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Comparative evaluation of the prophylactic effect of slow release devices containing homidium bromide and isometamidium on *Trypanosoma congolense* in rabbits

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Abstract

Two consecutive experiments were carried out to evaluate the prophylactic effect of biodegradable slow release devices (SRD), containing either isometamidium or homidium bromide. Rabbits subcutaneously implanted with SRD, were challenged with different *Trypanosoma congolense* stocks at regular intervals between 1 and 6.5 months after treatment. In a first experiment the efficacy of two types of isometamidium-SRD (poly(D,L-lactide) and poly(D,L-lactide-co-glycolide)) was compared with the classical intramuscular (i.m.) injection of the drug. Since the former polymer gave an average protection period, which was much longer than the other isometamidium formulation, a second experiment was carried out to evaluate the prophylactic effect of poly(D,L-lactide) SRD, containing either isometamidium or homidium bromide, with that of the i.m. injections of the same drugs at a dose of 1 mg kg^{-1}. The average protection period of the homidium bromide SRD was significantly longer than that of the i.m. injected drug (112 vs. 49 days). No significant difference was obtained, however, when isometamidium was administered either as a SRD or as an i.m. injection. The average protection periods were, respectively, 106 ± 37 days and 84 ± 18 days. When breakthrough isolates derived from SRD-treated animals were compared with the original stocks of *T. congolense*, the former showed some loss of sensitivity to homidium bromide. No difference in sensitivity was observed, however, for isometamidium.

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1. Introduction

In order to extend the prophylactic effect of currently available trypanocidal drugs and/or to avoid or minimize local toxicity at the injection sites, various alternative delivery systems have been tried, e.g. dextran or suramin complexes, liposomal formulations and carrier erythrocytes (Peregrine, 1994). Most of these formulations, however, were hampered by technical problems or seemed to be impractical for field use. Recently, promising results were obtained using different types of subcutaneously implanted slow release devices (SRD) containing homidium bromide (De Deken et al., 1989; Geerts et al., 1993). In both experiments a significant extension of the prophylactic effect was obtained using polycaprolactone or poly(caprolactone-L-lactide) SRD containing homidium bromide in comparison with the intramuscularly injected drug.

The purpose of the present experiment was to evaluate the prophylactic effect of a more readily biodegradable poly(D,L-lactide) or poly(D,L-lactide-co-glycolide) SRD containing either isometamidium chloride (ISMM) or homidium bromide (HBR) in comparison with the classical intramuscular (i.m.) injection of the same drugs.

2. Materials and methods

2.1. Slow release devices

Biodegradable slow release devices were prepared by extrusion of a physical mixture of poly(D,L-lactide) or poly(D,L-lactide-co-glycolide) (80:20) and ISMM (Trypamidium®, Rhone-Mérieux) or HBR (Ethidium®, Sigma). The SRD consisted of cylindrical rods of 1.7 mm diameter. They were loaded with 25 weight % of the above mentioned drugs. Some of them were coated by dipping them in a chloroform solution (10 wt. %) of the core polymer (coating thickness: 30 ± 10 μm). Since the amount of drug per unit of rod length was known, the length of the SRD was adjusted according to the weight of the rabbits by cutting the rod to the appropriate length, in such a way that each animal received a dose of 1 mg kg^{-1} of either drug. The SRD were implanted subcutaneously in the shoulder region by simple injection using a needle (internal diameter 1.8 mm) and a plunger. For each type of SRD, the in vitro release curves were determined. The results of incubation experiments carried out in phosphate buffer (pH 7.4) at 37°C demonstrated a slow release of the drug over a period of several weeks. Details will be described in a forthcoming paper.

2.2. Experimental design

Two successive experiments were carried out using, respectively, 15 and 20 adult female New Zealand rabbits with an average weight of 2.85 ± 0.22 kg (Experiment 1) and 3.55 ± 0.25 (Experiment 2). The animals were allocated at random to the different

treatment groups. They were kept in accordance with Belgian animal protection regulations and were housed in individual cages of appropriate size. Pellets, hay and water were available ad libitum. In a first experiment (Experiment 1) three groups of five rabbits were used to compare the efficacy of two types of uncoated SRD containing ISMM (1 mg kg^{-1})—poly(D,L-lactide) and poly(D,L-lactide-co-glycolide)—with the i.m. injection of 1 mg kg^{-1} ISMM (2% solution in distilled water) in the thigh (see Table 1). Four groups of five rabbits were included in the second experiment. Two groups received a poly(D,L-lactide) SRD, containing, respectively, ISMM (coated implant) or HBR (uncoated implant) both at 1 mg kg^{-1}. The other two groups were injected i.m. in the thigh with the same drugs at the same dosage (see Table 2). The prophylactic effect of the various drug formulations was evaluated by challenging the rabbits with different *Trypanosoma congolense* stocks at regular intervals from 1 month until 6.5 months after treatment. Blood samples were taken weekly at the ear vein and examined by the buffy coat technique (Murray et al., 1977). At the end of the experiment the rabbits were necropsied in order to look for the remnants of the implanted rods and to examine the tissue reaction at the implantation site.

2.3. *Trypanosome stocks*

Eight clones or stocks of *T. congolense* from different geographical origin were used to challenge the rabbits. They were used in the following order: IL 1180 (Tanzania), L 231 (Tanzania), EATRO 1157 (Uganda), Nkuiyi (Zaire), Djuma (Zaire), Kolo (Zaire), Agriumbe (Zaire) and Boma (Zaire). The rabbits were challenged by intraperitoneal injection of 0.5 ml heparinized rat blood containing antilog 7.2–8.1 trypanosomes per ml. At each challenge infection the infectivity of the trypanosomes was evaluated by intraperitoneal (i.p.) injection in one or two control rabbits.

2.4. *Evaluation of sensitivity of* T. congolense *isolates*

In order to assess possible changes in sensitivity of *T. congolense*, trypanosomes were isolated from the rabbits implanted with the ethidium SRD and the ISMM SRD (Experiment 2). The isolates were derived from breakthrough infections after challenge at 137 days post implantation and were compared with the original trypanosome stock. Sensitivity assessment was carried out as described by Sones et al. (1988). Five groups of five mice each were infected with each of the breakthrough isolates and the corresponding original stock. The mice were treated at different dosages (0.5, 1, 2, 5, 10 mg kg^{-1}) of either ISMM or HBR and followed up for 3 months. Effective (ED80) and curative doses (CD80) for 80% of the animals were calculated as the minimum doses, which resulted in temporary clearance or permanent cure respectively in at least four out of five mice.

2.5. *Statistical analysis*

Statistical analysis of the results obtained with the different treatments was carried out using the Kruskal–Wallis test. When significant differences were present, multiple comparisons between medians were used (Levy, 1979).

3. Results and discussion

Experiment 1 (Table 1) clearly showed that the average protection period in the rabbits treated with the poly(D,L-lactide) SRD was longer than in the animals which received the poly(D,L-lactide-co-glycolide) SRD containing ISMM. Therefore the second experiment (Table 2) was carried out using poly(D,L-lactide) SRD containing either ISMM or HBR, each with their corresponding controls (i.m. injection of the drug). A significant difference ($P < 0.05$) was observed between the average protection period provided by the HBR-SRD in comparison with the i.m. injected HBR. This was not the case for the ISMM-SRD versus the i.m. injected ISMM. Although in Experiment 1 a clearcut difference in the duration of the protection period was present between the uncoated poly(D,L-lactide) implant and the i.m. injection of ISMM, this difference was not statistically significant (P: 0.23) due to the large variation. The use of a coated ISMM implant in Experiment 2 did not improve the results. Although generally there was only a slight tissue reaction at the implantation site of the SRD, the use of the coated implant did not avoid the development of an inflammatory reaction at the implantation site in one out of five rabbits. This phenomenon did also occur in one rabbit implanted with an uncoated ISMM-poly(D,L-lactide) SRD (Experiment 1) and was probably caused by the direct contact of the ISMM with the tissue. It has to be noticed, however, that the coated ISMM-SRD was not end-coated, so that a direct contact of the ISMM with the surrounding tissue cannot be excluded completely.

Table 1
Comparison of the prophylactic effect of slow release devices and intramuscular injection of isometamidium (ISMM) in rabbits challenged with *T. congolense*

Drug	ISMM	ISMM	ISMM
Dosage (mg kg^{-1})	1	1	1
Formulation	SRD	SRD	Liquid
Administration	s.c.	s.c.	i.m.
SRD: – type	poly(D,L-lactide)	co-lactide/glycolide	–
– coating	A	A	–
No. of rabbits	5	5	5
No. of protected rabbits after challenge at days post treatment			
29	5	5	5
44	4	5	5
56	4	4	2
77	4	3	2
101	4	3	2
113	3	0	0
139	3		
167	0		
Average protection period (± SD) (days)	109 (± 43)	81 (± 25)	67 (± 28)

SRD, slow release device; A, absent.

Table 2
Comparative evaluation of the prophylactic effect of isometamidium (ISMM) or homidium bromide (HBR) injections and slow release devices in rabbits challenged with *T. congolense*

Drug	ISMM	ISMM	HBR	HBR
Dosage (mg kg^{-1})	1	1	1	1
Formulation	INJ	SRD	INJ	SRD
SRD: – type	–	poly (D,L-lactide)	–	poly (D,L-lactide)
– coating	–	P	–	A
No. of rabbits	5	5	5	5
No. protected rabbits after challenge at days post treatment				
30	5	5	5	5
49	5	5	5	5
64	5	4	0	5
91	3	4		5
112	1	3		5
137	0	1		0
164		1		
196		0		
Average protection period (±SD) (days)	84 (±18)	106 (±37)	49 (±0)	112 (±0)

INJ, intramuscular injection; SRD, slow release device; A, absent; P, present.

Another striking phenomenon was the important variation of the average protection periods in all the groups treated with isometamidium, either i.m. or implanted. This variation was not present in the rabbits treated with HBR. Part of the variation in both ISMM-poly(D,L-lactide) SRD implanted groups (Experiments 1 and 2) was due to the inflammatory reaction, which developed at the implantation site in one out of five rabbits and which shortened the protection period. In the majority of the rabbits, however, the implant had completely disappeared at the autopsy (5–6.5 months after the implantation) and macroscopically there was only very slight or no tissue reaction at the implantation site.

The possible development of drug resistance through the use of SRD containing trypanocides is a matter which should be examined thoroughly. A comparison of the sensitivity of a breakthrough isolate from ISMM-SRD treated rabbits with the original stock of *T. congolense* did not provide any indications of resistance development against ISMM. Indeed, as shown in Table 3 the ED80 and CD80 values were identical in both cases. When tested against HBR, the ED80 of the breakthrough isolate was higher (5 mg kg^{-1}) than that of the original stock (1 mg kg^{-1}). Unfortunately, no CD80 values could be calculated, because no cure at all was observed at the highest dose tested (10 mg kg^{-1}). Further studies should be carried out, in which trypanosome isolates (breakthrough infections) should be repeatedly exposed to drug concentrations as present in the blood of animals treated either by SRD or the classical i.m. injections in order to examine whether resistance would develop faster in one of both treatment methods. Although no definitive conclusions can be drawn from these experiments, it is premature to state that slow release devices are likely to increase the rate of development of drug resistance

Table 3
Sensitivity tests of the original stocks and the breakthrough isolates (from SRD-treated animals) of *T. congolense* (Kolo, Zaire) in mice

T. congolense	ED80 (mg kg^{-1})	CD80 (mg kg^{-1})
(A) Isometamidium		
Breakthrough isolate [a]	0.5	1
Original stock [b]	0.5	1
(B) Homidium bromide		
Breakthrough isolate [c]	5	NA
Original stock [b]	1	NA

[a,c] Isolated from ISMM-SRD and HBR-SRD treated rabbits, respectively, after challenge at 137 days post treatment (Experiment 2).
[b] Stock used to challenge the rabbits at 137 days post treatment (Experiment 2).
NA, not available.

(Peregrine, 1994), especially since the classical i.m. injections of some drugs like ISMM also give rise to a kind of slow release effect (Kinabo and Bogan, 1988), which might be less well controlled than in the polymer devices.

It can be concluded that the use of poly(D,L-lactide) SRD containing ethidium confers a significant extension of the protection period in comparison with the i.m. injection of the drug at the same dose. This confirms the previous observations using an ethidium SRD composed of a copolymer of ϵ-caprolactone and L-lactide (80:20), where also a clearcut improvement of the prophylactic period was obtained (Geerts et al., 1993). On the other hand coated or uncoated ISMM-SRD do not provide a significant extension of the prophylactic activity in comparison with i.m. injection of the drug. The reason for this discrepancy is probably the above-mentioned fact that there is a depot formation after i.m. injection of ISMM with a kind of slow release effect of the drug (Kinabo and Bogan, 1988). A similar effect seems not to be present or only to a lesser extent after i.m. injection of homidium bromide.

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