that the parasite should not be regarded as a strict plasma parasite.

On the other hand since 1967 cases of drug-resistance up to 7 mg/kg Berenil have been reported by MCLENNAN and JONES-DAVIES (13), and JONES-DAVIES (7) and others.

GRAY and ROBERTS (3) described a strain of *T. congolense* which retained its resistance to Berenil at a dose rate of 7 mg/kg during a series of eight transmissions by tsetse-flies in a period of 367 days, and other strains resistant to 3.5 mg/kg while being maintained by blood passage in a series of three cattle.

Berenil afforded an effective treatment against two different strains of trypanosomes (*T. congolense* and *T. brucei*) which were isolated from the brain and blood of two Zebus by us. However, we have observed that the effectiveness of the drug varies in the animals under treatment. After preliminary studies we were forced to apply 20-30 mg/kg dosages in order to completely cure the rats used in the study.

We were able to isolate *T. congolense* from the brain tissue of infected and treated Zebus, but only one out of four animals sheltered parasites in the brain. On the other hand, the

brain was the sole source of parasites in Zebu n° 2. We, therefore, assume that there might be similarities between our type of relapse results and the reappearance of *T. brucei* infections in mice reported by JENNINGS et al. (6). Once the parasites penetrate the CNS, they are beyond the blood-brain-barrier which prevents Berenil from reaching a sufficient concentration in the CNS to be effective.

Concerning the isolation of *T. brucei* in one rat after inoculation of blood of Zebu n° 1 our only interpretation is reinfection of the animal by tsetse-flies.

In African sleeping sickness due to *T. brucei gambiense* and *rhodesiense* infections parasitic invasion of the CNS occurs after varying periods of time. Trypanosomes have been shown to be present in the cerebrospinal fluid and in the substance of the brain. The treatment of patients with CNS involvement can be dangerous and difficult because of the adverse side-effects and toxicity of the compounds used. An additional serious problem evolves around the fact that these drugs do not attain a sufficient concentration in the cerebrospinal fluid.

Comprehensive studies involving the pathology of *T. congolense*, and especially its possible invasion of the CNS and reinvasion of the blood after treatment with short protection period drugs should be carried out in the future. Survival of the parasite in the brain seems feasible and should be kept in mind in cases where chemotherapy fails.

If mice are given an intracerebral injection of 10^3 *T. congolense* of our isolate, trypanosomes can be demonstrated in peripheral blood after 10 days. We, therefore, must assume that they infected the brain tissue, crossed the blood-brain-barrier to cause blood parasitaemia.

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Condensé de l'observation de l'auteur établi par la Rédaction de la Revue

Quatre zébus ouest-africains présentant une infection naturelle à *T. congolense* ont été

infectés par voie intraveineuse avec *T. vivax* et *T. brucei*. 16 jours plus tard ces animaux ont été traités à l'acéturate de Diminazène (Bérénil) à raison de 8 mg/kg, soit plus de deux fois la dose curative recommandée contre *T. congolense* et *T. vivax*. 16 jours après, alors qu'aucun parasite n'était présent dans leur sang, du sang total et des broyats de divers organes de chacun de ces zébus ont été injectés à des rats, par voie intrapéritonéale, et à deux chèvres par voie intraveineuse. *T. congolense* a été isolé chez deux rats ayant reçu une suspension de tissu cérébral d'un zébu ; *T. brucei* a également été isolé à partir de rats ayant reçu du sang d'un autre de ces zébus. Tous les autres passages ont été négatifs.

Les auteurs ont vérifié expérimentalement

que la présence de *T. congolense* chez les rats ayant reçu le broyat de tissu cérébral n'était pas due à un phénomène de chimio-résistance. Ils ont montré également que *T. congolense* injecté par voie intracérébrale provoque chez la souris une infection généralisée, avec présence de trypanosomes dans le sang de la queue moins de 10 jours après l'inoculation.

Ainsi, lorsque la réapparition de la trypanosomose chez des bovins traités ne peut être attribuée à de la chimio-résistance ou à un sous-dosage médicamenteux, il faut penser à la possibilité d'une réinfection à partir de trypanosomes réfugiés dans le système nerveux central, que le Bérénil n'a pu atteindre par suite de son incapacité à franchir la barrière hémato-méningée.

SUMMARY

A strain of *T. congolense* was isolated from two rats after injection of a brain tissue suspension from West African Zebu cattle previously infected with trypanosomes and treated with 8 mg/kg Diminazene aceturate (Berenil).

The reappearance of the strain, which was not due to drug-resistance, is experimentally elucidated and discussed. Its possible development in the brain substance was demonstrated by intracerebral injection of mice.

The brain as a source of reappearing trypanosomiasis in cattle after chemotherapy should be kept in mind when drug-resistance and under-dosage of the medication can be excluded.

ZUSAMMENFASSUNG

Trypanosomose bei Zeburindern. Das Gehirn als Reservoir von *Trypanosoma congolense* nach Berenilbehandlung.

Aus Gehirngewebe von westafrikanischen Zeburindern konnte über eine Rattenpassage ein *T. congolense*-Stamm isoliert werden, obwohl die Rinder zuvor mit 8 mg/kg Diminazene aceturate (Berenil) behandelt worden waren. Das Experiment, welches zur Isolierung des Stammes führte, wird beschrieben und diskutiert. Medikamenten-resistenz konnte ausgeschlossen werden.

Die Entwicklung des Trypanosomenstammes im Gehirn wurde durch intrazerebrale Infektion von Mäusen abgesichert.

Sofern bei der Chemotherapie der Trypanosomose der Rinder Medikamenten-resistenz und Unterdosierung ausgeschlossen werden können, sollte an das Gehirn als Reservoir für Reinfektionen gedacht werden.

RESUMEN

Tripanosomiasis en el cebú. Reparición de *T. congolense* a partir del tejido cerebral después de un tratamiento con Berenil.

Se aisló una cepa de *T. congolense* a partir de 2 ratas después de la inyección de una suspensión de tejido cerebral proviniendo de cebues oeste-africanos infectados por tripanosomos y tratados con 8 mg/kg de aceturato de Diminazene (Berenil).

Los autores comprobaron experimentalmente que ningún fenómeno de quimioresistencia era causa de la presencia de *T. congolense* en las ratas. Mostraron también que *T. congolense* inyectado par vía intracerebral provocaba en el ratón una infección generalizada con presencia de tripanosomos en la sangre de la cola menos de 10 días después de la inoculación. Así, cuando no se puede atribuir a la quimioresistencia la reaparición de la tripanosomiasis en bovinos tratados, hay que pensar en la posibilidad de una reinfeción a partir de tripanosomos presentes en el sistema nervioso central y no atacados por el Berenil.

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