MODELLING HUMAN FILARIASIS

CLINICAL STUDY OF THE OCULAR LESIONS INDUCED BY *MONANEMA MARTINI* IN ITS MURID HOSTS

AIMARD L.*, WANJI S.*, VUONG P.N.***, PETIT G.*, BAIN O.*

KEYWORDS : ocular lesions, ophthalmology, murids, filaria, *Monanema*, onchocerciasis.

The filaria *Monanema martini* with skin-dwelling microfilariae induces in its natural murid hosts lesions similar to those in human onchocerciasis. This was demonstrated by histo-pathological studies (Vuong *et al.*, 1991) but it appeared useful to evaluate the model by a clinical study. An ophthalmological analysis (Aimard *et al.*, 1993) was performed on the two species of hosts, inoculated by one, two or multiple doses of larvae, with patent infections since at least one year. A total of 140 eyes was examined (anterior and posterior segments) ; a system of values was established for the different types and intensities of lesions ; a file was prepared for each eye and an attempt at quantification was performed. The significant lesions were different in the two host species. In *Arvicanthis niloticus*, in which motile microfilariae were seen in the anterior segment, punctate keratitis was predominant. In *Lemniscomys striatus*, the posterior segment showed complete chorioretinal atrophy, similar to the ultimate stage of onchocercal chorio-retinitis.

The pathogenic mechanism is probably not unique and it may vary according to the species or individuals. It is noted for example that *L. striatus* has levels of skin microfilariae much higher than *A. niloticus*. *M. martini* represents in its natural hosts two complementary models for the study of pathogenesis and treatment of human onchocerciasis.

ACKNOWLEDGEMENTS

This investigation was supported by Science and Technology for Development Programme of European Community, Contract n° TSE-0067-F and TS3 CT9-0037, and by UNDP/World Bank/WHO programme for Research and Training in Tropical Diseases ID n° 9000535 and 910337.

REFERENCES

AIMARD L., WANJI S., VUONG P.N., PETIT G., BAIN O. : Ophthalmological study of the lesions induced by the filarial worm with dermal microfilariae, *Monanema martini*, in its murid hosts. *Current Eye Research*, 1993, 12, 885-891.

VUONG P.N., WANJI S., SAKKA L., KLAGER S. and BAIN O. : The murid filaria *Monanema martini* : a model for onchocerciasis. Part I : Description of lesions. *Ann. Parasit. Hum. Comp.*, 1991, 66, 109-120.

* Laboratoire de Biologie Parasitaire, Protistologie, Helminthologie, CNRS-URA 114, Museum National d'Histoire Naturelle, 61 rue Buffon, F-75231 Paris Cedex 05, France.

** Département d'Anatomie et de Cytologie Pathologiques, Hôpital Saint-Michel, 33 rue Olivier de Serres, F-75015 Paris, France.

<i>L.s</i>	Control	Mono-inoc	Bi-inoc
n	19	20	10
Mf/mm ²	0	78	101
Chor-ret atrophy Sc.	0.2	1.5	2.1
<i>A.n</i>	Control	Mono-inoc	Multi-inoc
n	7	9	10
Mf/mm ²	0	3.6	13.60
SPK-IPK Sc.	1.4	2.3	2.2

Table I. – Scores of significant ocular lesions in *L. striatus* and *A. niloticus* infected with *Monanema martini*.

Control : rodents not inoculated with infective filarial larvae (L3) ; Mono-inoc : inoculated with a single dose of 80 L3 ; Bi-inoc : inoculated twice with 80 L3 at twelve months intervals ; Multi-inoc : inoculated with a mean sum of 340 L3 given in bi-monthly doses of 15-20 L3 ; n : number of *L. s.* or *A. n.* per group ; Mf/mm² : mean microfilarial densities in the ear-skin lobe during the patent phase (based on the microfilarial counts performed each three months until 18 months) ; Chor-ret atrophy Sc : mean score of chorioretinal atrophy (maximal theoretical value : 5) ; SPK-IPK Sc. : mean score of superficial punctate keratitis and interstitial punctate keratitis (maximal theoretical score : 3).

DRUG TRIALS WITH *MONANEMA MARTINI* : EFFECT ON THE ADULT WORMS, THE DERMAL MICROFILARIAE AND THE NATURAL MURID HOST

WANJI S.*, VUONG P.N.***, GANTIER J.C.***, BOUGNOUX M.E.****, BRETON B.*, BAIN O.*

Technical assistance : PLATEAUX S.*, DUMARQUEZ E.***

KEYWORDS : macrofilaricides, side-effects, histopathology, filariasis with dermal microfilariae, onchocerciasis, murids.

SUMMARY :

The filaria *Monanema martini*, with skin-dwelling microfilariae, which induces onchocerca-like lesions, is well appropriate for drug trials. These are performed together with a histopathological study of side-effects on the murid host.

* Laboratoire de Biologie Parasitaire, Prostologie, Helminthologie, CNRS-URA 114, Museum national d'Histoire naturelle, 61 rue Buffon, F-75231 Paris Cedex 05.

** Laboratoire d'Anatomie et de Cytologie Pathologiques, 20 avenue de la Gare, 91570 Bièvres.

*** Laboratoire de Parasitologie, Faculté de Pharmacie, rue J.B. Clément, 92290 Chatenay-Malabry.

**** Laboratoire de Parasitologie et Microbiologie, Hôpital Ambroise Paré, 92100 Boulogne, France.

Drug	mg/day × n day	Mf/mm ²	F/L3	% Lu-He	Visc. les.	Cut. les.	% dead
Artemether sc	25 × 3 - 75 × 1	33 - 86	17.6	17.5	-	2+/3+/4+	
TFMGP sc	25 × 5	88 - 32	32.2	14	4/Ly	3/4+	
TCMTD sc	100 × 3 - 5	15 - 27	26.8	5	-	3+/4+/PNE	
UMF 289 sc	20 × 3	59 - 1.6	3.8	44	3	3/5+	
UMF 078 sc	50 × 3	41 - 0.6	2	100	3/4/Ly	3+/4/5+	
ALBZ sc	50 × 5	42 - 6.9	0.4	100	3/4/5/Ly PNE/Thr/inf	2/3+/4+/5	10
CGP 6140 po	100 × 5	107 - 80	13.8	23	3	2/3/4/5	6.7
CGI 18041 po	75 × 5	90 - 106	0.6	37	nephritis*/Thr	2/3/5	15
CGP 20376 po	12.5 × 5	104 - 62	12.5	0	3	3	
Levamisole po	15 × 5	-	19.6	3.5	2/3/4/5	5	
Suramin sc	40 × 5	18 - 4	17.1	28	Thr	-	20
Ivomec sc	5 × 5	124 - 181	27.4	0.2	2/3/4/5/Ly	3+/4/5+	
0		95 - 85	36.6 ± 10	3			

Table I. – Efficiency of 12 drugs on *Monanema martini* and their side-effects on *Lemniscomys striatus*, forty days after the beginning of treatment.

Note that the three benzimidazoles are micro and macrofilaricides, and that ivermectin does not reduce the microfiladermia. Frequently drugs which do not reduce significantly the microfiladermia have a slight effect, demonstrated by the lesions induced in the cutaneous tissue due to a small proportion of microfilariae driven outside the lymphatic vessels.

TFMGP : Trifluoromethylguanidinopyrimidine ; TCMTD : Trichloromethylthiadiazole, ALBZ : albendazole ; sc : drug administration by subcutaneous route ; 0 : not treated control rodents. – mg/day x n day : mg of drug¹ per kg of body weight per day, and number of days of drug administration. – Mf/mm² : microfiladermia at the ear-lobe, on day 0 (left number) and day 40 (right number). – F/L3 : the number of filariae recovered divided by the number of infective larvae inoculated x100. – % Lu-He : percentage of filariae located in the lungs and heart. – Visc. les. : more frequent and intense visceral lesions. – Cut. les. : more frequent and intense cutaneous lesions of the inflammatory process, with its different types : 2 : acute, 3 : sub-acute, 4 : granulomatous inflammation and 5 : lesions of scarring fibrosis (a + is added when the lesion is very intense ; nothing is written when the lesion is not frequent and weak) ; Ly : lymphagitis (type 3 associated to lymphatic vessels) ; PNE : eosinophilic polynuclear cells ; Thr : thrombosis of the pulmonary arteries ; inf : infarction ; nephritis* : observed on a treated rodent dead on day 16. – % dead : percentage of rodents treated and dead.

1. Drug amount per day expressed in mmol.10⁻³ : Artemether, 84.2 ; TFMGP, 39.6 ; TCMTD, 226.8 ; UMF 289, 49.4 ; UMF 078, about 153.4 ; ALBZ, 154.3 ; CGP 6140, 269.5 ; CGI 18041, 168.6 ; CGP 20376, 32.6 ; levamisole, 29.1 ; suramin, 27.4 ; ivermectin, 5.7.

To bring filariasis under control a search on macrofilaricides is being strongly stimulated (Macrofil Programme, WHO). The aim is to define a treatment with a correct equilibrium between its efficiency and its side-effects. Drug activities must be screened with very small amount of products, and a histopathological analysis of the infected treated hosts is necessary. Due to these two essential requirements, small rodent models in which parasitological cycle is completed are needed.

Monanema martini with dermal microfilariae belongs to that kind of model ; it is also the unique laboratory filaria which induces an *Onchocerca* like pathology, as demonstrated by histopathological (Vuong *et al.*, 1991) and ophthalmological studies (Aimard *et al.*, 1993). The biology of *M. martini* -rate of development of L3, distribution of filariae, evolution of microfiladermia- has been previously precised (Wanji *et al.*, 1990 ; Wanji, 1992).

METHODS

The filaria is maintained in its natural murid host, *Lemniscomys striatus*, 39 g of mean weight ; the infec-

tive dose of larvae is 80 L3, inoculated subcutaneously. Microfiladermia is measured on the left ear-lobe and expressed in mf/mm². Dissection of the rodents (intestine, mesentery, lumbar and renal lymph nodes, heart, lungs) and morphological study on fresh worms are performed. Each rodent head is fixed in formalin for eye and cutaneous (right ear) lesions ; some animals are fixed *in toto* for the histological study of internal organs. Localization (intra or extralymphatic) and density of parasites are noted ; lesions are identified (inflammatory reaction -types 1 to 5- and reactive lesions) and their intensity is noted ; a score of the cutaneous lesions is calculated. For each rodent, all the results are registered on a file.

RESULTS

1. *M. martini* has a very low susceptibility to ivermectin. It has been shown by *in vitro* study (microfilariae are immobilized with 50µg/ml, after 12 hours) and *in vivo* study (Bougnoux, 1987). The distribution of the drug in *L. striatus* is normal (analysis of Merck, Sharp and Dohme, 1987). This unusual behaviour presented by *M. martini* is of interest to

understand the genetic mechanisms of susceptibility to ivermectin and acquired refractiveness.

2. *M. martini* is normally susceptible to suramin, levamisole and albendazole.

3. Eight drugs provided by WHO have been tested : CGP 20376, CGP 6140, CGI 18041, Artemether, Trichloromethylthiadiazole, Trifluoromethylguanidinopyrimidine, and the two benzimidazoles, UMF 078 and UMF 289. Four principal data were shown :

a. The macrofilaricidal activity is first demonstrated by the abnormalities of the embryogenesis in the uteri of the female worms ; divided eggs are the most fragile stages ; uterine microfilariae persist (CGP 6140) or are destroyed (UMF 078 and 289, etc.).

b. A migration of the adult worm is generally observed. *M. martini* filariae are normally in the lymphatic vessels of the intestine wall. When treated, they are no more able to maintain themselves *in situ* and they are drawn passively towards the pulmonary blood system *via* the thoracic duct and right heart ventricle. This migration induces lymphangitis and sometimes thrombosis followed by infarction (albendazole, CGI 18041). Similar events may occur in all lymphatic filariae (*Brugia*, *Wuchereria*, and ? non nodular *Onchocerca volvulus*).

c. When a macrofilaricide is also microfilaricide, it may induce a Mazzotti reaction, as with DEC : microfilariae are driven from the lymphatic vessels, in which they normally inhabit, and they are destroyed in the perivascular connective tissue by a multitude of synchronous inflammatory reactions (albendazole, UMF 078, UMF 289).

d. A proportion of microfilariae and adult filariae do not migrate and are destroyed *in situ* in the lymphatic vessels, inducing lymphangitis.

On the basis of two trials performed, UMF 289 seems to link high macrofilaricide efficacy and more rapid recovery of the side-effects than the two other benzimidazoles.

ACKNOWLEDGEMENTS

This investigation was supported by Science and Technology for Development Programme of European Community, Contract n° TSE-0067-F and TS3 CT9-0037, and by UNDP/World Bank/WHO programme for Research and Training in Tropical Diseases ID n° 9000535 and 910337.

REFERENCES

- AIMARD L., WANJI S., VUONG P.N., PETIT G., BAIN O. : Ophthalmological study of the lesions induced by the filarial worm with dermal microfilariae, *Monanema martini*, in its murid hosts. *Current Eye Research*, 1993, 12, 885-891.
- BOUGNOUX M.E. : La filaire à microfilaïres dermiques, *Monanema martini*, modèle expérimental pour l'onchocercose ; compléments biologiques et essais thérapeutiques. *DEA Parasitologie* (Parasitologie : écologie, pathologie), Acad. Montpellier, Univ. Sc. Techn. Languedoc, 30 septembre 1987, 32 p.
- VUONG P.N., WANJI S., SAKKA L., KLAGER S. and BAIN O. : The murid filaria *Monanema martini* : a model for onchocerciasis. Part I : Description of lesions. *Ann. Parasit. Hum. Comp.*, 1991, 66, 109-120.
- WANJI S., CABARET J., GANTIER J.-C., BONNAND N., BAIN O. : The fate of the filaria *Monanema martini* in two rodent hosts : recovery rate, migration and localization. *Ann. Parasit. Hum. Comp.*, 1990, 65, 80-88.
- WANJI S. : Un nouveau modèle pour la thérapeutique de l'onchocercose : la filaire des muridés à microfilaïres dermiques, *Monanema martini*. Thèse de Doctorat de l'Université de Montpellier, 18 décembre 1992, 199p.

PARENTERAL ADMINISTRATION OF CYTOKINES AND DRUGS IN RODENT FILARIASIS VIA OSMOTIC PUMPS

RAPP J.*, DALLAH C.N.***, SCHULZ-KEY H.*

KEYWORDS : Interferon- γ , osmotic pump, *Lemniscomys striatus*, *Monanema martini*.

SUMMARY

Changes in the cytokine production profile are supposed to be responsible for the different courses of parasitic infections. Therefore, a big effort is made to understand the roles of cytokines produced by different cells during parasitic infections. In the following text we describe first experiments with Interferon- γ done in a natural model for filariasis, the filariae *Monanema martini* in *Lemniscomys striatus*. For the application of this cytokine to the animals we implanted osmotic pumps, which deliver IFN- γ continuously.

INTRODUCTION

Cytokines play an important role in the regulation of the immune response caused by parasitic infections. Two sets of cytokines are responsible for the activation of different effector mechanisms (Mosmann *et al.*, 1989). Interleukin-2 (IL-2) and interferon- γ (IFN- γ), produced by Th1-lymphocytes, are important for the activation of macrophages, cytotoxic T-lymphocytes and natural killer cells, whereas IL-4 and IL-5, produced by Th2-lymphocytes, induce an Ig E response and lead to the generation of eosinophilia. The type of cytokines produced depends on the host, the parasite, the site and the phase of infection. While Th1 responses are usually directed against intracellular parasites, the responses of Th2 often act against intestinal helminth infections. These patterns, however, are not uniform. Filarial infections often cause Th2 responses, but filariae possess different evasion strategies, especially the downregulation of the immune defense resulting in chronic infections (Behnke, 1987). Immunological hyperreactivity is associated with severe pathology, i.e. sowda in the case of onchocerciasis.

The dichotomy of the Th1 and Th2 cell population is based on multiple, subtle and dynamic equilibria. For studies on these mechanisms a suitable experimental filarial model is needed as well as new techniques for the constant and continuous application of cytokines and other immunological substances into the host. Methods to monitor changes in cytokine profiles and cell reactivity are also required.

Monanema martini in the striped mouse, *Lemniscomys striatus*, is at present the best natural rodent model for

* Institut für Tropenmedizin, University of Tübingen, Germany.

** Dept. of Pharmacology/Therapeutics, University of Nigeria, Enugu.