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Field Evaluation of the Prophylactic Effect of an Isometamidium Sustained-Release Device against Trypanosomiasis in Cattle

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In order to compare the prophylactic effect provided by a poly(D,L-lactide) sustained-release device (SRD) containing isometamidium (ISMM) with that provided by the classical intramuscular injection of the drug, a field trial was carried out at the Madina Diassa Ranch in Mali. One- to 3-year-old N'Dama cattle were randomly divided into three groups. The first group ($n = 42$) was treated with ISMM at a dose of 1 mg/kg of body weight, the second group ($n = 44$) received the same dose of the drug via an SRD, which was subcutaneously implanted in the shoulder region, and the third group ($n = 36$) was kept as an untreated control group. All animals were treated with diminazene aceturate (7 mg/kg of body weight) 2 weeks before the start of the experiment and were tested monthly by the buffy coat technique for a period of 8 months. *Glossina morsitans submorsitans* was the most important tsetse species, with apparent densities (number of catches/trap/day) varying between 11.9 and 38.7 over the experimental period. Eight months after treatment the cumulative infection rates were 27.7, 58.5, and 77.4% in the group with the SRD implant, the group receiving the intramuscular injection, and the control group, respectively. Statistical analysis showed that the incidence of trypanosomiasis was significantly lower ($P = 0.006$) in the group which received ISMM via the SRD than in the one which was treated with ISMM intramuscularly.

The number of drugs currently available for prophylaxis of bovine trypanosomiasis in Africa is limited. Isometamidium chloride (ISMM) and homidium, which are widely used, have narrow therapeutic indices and considerable variation in their prophylactic activities has been observed (16, 18). In order to extend the period of protection provided by these drugs and to decrease their local toxicities, different alternative delivery systems, such as suraminates, dextran complexes, liposomal formulations, carrier erythrocytes, and polymers, have been developed (16). Except for the polymers, very few of these formulations have been successful.

After some successful preliminary experiments with rabbits (6, 11, 13), Geerts et al. (12) reported that the prophylactic activities of ISMM and homidium in cattle could be extended significantly by incorporating the drugs in polymers in order to produce sustained-release devices (SRDs). Using a poly(D,L-lactide) SRD, an extension of the protection period of ISMM by a factor of 3.2 was obtained. This experiment was carried out under experimental conditions by exposing cattle to tsetse flies infected with drug-sensitive *Trypanosoma congolense* (clone IL 1180). The present experiment was undertaken in order to evaluate the efficacy of poly(D,L-lactide) SRDs containing ISMM in protecting cattle maintained under conditions of heavy tsetse fly challenge at the Madina Diassa Ranch in Mali. Regular prophylactic treatment of the N'Dama cattle at this ranch is necessary in order to avoid mortality due to trypanosomiasis (7, 8).

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MATERIALS AND METHODS

SRDs. Biodegradable SRDs were prepared by extrusion of a mixture of poly(D,L-lactide) and ISMM (Trypamidium; Rhône-Mérieux). The SRDs consisted of cylindrical rods that were 3 mm in diameter and about 3 cm in length and that were loaded with 25% (wt/wt) ISMM. They were coated by dipping them in a poly(D,L-lactide)–chloroform solution (10% [wt/vol]). Dexamethasone (1% [wt/wt]) was added to the coating of the SRDs to reduce the tissue reaction at the implantation site.

Experimental site. The experiment was carried out at the Madina Diassa Ranch, about 300 km south of Bamako, Mali. The climate is tropical and semihumid and is of the soudano-guinean type, with a wet season of about 6 months (May to October) and a dry season from November to April. The N'Dama cattle were kept in herds, which had access to the pasture during the hours of daylight (between 0800 and 1700 h). At night they were kept inside a kraal. Cotton grains, vitamins, and minerals were given as a feed supplements. The animals were vaccinated against the most important diseases, such as rinderpest, contagious pleuropneumonia, anthrax, blackleg, and pasteurellosis. Acaricidal treatment was given once a month during the dry season and twice monthly during the wet season. No tsetse control operations had been carried out at the ranch since 1983.

Two herds of 1- to 3-year-old N'Dama cattle, consisting of 65 female and 57 male animals, respectively, were included in the experiment. Each of the two herds was randomly divided into three groups, two of which received ISMM either via an SRD or intramuscularly (i.m.). The third group served as a control group. The male and the female herds grazed separately, but in close proximity. Within each herd all groups of animals grazed together. Hence, all cattle were exposed to similar tsetse challenges.

Experimental protocol. The study began in November 1995. Two weeks before the start of the experiment all animals were treated with 7 mg of diminazene aceturate (Berenil; 7% aqueous solution; Hoechst) per kg of body weight in order to clear all trypanosome infections. The first group (21 males and 21 females) was treated by i.m. injection of ISMM (Trypamidium; 2% [wt/vol] aqueous solution; Rhône Mérieux) at a dose of 1 mg/kg of body weight. The second group (22 males and 22 females) received the same dose of the drug via a subcutaneously implanted SRD. The SRDs were administered in the shoulder region with an implanter (Crestar; Intervet). Each animal received two to three rods, according to the animal's body weight. The third group (14 males and 22 females) consisted of untreated control animals. At the start of the trial the average weight of the three groups of cattle varied between 117 and 125 kg.

Parasitological examination was carried out by the dark-background buffy coat technique (14) 2 weeks prior to treatment and every month until 8 months

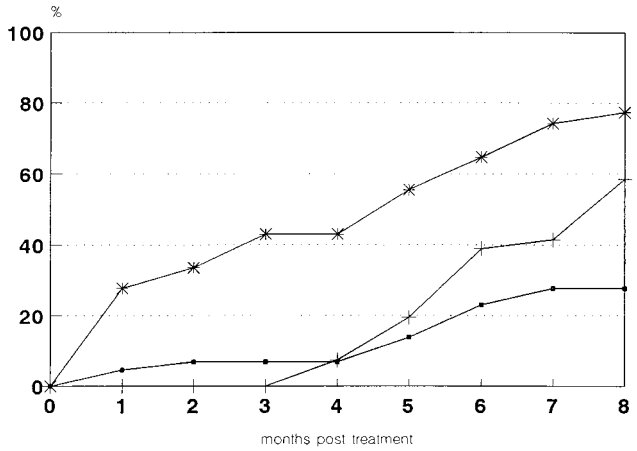

FIG. 1. Cumulative trypanosome infection rate in two treatment groups which received ISMM either via an implanted SRD (■) or as an i.m. injection (+) compared to that in the control group (∗).

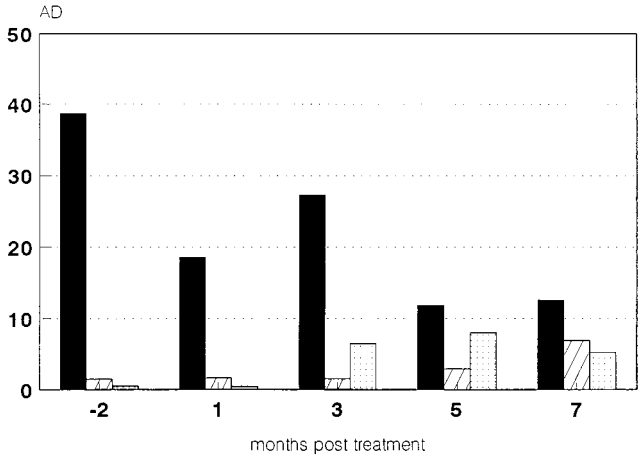

FIG. 2. Apparent densities (AD) of *G. morsitans submorsitans* (at pasture) (■) and of *Glossina palpalis gambiensis* (▨) and *Glossina tachinoides* (▩) (both at cattle watering sites) at the Madina Diassa Ranch.

posttreatment. Simultaneously, thin blood smears were prepared; these were then stained with Giemsa stain and were examined to identify the trypanosome species. Any animal which became parasitemic during the trial was immediately treated with diminazene aceturate at 7 mg/kg of body weight.

Tsetse fly challenge. The apparent density of the tsetse flies at the site of the trial was measured before the start of the experiment and, beginning 1 month after treatment, at 2-month intervals during the experiment. Ten biconical traps (4) were used at the grazing site (savannah) and 10 were used at the watering site (forest gallery). They were placed at intervals of about 100 to 200 m. The flies were collected after 24 and 48 h. The apparent density was calculated as the mean number of flies per trap per day. A total of 257 *Glossina morsitans submorsitans* flies obtained at 3, 5, and 7 months after the start of the experiment were examined. The proboscis, intestine, and salivary glands were dissected as described by Pollock (19) in order to determine the degree of infection and to identify the trypanosome species.

Drug sensitivities of trypanosomes. In order to examine the drug sensitivities of the trypanosomes circulating at the ranch, nine stocks of trypanosomes (four *Trypanosoma vivax* stocks and five *Trypanosoma congolense* stocks) were isolated from local cattle 2 months before the start of the experiment; the isolates were either cryopreserved in liquid nitrogen or blood from infected animals was inoculated into mice. Two pools were prepared by using two and three *T. congolense* stocks, respectively. Another pool contained all four *T. vivax* stocks. Each pool was inoculated into three young bovines (Zebu and Zebu crosses) originating from a tsetse fly-free area in Mali (Sotuba). These animals were kept in a stable during the course of the experiment and were treated with deltamethrin (Butox) pour-on at 2-week intervals. Once the animals became parasitemic, two of them were treated with ISMM at 0.5 mg/kg of body weight and one was treated with diminazene aceturate at 3.5 mg/kg of body weight. These doses were chosen because they are the lowest doses which are efficacious against both trypanosome species for prophylactic and curative purposes, respectively. Buffy coat examination of the blood (14) was carried out three times a week for a period of 100 days posttreatment.

Statistical analysis. The incidence of trypanosomiasis in the three groups of N'Dama cattle in the field was compared by the method of survival analysis (5). The Cox-Mantel Two-Sample test was used to compare the two treatment methods.

RESULTS AND DISCUSSION

Parasitological results. The parasitological results for cattle exposed to natural tsetse fly challenge at the Madina Diassa Ranch are presented in Fig. 1. Eight months after treatment the cumulative rates of infection were 27.7, 58.5, and 77.4% for the cattle with implants, the i.m. treated cattle, and the control group, respectively. Survival analysis showed a highly significant difference between treated and nontreated animals over the whole of the experimental period ($P < 0.001$). The risks of infection (hazard rate) for the group with the implants and the i.m. treated group compared to that for the control group were 0.24 and 0.54, respectively. The Cox-Mantel Two-Sample test showed a significant difference in the incidence of trypanosomiasis between the two treatment groups ($C = 2.504$; $P = 0.006$). These data confirm the results of Geerts et al. (12), who showed that under experimental conditions a significant extension of the protection period of ISMM could be obtained with poly(D,L-lactide) SRDs. It is noteworthy that during the first 3 months after treatment none of the animals in the group treated i.m. became positive, whereas 3 of 44 (6.8%) cattle which received the SRD became infected. These 3 animals were all infected with *T. vivax*, unlike the 15 animals in the control group with infections which occurred over the same period, of which 6 were infected with *T. vivax* and 9 were infected with *T. congolense*. Previous experiments with the same type of SRD under controlled conditions have shown that the peak concentrations of ISMM in serum (0.8 ng/ml) were reached only 3 to 4 weeks after implantation (12). It is possible, therefore, that in some animals the level of the drug during the first weeks after the implantation might have been insufficient to prevent *T. vivax* infections. Several investigators (10, 15, 17, 21) have already suggested that ISMM might be less effective against *T. vivax* than against *T. congolense.*

Tsetse fly challenge. The apparent densities of the different species of tsetse flies at the ranch are presented in Fig. 2. It shows that *G. morsitans submorsitans* was the most important species, which confirms earlier observations (9). During the course of the experiment the proportion of *G. morsitans submorsitans* flies infected with trypanosomes varied between 22.4 and 24.4%, of which 46 to 68% harbored metacyclic trypanosomes. *T. vivax* was the most common parasite and was found in 61.2% of the infected flies examined, followed by *T. congolense* (14.9%) and *Trypanosoma brucei* (1.8%). *Trypanosoma grayi* was identified in 1.5% of the flies, and 20.6% were immature infections. These data suggest that the cattle at the site of the experiment were exposed to a high level of tsetse fly challenge.

Drug sensitivity of local trypanosome populations. No breakthrough infections were observed for a period of 100 days after ISMM or diminazene aceturate treatment of the animals inoculated with the pools of *T. congolense* or *T. vivax*. Since it has been possible for investigators to identify resistant trypanosome isolates by a similar approach in Somalia (1) and since no problems of resistance to trypanocidal drugs were reported at the ranch during the previous years, resistant trypanosome strains are probably not present at the ranch.

Other parameters. Although the SRDs were coated with dexamethasone (1% [wt/wt]), some swelling was observed at the implantation site in most of the animals. The dimensions of these nodules varied from 1 to 3 cm in diameter, and the nodules decreased in size during the course of the experiment. In most of the animals the nodules had disappeared by 8 months posttreatment. In contrast to the necrotic lesions observed after i.m. injection of ISMM (3), these tissue reactions were subcutaneous and therefore unlikely to affect the quality of the carcass.

From the results of this experiment it can be concluded that the use of poly(D,L-lactide) SRDs allows the period of prophylaxis afforded by ISMM to be extended significantly, even under conditions of heavy tsetse fly challenge in a field situation. Further potential advantages of these devices over the classical i.m. use of the drug are as follows: (i) there is less of a possibility of diluting the product or underdosing the animal, (ii) there is no requirement for sterile water, (iii) there are no potentially toxic residues at the injection sites in the muscles, and (iv) veterinary services are easier to control since SRDs can be administered only with special implanters, which are not widely available to farmers. In order to avoid the breakthrough infections immediately after the implantation of the SRD, the devices could be modified to improve the release rate during the first weeks after implantation. This could be achieved by improving the permeability of the polymer matrix either by copolymerization with another suitable comonomer or by the addition of a plasticizer, e.g., low-molecular-weight poly(D,L-lactide). Alternatively, combining the administration of the SRDs with a sanative treatment with diminazene aceturate might also reduce the number of early breakthrough infections. Possible toxic reactions due to this drug combination should be carefully evaluated, but none is anticipated because the high peak concentrations of ISMM immediately after the i.m. injection, which are mainly responsible for acute toxicity, are absent when SRDs are used (12). Moreover, the pharmacokinetics of diminazene (2) suggest that most of the dose administered would be eliminated by the time that a significant quantity of ISMM had been released. Further improvement of the devices could also be realized by incorporating a higher concentration of anti-inflammatory product in the coating to avoid the development of tissue reactions at the implantation site.

Although the possibility of development of drug resistance with the use of SRDs cannot be excluded, preliminary results obtained by Geerts et al. (12) indicate that the rate of development of resistance is no faster after administration of an SRD than after the classical i.m. injection. By comparing breakthrough isolates of *T. congolense* collected from animals treated with homidium bromide i.m. or via an SRD, those investigators could not detect any loss of drug sensitivity in the latter isolates compared to that in the former isolates. By the mouse test described by Sones et al. (20), the dose which resulted in permanent cure of 80% of the mice was 2 mg/kg for both isolates. Further research is necessary, however, in order to examine this aspect in more detail.

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