In experiment B, where the pumps were removed, the leucocyte counts increased again.

These animals, treated with interferon- γ prior to infection, showed higher immunoglobulin titers (IgG and IgM) in the early phase of infection. After one week of treatment, at the day of infection, the neutrophil counts were significantly increased, whereas the eosinophils were reduced during the first 14 days. Up to week 20 p.i. microfilaridemia reached titers which were twice as high as in the control group, but this difference was not significant. Four of the treated and two of the control animals were dissected 35 weeks post infection in order to carry out lymphocyte transformation tests. No differences were seen between the treated and the untreated control group.

Macrophages (M Φ) isolated from the peritoneum of patent animals showed a high baseline proliferation, whereas cocultivation of M Φ 's with spleen cells resulted in a lower proliferation. In both cases proliferation could be increased by the addition of filarial antigen or Phytohemagglutinin (PHA). However, the presence of IFN- γ reduced the proliferation to baseline or lower levels. Despite this proliferation inhibiting effect, IFN- γ enables macrophages to kill microfilariae *in vitro* within one to three days. The production of nitric oxide by IFN- γ activated M Φ 's could be shown.

DISCUSSION AND CONCLUSION

Implantation of osmotic pumps is a suitable method for the application of short lived substances like IFN- γ or other cytokines. Drugs can be delivered satisfactorily as well. Drugs and cytokines administered in this way had the same or an even better efficacy than injected ones (Murray, 1990, own observation). Other implantation sites in the animal can be used, if there is sufficient space for the pump. The pocket should be large enough and about 1 cm longer than the pump. This prevents the pump from resting directly on the wound. In addition, the pump should be inserted with its delivery portal first to minimize interaction between the delivered compound and the healing of the wound. Normally the incision heals within three days, and the animals show no sign of discomfort. Therefore, it is a useful alternative to repeated injections especially for rodents which are sensitive to frequent manipulation and are not well adapted to laboratory maintenance.

This investigation also shows that recombinant murine IFN- γ is effective in *L. striatus*. The increasing cell counts in the spleen of IFN- γ treated animals do not correspond with *in vitro* assays carried out in parallel, where the proliferation of spleen cells isolated from infected and naive animals was suppressed in the presence of IFN- γ , even when stimulated with filarial antigen.

Although there was no great effect on the course of the filarial infection, some of the results suggest that cytokine work in this model should be continued. Encouraging results include the reduced leucocyte counts, the changes between the different types of leucocytes and the raised microfilaridemia. These results also support the hypothesis that IFN- γ , a Th1-cytokine, counteracts a Th2 response, which is supposed to be involved in protection.

The different effects of IFN- γ to lymphocytes *in vivo* and *in vitro* show that this cytokine has different features, depen-

ding on different factors like other cytokines and immune cells. In an organism cytokines as well as immune cells belong to a highly crosslinked immunological network. More significant results could perhaps be obtained by using combinations of cytokines or with depletion of different cytokines with the help of antibodies against them.

For further investigations, in addition to the administration of cytokines, it is necessary to monitor the changes of cytokine profiles during the course of filarial infection. Tests based on PCR and ELISA are being established.

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THE EFFICACY OF UMF 078 ON *ACANTHO-CHEILONEMA VITAE* AND *LITOMOSOIDES SIGMODONTIS* IN *MERIONES UNGUICULATUS*

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KEYWORDS : UMF 078. *Acanthocheilonema viteae*. *Litomosoides sigmodontis*.

SUMMARY

UMF 078, a new benzimidazole analogue of Flubendazole, was tested on jirds infected with the filarial parasites *Acanthocheilonema viteae* and *Litomosoides sigmodontis* respectively. A total of 19 jirds, eight subcutaneously inoculated with infective larvae of *A. viteae* and eleven with *L. sigmodontis* infective larvae, were used in this study. UMF 078 had both microfilaricidal and macrofilaricidal activities against both parasites. Blood microfilariae were completely eliminated in the two treated groups by day 56 post-treatment. Very few adult worms were recovered, and in both groups all the worms recovered were abnormal. In *L. sigmodontis* however, microfilariae were detected in various organs/fluids (peritoneal fluid, pleural cavity fluid, lungs) on autopsy of the jirds.

INTRODUCTION

A. viteae in jirds is one of the accepted filarial models for the screening of drugs targeted against *Onchocerca volvulus*. Adult *O. volvulus* has proved a big challenge to researchers, because of its host specificity and the absence of an effective macrofilaricide. There is still an urgent need for a non-toxic drug with a macrofilaricidal or a permanent sterilizing effect on the adult *O. volvulus*. UMF 078 (develo-

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ped by Townsend, Michigan) is a new Benzimidazole analogue of Flubendazole "[5-(4-fluorobenzoyl)-1H-benzimidazol-2-yl]carbamic acid methyl ester". UMF 078 is modified from Flubendazole and Mebendazole (Benzimidazole derivatives), which were used against adult *O. volvulus*, but have been discarded due to their toxicity and transient effects (WHO 1991). UMF 078 has been found to have both microfilaricidal effects and macrofilaricidal on *Monanema martini*, a filaria that closely resembles *O. volvulus* in having skin dwelling microfilariae (mf) (Wanji, 1992). This paper reports our observations on the effect of UMF 078 on both the microfilariae and macrofilariae of *A. viteae* and *L. sigmodontis*.

MATERIALS AND METHODS

A. VITEAE IN *M. UNGUICULATUS*

Eight *M. unguiculatus*, subcutaneously inoculated with 60 L3 of *A. viteae* were used for the study six months post inoculation. Five jirds were given subcutaneous injections of UMF 078 once daily for three days with a dosage of 50 mg/kg, and at a concentration of 25mg/ml, while three jirds were used as untreated controls.

Blood samples were taken pre-treatment, and at weekly intervals post-treatment from the retro-orbital vein plexus. The microfilarial densities were determined by mixing the blood with acetic acid in blood mixing pipettes and counting the fixed microfilariae in a Fuchs-Rosenthal counting chamber. If no microfilariae could be detected by this method a more sensitive test with a drop of native blood was done to confirm this result. Autopsy of the jirds was carried out 56 days post-treatment.

Adult worms of *A. viteae* were recovered by stripping the jirds of their pelts, and incubating the pelts with the carcass in PBS at 37° C for four hours (Court *et al.*, 1986). The adult worms recovered were fixed in 70 % ethanol + 5 % glycerol and morphologically examined. Female worms were further subjected to the embryogram technique (Schulz-Key *et al.*, 1980).

L. SIGMODONTIS IN *M. UNGUICULATUS*

Eleven jirds inoculated with 50 L3 of *L. sigmodontis* were studied three months post inoculation. Seven jirds were treated with 50 mg/kg dosage of UMF 078 administered through different modes while four jirds served as untreated control.

The different administration modes were :

number of jirds	day 0	day 1	day 2
3	subcutaneously	subcutaneously	subcutaneously
2	intrapleurally	subcutaneously	subcutaneously
2	intrapleurally	—	—
4	—	—	—

The microfilaremia and the adult worms, found by washing the pleural cavity, were examined as described above for *A. viteae* in *M. unguiculatus*. Additionally the peritoneal fluid, pleural cavity fluid and lungs were checked for the presence of microfilariae.

RESULTS

EFFECTS OF UMF 078 ON *A. VITEAE*

Microfilariae : UMF 078 was found to reduce the blood microfilariae densities of the treated jirds seven days post-treatment and eliminated the blood mf in most of the jirds by day 21 (Fig. 1).

Macrofilariae : Very few adult worms were recovered from the treated jirds. Encapsulated adult worms were also recovered as well as worm fragments. The encapsulated worms and fragments were not counted. Studies on the embryonic state of the female worms from the treated jirds showed a great number of abnormal intra-uterine embryonic stages. All the different stages were affected. From the control group, however, the mean percentage recovery rate was 36,7 % and the embryogram of all the female worms was normal.

Fig. 1. – Microfilaricidal effect of UMF 078 on *Acanthocheilonema viteae* in jirds treated on three consecutive days with a dose of 50 mg/kg given subcutaneously.

EFFECT OF UMF 078 ON *L. SIGMODONTIS*

Microfilariae : In all the jirds, except one treated once through intrapleural injection, there was a remarkable decrease of microfilariae in the peripheral blood seven days post treatment and on day 21 no microfilariae were found in most of the jirds. The microfilariae reappeared, however, in some of the jirds after the day 21 at a low level and by the day 56 no jirds had microfilariae in the peripheral blood (Fig. 2). On autopsy, however, microfilariae were observed in the peritoneal fluid, lungs, and pleural cavity fluid, but none were found in the peripheral blood.

Effect of the different modes of drug administration on mf :

In jirds treated subcutaneously only, the mf density decrease remarkably by the day seven, and by day 21 no jird had microfilariae in the peripheral blood. Mf reappeared in one of the jirds on the day 28, and in another on the day 35 at a low level, but they decreased again to zero and remained there till autopsy.

Fig. 2. – Microfilaricidal effect of UMF 078 administered through different modes on *Litomosoides sigmodontis* (administration modes : three times subcutaneously, once intrapleurally and twice subcutaneously, once intrapleurally ; administration on consecutive days).

The jirds treated once intrapleurally, and twice subcutaneously, showed similar results. In the jirds treated once intrapleurally, and received the lowest dose of the drug, a decrease in blood mf level was seen in one animal, whilst the other had an increase in mf level, more than twice the initial level at day seven. By the day 14, both animals had a remarkable decrease in the mf level. The jird with the initial decrease in mf level had no more mf in the blood at the 28th and 35th day, mf reappeared a little on the 42nd day, but cleared completely by the end of the experiment. The jird with the initial increase in the mf level, had mf up to the day 49. Even though the mf level decreased all the time, and was considerably very low by the 21st day. No mf was found in this animal by the day 56.

Macrofilariae : Despite the presence of microfilariae in peritoneal, pleural cavity fluids and lungs of the jirds, only four female adult worms were recovered from one of the jirds (treated once intrapleurally). From the rest of the jirds, encapsulated dead worms were recovered. It was therefore difficult to calculate the percentage recovery rate of the adult worms, but from the control group, an average of 60 % adult worms were recovered. On examination of the embryonic state of the four female worms recovered, all the worms were found to have empty uteri.

DISCUSSION

UMF 078 appears to have both microfilaricidal and macrofilaricidal activities on *A. viteae* and *L. sigmodontis*. Its mode of action is still not clear but Flubendazole, its analogue, is known to interfere with the microtubulin formation. This could explain the high number of abnormal embryonic stages found in female *A. viteae*. The benzimidazoles as a whole are more macrofilaricidal than microfilaricidal (Zahner and Schares, 1993). The persistence of the microfilariae of *L. sigmodontis* in the peritoneum, pleural cavity and lungs may be a result of a delayed effect of the drug.

The formation of worm capsules is related to immune reactions which result in the death of the worms. This sometimes occurs in the late phase or postpatent infections, but the rate of worm capsules recovered shows that this is a clear-cut drug effect. UMF 078 has some macrofilaricidal activity and could be effective against adult *O. volvulus*. Furthermore, no side effects were observed on the treated jirds. The results from this study recommend the drug for the next phase of screening against adult *O. volvulus*.

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A COMPARISON OF GROSS PATHOLOGICAL CHANGES IN PEOPLE INFECTED WITH *WUCHERERIA BANCROFTI* AND CATS REPEATEDLY INFECTED WITH *BRUGIA PAHANGI*

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KEYWORDS : *Brugia pahangi*, *Wuchereria bancrofti*, cats, human, filariasis, lymphadenitis, lymphangitis, lymphoedema.

People in an area endemic for *Wuchereria bancrofti* usually become microfilaraemic (mf + ve) during early life but to start with have no clinical signs of infection. If cats are repeatedly inoculated with infective larvae (L3) of *Brugia pahangi* they also become mf + ve. Because cats can be examined frequently the onset of clinical disease can be detected early.

Our cats were infected about every ten days with L3 into the surface of the foot of one rear leg. Very soon after infec-

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