ACTIVITY OF ALBENDAZOLE-IVERMECTIN COMBINATION AND OTHER FILARICIDAL DRUGS AGAINST INFECTIVE LARVAE, PREADULT, MICROFILARIAE AND ADULT WORMS OF *MOLINEMA DESSETAE* IN THE RODENT *PROECHIMYS ORIS*.

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Summary :

The efficacy of albendazole-ivermectin combination was tested on adult and developing stages of *Molinema dessetae* in the rodent *Proechimys oris*. Albendazole and ivermectin, both given alone, suramin and diethylcarbamazine were used as reference compounds. The drug combination (albendazole at 10 mg/kg/day × 5 days and ivermectin at 0.04 mg/kg/day × 5 days) was effective against infective larvae and preadult worms, and substantially reduced the number of live adult worms. The known filaricidal agents, diethylcarbamazine (400 mg/kg twice daily × 5 days), ivermectin (0.2 mg/kg/day × 5 days), and suramin (40 mg/kg/day × 5 days), as well as albendazole (50 mg/kg/day × 5 days) were active on infective larvae, preadult worms, microfilariae and adult worms. All drugs had the same level of efficacy on infective larvae. Albendazole had the highest efficacy against adult and preadult worms and diethylcarbamazine was the most active on microfilariae. Although the drug combination was not as effective against preadult and adult worms as albendazole alone, the results indicate that albendazole-ivermectin combination at a low dose had prophylactic effect and suggest a possible macrofilaricidal activity.

KEY WORDS : *Molinema dessetae*, filaricides, chemotherapy, chemoprophylaxis, albendazole-ivermectin combination, albendazole, *Proechimys oris*.

MOTS CLES : *Molinema dessetae*, filaricides, chimiothérapie, chimioprophylaxie, association de l'albendazole avec l'ivermectine, albendazole, *Proechimys oris*.

Résumé : ACTIVITÉS DE L'ASSOCIATION ALBENDAZOLE-IVERMECTINE ET D'AUTRES PRODUITS FILARICIDES SUR LES LARVES INFECTANTES, PREADULTES, MICROFILAIRES, ET VERS ADULTES DE *MOLINEMA DESSETAE* CHEZ SON HÔTE DÉFINITIF NATUREL, LE RONGEUR *PROECHIMYS ORIS*.

L'effet de l'albendazole et de l'ivermectine administrés seuls ou en association, ainsi que celui de la diéthylcarbamazine citrate et de la suramine, sur les larves infectantes, stades preadultes, microfilaries et les adultes de *Molinema dessetae* ont été étudiés in vivo, chez son hôte définitif naturel, le rongeur *Proechimys oris*. L'association albendazole-ivermectine (albendazole 10 mg/kg/jour × 5 jours, ivermectine 0.04 mg/kg/jour × 5 jours) a montré une activité complète sur les larves infectantes ainsi qu'un effet significatif sur les jeunes adultes qui a été légèrement inférieur à celui de l'albendazole. En outre l'association albendazole-ivermectine a causé une réduction du nombre des vers adultes vivants, ainsi qu'une augmentation du nombre des vers adultes morts, par rapport au groupe témoin. Tous les produits de référence, la diéthylcarbamazine (400 mg/kg/jour × 5 jours), l'ivermectine (0.2 mg/kg/jour × 5 jours) et la suramine (40 mg/kg/jour × 5 jours), ainsi que l'albendazole (50 mg/kg/jour × 5 jours), se sont révélés actifs sur tous les stades du développement parasitaire évalués. Tous les produits ont eu un effet similaire sur les larves au troisième stade. L'albendazole a été le produit le plus efficace sur les adultes et les jeunes adultes. La diéthylcarbamazine a montré la plus forte activité sur les microfilaries. Bien que l'activité de l'association albendazole-ivermectine ait été inférieure à celle de l'albendazole administrée individuellement sur le stade preadulte et les vers adultes, le résultat de cette étude met en évidence l'effet prophylactique de l'association albendazole-ivermectine à faible dose et suggère un probable effet macrofilaricide.

INTRODUCTION

At present the drugs available for treating and controlling human filariasis are unsatisfactory and limited. Hence there is an urgent need to find an effective, safe and easily administered drug that affects not just the adult worms and microfilariae but also the other immature stages (W.H.O., 1987).

Suramin is the only proven onchocerca macrofilaricidal agent (Duke, 1991 ; Ottessen, 1985), but its toxicity and intravenous dosing limit its use.

Several clinical trials have shown that ivermectin and diethylcarbamazine are useful for treating onchocerciasis and lymphatic filariasis respectively (Duke *et al.*, 1990 ; Greene *et al.*, 1990, 1991 ; Prod'hon *et al.*, 1991 ; Sharma *et al.*, 1987). Both are good microfilaricides but

they have little or no effect against adult worms (Albiez *et al.*, 1988 ; Eberhard *et al.*, 1988, 1991). Their activity on developmental stages varies with the filarial species and the stage within the same species (Campbell, 1981, 1982 ; Duke, 1968 ; Fujimaki *et al.*, 1990 ; Kevin Baird *et al.*, 1991 ; Paul *et al.*, 1986).

Flubendazole and mebendazole are active on third and fourth stages of different filarial species (Burbure *et al.*, 1986 ; Katiyar *et al.*, 1987), as well as embryonic stages of *O. volvulus* (Rivas Alcala *et al.*, 1981 a, b). However, they are poorly absorbed orally.

There is considerable interest in using albendazole in combination with ivermectin for the treatment of onchocerciasis as such a drug interaction may improve efficacy against worms (Awadzi *et al.*, 1991 ; Hoaksey *et al.*, 1990). Albendazole is a powerful benzimidazole carbamate involved in carbohydrate catabolism and tubulin synthesis. Ivermectin acts on the neuromuscular level (Vande-Waa, 1991). Both products are intestinally well absorbed in man.

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We carried out a multidisciplinary study to investigate the host parasite relationship of the filaria *Molinema dessetae* Bain, 1973 and the rodent *Proechimys oris* under the influence of several different filaricidal drugs. This experimental model of filariasis has been showed to be a useful model for screening filaricidal compounds (Gayral *et al.*, 1976, 1982 b, 1989).

The aim of this paper is to report on the effects of albendazole-ivermectin combination against infective larvae, preadult worms, microfilariae and adult worms of *M. dessetae*. Also studied were the effects of diethylcarbamazine and suramin, as well as albendazole and ivermectin when administered alone. The influence of these drugs on macroscopic lesions due to filarial infection in *P. oris* is also reported.

MATERIALS AND METHODS

The rodents (*Proechimys oris*), mosquitoes (*Aedes aegypti*) and parasites (*Molinema dessetae*) were bred and maintained at the Parasitology Laboratory of the Pharmacy Faculty, Paris XI University, Châtenay-Malabry, France.

EXPERIMENTAL INFECTION

Male *P. oris* aged between six and ten weeks were infected by subcutaneous injection of 200 third stage *M. dessetae* larvae at the abdominal lower right quadrant. Larvae were isolated from infected *A. aegypti* 21 days after an infective blood meal (Gantier *et al.*, 1979; Gayral *et al.*, 1982 a; Petit, 1978).

DRUGS AND DOSES

Four of the drugs were administered subcutaneously (Sc) once a day over 5 successive days (albendazole, ivermectin, albendazole-ivermectin combination and suramin) and one drug was administered orally (Or) twice a day over 5 successive days (diethylcarbamazine) (Gayral *et al.*, 1982 b; Kani *et al.*, 1986).

The drugs were suspended at 5 mg/ml in 1% carboxymethyl cellulose (CMC 1%) in distilled water, in which some 50 µl drops of polysorbate-80 (Tween) had been added.

Doses of drugs administered by subcutaneous route

1. Albendazole was administered at 50 mg/kg/day × 5 days. Concentration 25 mg/ml. Supplied by SKF (Lima, France), lot 359/80.
2. Ivermectin was administered at 0.2 mg/kg/day × 5 days. Concentration 0.1 mg/ml. Supplied by MSD-AGVET (Le Puy, France), lot 73350034.
3. Albendazole-Ivermectin combination was adminis-

tered at: Albendazole 10 mg/kg/day × 5 days ; concentration 5 mg/ml. Ivermectin 0.04 mg/kg/day × 5 days ; concentration 0.02 mg/ml.

4. Suramin was administered at 40 mg/kg/day × 5 days. Concentration 20 mg/ml. Supplied by Rhône Poulenc Santé (Gien, France), lot 126.

Doses of drugs administered by oral route

5. Diethylcarbamazine citrate was administered at 400 mg/kg/day × 5 days. Concentration 200 mg/ml. Supplied by Rhône Poulenc Santé (Gien, France), lot HC 5548.

DESIGN OF TREATED AND CONTROL GROUPS

Five rodents were used per drug. For the rodents given the drug subcutaneously (4 drugs: 20 animals) there was one control group of 10 animals and for the rodents given the drug orally (1 drug: 5 animals), five animals were used as control. The number of rodents used as control was calculated according to the total number of *P. oris* treated by Sc and Or routes (Lellouch & Lazar, 1974). Rodents were allocated at random into treated and control groups.

The control groups of rodents, which were infected but left untreated, received a volume of CMC 1% with Tween equivalent to that received by the treated groups.

The activity of the drugs was evaluated on four filarial stages : infective larvae, preadult worms, microfilariae and adult worms. The drugs were administered four days post infection to evaluate the effects against infective larvae. To assess the efficacy on preadult worms, rodents were treated 47 days after inoculation. The drugs were given 189 days after infection to investigate their activity on microfilariae and adult worms. In this group, only rodents with 10 mf or more per 10 µl of blood were selected to assess micro- and macrofilaricidal efficacy. Dates of treatment were selected according to Gayral *et al.*, 1982 a, b.

COUNTING MICROFILARIAE

Blood was taken from the retro-orbital venous plexus between 2 and 4 p.m. Giemsa-stained thick smears were prepared with ten microliters of blood and examined microscopically to determine the number of microfilariae.

The microfilaremia counts of rodents treated 189 days post infection were measured one week before treatment and then weekly for six weeks following treatment.

As microfilariae begin to appear in the blood on the 90th day after larvae inoculation, the microfilaremia of animals treated 47 days post inoculation was measured one day before or at the moment of the necropsy only.

Recovery of adult worms

Rodents were euthanized with ether six weeks after treatment and a complete autopsy was performed. Intraperitoneal live worms were recovered. Haematological, biochemical and complete pathology studies were also performed. These results will be reported at a later date. The macroscopic findings of intraperitoneal nodules are reported here.

Statistical analysis

The nonparametric Wilcoxon rank sum test was used to compare the data obtained in each treated group with its corresponding control group. The differences were considered significant for a 5% risk (Schwartz, 1990). Data are presented as arithmetic means and percentages of reduction.

RESULTS

EFFECTS ON MICROFILARIAE

The microfilaremia counts obtained each week during the six weeks following treatment are expressed as a percentage of the initial pre-treatment counts. Most of the drugs produced a sustained reduction in microfilaremia levels following treatment (Fig. 1).

Albendazole caused a moderate microfilaremia reduction during the first two weeks (60% reduction by week two) that became very pronounced by week three (98% reduction) and remained stable from week four (99% reduction).

In animals treated with ivermectin, the microfilaremia counts fell moderately (69% by week one) and remained relatively stable over the six weeks following treatment (59% by week six).

With albendazole-ivermectin combination, there was an increase in microfilaremia levels of the rodents from the third week after treatment, which peaked by week four (84% increase), followed by a gradual reduction that remained higher than the pre-treatment counts (55% increase by week six).

The microfilaremia counts in rodents treated with suramin fell progressively. The levels were reduced by 14% at the first week post-treatment and gradually decreased to 98% by the end of the study.

Microfilaremia levels fell rapidly in rodents treated with diethylcarbamazine. By the first post-treatment week, no microfilariae were found. The levels increased (1-2%) over weeks two to five but by week six the levels measured had fallen back to zero.

In the two control groups of rodents, the microfilaremia counts increased over the six weeks post-treatment. The levels were similar for the two groups: they increased from 5% at the first post-treatment week to 36% by the end of the study.

Fig. 1. Microfilarial percentages one-week before treatment (0 week) and during the six weeks following treatment (therapy at 189 days post-infection).

The number of rodents that became free of microfilariae after treatment varied according to the drug administered. Diethylcarbamazine completely cleared microfilariae in all rodents. Albendazole, suramin and ivermectin caused complete clearance of microfilariae in 4 of 5, 3 of 5 and 2 of 5 rodents, respectively. In albendazole-ivermectin combination and control groups no rodents became amicrofilaraemic during the study period.

EFFECTS ON ADULT WORMS

LIVE WORMS

The mean number of live adult worms recovered from control and treated rodents is shown in Fig. 2. Also shown is the percentage of reduction in the number of worms recovered compared to controls. The mean number of adult worms recovered after treatment from both control groups was similar, between 8.9 and 9.4 worms. The two control groups also had similar mean counts for adult female worms (5.3 and 6.0 worms) and adult male worms (3.4 and 3.6 worms).

All of the drugs tested in the *Molinema dessetae-Proechimys oris* model reduced the worm burden by between 37% and 100%, relative to the number of worms recovered in the control groups. Albendazole was the most effective substance against

the adult worms. No adult filariae were detected at autopsy. Suramine and diethylcarbamazine were both strongly active, producing similar reductions of 93%-94%, respectively, in the number of live worms when compared to controls. No male live worms were recovered in rodents treated with diethylcarbamazine. In contrast, suramine caused a reduction of 90% in the number of male worms compared to the control. Ivermectin caused a 71% reduction in the total number of live worms recovered after treatment when compared to control.

The live worm burden in rodents treated with albendazole-ivermectin combination was reduced to 37% compared to the control group. There was a larger reduction in the number of male worms (44%) compared to female worms (32%).

The differences between treated and control groups in the mean number of live adult worms recovered after treatment were statistically significant in all groups except the rodent group given albendazole-ivermectin combination.

DEAD WORMS

An important finding was the presence of dead adult filariae surrounded by a yellow material in the abdominal cavity of treated rodents. Two rodents in each of the five treated groups showed dead filariae. Of interest was the observation that in the group which

IVM : ivermectin, ABZ : albendazole, ABIV : albendazole-ivermectin combination, SA : suramine, DEC : diethylcarbamazine.

Fig. 2. – Mean number and percentage reduction of live adult worms recovered from rodents six weeks after treatment (therapy at 189 days post-infection).

IVM : ivermectin, ABZ : albendazole, ABIV : albendazole-ivermectin combination, SA : suramine, DEC : diethylcarbamazine.

Fig. 3. – Mean number and percentage reduction of live adult worms recovered from rodents six weeks after treatment (therapy at 47 days post-infection).

received albendazole-ivermectin combination one rodent had two dead worms. Only one dead worm per rodent was found in the other nine animals. No dead adult filariae were found in the control groups.

Effects on preadults :

Figure 3 shows the mean number of live adult worms recovered from treated and control groups. Also shown is the percentage decrease in live adult worms recovered from treated groups compared to the live worm burden for control groups. The mean number of live adult worms recovered in the control groups was considered to be 100%.

Albendazole showed the highest activity, as no live adult worms were found in the five treated rodents at necropsy. The other four drugs showed high activities against preadult worms, the overall percentage reduction ranged from 70% to 95%, with diethylcarbamazine (95%) and albendazole-ivermectin combination (85%) being the most active and ivermectin (77%) and suramine (70%) the least active. In rodents treated with albendazole-ivermectin combination, as well as those treated with diethylcarbamazine, no live male worms were found. This was in contrast to animals treated with suramin or ivermectin where some live male and female worms were recovered.

The lower mean numbers of live adult worms in treated groups were statistically significant. Moreover, no

microfilariae were seen in the thick smear prepared from blood of the treated rodents, unlike with control rodents where a mean of 2.3 microfilariae per 10 μ l of blood was observed.

Effects on infective larvae :

All the drugs were active against third stage larvae, as all treated rodents were negative for live worms at autopsy. In contrast, an average of three worms per rodent was found in the control groups. The difference between the live worm recovery means of each treated group and its control group was statistically significant.

Effects on gross pathological lesions :

Intra-abdominal granulomas were the most common macroscopic finding seen at autopsy. They were found in all control animals and in those treated at 47 and 189 days post-infection (Table I). Also, more granulomas were present in these treated rodents than in the controls. Interestingly, no granulomas were found in rodents treated four days after inoculation.

Most granulomas were found in the omentum. They were also seen, though less frequently, in the pelvis, diaphragm, retroperitoneum and free in the cavity. Their size ranged from 2 mm to 15 mm, but most were less than 5 mm. All granulomas were yellowish and friable.

PARASITE STAGE EVALUATED	RODENT no.	LOCATION					TOTAL MEAN
		omentum	pelvis	diaphragm	retro-peritoneum	free	
ADULT WORMS							
control	15	4,2	1,2	0,1	0,1	0,5	6,2
treated	25	6,3	1,2	0,3	0,4	0,3	8,5
PREADULT WORMS							
control	15	2,5	0,2	0,1	0,2	0,2	3,3
treated	25	3,4	0,9	0,3	0,2	0,3	5,4
INFECTIVE LARVAE							
control	15	3,8	0,6	0	0	0,2	4,9
treated	25	0	0	0	0	0	0

Table I. – Mean of intra-abdominal granulomas number by location six weeks after treatment.

DISCUSSION

All the drugs administered alone were active on developing stages and adult worms, showing the high sensitivity of *M. dassetiae* to filaricidal agents, such as suramin, diethylcarbamazine and ivermectin. These drugs are well-known filaricides in humans (Schulz-Key *et al.*, 1985 ; Schuurkamp *et al.*, 1990 ; Vingtaing *et al.*, 1988).

The clearance of circulating microfilariae was complete and quick in animals receiving diethylcarbamazine. It was slow and progressive in rodents given suramin and albendazole, with a reduction in microfilaremia between 98% and 99% in each group respectively by the sixth week after treatment. Ivermectin was less active against microfilariae than the three above mentioned drugs. It produced a 59% reduction in the microfilaremia count by the end of the study.

Albendazole produced a complete reduction in the number of live adult worms recovered at autopsy. Suramin, diethylcarbamazine and ivermectin were less active than albendazole on adult worms.

Albendazole-ivermectin combination caused a 37% reduction in the number of live adult worms compared to control groups. The reduction was however non significant. The increase in the microfilaremia level seen in these animals cannot be explained from results obtained here. There are two possible explanations for this increase. It may be the result of natural variation in microfilaremia. Another possible reason is a still poorly understood non-lethal, toxic effect on adult female worms that induces a release of microfilariae from the uteri.

All drugs given alone showed complete activity on infective larvae. Albendazole was the most effective against preadult worms, as no live adult worms were seen at autopsy. The remaining drugs produced smaller but significant activity on preadult worms.

Albendazole-ivermectin combination showed a complete effect against infective larvae. When given 47

days after inoculation, there was complete activity against males and an incomplete but significant effect on females. The drug combination was completely active on both male and female preadult worms in four rodents. In the fifth rodent, the drug combination had a complete effect on males and an incomplete effect on females.

The effect of the drug combination on preadult worms was greater than that of ivermectin when administered alone and slightly less than albendazole when used alone. Furthermore, ivermectin when given alone to chimpanzees, had a partial effect against infective larvae and no effect on later larval stages of *O. volvulus* (Taylor *et al.*, 1988).

The presence of intra-abdominal granulomas in *P. oris* infected with *M. dassetiae* has been associated with dead worms (Gantier *et al.*, 1987). The increase in the number of granulomas in treated groups when compared to control groups could be interpreted as a filaricidal effect. However, a histological examination of nodules is required to determine their nature. The lack of granulomas in rodents treated four days after inoculation could be due to infective larvae dying before arriving to the peritoneal cavity, as it takes around 14 days for the larvae to reach the cavity (Gayral *et al.*, 1982 a).

Albendazole used alone and administered by subcutaneous route was the most potent filaricidal drug tested. It had a complete effect on three developmental stages: adult, preadult and infective larvae. It was extremely active on microfilariae. The efficacy of this drug against infective larvae and on later larval stages has not been assessed in other animal models or human filariasis. In the *Monanema martini* model of onchocerciasis (Vuog-Ngoc, 1992), the compound administered subcutaneously at 50 mg/kg daily over 5 days had incomplete activity on microfilariae and complete activity on adult worms in two animals but incomplete in the third rodent. Moreover, a clinical study carried out on 92 people infected with *O. volvulus* showed that the principal effect of albendazole when given orally at 800 mg daily for 3 days was on all intra-uterine stages (Awadzi *et al.*, 1991).

The filaricidal effects of the albendazole-ivermectin combination has not been previously examined *in vivo*. In the *M. dessetae* model, the effects of the drug combination on adult worms were not greater than that of both drugs when administered separately. This result should be interpreted with care as the dosage (which corresponded to 1/5 of the albendazole and ivermectin dosage when administered alone) was low and may have been borderline or inferior to the minimal effective dosage against adult worms. Also no activity was observed against microfilariae. It is probable that a higher dose could be effective against both microfilariae and adult *M. dessetae*. In this case, the microfilaricidal effect would probably be slow and progressive. This may be advantageous as it could be associated with few or no secondary effects. In contrast, the most serious side effects, like Mazzotti reaction, are due to the massive and rapid death of microfilariae. Such an effect occurs with diethylcarbamazine (Ackerman, *et al.*, 1990).

This study shows that the albendazole-ivermectin combination has prophylactic effect against *M. dessetae* infection and a likely macrofilaricidal activity. On the basis of these results, and the fact that ivermectin and albendazole are known safe drugs in man, it may be of considerable interest to examine the filaricidal activity of the albendazole-ivermectin combination in humans.

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