

Laboratory and Field Evaluation of Biodegradable Polyesters for Sustained Release of Isometamidium and Ethidium - Minireview

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An overview is presented of the results obtained with biodegradable sustained release devices (SRDs) containing a mixture of polymers and either isometamidium (ISMM) or ethidium. Under controlled laboratory conditions (monthly challenge with tsetse flies infected with Trypanosoma congolense) the protection period in SRD treated cattle could be extended by a factor 2.8 (for ethidium) up to 4.2 (for ISMM) as compared to animals treated intramuscularly with the same drugs. Using a competitive drug ELISA ISMM concentrations were detected up to 330 days after the implantation of the SRDs, whereas after i.m. injection the drug was no longer present three to four months post treatment. Two field trials carried out in Mali under heavy tsetse challenge showed that the cumulative infection rate was significantly lower in the ISMM-SRD implanted cattle than in those which received ISMM intramuscularly. Using ethidium SRD, however, contradictory results were obtained in field trials in Zambia and in Mali. The potential advantages and inconvenients of the use of SRDs are discussed and suggestions are made in order to further improve the currently available devices.

Key words: review - isometamidium - ethidium - sustained release - prophylaxis - trypanosomiasis - cattle

In order to extend the prophylactic activity of isometamidium (ISMM) and ethidium (homidium bromide) and to decrease their toxic effects at the injection site, several alternative delivery systems have been developed for the treatment of trypanosomiasis of livestock. Peregrine (1994) did review the use of liposomes, carrier erythrocytes, suraminates and dextran complexes. Generally these new formulations were evaluated in laboratory animals and the few experiments in cattle usually gave unsatisfactory results. The use of polymers was also briefly mentioned by Peregrine (1994), but at that time no data were yet available about their application in cattle. The purpose of this minireview is to present and discuss the results obtained during several laboratory and field trials in cattle using sustained release devices containing a mixture of different polyesters and ISMM or ethidium.

SUSTAINED RELEASE DEVICES

Biodegradable sustained release devices (SRDs) were prepared by extrusion of a physical mixture of the homopolymer poly(D,L-lactide) or the copolymer poly(caprolactone-L-lactide) (80/20 w/w) and the drugs as described by Lemmouchi et al. (1996). The SRDs which consisted of cylindrical rods of 3 mm diameter and up to 3 cm long, loaded with 25% (w/w) of ISMM or ethidium, were implanted subcutaneously in the shoulder region. Except for the poly(D,L-lactide)-ISMM-SRD, dexamethasone (0.5-1%, w/w) was added to the coating of the SRDs to reduce the tissue reaction at the subcutaneous implantation site.

In vitro release experiments (Lemmouchi et al. 1996, Lemmouchi & Schacht 1997, Lemmouchi et al. 1998) and several trials using SRDs in rabbits (De Deken et al. 1989, Geerts et al. 1993, Kageruka et al. 1996) gave very promising results, justifying their application in cattle.

EVALUATION OF ISMM- AND ETHIDIUM-SRD UNDER EXPERIMENTAL CONDITIONS IN CATTLE

The evaluation of the efficacy of the SRDs was carried out under controlled conditions in three experiments by exposing cattle once a month to an average of eight *Glossina morsitans morsitans* (line MALL), of which 50 to 90% were infected with *Trypanosoma congolense* (clone IL 1180). Blood samples were taken weekly and examined by the buffy coat technique (Murray et al. 1977). The re-

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sults of three successive experiments are summarized in Table I. The prophylactic period using the SRD was extended by a factor 2.8 up to 4.2 for ethidium and ISMM (copolymer) respectively as compared to the i.m. injection of the drugs. At the implantation site of the SRDs nodules of up to 3 cm diameter developed, which gradually disappeared afterwards.

The kinetics of the concentration of ISMM and ethidium in the serum of the poly(D,L-lactide)-SRD implanted cattle was studied using the ISMM-ELISA as described by Eisler et al. (1993,1996) or the ethidium-ELISA according to Murilla (1996). In the SRD-implanted cattle the pharmacokinetics of both drugs were completely different from that observed in i.m. treated animals. In the latter cattle, peak levels of ISMM or ethidium were present immediately after injection, which dropped rapidly to reach undetectable levels at about 85 or 30 days post treatment respectively. In the SRD-implanted animals, however, the peak levels which were about 10 times lower than in the i.m. treated cattle, were reached only after one to four weeks. Afterwards the drug concentrations remained quite stable until 150 days post treatment. Low concentrations of ISMM in serum could be detected up to 200 and even 330 days after the implantation of the poly(D,L-lactide)-SRD and the poly(caprolactone-co-L-lactide)-SRD respectively. Thereafter the drugs could no longer be detected, although the animals were still protected during several months against challenge with infected tsetse flies (Geerts et al. 1997). A possible explanation for this phenomenon could be that - in spite of the undetectable serum levels - ISMM concentrations in the subcutaneous tissue and the skin, where the try-

panosomes are inoculated by the tsetse flies, remained at levels which are high enough to kill trypanosomes over much longer periods. In none of the experiments trypanolytic antibodies could be detected, which indicates that it is the drug and not the immune response, which is responsible for the prolonged protection of the animals.

The possible development of drug resistance after the use of trypanocidal drugs and especially after the use of SRDs is an important factor to be taken in consideration (Peregrine 1994, Geerts & Holmes 1998). In order to assess possible changes in drug sensitivity of *T. congolense* following treatment, isolates were made from breakthrough infections (from animals treated i.m. or by SRD) and compared with the original trypanosome stock or clone IL 1180. Sensitivity assessment was carried out using the mouse assay as described by Sones et al. (1988). Although the mouse test generally indicated some loss of sensitivity of the breakthrough isolates as compared to the original clone (IL 1180), no difference in sensitivity could be observed between breakthrough isolates derived from i.m. injected and those from SRD-treated animals (Geerts et al. 1997).

FIELD EVALUATION OF ISMM- AND ETHIDIUM-SRD

Field trials were carried out in Mali (Madina-Diassa ranch, 300 km south of Bamako) and in Zambia (Chipopela region, Eastern Province). In a first trial in Mali by Diarra et al. (1998) two groups of N'Dama cattle were treated respectively with ISMM either as a subcutaneously implanted poly(D,L-lactide)-SRD or i.m., both at 1 mg/kg. A third group served as an untreated control group. Two weeks before the start of the experiment all cattle were treated with diminazene aceturate

TABLE I

Prophylactic effect of a sustained release device (SRD) or an intramuscular injection of isometamidium (ISMM) or ethidium in cattle monthly exposed to *Glossina morsitans morsitans* experimentally infected with *Trypanosoma congolense*

| Group ^a | Experiment 1 | | Experiment 2 | | Experiment 3 | |
|----------------------------|--------------|---------|------------------|---------|--------------|----------|
| | 1 | 2 | 1 | 2 | 1 | 2 |
| Drug | ISMM | ISMM | ISMM | ISMM | Ethidium | Ethidium |
| dosage (mg/kg) | 0.5 | 0.5 | 0.5 | 0.5 | 1 | 1 |
| formulation | SRD (hp) | 2% sol. | SRD(cp) | 2% sol. | SRD (hp) | 2% sol. |
| administration | s.c. | i.m. | s.c. | i.m. | s.c. | i.m. |
| Protection period (months) | | | | | | |
| average | 20 | 5.7 | >24 ^b | 5.7 | 8.3 | 3 |
| range | 18-22 | 4-7 | 20->24 | 3-10 | 5-10 | 2-4 |

a: each group consisted of three cattle; b: experiment 2 (group 1) was stopped at 24 months post treatment; i.m.: intramuscular; s.c.: subcutaneous; hp: homopolymer, i.e. poly(D,L-lactide); cp: copolymer, i.e. poly(caprolactone-co-L-lactide). Source: Geerts et al. (1997) Geerts et al. (1999).

(Berenil®, Hoechst) at 7 mg/kg. All animals were examined parasitologically using the darkground buffy coat technique (Murray et al. 1977) before the start of the trial and afterwards at monthly intervals. The tsetse challenge was measured by the number of tsetse flies caught per trap per day at two-monthly intervals during the course of the experiment. The apparent density of *Glossina morsitans submorsitans*, the most important tsetse species, varied between 11.9 and 38.7. Forty six to 68% of the flies harboured metacyclic trypanosomes. *T. vivax*, *T. congolense* and *T. brucei* were found respectively in 61.2%, 14.9% and 1.8% of the flies. Eight months after treatment the cumulative infection rate was 27.7%, 58.8% and 77.4% in the SRD-implanted, the i.m. injected and the control group respectively (Table II). Statistical analysis showed a significant difference of the trypanosomiasis incidence between the two treatment groups, which clearly proves that even under a high tsetse and trypanosomiasis challenge the ISMM-SRD gave a much better protection than the i.m. injection of the drug. During the first months after the implantation, however, a small number of cattle became infected with *T. vivax* probably due to an insufficient release of the drug during the first weeks after the implantation of the device (Diarra et al. 1998).

A second trial in Mali, in similar conditions as the previous one, included five groups of N'Dama cattle: two groups were treated with ISMM, either as a poly(caprolactone-co-L-lactide)-SRD or i.m.; two other groups with ethidium either as a poly(D,L-lactide)-SRD or i.m., all at 1 mg/kg. A fifth group served as an untreated control group. The results of this trial, which are summarised in Table II, confirmed the ISMM results obtained in the first trial.

For ethidium, however, the cumulative infection rate in the implanted and the i.m. injected animals was very similar over the whole observation period (Geerts et al. man. prep.). This latter result is in contradiction with the results obtained in Zambia, where 50% of the Angoni (Sanga type) cattle which received the ethidium-SRD remained protected up to the fourth month after treatment, whereas in the group treated i.m. with ethidium 50% of the cattle were protected up to the second month (Mubanga 1996). The breakthrough infections during the first weeks after the implantation of the SRDs, which were reported by Diarra et al. (1998), were also observed in this experiment.

CONCLUSIONS

Analysis of the experiments described above shows that the results obtained with ISMM-SRD were the most promising, since it was possible to obtain significant extension of the protection period both under laboratory and field conditions. Although the ethidium-SRD gave good results under laboratory conditions and in field trials in Zambia, contradictory results were obtained in Mali. Further experiments will be necessary before definitive conclusions on the efficacy of ethidium-SRD can be drawn.

Both polymers - poly(D,L-lactide) or poly(caprolactone-co-L-lactide) (80/20 w/w) - have given very good results. The latter polymer, however, is cheaper and more flexible than the former and provides a longer protection period, at least under experimental conditions. However, it is less biodegradable than the homopolymer.

Further potential advantages of the ISMM-SRDs over the classical i.m. use of the drug are: (1) more certainty about the use of the correct dose (less possibility to dilute the product or to

TABLE II

Prophylactic effect of isometamidium or ethidium administered as sustained release devices or as i.m. injection to N'Dama cattle under field conditions in Mali

| Group | Experiment 1 | | | Experiment 2 | | | | |
|------------------------|--------------|------|-------|--------------|------|-------------|-------|-------|
| | 1 | 2 | 3 | 1 | 2 | 3 | 4 | 5 |
| Treatment ^a | ISMM | ISMM | CONTR | ISMM | ISMM | ETHID | ETHID | CONTR |
| Formulation | SRD (hp) | sol | - | SRD (cp) | sol | SRD (hp) | sol | - |
| No. of animals | 44 | 42 | 36 | 23 | 24 | 23 | 24 | 24 |
| CIR | | | | | | | | |
| 8 mptr | 27.7 | 58.5 | 77.4 | 26.1 | 41.6 | 49 | 57.1 | 70.8 |
| 12 mptr | NA | NA | NA | 56.5 | 87.8 | 94.4 | 92.7 | 91.6 |

^a: all treatments were at a dosage of 1 mg/kg; NA: not available; CIR: cumulative infection rate; mptr: months post treatment. Source: Diarra et al. (1998), Geerts et al. (1999).

underdose); (2) no requirement for sterile water; (3) no potentially toxic residues and necrotic lesions at the injection sites in the muscles and (4) better control by the veterinary services since SRDs can only be administered using special implanters, which are not commonly available to farmers.

In order to avoid breakthrough infections immediately after the implantation of the SRDs, the devices could be modified to improve the release rates during the first weeks. Alternatively combining the administration of the SRD with a sanative treatment of diminazene aceturate might also reduce the number of early breakthrough infections. Possible toxic reactions due to the combined use of both drugs should be carefully examined. Such are not expected, however, because the high concentrations of ISMM immediately after the i.m. injection, which are mainly responsible for the acute toxicity, are absent when SRDs are used (Geerts et al. 1997).

Preliminary results indicate that the risk for development of drug resistance is not higher in trypanosomes exposed to ISMM- or ethidium-SRD than in those exposed to the i.m. injected drugs. Further research is necessary, however, in order to examine this aspect in more detail.

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