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Ivermectin and moxidectin in two filarial systems: resistance of *Monanema martini*; inhibition of *Litomosoides sigmodontis* insemination

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Abstract. Effects of ivermectin and moxidectin were compared on two filarial species: *Monanema martini* which presents dermal microfilariae and induces *Onchocerca*-like lesions in its natural murid host *Lemniscomys striatus*, and *Litomosoides sigmodontis* (= *L. carinii*). *M. martini* microfilariae showed an unusual resistance to ivermectin, *in vitro* and *in vivo*; moxidectin was no more efficient. However, the two drugs used at high concentrations deeply altered the uterine embryogenesis, but had no lethal effect on adult filariae. *L. sigmodontis* blood microfilariae showed a great susceptibility to moxidectin, similar to that previously described for ivermectin. The two drugs also induced a long term effect because they inhibited the insemination of the female filariae. This result reinforces the observations made by other authors on the human parasite, *Onchocerca volvulus*.

Key words: ivermectin, moxidectin, resistance, *Monanema martini*, *Litomosoides sigmodontis*, dermal microfilariae, blood microfilariae, inhibition of insemination, histopathology.

The macrocyclical lactones are efficient drugs against the majority of nematode parasites, the filarial species included. Ivermectin belongs to this family and is a molecule particularly interesting in the treatment of onchocerciasis, because it is the most efficient against the microfilariae and, in contrast to diethylcarbamazine, its side-effects are limited (Aziz *et al.*, 1982; Awadzi *et al.*, 1985; Darge *et al.*, 1991; Duke *et al.*, 1991; Vuong *et al.*, 1992).

Moxidectin, a macrocyclical lactone partially related with ivermectin, has demonstrated a good efficacy against a spectrum of nematode parasites. In some case, experimental trials on ivermectin-resistant strains have been successful with moxidectin (Pankavich *et al.*, 1992; Craig *et al.*, 1992; Sivaraj *et al.*, 1994).

In this study, the activity of ivermectin and moxidectin is compared on two experimental filarial species: *Monanema martini* and *Litomosoides sigmodontis*. The first species has dermal microfilariae; as a consequence, it induces *Onchocerca*-like lesions in its host, a murid rodent (Vuong *et al.*, 1985, 1991; Aimard *et al.*, 1993). This filaria is normally susceptible to a number of molecules such as suramin, levamisole, albendazole, amocarzine (Wanji *et al.*, 1994a). The second species is already well known for the susceptibility of its microfilariae to ivermectin (McCall *et al.*, 1979; Söffner *et al.*, 1985; Zahner *et al.*, 1987; Rao *et al.*, 1990) and to moxidectin (Shares *et al.*, 1994). The present study is particularly aimed at the adult filarial stage, alterations of which could induce a long term effect.

MATERIALS AND METHODS

Filaria-host pairs

M. martini is maintained in its natural host, the African murid *Lemniscomys striatus*. The experimental vector is the hard tick *Hyalomma truncatum*. Rodents are inoculated with 80 infective larvae (L3), exceptionally with 15, 30 or 60 L3. The filarial worms live in the lymphatic vessels of the large intestine and caecum, however a small proportion of about 4% can be recovered in the right heart and pulmonary arteries (Wanji *et al.*, 1990). Microfilariae are concentrated in the ear pinnas. The prepatent phase lasts two months, and the patent phase at least one year. Microfilariae densities are maximal between the sixth and ninth month post-inoculation (pi) (Wanji *et al.*, 1994b).

Litomosoides sigmodontis, well known as *L. carinii* (see Bain *et al.*, 1989), is maintained in the experimental host, the jird *Meriones unguiculatus*. The experimental vector is the mite *Ornithonyssus bacoti*. Rodents are inoculated with 25 to 200 L3. Filariae live in the pleural cavity, and less commonly in the peritoneal cavity. The prepatent phase lasts over six months, and blood microfilarial densities are high from the second month pi.

Choice of rodents

Treated and control infected rodents were selected for experimentation when the microfilarial density is increasing or high and stable, ie. five months ± one

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month pi for *M. martini* and four months pi for *L. sigmodontis*.

Treatments

Ivomec(R) Merck Sharp & Dohme is an injectable solution of 1% ivermectin in glycerol formaldehyde and propylene glycol (1-2-propanediol Prolabo). Cydectin(R) American Cyanamid Company is an injectable solution of moxidectin at 1%. The two drugs were injected subcutaneously (except one rodent *per os*). Doses were calculated for 40 g live body weight (bw). *L. striatus* were treated with 5, 50 and 200 mg/kg ivermectin in 0.38 ml of solution. The volume injected was 0.02 ml for ivermectin, when several doses were administered, and 0.2 ml for moxidectin. The jirds received the two drugs in under 0.1 ml (Tables 1 and 3).

The *L. striatus* ivermectin controls received propylene glycol (PPG) in 0.38 ml for 40 g bw, and moxidectin controls received 0.2 ml PPG. The jird controls received 0.1 ml PPG for 40 g bw.

Measurement of microfilarial densities

Microfilarial densities were measured before treatment (D0) and at least once a week until necropsy.

For *M. martini*, cutaneous samples were taken at the left ear pinna, the surface of the samples was drawn at the microscope with a camera lucida and measured. Then they were teased in RPMI 1640 supplemented with 20% newborn calf serum. Microfiladermia is the number of microfilariae per mm^2 (mf/mm^2).

Microfilaraemia of *L. sigmodontis* is the number of microfilariae per 10 µl of blood drawn from the retro-orbital sinus (Chandre *et al.*, 1993).

Search for adult filariae and morphological study

Necropsies were performed 42 to 90 days after treatment, in RPMI supplemented with 20% newborn calf serum. The large intestine, mesentery, heart and lungs of *M. martini* were dissected separately. The coelomic cavities of *L. sigmodontis* were examined.

Filariae were counted, their sex and localization were noted. The sex ratio and the recovery rate (F/L3) were calculated. The state of the filariae and their uterine contents were studied after fixation in hot 70% alcohol or on live worms, each observed *in toto* from the tail to the head.

Particular techniques used with *M. martini*

In vitro protocol

The culture medium was composed with RPMI supplemented with 20% newborn calf serum, 2% Hepes buffer, 100 UI/ml Penicillin, 100 µg/ml Streptomycin, and 150 µg/ml Amphotericin B. Ivomec(R) solution 10 mg/ml was diluted in the culture medium. The control medium was 1% PPG in survival medium.

Microfilariae were recovered from cutaneous samples of infected untreated *L. striatus*. They were washed twice in the culture medium. After centrifugation at 2000 rpm for 5 min, the pellet with the microfilariae was resuspended in RPMI. The suspension was distributed in Eppendorf tubes, one for each concentration. After another centrifugation at 2000 rpm for 5 min, pellets were resuspended in different concentrations of ivermectin (per 1-ml): 5 ng, 50 ng, 500 ng, 5 µg, 50 µg. Microtitration plates of 24 wells of 2-cm diameter (Nunc Prolabo) were used. Each concentration was tested in 3 wells (150 mfs). The volume of each well was 0.5 ml and each contained about 50 microfilariae. Each plate was placed in the incubator at 37°C, in 5% CO_2 atmosphere for 48 hours.

Histopathology

Anaesthetized treated and control infected rodents were fixed in 10% formaldehyde *in toto*, or the head only when animals were dissected. Organs and tissue samples were taken and blocked in paraffin. A few serial sections 5-µm thick were stained with Hematoxylin-Eosin-Safran. Cutaneous lesions (of the right ear), ocular lesions and visceral lesions were identified, and parasites localized and counted. The five types of inflammatory reactions and reactive lesions were identified. Densities of parasites and intensities of lesions were expressed by one, two or three + and their absence by –. An attempt at quantification was made for the dermal and visceral lesions by assigning a score 1, 2 or 3 to increasing intensities, and the sums of the scores were compared (Vuong *et al.*, 1991).

Particular techniques used with *L. sigmodontis*

Chromosomes staining

Filariae recovered from treated jirds (0.2 mg/kg ivermectin) and controls were dissected in physiological saline. They were placed 15 seconds in 0.37% sodium citrate, in order to burst the cuticle and better squash the specimen. The posterior part of the uteri containing ovula and eggs were used to study the chromosomes (technique in Adamson and Petter, 1983 a and b).

Transplantation of filarial worms

Treated and control filariae were transferred to the peritoneal cavity of naive jirds. Each rodent received 3 male and 3 female worms. Untreated immature worms were recovered on D22 pi. Treated worms were recovered from rodents inoculated four months earlier and treated for 90 days. The following transplantations were made: ivermectin (iv) male x female iv (2 rodents); male control x female iv (1 rodent); male iv x female control (2 rodents); male control x female control (1 rodent).

Table 1. *Monanema martini/Lemniscomys striatus*: ivermectin and moxidectin treatments; experimental materials. Dose: expressed in mg/kg/bw and administered subcutaneously once (x1), or 5 consecutive days (x5), or 4 consecutive weeks (/w x4), or 5 consecutive weeks (/w x5). DpT: number of days between the first drug administration and observation. nR: number of rodents used to study microfiladermia (Mf), or adult worms (Ad). nL3 : number of infective larvae inoculated. po: *per os*. PPG: propylene glycol.

Parameters	Treated														Controls			
Drug	Invermectin											Moxidectin			PPG		water	–
Dose	5 mg × 1				5 mg × 1 po		5 mg × 5	5 mg/w × 5	5 mg/w × 4 +25 mg	50 mg	200 mg	5 mg						
DpT	–				–		D42	D60	D60			D42	D60	D80	D42			
nR Mf	1	1	1	6	1	1	2	1	1	–	1	1	2	1	1	2	2	4
nR Ad							1	1	1			1	1	1		1	2	4
nL3	40	30	60	80	15	30		80		30	15		80		30	80	80	80

RESULTS

Effect of ivermectin and moxidectin on *M. martini*

In vitro resistance of microfilariae to ivermectin

In vitro ivermectin trials were performed with 5 ng to 200 µg/ml and observations made at H0, H2, H18, H24 and H48; microfilariae were classified according to their motility: normal, slow, head alone motile, totally inmotile.

With 5 to 500 ng/ml, no effect was observed; all treated microfilariae and controls were slowed from H36. With 5 µg/ml, all treated microfilariae were slowed at H18 and head alone motile from H24 to H48. With 50 or 200 µg/ml, all microfilariae were immotile from H12.

A trial to verify the *in vitro* technique was performed with *Brugia malayi*, maintained in the laboratory. It showed a microfilaricidal effect from 50 ng/ml, similar to the results of Devaney *et al.* (1985) on the related species, *B. pahangi*.

In vivo resistance of microfilariae to ivermectin

Due to the very low susceptibility of microfilariae *in vitro*, the first trial was made with a single dose of 5 mg/kg inoculated to 9 *L. striatus* (Table 1). Microfiladermia of controls showed the usual great individual variation during the longitudinal study (Wanji *et al.*, 1994b). The variation was similarly great in the treated rodents and no reduction of microfiladermia was observed during the following 30-40 days (Fig. 1).

Other protocols were then tried (Table 1): a *per os* dose of 5 mg/kg to a rodent; inoculation of 5 mg/kg over 5 consecutive days, or once a week during five consecutive weeks or once a week during four consecutive weeks and a final dose of 25 mg/kg the fifth week; inoculation of a dose of 25, 50 or 200 mg/kg to the rodent previously treated with the single 5 mg/kg dose. In no case was a decrease of the microfilarial density observed. The 200 mg/kg dose was lethal, twelve hours later.

The analysis of the bioavailability of ivermectin metabolites was performed in two *L. striatus*, which received previously 200 and 5 mg/kg of drug, by Merck Sharp & Dohme, via Dr P. Robin, and it showed normal distribution of ivermectin in different tissues of the body.

In vivo resistance of microfilariae to moxidectin

Four *L. striatus* were treated with a dose of 5 mg/kg of moxidectin. No reduction of microfiladermia was observed (Fig. 1).

Effect of ivermectin and moxidectin on adult filariae: embryogenesis alterations

Results of necropsies are shown in Table 2.

Untreated controls: The mean sex ratio male/female was 1 and the mean recovery rate was 34.5%. Most filariae were in the lymphatic vessels of the caecum-colon walls. A few were in the lungs. A very small percentage of female filariae showed abnormal embryogenesis. Male worms were normal.

Ivermectin: The two *L. striatus* treated with 5 mg/kg×5 ivermectin and necropsied at D42, pre-

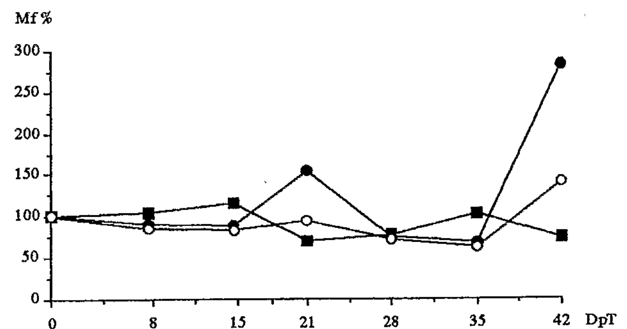

Fig. 1. *Monanema martini/Lemniscomys striatus*: time course of microfiladermia in rodents treated with ivermectin or moxidectin, and in controls. mf%: percentage of pretreatment microfiladermia. DpT : number of days between the first drug administration and observation. Ivermectin treatment (●): 5 mg/kg x1, six rodents, mean initial microfiladermia at D0: 149.7 ± 106.1 mf/mm2. Moxidectin treatment (■): 5 mg/kg x1, four rodents, 115.5 ± 101 mf/mm2 at D0. Control (o): placebo, eight rodents, 101.4±189.6 mf/mm2 at D0. Rodents are inoculated with 80 L3.

Table 2. *Monanema martini/Lemniscomys striatus*: effects of ivermectin and moxidectin on adult worms. DpT: number of days between the first drug administration and necropsy. %F/L3: percentage of filariae recovered compared to the number of L3 inoculated. % abn f: percentage of abnormal female worms. % lung, % mes, % colon: percentage of filariae present respectively in the lungs, mesentery, and colon-caecum. nR: number of rodents used. ± : standard deviation. Rodents are inoculated with 80 L3.

Parameters	Ivermectin 5 mg × 5	Ivermectin 5 mg/w × 4+ 25 mg	Moxidectin 5 mg × 1			Controls
DpT	D42	D60	D42	D60	D80	D42
%F/L3	21.5-33.3	4.4	18.7	7.5	18.8	34.5 ± 7.3
Sex ratio	1.4	1	1.5	2	0.3	1 ± 0.2
% abn f	71.4-91.7	100	100	100	100	2.5 ± 3.8
% lung	0-6	25	40	100	0	5.1 ± 7.6
% mes	15.8-27.3	0	0	0	0	4.9 ± 6
% colon	84.2-66.6	75	60	0	100	89.9 ± 9.7
nR	2	1	1	1	1	7

sented normal sex ratio, recovery rate and localization, although a slight increase of mesenteric localization was noted. Male worms were normal. Abnormal embryogenesis (aborted eggs and lysed microfilariae) were observed in 80% of the female worms.

The *L. striatus* treated with 5 mg/kg×4+25 mg/kg ivermectin and necropsied at D60, showed normal sex ratio and male worms. However the recovery rate was very low (4.4%). One of the four filariae recovered was in the pulmonary arteries. Embryogenesis was altered and degenerative lipidic drops in the uterine walls were seen. The search for male spermatozoa in the female worms was not done.

Moxidectin: The four *L. striatus* treated with 5 mg/kg and necropsied at D42, D60 or D80 showed variability in sex ratio, recovery rate and filaria location, and these variations were not related to the date of necropsy. Male worms were normal. Whatever the time post-treatment may be, all female worms presented aborted eggs, and no microfilariae or a few which had migrated to the posterior of the empty uteri. No spermatozoa were seen in the uteri.

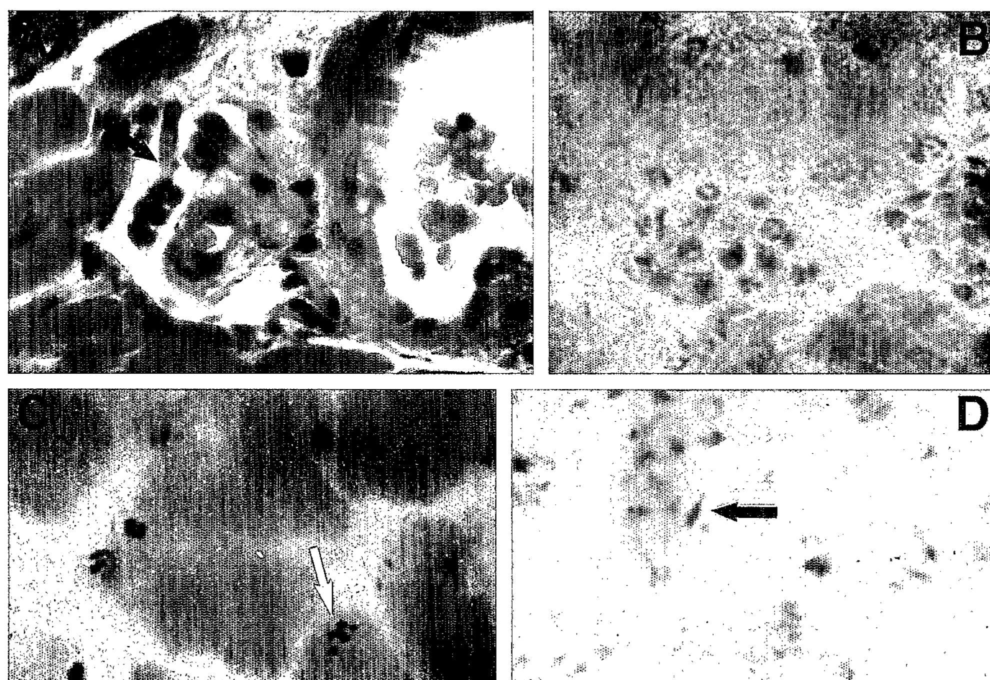

Fig. 2. A: *Monanema martini/Lemniscomys striatus*: persistance of normal microfilariae in the lymphatic vessels after moxidectin treatment (myocardium, D60 pT). Arrow: microfilariae. Scale: 1 cm = 25 µm. B, C, D: *Litomosoides sigmodontis/Meriones unguiculatus*: effect of ivermectin treatment (0.2 mg/kg) on intra-uterine content in treated female worms and in controls at D42. B: normal stages 3 (morula) in a control female worm. C: normal ovums presenting apical nucleus with n chromosomes (arrow), in a treated female worm. D: shrunked embryonic stages, with picnotic nucleus (arrow), in a treated female worm. Scale: 1 cm = 5 µm.

Table 3. *Litomosoides sigmodontis/Meriones unguiculatus*: ivermectin and moxidectin treatments; experimental materials. Dose: expressed in mg/kg/bw and administered once subcutaneously. DpT: number of days between the first drug administration and observation. nR: number of rodents used to study microfilaraemia (Mf) or adult worms (Ad) or chromosomes (Chrom). nL3: number of infective larvae inoculated. PPG: propylene glycol.

Parameters	Treated								Controls			
Drug	Ivermectin						Moxidectin		PPG			
Dose	0.05	0.2			2		0.2		–			
DpT	D42	D42	D63	D90	D42	D63	D42	D90	D42			D90
Mf	1	9	4	3	3	1	4	2	1	1	1	2
nR Ad	1	2	1	0	1	1	4	2	1	1	1	2
nR Chrom	0	3	0	3	0	0	4	2	1	1	1	2
nL3	60						60		25	60	200	60

Ivermectin and moxidectin side-effects on infected treated L. striatus

Although rodents showed important variability in histopathological scores, all treated and control rodents had similar lesions and microfilarial localizations (mainly intralymphatic) and densities (Fig. 2, A). Most of the lesions belonged to the inflammatory process (Vuong *et al.*, 1991) of high intensity in the ear subcutaneous tissue (mean scores of 9.5 and 7.5 respectively in the treated and control rodents), of lower intensity and less frequent in the irido-corneal angle and visceral organs.

Effects of ivermectin and moxidectin on *L. sigmodontis*

Drug doses, duration of the survey of microfilaraemia and dates of necropsies are presented in Table 3.

Suppression and renewal of microfilaraemia following ivermectin or moxidectin treatment

Untreated controls: Microfilaraemia was stable, except in the samples performed on the first day which presented a small variation (Fig. 3, A and B).

Ivermectin: a) Treated rodents: Microfilaraemia was suppressed 1 to 4 days post-treatment (pT) relative to the decreasing concentration of the drug (2 or 0.2 or 0.05 mg/kg). It appeared again three weeks later with the highest dose and one week later with the lowest dose. With 0.2 mg/kg, the mean density of the new microfilaraemia was 25% of the initial one at D42-63 pT, and only 12% at D90 pT (Fig. 3, A and B). b) Naive recipient rodents of treated filariae: Six uninfected and untreated jirds received each the following combination of worms: ivermectin (iv) male × female iv (2 rodents), male × female iv (1 rodent), or male iv × female (2 rodents), male × female (1 rodent).

A positive microfilaraemia appeared in the combinations male iv x untreated female and *viceversa*, respectively 48 and 35 days following the implantation. In the first case, it remained positive until the necropsy, 90 days later. In the second case, the microfilaraemia decreased to 0 at D63 (Fig. 5). Microfilaraemia was negative in the 4 other rodents.

Moxidectin: The microfilaraemia fell to 0 as soon as D2 pT with a dose of 0.2 mg/kg and was again positive at D14. The new microfilaraemia remained at about 25% of its initial value until D70, then decreased to 6% at D90 (Fig. 3, B).

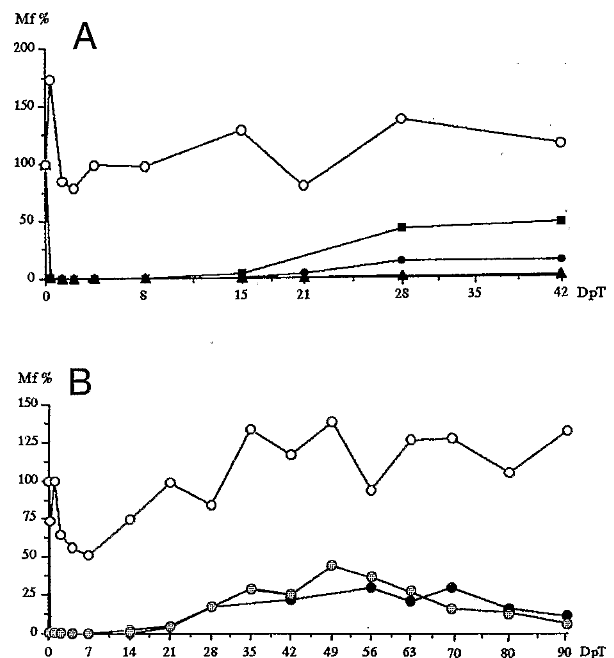

Fig. 3. *Litomosoides sigmodontis/Meriones unguiculatus*: time course of microfilaraemia in rodents treated with ivermectin or moxidectin, and in controls. A: 42 days of follow up after different treatments with ivermectin. Mf%: percentage of pretreatment microfilaraemia. DpT: number of days between the first drug administration and observation. 0.05 mg/kg (■), one rodent, microfilaraemia at D0: 1860 mf/10 µl; 0.2 mg/kg (●), nine rodents, 4212 ± 3008 mf/10 µl at D0; 2 mg/kg (▲), three rodents, 6910 ± 4154.6 mf/10 µl at D0; control (o), five rodents, 4086 ± 2578.7 mf/10 µl at D0. B: 90 days follow up after ivermectin or moxidectin treatment, with 0.2 mg/kg. Ivermectin (●): nine rodents, 4212 ± 3008 mf/10 µl at D0. Moxidectin (◎): six rodents, 3070 ± 1199 mf/10 µl at D0. Controls (o), five rodents, 4086 ± 2578.7 mf/10 µl at D0.

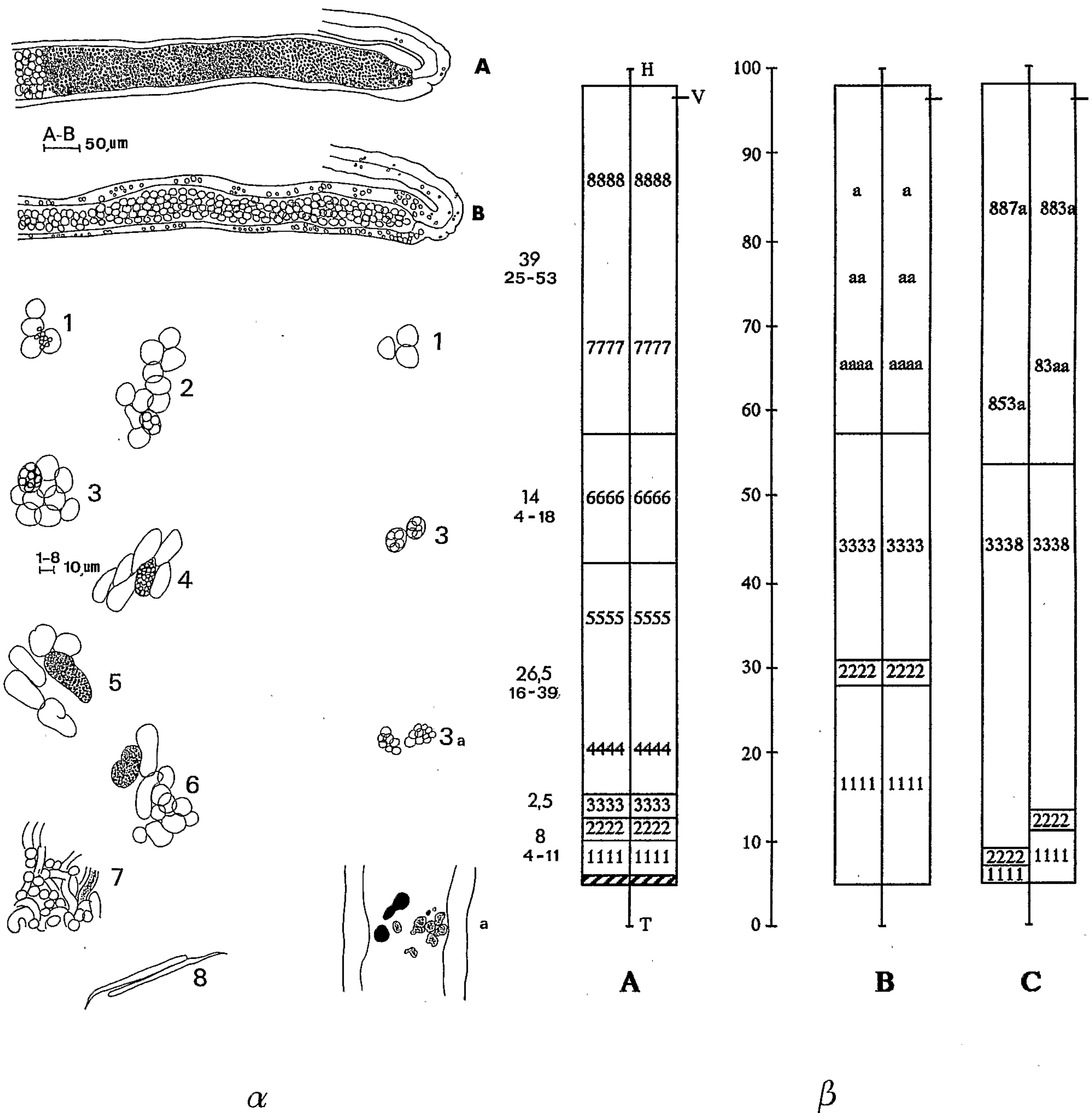

Fig. 4. *Litomosoides sigmodontis/Meriones unguiculatus*: morphology of the female filariae. α) Detail of the uterine contents. Above, distal part of the uterus containing spermatozoa (A), and of uterus without spermatozoa (B) (note in this case the refringent parietal granules). On left, normal embryogenesis with the principal embryonic types recognizable: type 1, round shape and clear aspect (ovule and undivided egg); type 2: first and second cell divisions, clear aspect (it may be confused with type 1); type 3, round shape with bulging cells, size slightly greater than types 1 and 2 (morula); type 4, angular shape, cells small and distinct from each other; type 5, not angular, more elongated than type 4 and slightly folded, with small distinct cells; type 6, with cells no more identifiable and unequal round or elongated worm sections; type 7, with section diameters two to three times reduced compared to type 6 (bredzel stage); type 8, mature stretched microfilaria. On right, abnormal embryogenesis with type 1, 3, abnormal 3 and not identifiable altered forms (a) (scales: A, B, 50 µm; other drawings, 10 µm). β) Schematic representation of the female categories A, B and C with their two uteri. T: tail; H: head; V: vulva; hatches: spermatozoa (they occupy 1 to 1.5 mm at the end of the uteri in category A). In the uteri the numbers represent the embryonic stages and their repetition expresses their density; the position of the embryonic stages and their relative extension is presented after the scale (in the middle); their mean value (above) and extremes of their extension (below) are written on the left.

Table 4. *Litomosoides sigmodontis/Meriones unguiculatus*: effect on female worms of ivermectin (A) or moxidectin (B). Dose: expressed in mg/kg/bw and administered once subcutaneously. DpT: number of days between the first drug administration and necropsy. nR: number of rodents used. nf: total number of female worms recovered and studied. Category A, B, C: percentage of female of category A (presenting spermatozoa and normal embryogenesis), B (without spermatozoa, with abnormal embryogenesis and without microfilariae), C (without spermatozoa, with abnormal embryogenesis and with microfilariae). Mf nec: mean microfilaraemia at necropsy. Observations made on fixed female worms for ivermectin and live female worms for moxidectin.

Treatment	Doses	DpT	nR	nf	Category A	Category B	Category C	Mf nec
Controls iv	–	D42	3	29	77.4	16.7	5.9	3140.5
Invermectin	0.05	D42	1	14	0	14.3	85.7	870
	0.2	D42	2	19	0	31.5	68.5	415.5
		D63	1	5	0	60	40	865
	2	D42	2	30	0	36.7	63.3	22.5
		D63	1	5	0	60	40	4
Controls mo	–	D90	2	41	80.7	12.2	7.1	9736
Moxidectin	0.2	D42	4	75	0	60.4	39.6	712.5
		D90	2	31	0	40	60	197

Effect of ivermectin and moxidectin on adult filariae; inhibition of insemination and embryogenesis alteration

Male filarial morphology was stable and no differences were observed between control and treated specimens.

In contrast, female filariae presented important variability in the uterine contents. A hundred of fixed female specimens from control and treated rodents were studied. The results are summarized in Table 4. Eight embryonic stages were determined. Their density (0 to 4+) and relative extension were precised for each female (Fig. 4, A). Three categories of female worms were distinguished (Fig. 4, B). Category A: the embryonic stages 1 to 8 were present in the two uteri (or only one), regularly distributed and the density of each stage was 4+. The spermatozoa were present in the two uteri (rarely in one); they were accumulated at the bottom of the uteri of a total length of 1000-1500 µm. Category B: the uteri did not contain microfilariae of stages 7 and 8; at their normal place, altered stages "a" were present; these became fewer towards the anterior part of the uteri or this part of the uteri was empty. The embryonic stages 4, 5 and 6 were absent or too modified to be identifiable. The stages 1 to 3 occupied mainly the uteri and their density was high (4+). The stage 3 (morula) had an irregular shape, and was progressively altered. The spermatozoa were absent in the uteri. The uterine wall contained numerous large refringent granules. Category C: the uteri, or at least one of them, contained microfilariae of stages 7 and 8 in the anterior part, generally at low density, sometimes at 3+, exceptionnally at 4+, and often scattered all along the uteri. The more posterior contents were similar to those of the category B: stages 1, 2, 3 in normal density; altered stage "a" present; stage 5 was sometimes identifiable. The spermatozoa were absent in the uteri. The uterine wall contained refringent granules like category B.

In the experiments with moxidectin (Table 4), live filariae were observed (before the chromosome staining). It was not possible to determine the relative extension of the different embryonic stages, but the presence/absence of spermatozoa and embryonic stages, and the aspect of the microfilariae were noted. A small proportion of microfilariae from category C appeared more or less altered (this was not detectable in fixed worms).

After staining, the ovula had the same aspect in the three categories of female worms: a high proportion of them had an apical nucleus with the n chromosomes identifiable (Fig. 2, C). The aspect of the divided eggs differed according to the categories. In category A (Fig. 2, B), the cells with 2n chromosomes were frequent; in the categories B and C, they were exceptional and were observed only when some rare spermatozoa were present. In general, the cells were lyzed, with a picnotic nucleus, and were shrunked inside the egg envelope (Fig. 2, D).

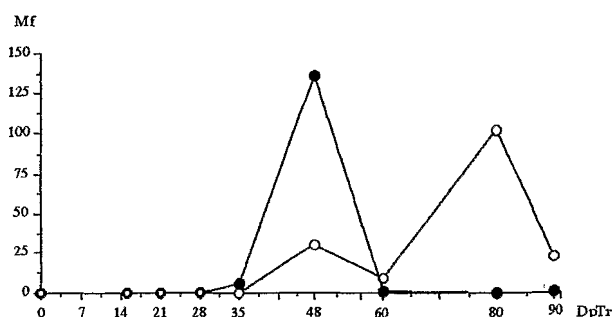

Fig. 5. *Litomosoides sigmodontis/Meriones unguiculatus*: microfilaraemia following implantation in naive jirds of treated filariae crossed with control ones. Mf: microfilaraemia. DpTr: days post-transfer (o): 3 control males 22 days old and 3 treated females 210 days old; (•): 3 treated males 210 days old and 3 control females 22 days old.

Table 5. *Litomosoides sigmodontis/Meriones unguiculatus*: results of crossings between treated and control filariae implanted in naive rodents. m x f: crossing male with female. iv: ivermectin. Category: number of female worms in the three categories. A*: female worms inseminated with normal embryogenesis but without microfilariae. Mf: microfilaraemia (maximum value, on the left; at necropsy, on the right).

m × f	Category			m	Mf
	A*	B	C		
3m iv × 3f	3	0	0	1	100-25
3m × 3f iv	0	1	1	1	125-0

The proportion of the three categories of female worms differed between controls and treated rodents.

Untreated controls: Almost all the female worms belonged to category A, and a few to category B or C (29 female worms fixed recovered from 3 rodents; 41 fresh worms recovered from 2 rodents, Table 4).

Ivermectin: a) Female worms from treated rodents: No female worm belonged to category A; none had spermatozoa and their embryogenesis was abnormal whatever the dose of drug (0.05 to 2 mg/kg) and the date of necropsy (D42 or D63). With the lowest dose, the proportion of B was 14% instead of 30% with the two higher doses. b) Transplanted female filariae in naive rodents: Necropsies were performed 90 days following the worm implantation and observations were made on fresh material. In the combination males iv × untreated female, the three female worms were peculiar because they presented spermatozoa, normal divided eggs, however no microfilariae (Table 5). In the inverted combination, the two surviving female worms had no spermatozoa, a low density of altered divided eggs, and one presented a few microfilariae (category C). In the other rodents, only one sex survived or worms were all destroyed.

Moxidectin: Following a treatment with 0.2 mg/kg, the fresh female worms were studied at D42 or D90 (respectively 75 and 31 female worms). They were distributed in categories B and C, except one in category A at D42 (Table 4).

DISCUSSION

Ivermectin and moxidectin had no discernible microfilaricidal effect *in vivo* against *M. martini* (Fig. 1) and did not induce any increase of skin lesions, even at the high concentrations used (5 to 45 mg/kg). However, in these conditions, they altered the uterine embryogenesis. It was shown with ivermectin that this inefficacy was not due to a poor biodistribution in the rodent host, *L. striatus*, but to a natural resistance of *M. martini*. Drug concentrations necessary to immobilize its microfilariae *in vitro* were ten times higher than for *O. lienalis* dermal microfilariae (in Devaney *et al.*, 1985).

In contrast, the susceptibility of *L. sigmodontis* blood microfilariae to ivermectin was great (Fig. 3), a feature which has been shown many times (Söffner and Wenk, 1985; Zahner *et al.*, 1987; Rao *et al.*, 1990, Chandre *et al.*, 1993). The same is true of moxidectin (Fig. 3, and Shares *et al.*, 1994). However, with the two drugs, the microfilaraemia becomes rapidly positive again, a feature also observed by Nakamura *et al.* (1986) with the related milbemicyn D.

This new microfilaraemia was much lower than before treatment and diminishes with time. According to Söffner and Wenk (1985), it results from the microfilariae which remained alive in the uteri.

These authors also observed the female filariae recovered up to 3 weeks post-treatment. They found a uterine microfilarial density particularly high at D21 pT and they supposed that the egg laying was temporarily interrupted. Our observations were performed later, at D42 to D90 post-treatment, with rodents having their microfilaraemia renewed. The female worms recovered during that period had a different morphology. The uterine embryogenesis was discontinuous, the density of the advanced stages was low or nil, microfilariae were absent (category B), or present but not abundant (category C; Fig. 4, B). The female filariae had emptied themselves of their microfilariae, more or less completely (respectively B or C morphology) and these microfilariae, at least those which arrived in the blood circulation, were not altered, as assessed by their normal transmission rate in the mite vector (Chandre *et al.*, 1993).

Moreover, it was noted that the treated female filariae had a common feature: the bottoms of the uteri did not contain the large masses of spermatozoa which, in contrast, were present in the majority of the control filariae (Fig. 4, B). This absence was surprising since the treated male worms were present in normal quantity, and the mature gametes were normally present in their ejaculatory duct. It was explained by an inhibition of the insemination.

No embryogenesis being possible, the new microfilaraemia remained at a level much lower than its initial value and tended to decrease (20% at D42 and 12% at D90 pT). However, the treated male worms restored their ability to inseminate and fecundate the female worms, as assessed by the positive microfilaraemia obtained in the combination of very young control females with treated males, in a naive rodent.

However, it was not possible to state if the microfilaraemia obtained in the combination treated females and very young control males resulted from reinsemination followed by a new embryogenesis, or from uterine microfilariae which had survived the treatment and were produced in the naive rodent.

The rare untreated filariae which were not inseminated had uteri similar to those of treated worms (categories B or C). As in the treated females, the quantity of ovula was normal or increased, showing that they were continuously laid, even when spermatozoa were absent.

The divided eggs were deeply altered by the two drugs, and this effect was prolonged and reinforced by the inhibition of insemination.

CONCLUSION

Moxidectin has similar effects to those of ivermectin: *M. martini* is a remarkable case of natural resistance to the two drugs. On the other hand, *L. sigmodontis* microfilariae are immediately cleared from the blood. However a positive microfilaraemia is restored when the uterine microfilariae, which are mainly in normal condition contrary to the divided eggs, are released. The new microfilaraemia is lower than the previous one due to the drug inhibition of insemination.

The features observed with *L. sigmodontis,* which is very susceptible and easy to analyse, allow an explanation of the main aspects of the ivermectin treatment of onchocerciasis. In this filariasis too, the circulating microfilariae disappear rapidly and appear again several weeks later (Schulz-Key, 1987), at a lower density (Prod'hon *et al.*, 1991; OMS, 1995). Concerning the adult filariae, Schulz-Key *et al.* (1986, 1987) observed a phase of accumulation of uterine microfilariae, followed by a phase with a high proportion of female worms with empty uteri (Renz *et al.*, 1995, described similar events with the closely related species, *O. ochengi,* after ivermectin treatment). Duke *et al.* (1991) and Chavasse *et al.* (1993) observed an increase of non inseminated *O. volvulus* female worms, and consequently alterations of embryogenesis more and more severe. As in the experimental system, the uterine microfilariae which disappear, instead of being absorbed by the uterine wall (Schulz-Key *et al.*, 1986), are laid and renew the circulating microfilariae. The absence of spermatozoa in the female worms explains the maintenance of their density at a low level.

From a general point of view, the consequences of the treatment with ivermectin (and probably with moxidectin) may all been explained by its action on the neuro-muscular transmission (Fink and Porras in Campbell, 1983; Subrahmanyam, 1987): retention in the liver and spleen of the less motile microfilariae (*L. sigmodontis*), or their passive drainage in the regional lymph nodes (*O. volvulus,* in Darge *et al.*, 1991; Vuong *et al.*, 1991), temporary blockage of the laying of microfilariae, due to a paralysis of the vagina and ovijector (and microfilariae ?), absence of insemination, due to this paralysis of the vagina and to a reduction of the male worm activity, an event which has been noted *in vitro* on *L. sigmodontis* by Rao *et al.* (1990) and Mössinger and Wenk (1988).

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