Evaluation of microfilaricidal effects in the cornea from topically applied drugs in ocular onchocerciasis: trials with levamisole and mebendazole

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SUMMARY Increasing concentrations of levamisole and of mebendazole were applied to 1 eye in groups of 4 patients with ocular onchocerciasis in northern Cameroon. No effects resulted from up to 3.0% mebendazole suspensions, but 3.0% levamisole solutions rapidly caused entry of microfilariae, straightening out and subsequent opacification of previously curled-up living microfilariae, the rapid formation of typical limbal globular infiltrates, and the subsequent formation of fluffy opacities around the microfilariae. These changes are typical of all other drugs so far studied that have a microfilaricidal action on *O. volvulus*—diethylcarbamazine citrate (DEC), suramin, and metrifonate. The efficacy of 3.0% levamisole approximated to that of 0.03% DEC. This is in keeping with published observations on the filaricidal activity of these 2 compounds. It is suggested that this system of drug testing should be considered for systematic use in the search for more effective and safer drugs for onchocerciasis.

The treatment of ocular onchocerciasis is still unsatisfactory (Anderson and Fuglsang, 1977), and the World Health Organisation is urging the search for new drugs and optimum utilisation of existing drugs alone or in combinations.

The clinically observable effects of diethylcarbamazine citrate (DEC) on microfilariae (MFS) of *Onchocerca volvulus* are well defined, whether the drug is administered systemically (Anderson *et al.*, 1976a) or topically (Ben-Sira *et al.*, 1970; Aviel and David, 1972; Anderson and Fuglsang, 1973). The last authors have shown that drug effect can be judged by the entry of MFS into the cornea, the straightening out of previously curled-up living MFS, the formation of typical limbal globular infiltrates, and the subsequent formation of fluffy opacities around the MFS (Anderson and Fuglsang, 1973; Anderson *et al.*, 1976a). These effects have also been observed during systemic treatment with suramin (Anderson *et al.*, 1976b) and with metrifonate (Fuglsang and Anderson, 1977), both of which drugs have a microfilaricidal action.

To test the value of observing effects from drugs

applied topically to 1 eye, preparations of levamisole and mebendazole (Fig. 1) were instilled in a small number of patients with ocular onchocerciasis.

Materials and methods

The trial was carried out at Toubourou in north Cameroon. All the patients were infected with the savanna strain of *O. volvulus*. Both levamisole and mebendazole have low water solubilities, so that a range of concentrations could not be prepared in simple aqueous solution. Antifungal imidazole drugs of low water solubility have been successfully formulated for use in the eye by dissolving the compound in chloroform, diluting in arachis oil

Fig. 1 Structural formula of (a) levamisole and (b) mebendazole

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(BP) to the desired concentration and driving off the chloroform by heat or vacuum (Jones, 1975). This method was used for preparing levamisole drops 0.01, 0.03, 0.1, 0.3, 1.0, and 3.0%.

Mebendazole is not sufficiently soluble in either water or arachis oil for simple formulation. It was dissolved in dimethyl sulphoxide (DMSO) and diluted in arachis oil to give suspensions of mebendazole (0.01, 0.03, 0.1, 0.3, 1.0, and 3.0%) each with a final concentration of 1% DMSO.

After testing to exclude immediate irritancy and toxicity in the rabbit eye by the Draize method, 8 patients were given levamisole and 4 mebendazole. The drops were instilled in the right eyes only, while the left eyes served as controls. The patients were examined with a Haag-Streit 900 slit lamp before treatment and as far as possible before each instillation. Drug effect was judged by the above-mentioned changes, which in the case of DEC therapy are usually obvious within 6 hours of the initial application of a 0.01% solution (Jones *et al.*, 1978).

Photographs of corneal and limbal lesions were taken with a portable photographic system (Jones, Sheen, and Minassian, in preparation) using a reversed Canon FL 35 mm f2.8 automatic diaphragm lens (Fig. 2) and a preset Leitz UM 10/0.22 geological microscope lens (Fig. 3), in each case with an extension tube to give the magnification indicated. Diffuse oblique illumination was from a modified Vivitar 283 flash unit. Positive transparencies taken on Kodachrome 64 were enlarged on Cibachrome.

Results

EXPERIMENT 1

At midday 4 patients were given a drop of 0.01% levamisole to the right eye and 1% aureomycin ointment to the left. Six hours later there was no detectable effect, and the levamisole solution was increased to 0.3% (1 drop). The next morning there was still no visible effect, so the strength of levamisole was increased to 3.0%, which was repeated at midday without re-examination. At 1500 h the previously described drug effects were obvious, and in particular 10 to 30 new discrete globular limbal infiltrates were present in the right eyes of each patient (Fig. 2). At the cessation of therapy 3 days later, most of these globular infiltrates had largely diffused away, but in 1 patient, a boy of 14, 1 still well-defined infiltrate showed a haemorrhage within it (Fig. 3). Photomicrographs with magnification $\times 64$ revealed that erythrocytes had broken up the creamy, rather solid-looking exudate within the globule. Straightening of corneal MFS and subsequent development of fluffy opacities were seen in the right eyes of all

these patients, but no changes were observed in the left control eyes.

EXPERIMENT 2

In order to compare the effects of levamisole with those of DEC 4 other patients were given 3% levamisole to the right eye and 1% DEC to the left eye. The drops were instilled at midday and again in the afternoon on the first day. When the patients were examined the following morning drug effect was obvious in both eyes of all 4 patients. However, the DEC effects were considerably more pronounced than those of levamisole, inasmuch as there were 10 to 20 times as many new globular infiltrates in the left eyes, which also showed far more inflammatory response at the limbus on the second and third days of therapy.

EXPERIMENT 3

Mebendazole was given to 4 other patients at the same dilutions and at the same time intervals as described for levamisole in experiment 1; 1% aureomycin ointment was given to the left control eyes. There were no visible drug effects even after 3 days of treatment by the 3% suspension. Subsequent administration of 1% DEC drops to both eyes of 2 of these patients was rapidly followed by corneal changes typical of microfilaricidal effect.

Discussion

Levamisole (Fig. 1a) is a wide-spectrum anthelmintic given at doses ranging from 2.5 to 5.0 mg/kg daily for 1 to 3 days (Davis, 1973). It has also been shown to have immunopotentiating activity (Janssen, 1976) and antifilarial activity against both MFS and adult *Dirofilaria immitis* in dogs (Tulloch and Anderson, 1971); and also against MFS and at higher dose levels against adult *Breinelia sergenti* in the slow loris (Zaman and Natarajan, 1973). Lämmler *et al.* (1971; see also World Health Organisation, 1974) found that the minimum effective dose against the microfilariae of *Litomosoides carinii* infection in the multimammate rat, *Mastomys natalensis*, was 25 mg/kg for levamisole and 60 to 125 mg/kg for DEC, each given 5 times daily by mouth. The respective maximum tolerated doses were 160 mg/kg $\times 5$ for levamisole and 2000 mg/kg $\times 5$ for DEC. Levamisole also had activity against the adult worms at high dose levels, whereas DEC had none. Maertens and Wéry (1975) found that 100 mg mebendazole given orally twice daily for 14 days had no effect on *Dipetalonema perstans* in a series of approximately 10 patients, whereas this dosage with 50 mg levamisole daily in addition had a significant microfilaricidal effect.

Fig. 2 *Peripheral corneal and limbal globular infiltrates after 7 hours of topical treatment with levamisole (3%) ($\times 18.8$: primary magnification $\times 4.7$, enlarged $\times 4$)*

Fig. 3 *Limbal globular infiltrate after 3 days of treatment with topical levamisole (3%). Haemorrhage into the globular infiltrate has broken up the rather solid exudate within the globule ($\times 64$: primary magnification $\times 16$, enlarged $\times 4$)*

However, the same combined dosage with both levamisole and mebendazole had no demonstrable effect on microfilaria counts in 9 patients with onchocerciasis; nor did 400 mg mebendazole combined with 300 mg levamisole daily for 6 weeks have any effect on microfilaria counts, nor any side effects in a further 5 patients. Duke (1974a) treated a chimpanzee infected with *O. volvulus* with levamisole at a dose of 10 mg/kg intramuscularly daily for 15 days, and showed that it was both micro- and macrofilaricidal at this high dosage, which was too toxic to be considered suitable for man. The maximum dose tolerated by a single human volunteer was 2 mg/kg intramuscularly daily for 15 days, but at this level the drug had no action on MFS or adult worms (Duke, 1975).

The observation that 0.01 and 0.3% solutions of levamisole gave no effect when applied directly to the eye and that the 3% preparation was less effective than 1% DEC, together with other observations on the effect of various concentrations of DEC (Jones *et al.*, 1978), suggest that the topical efficacy of 3% levamisole approximates to that of 0.03 to 0.1% DEC. This ratio of therapeutic activity is in keeping with the observations of Duke in the chimpanzee. There was strikingly close similarity in all respects between the changes in MFS and the associated inflammatory reactions induced by corresponding concentrations of either levamisole or DEC applied topically. This suggests that the levamisole effect is initiated by a direct action on the MFS comparable with that of DEC rather than by an immunomodulation of host defence mechanisms.

Depending on the pharmacokinetic behaviour of levamisole, it is possible that the adverse sequelae to killing MFS in the eye might be easier to regulate than is the case with DEC, but further investigation is necessary to elucidate the possibilities of useful therapy of ocular onchocerciasis with levamisole.

Mebendazole (Fig. 1b) is also a wide-spectrum anthelmintic given at a dose of 100 mg twice daily for 3 days (Davis, 1973). Maertens and Wéry (1975) observed reduction in filariaemia due to *Dipetalonema perstans* in a group of 6 patients given mebendazole 100 mg twice daily for 30 days in a randomised placebo-controlled coded trial, but no effect was seen in another group treated for only 14 days. There was complete and apparently permanent clearing in a further patient given 7 weeks' treatment. On the other hand Aguilar and Manzo (1973) were unable to detect any effect on either microfilarial or adult forms of *O. volvulus* in 19 patients treated with 400 mg of mebendazole daily for 10 days. Similarly, Duke (1974b) found neither micro- nor macrofilaricidal effect when he administered meben-

dazole tablets 27 mg/kg daily for 7 days to an experimentally infected chimpanzee. Duke suggested that the lack of action against *O. volvulus* was partly due to poor absorption of drug from the gut: only 5 to 10% is absorbed. However, the absorption from similar oral dosing (400 to 600 mg 3 times daily) for 21 to 30 days gave unequivocal ultrasonic echotomographic, immunological, and clinical evidence of destruction of *Echinococcus granulosus* cysts in hepatic hydatid disease in each of 4 patients treated. This effect was apparently mediated by damage to the germinal layer of the cysts (Bekhti *et al.*, 1977).

The present investigation gave no evidence of effect on *O. volvulus* microfilariae in the cornea from 3% mebendazole applied topically as a suspension in arachis oil with 1% DMSO. It is possible that poor penetration into the cornea may have contributed to or been responsible for the lack of effect. Although 40 mg/kg mebendazole has been given daily for up to 2½ years in treatment of alveolar hydatid disease without adverse side effects (D. Thienpont, personal communication) there is no clear evidence that this drug affects the adult *O. volvulus*.

Since the microfilaricidal effects of DEC, suramin, metrifonate, and now levamisole can be easily demonstrated in the cornea, it is suggested that this model should be considered for systematic drug testing in onchocerciasis. When absence of topical irritancy and toxicity, and the solubility and absorption allow, the drug should ideally be applied topically to 1 eye, ascending cautiously up a half-log dilution series, leaving the fellow eye to act as a control. In this way the threshold of effective drug concentration can be determined and the dose-response relation defined (Jones *et al.*, 1978). It is also practicable to observe and measure unwanted side effects or sequelae to killing MFS and to study ways of minimising these.

The way in which the above drugs act on the MFS is still not understood, but it is a help to be able to observe the sequence of events in the cornea. The appearance of the typical globular infiltrates at the limbus which occur in microfilaria-loaded corneas after the administration of microfilaricidal drugs requires further studies with immunopathological examination. Detailed examination of patients with ocular onchocerciasis sometimes reveals the presence of occasional globular infiltrates in scanty numbers in untreated persons. It may be that they are related to the spontaneous death of microfilariae or to the immunopathological processes which are involved in the killing or absorption of the MFS. The question remains whether or not they are unique to onchocerciasis.

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