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The skin-dwelling microfilariae of *Monanema martini* in *Lemniscomys striatus* as potential drug screening model for onchocerciasis: midazolam effect in vitro

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Summary

The murid model of *Monanema martini* in *Lemniscomys striatus* was used to evaluate its potential as drug screening model in onchocerciasis. It had been described that the histopathology and the reaction to diethylcarbamazine treatment of this model closely resemble human onchocerciasis. To study further similarities the in vitro effect of midazolam was examined. Skin-dwelling microfilariae (mf) of *M. martini* were taken by skin snips and placed in either plain phosphat buffered saline or midazolam. Concentrations of 50 µg/ml midazolam significantly reduced motility within 15 min. The percentage of fully motile mf dropped to 9.2 and 1.4 after 15 and 30 min, respectively. In contrast to this finding fully motile mf were obtained after intraperitoneal injection of 60 mg/kg BW; but the technique used did not allow to evaluate the in vivo effect of midazolam. The similarities with the human disease and the finding that midazolam paralyses mf of *M. martini* like mf of *O. volvulus* in vitro indicate the potential of the model for simulating human onchocerciasis.

Key words: microfilariae; *Monanema martini;* midazolam; onchocerciasis.

Introduction

A particular need for an animal model exists in onchocerciasis. Various models have been tried without success (Kozek and Figueroa Marroquin, 1982; Townson et al., 1981), others have severe limitations (Duke, 1962; Townson and Bianco, 1982; Greene, 1987). Promising models have been proposed using

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Table 1. Effect of 50 µg/ml midazolam on the motility of *M. martini* microfilariae in vitro*, defined as full, half, and zero motility as described in text

Time (min)	Motility	Controls		Midazolam	
		n	%	n	%
0	full	199	88.4	183	83.6
	half	10	4.4	4	1.8
	zero	16	7.1	32	14.6
15	full	228	86.7	26	9.2**
	half	14	5.3	118	41.7**
	zero	21	8.0	139	49.1**
30	full	301	94.4	4	1.4**
	half	8	2.5	112	39.2**
	zero	10	3.1	170	59.4**
45	full	336	84.6	9	2.7**
	half	29	7.3	140	41.8**
	zero	32	8.1	186	55.5**
60	full	294	89.6	2	0.8**
	half	18	5.5	78	30.8**
	zero	16	4.9	173	68.4**

* Summary of 4 experiments
** Significant differences to controls ($p < 0.005$)

rodents with skin-dwelling microfilariae (mf) (Bianco, 1975; Bain et al., 1985a, 1985b). The pathologic lesions of the model *Monanema martini* in murids *Lemniscomys striatus* are caused by mf in skin and eyes and resemble closely human onchocerciasis (Vuong-Ngoc et al., 1985, 1986).

In a previous paper it has been shown that midazolam, a benzodiazepine derivative, was able to paralyze mf *Onchocerca volvulus* in vitro (Laukamm-Josten, 1987). We evaluated the in vitro effect of midazolam on mf of *M. martini* from the skin of *Lemniscomys striatus* to look for further similarities of these mf with mf of *O. volvulus*.

Material and Methods

Two *Lemniscomys striatus* were infected with 35 L_3-larvae of *M. martini* which were developed in hexapod larvae of *Hyalomma truncatum*. These hexapod larvae had been infected on a *L. striatus* having 60 mf in the ear snip; a mean number of 8 L_3-larvae/nymph had been obtained twelve days later at 27°C. Skin snips were taken 12 and 22 weeks after inoculation. The experiments were performed 30 weeks after infection which corresponds to the period of increasing microfiladermia.

Skin snips from the edges of the ears were taken by a Walser-Touffie pinch of 2 mm diameter. Skin snips were placed in flat bottomed microtitration plates filled with 100 µl phosphate buffered saline (PBS) with a pH of 7.4. Emerged mf were counted after 30 min and categorized in three groups of motility pattern: a) full, b) half, or c) zero. In group a mf showed typical continuous rapid

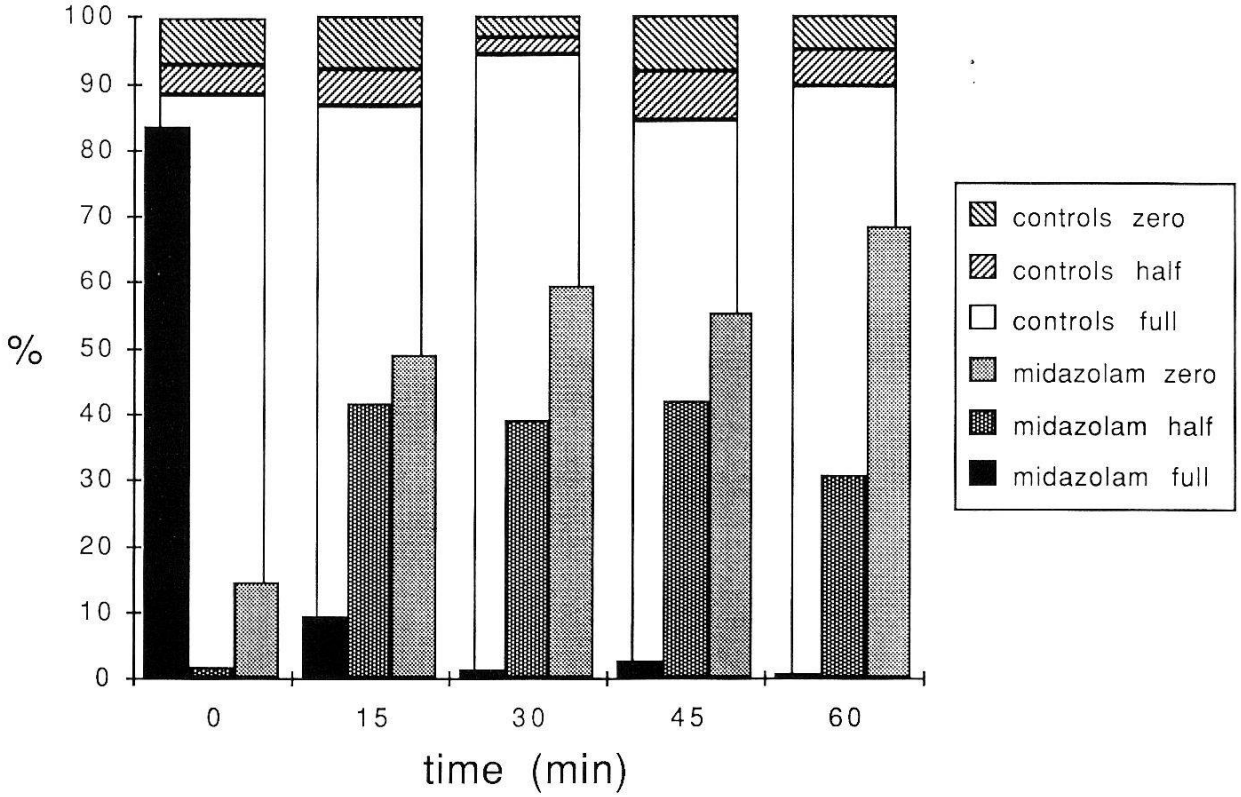

Fig. 1. Percentage of motile mf of *M. martini* after 50 μg/ml midazolam in vitro (motility defined as full, half, and zero as described in text).

contractions, in group b abnormal contractions either of only parts (e.g. tail or head) or with longer intervals than 5 sec, in group c absence of motility was noted. Either 100 μl plain PBS or 100 μl midazolam was added so that a concentration of 50 μg/ml was obtained. Counting was repeated in intervals of 15 min. Four experiments were performed and evaluated together. One animal was given 0.4 ml midazolam intraperitoneal (i.p.), corresponding to 60 mg/kg BW. Skin snips taken between 15 min and 1 h were evaluated for motility of mf.

Student's t-test was used for calculating significance levels.

Results

The two *L. striatus* had 190 and 33 mf per skin snip at the outer edge of the ears when they were selected for the experiments at week 22. The previous skin snips at 12th week had shown 4 and 4 mf.

A maximum number of 732 mf in 4 experiments were counted, 397 in controls, 335 with midazolam (Table 1). Mf survived well over 3 h in PBS without loss of activity (data not shown). Some of them had lost their sheath when they escaped from the snip; the others were sheathed. The proportion between unsheated and sheathed mf was 1:3. No obvious differences with regard to the drug effect could be detected. Within 15 and 30 min the percentage of mf with full motility dropped to 9.2 and 1.4, after 1 h 68.4% of mf were without any sign of motility. All observed changes were significant ($p < 0.005$) already after 15 min when compared to controls (Table 1). In controls there

were no significant changes in motility patterns during the observation period (Fig. 1).

In the animal treated with midazolam i.p. skin snips taken at 15, 30, 45, 60 min after administration of the benzodiazepine revealed fully motile mf when the snips were placed in PBS and allowed 30 min of escape.

Discussion

The animal model of *L. striatus/M. martini* yields mf densites in the skin of the ears which are proportional to the infective dose of inoculated L_3-larvae (Bain, unpublished). While the adult worms are located in the lymphatics of the colon, the mf live in the lymphatic capillaries of the skin preferably at the ears (Bain et al., 1986). It has been suggested that in human onchocerciasis the skin-dwelling mf predominantly live in the lymphatic capillaries also (Bain et al., 1985b, 1987). The adverse reaction to diethylcarbamazine (DEC) in *L. striatus* resemble the Mazzotti reaction (MR) in man: after DEC the mice start scratching. Histopathologically the rapid escape from lymph capillaries within 1 h after DEC administration into the surrounding tissue and consecutively an inflammatory reaction as seen in the MR in man are the important features (Bain et al., 1987). Whether an increased motility of mf is the leading factor or a change in the endothelium of lymph vessels, or a combination of both can not be decided yet (Bain et al., 1987).

We report here that the mf of *M. martini* can be paralyzed with 50 µg/ml midazolam in vitro similarly as shown with mf of *O. volvulus*. The effect seemed to be less rapid and complete as observed in the experiments with *O. volvulus* (Laukamm-Josten, 1987). Since 3/4 of mf *M. martini* have a sheath in contrast to all mf *O. volvulus* are without a sheath this difference may be important. But obvious differences in motility between the two populations of mf *M. martini* could not be detected.

The benzodiazepines potentiate the inhibitory effect of gamma-aminobutyricacid (GABA) suggesting that both species of mf have GABA dependent neuromuscular synapses. This has been taken as evidence that the model used may be a valuable experimental device for screening drugs such as ivermectine or benzodiazepines.

After administration of midazolam i.p. skin snips still produced motile mf. As mf need some time to emerge from the skin snips this time may be sufficient to reverse any paralyzing effect of midazolam possibly present in vivo. It was shown in previous experiments that in plain PBS mf regained motility within very short time (Laukamm-Josten, 1987). So the technique of skin snips seemed not to allow concluding conclusively about the effect of midazolam on mf in vivo. After ivermectine treatment in humans a similar technique has been used and a reduced motility index of mf reported (Soboslay et al., 1987).

The murid model of *M. martini* seems to be an appropriate system for simulating human onchocerciasis. The similarities with the human disease in pathology and the finding that midazolam paralyses mf of *M. martini* in vitro demonstrate the potential for further experiments.

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