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The safety, tolerability and pharmacokinetics of levamisole alone, levamisole plus ivermectin, and levamisole plus albendazole, and their efficacy against *Onchocerca volvulus*

K. AWADZI*, G. EDWARDS†,‡, N. O. OPOKU*, A. E. ARDREY†, S. FAVAGER†, E. T. ADDY*, S. K. ATTAH*, L. K. YAMUAH§ and B. T. QUARTEY*

**Onchocerciasis Chemotherapy Research Centre (OCRC), Hohoe Hospital, P.O. Box 144, Hohoe, Ghana*
†*Department of Pharmacology and Therapeutics, The University of Liverpool, Ashton Street Medical School, Liverpool L69 3GE, U.K.*
‡*Molecular and Biochemical Parasitology Research Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, U.K.*
§*Armauer Hansen Research Institute (AHRI), P.O. Box 1005, Addis Ababa, Ethiopia*

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Two randomized, double-blind, placebo-controlled trials, in which levamisole (2.5 mg/kg) was given alone or co-administered with ivermectin (200 μg/kg) or albendazole (400 mg), were conducted. In Trial 1, safety and drug–drug interaction were explored in 42 healthy male volunteers. During Trial 2, the safety of the same treatment regimens and their efficacy against the adult worms and microfilariae of *Onchocerca volvulus* were investigated in 66 infected subjects of both sexes. Safety was determined from the results of detailed clinical and laboratory examinations before treatment, during hospitalization and on day 30. The pharmacokinetic parameters for levamisole alone and the combinations were determined in Trial 1 and then compared with historical data for ivermectin and albendazole, given as single agents, to determine if drug–drug interaction had occurred. The level of efficacy against the adult worms was determined by the examination of histology sections of nodules excised 6 months post-treatment and from the changes seen in the levels of microfilaridermia within a year of treatment. Microfilaricidal efficacy was estimated from the reductions in the levels of microfilaridermia between day 0 (1 day pre-treatment) and day 30. Although the regimens were generally well tolerated, there were unexpected adverse effects in both healthy volunteers and infected subjects. Clinically significant drug–drug interactions resulted in an increase in the bio-availability of ivermectin but a reduction in that of albendazole when these drugs were co-administered with levamisole. Levamisole given alone or with albendazole had little effect on *O. volvulus*. The combination of levamisole with ivermectin was neither macrofilaricidal nor more effective against the microfilariae and the adult worms than ivermectin alone. The pathogenesis of the adverse events and the drug–drug interactions are discussed.

Levamisole hydrochloride is a broad-spectrum anthelmintic and an immunostimulant that restores cell-mediated immune mechanisms that have become depressed in peripheral T-lymphocytes (Renoux, 1980). Awadzi *et al.* (1982) found that levamisole had little effect on the microfilariae (mff) or the adult worms of *Onchocerca volvulus* when given, at 2.5 mg/kg, on two occasions during the first week and then weekly for 3 weeks, to patients infected with the parasites. When the same dose was administered on two occasions in the week preceding a 21-day treatment with mebendazole, however, levamisole was seen to augment the modest effects of the mebendazole against the mff and the

Reprint requests to: K. Awadzi.
E-mail: awadzi@ghana.com; fax: +233 21 668871.

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intra-uterine embryos of *O. volvulus* (Awadzi *et al.*, 1982). Although the mechanism was unclear, this benefit was attributed to a 'priming' of the immune system by pre-treatment with levamisole, and not to any alteration in the pharmacokinetics of the mebendazole (Awadzi *et al.*, 1982). Albendazole, another benzimidazole carbamate derivative, has little activity against the mff of *O. volvulus* but has been found to disrupt severely all the intra-uterine stages in the adult, female worms (Awadzi *et al.*, 1991). Moreover, as albendazole is much more potent than mebendazole, significant activity against *O. volvulus* is achieved with a single dose, and prolonged treatment, like that required with mebendazole, is unnecessary (Awadzi *et al.*, 1994). Should the activity of albendazole against adult *O. volvulus* be augmented, like that of mebendazole, by levamisole, then treatment with levamisole followed by albendazole may possibly lead to a more prolonged embryotoxic effect than seen with levamisole/mebandazole, or even a permanent sterilization of the adult, female worms.

Ivermectin, the only drug currently recommended for the treatment of onchocerciasis, neither kills nor sterilizes the adult worms of *O. volvulus* when given in single doses of 150–800 μg/kg bodyweight (Awadzi *et al.*, 1999). Given repeatedly, however, ivermectin causes a variable level of mortality among the adult, female worms (Chavasse *et al.*, 1992; Duke *et al.*, 1992; Klager *et al.*, 1996; Gardon *et al.*, 2002). If a single dose of ivermectin has a significant but hidden macrofilaricidal potential that could be revealed by the immunomodulating properties of levamisole, the co-administration of the two drugs should radically improve the control of onchocerciasis.

Combinations of levamisole with albendazole or ivermectin have not previously been used in humans. The aims of the present study were to determine the safety and tolerability of levamisole, when used alone or in combination with albendazole or ivermectin, in healthy volunteers, to explore the pharmacokinetic interactions of the two drug combinations in the same volunteers, and then to determine the safety and efficacy of the same three treatment regimens in patients infected with *O. volvulus*.

SUBJECTS AND METHODS

Ethical Aspects

In each of the communities in which the subjects of the present study resided, the aims of each trial, the procedures involved and any potential risks and benefits were explained in detail to all the participants, the village co-ordinators who are supported by the Onchocerciasis Chemotherapy Research Centre (OCRC) at Hohoe, Ghana, and the community leaders, prior to any investigative procedures and admissions to hospital. Each subject's consent to participate in the study, given verbally, was witnessed and documented on a form by the local OCRC village co-ordinator and by an impartial witness from the community. The form was then countersigned and dated by one of the investigators. The study protocol was approved by the Ethics Committee of the Ministry of Health, Ghana, and by the Secretariat Committee for Research Involving Human Subjects (SCRIHS) of the World Health Organization. The trials took place at the OCRC between February 1998 and June 2000. They were conducted in accordance with good clinical practices and the Declaration of Helsinki (1996 revision).

Subjects

All 108 participants in the two trials that were conducted came from onchocerciasis-endemic communities in the basin of the River Tordzi, in the forested region of south–eastern Ghana. These communities lie in a focus that has not been under vector control and is not part of any ivermectin-treatment programme. The subjects were aged 18–60 years and in good general health, and each weighed at least 40 kg and had no significant, concurrent, clinical,

haematological or biochemical abnormalities (see details below).

Trial 1

In Trial 1, 42 male subjects who appeared free from the filarial parasites prevalent in the area (i.e. *O. volvulus*, *Mansonella streptocerca* and *Wuchereria bancrofti*) were cautiously exposed to the treatment regimens that had never before been given to humans. Only six men were initially enrolled, with two assigned to each treatment regimen. After close observation of this first group revealed no serious untoward effects, a second group of nine subjects, followed by third of 12 subjects, and finally a fourth group of 15 subjects were investigated, giving a final total of 14 subjects in each of the three treatment arms. The time intervals between the treatments of successive groups of subjects were 12, 14 and 21 days.

Trial 2

For Trial 2, 66 onchocercal nodule carriers of both sexes were enrolled, with five groups (of 12–15 subjects each) tested one after the other and 22 subjects eventually assigned to each treatment regimen. None of the women investigated was breastfeeding and all gave negative results in pregnancy tests. Although the initial group consisted of lightly infected subjects (with <10 mff/mg skin-snip), more heavily infected individuals were enrolled in the subsequent groups.

Study Design and Blinding

Each trial was randomized, double-blind and placebo-controlled. Randomization to treatment regimens, blinding, and the monitoring for drug safety, were conducted in a very similar manner in the two trials; the treatment regimens were identical. In each trial, subjects satisfying the protocol requirements were given code numbers and randomized into three treatment arms, using three columns of random integers generated by the MINITAB computer programme (MINITAB Inc., State College, PA). The drugs were packaged individually into envelopes and labelled with the participant's name, code number and date of administration. The code number and treatment schedule for each participant were recorded on a piece of paper which was then deposited in a sealed, opaque envelope. The sealed envelopes were kept in a locked cabinet for the duration of the trials. The investigators who monitored the trials were different to those who arranged the randomization. All subjects received the same number of tablets; their nature was unknown to the participants and to those who administered them. Individuals who were unaware of the treatment regimens performed the laboratory tests and drug analyses. All assessors were un-blinded only after all the data collected over the trial period had been verified, validated and locked by a clinical monitor, who also closely examined the procedures during Trial 2.

Primary and Secondary Endpoints

The primary endpoint in Trial 1 was the safety of the novel drug combinations (levamisole–ivermectin and levamisole–albendazole). The secondary endpoint was an assessment of drug–drug interaction. The primary endpoints in Trial 2 were the efficacy against the adult worms and the tolerability of the combinations in onchocerciasis patients. The secondary endpoint was the efficacy against the mff in the skin and eyes.

Treatments

Merck (Merck & Co., Inc., Whitehouse Station, NJ) provided 6-mg tablets of ivermectin and matching placebo tablets, GlaxoSmithKline (Brentford, U.K.) provided 200-mg tablets of albendazole and matching placebo tablets, and Janssen Pharmaceutica (Beerse, Belgium) provided 50-mg tablets of levamisole. All participants received levamisole (at approximately

2.5 mg/kg). In addition, the subjects in the levamisole-only (LO) treatment arm received two placebo tablets to match the ivermectin and two placebo tablets to match the albendazole, those in the levamisole–ivermectin (LI) treatment arm received two ivermectin tablets (12 mg) and two placebo tablets to match the albendazole, and those in the levamisole–albendazole (LA) treatment arm received two albendazole tablets (400 mg) and two placebo tablets to match the ivermectin. The tablets were given after an overnight fast, under the direct observation of a medical officer and two nurses, and from 07.00 hours ('hour 0') on day 1. The date and time of administration and the number of the tablets given were recorded in the notes for each participant. In Trial 1, a standard breakfast was taken 2 h after drug administration whereas in Trial 2 the tablets were given with a standard breakfast, in order to enhance the absorption of albendazole (Awadzi *et al.*, 1994).

Monitoring Safety Variables

Each subject was admitted 3–5 days before the administration of the treatment regimens and hospitalized for 8 days afterwards. Adverse events (AE) were determined from changes in symptoms and vital signs, and the results of physical and ocular examinations, electrocardiogrammes (ECG), and laboratory tests (haematological, biochemical and urine). Each ocular examination included the taking of an opthalmic history, visual acuity tests using an illiterate-E chart, visual-field measurement using a standardized Goldmann perimeter, an external ocular examination, and tests for ocular motility. The anterior segment of each eye was examined using a Haag-Streit 900 slit-lamp (Haag-Streit, Könitz, Switzerland) after head-down positioning for at least 5 min. Microfilariae in the anterior chamber, living and dead corneal mff, and onchocercal punctate opacities were counted and their positions noted. The ocular fundi were examined by direct and indirect ophthalmoscopy after pupillary dilatation. A TRC-50VT retinal camera (Topcon America, Paramus, NJ) was used for fundus photography (limited to the initial examination) and fluorescein angiography (using 20% fluorescein sodium). The haematological variables recorded were haemoglobin concentrations, haematocrits, total and differential leucocyte counts, platelet and reticulocyte counts, erythrocyte sedimentation rates, presence or absence of glucose-6-phosphate-dehydrogenase (G6PDH) deficiency, and the results of haemoglobin electrophoresis (HbE). Biochemical tests included total and direct bilirubin, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase, γ glutamyl transferase (GGT), lactate dehydrogenase (LDH), albumin, urea, creatinine, sodium, potassium, bicarbonate and chloride. Urine was examined for pH, specific gravity, protein, glucose, ketones, bilirubin, urobilinogen, blood, nitrites and leucocytes, using a reagent strip, and a centrifuged deposit was examined for cells, casts and parasite ova.

Symptoms were documented whenever they occurred and during formal enquiries at 06.00, 14.00 and 21.00 hours each day throughout the admission period. Vital signs were recorded at the same time and a detailed physical examination was done at least once daily. Ocular examinations were repeated on day 8. ECG were repeated approximately 4 h after dosing and on days 2 and 3. The haematological (except G6PDH and HbE), biochemical and urine tests were repeated on days 4 and 8. In Trial 1, the subjects were visited in their villages on day 18 or 20, and all AE that had occurred since discharge were then recorded. On day 30, all the participants were asked to report to the OCRC, when they were given detailed systemic, clinical and ocular examinations and the haematological, biochemical and urine tests were repeated. Each type of AE observed was listed on a standard form for each subject, together with its date of onset,

duration, severity, number of episodes and causality. The AE were graded and causality attributed using modifications of the criteria published by Awadzi (1980) and Hero *et al.* (1992). The data collected up to day 30, when Trial 1 was terminated, determined the safety of the test compounds.

Drug-concentration Measurements and Definition of Pharmacokinetic Parameters

Samples (10 ml) of heparinized blood were collected from each subject, pre-treatment and 1, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 72 h post-treatment, through an indwelling butterfly needle kept patent with isotonic saline. Each sample was centrifuged ($2000 \times \boldsymbol{g}$ for 15 min) within 30 min of its collection, so that the plasma could be recovered, split between two, 1.8-ml cryotubes, and then frozen at -70°C. One of each pair of tubes was shipped on dry ice to the Department of Pharmacology and Therapeutics at Liverpool University (Liverpool, U.K.) but the other was kept at Hohoe, as a standby, until all the drug analyses were complete. In Liverpool, each plasma sample was extracted with chlorobutane before the concentrations of levamisole were determined by reverse-phase HPLC with ultra-violet detection (at 225 nm), using quinidine as an internal standard, and a Hypersil® BDS C18 column (Thermo Electron, Waltham, MA; 4-μm particle size; 15 cm long, with an internal diameter of 4.6 mm). The mobile phase consisted of CH_3CN (15%) and water (85%) containing KH_2PO_4 (0.05 M) and 1-pentane sulphonic acid sodium salt (5 mM; pH 3.0), flowing at 1.0 ml/min. Under these conditions, the coefficient of variation was $<15\%$ and the limit of quantification was 20 ng/ml. Plasma concentrations of albendazole sulphoxide (Hoaksey *et al.*, 1991; Rawden *et al.*, 2000) and ivermectin (Edwards *et al.*, 1988) were also determined by reversed-phase HPLC, using previously validated methods. The area under the curve (AUC) of plasma concentration *v.* time, to the last experimentally determined concentration, was determined by the linear trapezoidal rule. The maximum plasma concentrations (C_{max}), and the times at which they were achieved (T_{max}) were noted directly.

Monitoring Efficacy variables

The primary efficacy variable was the death of the adult worms (macrofilaricidal effect) or their permanent sterilization. The viability and reproductive activity of the adult worms were determined by the examination of histology sections prepared from onchocercal nodules removed on day 180. At this time, all located nodules were aseptically excised, under local anaesthesia with 2% xylocaine, and processed as described previously (Awadzi *et al.*, 1997). The features examined are listed in Table 4. Additional information was obtained from the repopulation of the skin by mff, assessed on days 180, 270 and 365. The secondary efficacy measurements were the percentage reductions seen in the levels of microfilaridermia (mff/mg skin-snip) between day 0 and day 30 (microfilaricidal effect). In each subject, skin-snips were taken from four sites (both iliac crests and both calves) with a Walser-type corneo–scleral punch. Punches were sterilized between subjects in an autoclave. Each snip was weighed immediately, on a sensitive balance, and then incubated for approximately 24 h in isotonic saline in a well of a flat-bottomed microtitre plate. The mff that had emerged were then counted using an inverted microscope. The density of mff at each site was expressed as the number of mff/mg skin. The 'count' for each individual was the sum of the densities at the four sites (mff/4 mg of skin).

Data Management and Analysis

When the trial codes were broken, all the data collected for individual subjects were arranged by treatment regimen. The AE were listed by body system or category and then their incidences and severities were tabulated. All three treatment arms were initially compared, to identify variables with

significant inter-arm differences, which were then explored in paired comparisons, using χ^2 test or Fisher's exact tests and version 6 of the Epi Info software package (Centers for Disease Control and Prevention, Atlanta, GA). The drug- and non-drug-related AE were compared separately. The pharmacokinetic parameters (AUC, C_{max} and T_{max}) were used to determine if a drug–drug interaction had occurred. Briefly, the geometric means, geometric mean ratios (value of the parameter for the drug when used in a combination:corresponding value for the drug used alone), and the 90% confidence intervals (90%CI) about the geometric mean ratios (GMR) were determined, using the computer programme Confidence Interval Analysis (CIA; British Medical Journal, London, U.K.). The pharmacokinetic parameters defined previously, for ivermectin used alone and albendazole used alone (Awadzi *et al.*, 2003), were used as historical controls. It was concluded that a clinically significant interaction had occurred whenever the 90%CI for a systemic exposure ratio fell entirely outside the equivalence range of 0.8–1.25 (Anon., 1999). The percentages of adult worms and nodules with each of the various features listed in Table 4 were compared across all three treatment arms, with subsequent paired comparisons being limited to the areas of difference so identified.

The densities of mff in the skin were logarithmically transformed prior to analysis. Analysis of variance (ANOVA) was used to compare the three treatment arms, the unpaired *t*-test to compare geometric means at various time-points for the LO and LA treatment arms, and the paired *t*-test to compare adjacent counts in the LI treatment arm, in order to define the pattern of response (i.e. the microfilarial kinetics). The ocular counts of mff were generally low and highly variable within and between treatment arms; analysis of the effect of treatment on these counts was thus limited to a description of the pattern of response within each treatment arm. For all comparisons, the null hypothesis was rejected at $P<0.05$.

RESULTS

Background Characteristics

The 42 healthy volunteers of Trial 1 were all adult males aged 18–56 years (mean = 34 years). All but six weighed $\geqslant$ 50 kg. Two had choroido-retinal lesions of non-onchocercal origin. The baseline characteristics of the 66 subjects in Trial 2 are summarized in Table 1. Overall, the subjects in the three treatment arms in Trial 2 were similar with respect to age, gender distribution, physical characteristics and the paucity of onchocercal lesions, of which the main manifestations were subcutaneous nodules. The percentage of subjects in the LA treatment arm who had mff in the anterior chamber of the eye (54.5%) was, however, significantly higher than the 18.2% in the LI treatment arm ($\chi^2=6.29$; $P=0.012$) and higher, but not significantly so, than the 36.4% in the LO treatment arm ($\chi^2=1.47$; $P=0.226$). In Trial 2, there were no significant gender differences in the numbers of nodule sites or in the intensities of infection with *O. volvulus*. Nineteen (54.2%) of the 35 male subjects and 22 (71.0%) of the 31 females each had two or more nodule sites ($\chi^2=1.94$; $P=0.16$). The geometric mean values and 95% confidence intervals (95%CI) for the initial levels of microfilaridermia were 86.4 (66.1–112.8) for the male subjects and 74.4 (52.4–105.6) for the female subjects (95%CI for the difference = -0.126–0.253; $T=0.67$; degrees of freedom = 57; $P=0.51$). On average, the women were 5 years older, 5 kg lighter and 10 cm shorter than the men.

Adverse Events

HEALTHY VOLUNTEERS

Adverse events were pooled for the 42 persons included in Trial 1, as the frequencies appeared unrelated to the treatment

TABLE 1. *Baseline characteristics of the 66 infected subjects of Trial 2, who were treated with levamisole only (LO), levamisole plus ivermectin (LI) or levamisole plus albendazole (LA)*

Variable	Treatment		
	LO	LI	LA
No. of subjects	22	22	22
% of subjects male	50.0	45.5	63.6
Mean age and (range) (years)	38 (18–58)	40 (23–60)	43 (19–60)
Mean weight and (range) (kg)	50.1 (40.0–65.5)	55.1 (42.0–75.0)	53.4 (43.0–65.0)
Mean height and (range) (cm)	161.6 (152.0–180.0)	162.3 (147.5–180.5)	162.1 (142.5–177.5)
NO. AND (%) OF SUBJECTS WITH:			
One or two nodule sites	20 (90.9)	15 (68.2)	18 (81.8)
More than two nodule sites	2 (9.1)	7 (31.8)	4 (18.2)
Normal visual function	19 (86.4)	20 (90.9)	18 (81.8)
Microfilariae in the anterior chamber	8 (36.4)	4 (18.2)	12 (54.5)
Ocular pathology	2 (9.1)	0 (0)	4 (18.2)
Geometric mean count of microfilariae in the skin and (95% confidence interval) (microfilariae/4 mg skin)*	82.1 (56.9–118.3)	71.9 (45.1–114.2)	88.5 (66.6–117.5)

*Each 'count' was the sum of densities, in microfilariae/mg, in snips from both iliac crests and both calves.

regimen. The most common drug-related events were general weakness and headache, each reported by nine subjects (21.4%), followed by itching in six subjects (14.3%), rash in five (11.9%), and joint pains in four (9.5%). With the exception of grade-2 itching in one subject in the LA treatment arm, and grade-2 headache and fever resulting from malaria in another, all the AE recorded in Trial 1 were of grade-1 severity.

Itching began relatively early in two subjects: at 8.25 h in one subject from the LO treatment arm, and at 12.25 h in one subject in the LI arm. In one other subject from the LI treatment arm, one from the LO, and two from the LA, itching was not noted until at least 23 h post-treatment. Itching lasted for 8–25 h except in one subject in the LA treatment arm, who had mild itching throughout most of the admission period. A papular rash developed over the trunk in four subjects who developed itching. One subject without itching developed papules on the right arm on day 3. Swelling of the foot or leg, beginning on day 1 or day 3 and lasting for 9 h, occurred in two subjects (one LA and one LI). During Trial 1, no important changes in the ECG or in visual function were observed and there were no abnormalities of clinical significance in any of the laboratory tests.

SUBJECTS INFECTED WITH *O. volvulus*

The most common drug-related events occurring in the 66 infected subjects of Trial 2 were itching and rash, occurring in 36 (54.5%) and 31 (47.0%) of the subjects, respectively. Other AE, occurring with moderate frequency, were headache in 20 subjects (30.3%), arthralgia in 11 (16.7%), and other body pains in 11 (16.7%). None of the AE was serious and most were of grade-1 severity. The commonest grade-2 AE were itching (17 subjects), followed by headache (six), rash (three) and dizziness (three). Lymphadenitis, myalgia, fever and anorexia of grade 2 were each observed in one subject. Two subjects in the LI treatment arm had grade-3 itching. Lymphadenitis (five subjects) and swelling of the face or limb (five subjects) were limited to the subjects treated with ivermectin. The other drug-related AE that each occurred in at least five of the 66 subjects are tabulated, and the frequencies of all such events among the three treatment arms are compared, in Table 2. This shows that the three treatment arms

TABLE 2. *Comparison of the frequencies of the more common drug-related adverse events (each of which occurred in at least five subjects) among the 66 subjects infected with* Onchocerca volvulus *who were treated with levamisole only (LO), levamisole plus ivermectin (LI) or levamisole plus albendazole (LA)*

	Treatment			
	LO	LI	LI	*P*
No. of subjects investigated	22	22	22	–
NO. OF SUBJECTS DEVELOPING:				
Pruritus	8	18	10	0.01
Rash	10	12	9	0.653
Headache	7	8	5	0.605
Arthralgia	1	7	3	0.047
Other body aches	2	9	0	<0.0001
Ocular pain/discomfort	2	4	0	0.111
Myalgia	1	3	1	0.421
Fever	1	7	0	0.002
Dizziness	2	3	0	0.22
Sinus bradycardia	3	2	1	0.577
Elevated aminotransferases	3	2	4	0.680

were dissimilar in the frequencies of pruritus, arthralgia, other body aches, and fever. Further examination revealed that all of these AE were more frequent in the LI treatment arm than in the LO or LA, the latter two treatment arms being similar in the frequencies of all the AE listed.

In view of the unexpectedly high incidence of itching and rash recorded in Trial 2, the time of onset, severity and duration of the itching and the type and anatomical distribution of the rash were compared between the treatment arms. The itching began 'early' (i.e. within 10 h of treatment) in all eight subjects reporting this AE in the LO treatment arm, in nine of the 18 subjects who reported it in the LI treatment arm, and in eight of the 10 subjects reporting it in the LA arm. Nine subjects given LI and two given LA began itching 'late' (i.e. at least 22 h post-treatment). The three treatment arms differed significantly in terms of when itching began ($\chi^2=7.25$; $P=0.027$). The main factor was the much earlier onset of this AE among the subjects given levamisole alone, as compared with the LI ($\chi^2=4.11$; $P=0.043$) or LA treatment arms. Itching was, however, more severe with LI than LO or LA, being scored grade-2 or -3 for only three subjects given LO and only five of those given LA but 13 of those given LI ($\chi^2=11.73$; $P=0.003$).

The onset of rash, which was mainly papular, parallelled the onset of itching. The rash appeared in <10 h in nine of the 10 LO subjects who developed this AE, five of the 12 LI, and seven of the nine LA. The rash seen on the LO subjects involved mainly the posterior part of the upper trunk whereas that on the LI and LA subjects was evenly distributed between the upper limbs and the upper trunk. The three treatment arms did not differ significantly, however, in the frequency of rash or the extent of the rash.

As in Trial 1, no important changes in the ECG or in visual function were observed during Trial 2, and there were no abnormalities of clinical significance in any of the laboratory tests. Many AE that did not appear to be drug-related (46, 33 and 32 in the LO, LI and LA treatment arms, respectively; $P>0.05$) were recorded during the 30-day safety assessment.

Concomitant Medication

The main therapeutic intervention was the administration of acetaminophen for headaches (four subjects), gland pain (one subject), myalgia (one subject) or diffuse

aches and pains (one subject). Two patients who had malaria also received chloroquine. Three patients received tincture of belladonna and one magnesium trisilicate, for abdominal pain. Chlorpheniramine was given to 12 subjects during the first 30 days, for the prevention and treatment of reactions to intravenous fluorescein sodium. These reactions manifested as itching, urticaria, nausea, vomiting, abdominal pain and palpitation. The treatment arms did not differ in their requirement for additional medication.

Pharmacokinetic Parameters in Healthy Volunteers

Plots of mean plasma concentrations *v.* time are shown for ivermectin, in the presence and absence of levamisole, in Figure 1. Similar plots for albendazole sulphoxide and levamisole are shown in Figure 2, and those for levamisole, levamisole plus albendazole, and levamisole plus ivermectin appear as Figure 3. (Error bars have been omitted to avoid overcrowding of the figures.) Levamisole and ivermectin were both rapidly absorbed, as indicated by the short times taken to reach their peak plasma concentrations. Albendazole was not detected in any plasma sample, reflecting this drug's rapid conversion to albendazole sulphoxide. In nearly all subjects, the concentrations of levamisole, ivermectin and albendazole sulphoxide fell below the lower limits of their quantification by 72 h post-treatment. The number of subjects in each treatment regimen, and the pharmacokinetic variables for levamisole (when given alone or with albendazole or ivermectin), albendazole sulphoxide when albendazole is given with levamisole, and ivermectin when given with levamisole, together with those for

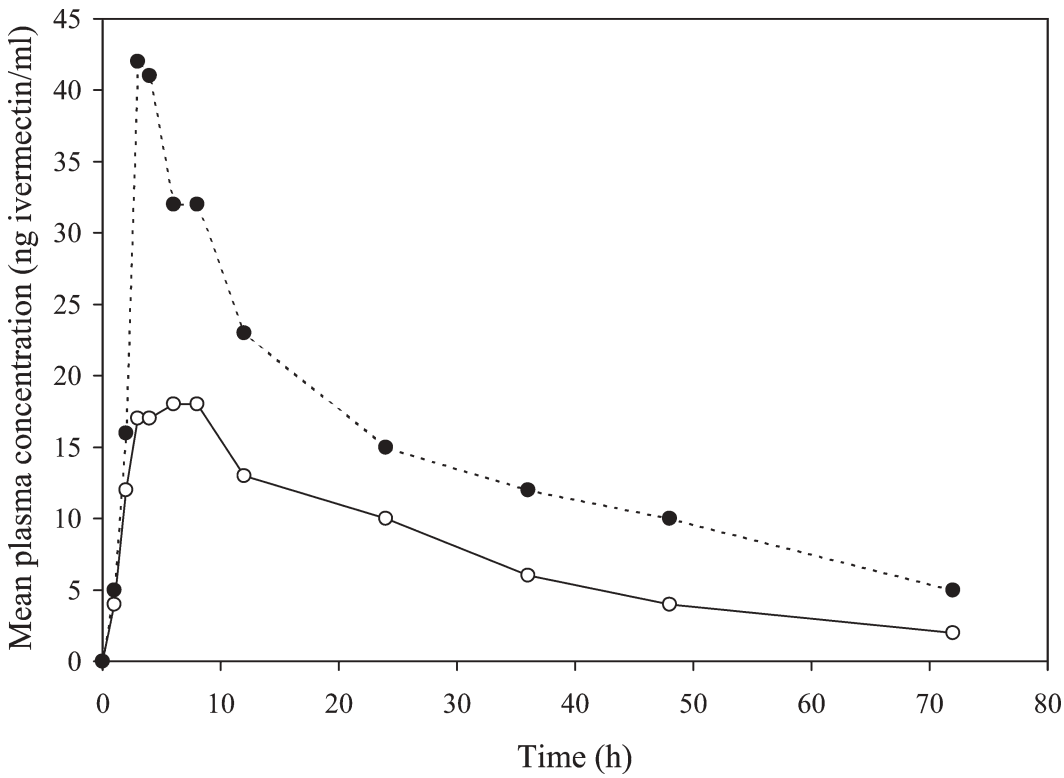

FIG. 1. The mean plasma concentrations of ivermectin in 11 subjects receiving ivermectin plus levamisole (●; present study) and 13 receiving ivermectin alone (○; Awadzi *et al.*, 1993).

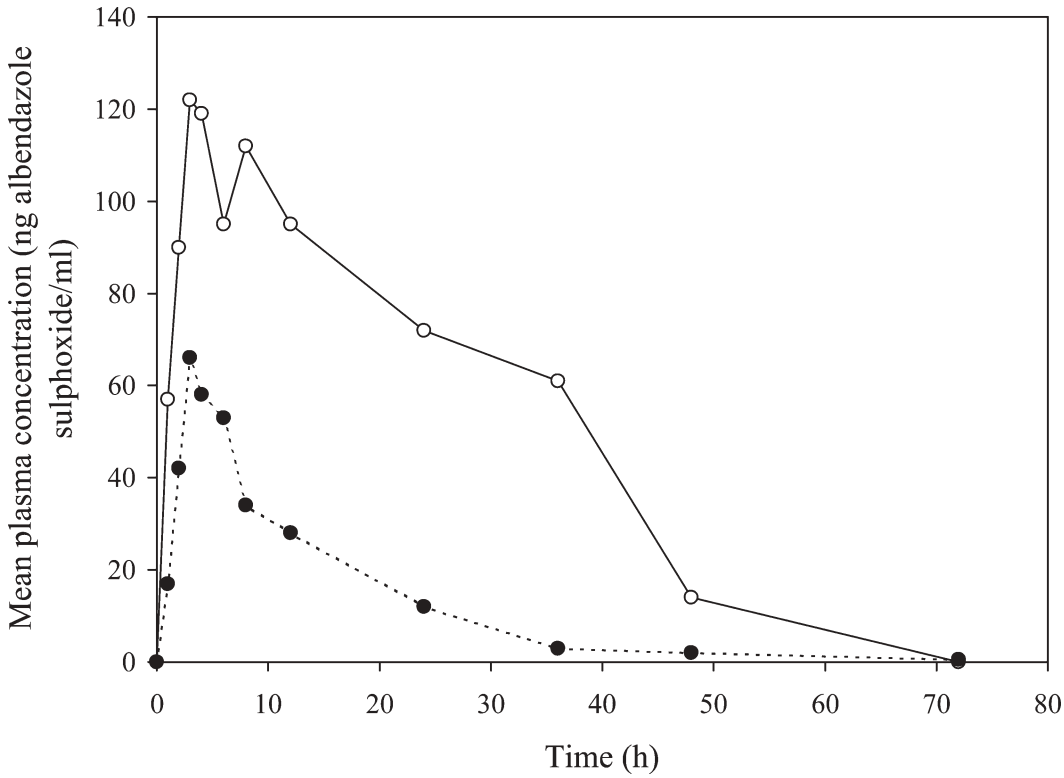

FIG. 2. The mean plasma concentrations of albendazole sulphoxide in 14 subjects receiving albendazole plus levamisole (●; present study) and 14 receiving albendazole alone (○; Awadzi *et al.*, 1993).

ivermectin used alone and for albendazole sulphoxide when albendazole is used alone [obtained from historical data (Awadzi *et al.*, 2003)], are listed in Table 3.

The geometric mean ratio (GMR), and the related 90%CI for the AUC, $C_{max,}$ and T_{max}, are summarized for each paired drug combination, together with the equivalence range, in Figure 4. Levamisole produced a clinically significant increase in the AUC and C_{max} of ivermectin. The GMR was >2 in each case, whereas the 90%Cl were all outside the 'no effect' range (of 0.8–1.25); there was a modest reduction in the T_{max}. When combined with albendazole, levamisole significantly reduced the AUC, C_{max}, and T_{max} of albendazole sulphoxide. The GMR for the AUC and C_{max} were <0.5, with the 90%Cl well outside the 'no effect' range. With the T_{max}, the upper limit of the 90%CI (0.875) barely crossed the lower limit of the no effect range (0.800). Ivermectin had no influence on the PK parameters of levamisole, whereas albendazole sulphoxide produced a modest reduction in the AUC (but not in the other parameters) of levamisole. Thus, the only clinically significant drug–drug interactions were levamisole-attributable increases in the AUC and C_{max} of ivermectin, and decreases in the AUC and C_{max} of albendazole sulphoxide.

Efficacy Against the Adult Worms

Overall, 278 onchocercomata were excised from 64 of the subjects of Trial 2 (the nodules from the other two subjects contained no worm material). Table 4 summarizes the effects of the three drug regimens on the viability, reproductive activity and microfilarial output of the adult worms (as represented by the presence of mff in nodular tissue). The treatment arms were similar in

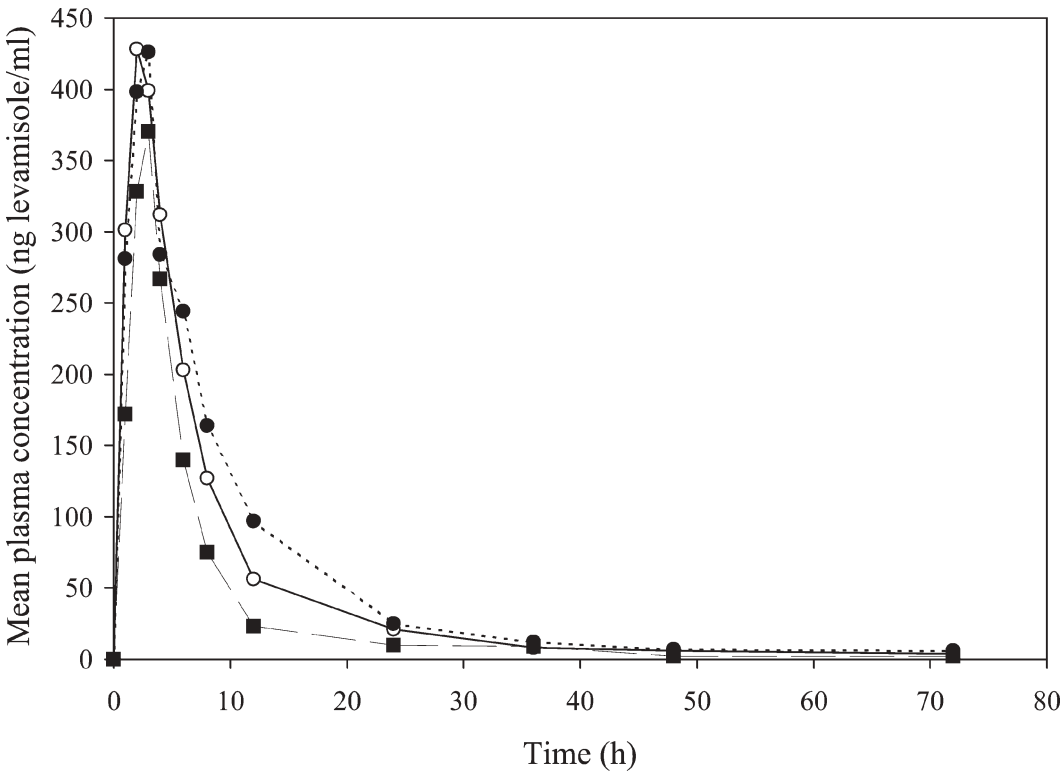

FIG. 3. Mean plasma concentrations of levamisole in 14 subjects receiving levamisole alone (○), 14 receiving levamisole and albendazole (■) and 14 receiving levamisole and ivermectin (●).

terms of the low percentages of the female worms considered dead or moribund, or dead and calcified, and of the low percentages of male worms found dead, indicating the lack of a macrofilaricidal effect. The major differences were in the effects on reproductive activity and microfilarial output ($P<0.0001$). In the LI treatment arm, there was a suppression of embryo production, with only 40% of the adult female worms showing active embryogrammes, as compared with approximately 66% in the LO and LA treatment arms. Moreover, those female worms producing embryos in the LI treatment arm contained predominantly degenerate forms, with the result that $<25\%$ of the onchocercomata from this treatment arm contained mff. This treatment arm differed significantly from each of the two other treatment arms in all these respects, whereas LO and LA produced similar effects on the adult female worms. There was no apparent effect on spermatogenesis in any treatment arm.

Efficacy Against the Microfilariae

The initial levels of microfilaridermia and the changes observed in the year post-treatment are presented in Table 5. The initial densities were similar in all three treatment arms (ANOVA; $F=0.32$; $P=0.724$). In the LO and LA arms, the levels of microfilaridermia observed post-treatment were always similar to the pre-treatment values ($P>0.05$) and the counts remained similar in the two treatment arms at each time-point. In contrast, massive reductions in the level of microfilaridermia occurred rapidly in the LI treatment arm, and it seems more appropriate to describe the pattern of response within this treatment arm than to

TABLE 3. *The values of three pharmacokinetic parameters determined for apparently healthy volunteers treated with ivermectin (alone or with levamisole), albendazole (alone or with levamisole) or levamisole alone*

	Ivermectin, when given:		Albendazole sulphoxide, when albendazole was given:		Levamisole, when given:		
Parameter	Alone★	With levamisole	Alone★	With levamisole	Alone	With ivermectin	With albendazole
No. of subjects	13†	11‡	14	14	14	14	14
ARITHMETIC MEAN VALUE AND (S.D) FOR:							
AUC_{0-72h} (ng.h/ml)	527 (309)	1056 (453)	3581 (1432)	913 (514)	3298 (1742)	3696 (2263)	2189 (1211)
C_{max} (ng/ml)	23 (12)	57 (53)	153 (52)	73 (38)	482 (223)	534 (280)	449 (244)
Median and (range) for T_{max} (h)	2 (3–12)	4 (2–6)	3 (1–6)	4 (3–24)	2 (1–4)	2.5 (1–6)	3 (1–4)

★The 'historical' data of Awadzi *et al.* (2003).
†Ivermectin was not detected in one subject.
‡Ivermectin not detected in three subjects.
AUC, Area under the plot of plasma concentration *v.* time; C_{max}, maximum plasma concentrations; T_{max}, time to C_{max}.

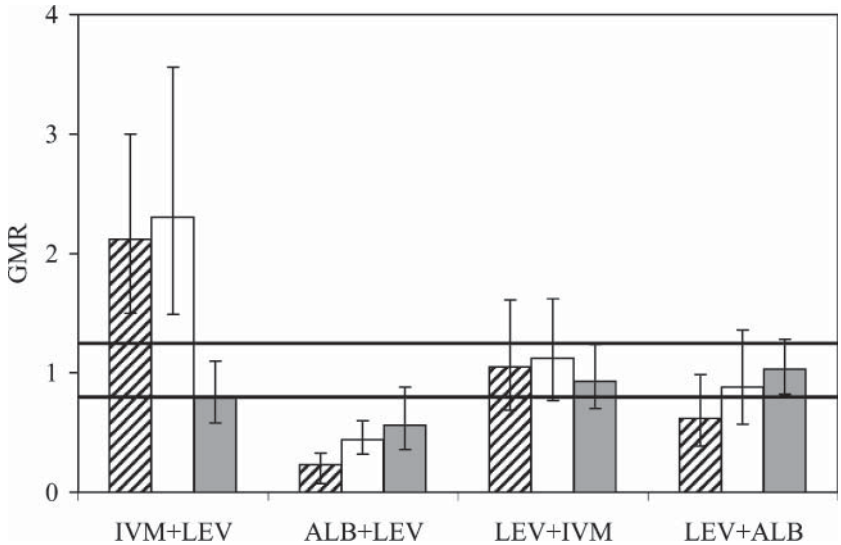

FIG. 4. Geometric mean ratios (GMR) for the pharmacokinetic parameters AUC (▨), C_{max} (□) and T_{max} (■) for ivermectin when given with levamisole:alone (IVM + LEV), albendazole when given with levamisole:alone (ALB + LEV), levamisole when given with ivermectin:alone (LEV + IVM), and levamisole when given with albendazole:alone (LEV + ALB). The horizontal lines indicate the equivalence range (0.8–1.25). Whenever the vertical lines, which indicate 90% confidence intervals, lie entirely outside of the equivalence range, a clinically significant drug–drug interaction is indicated.

TABLE 4. *The effect of treatment with levamisole only (LO), levamisole plus ivermectin (LI) or levamisole plus albendazole (LA) on the viability and reproductive activity of adult* Onchocerca volvulus *collected from nodules 180 days post-treatment*

	Treatment			
Variable	LO	LI	LA	*P*
No. of onchocercomata excised	84	112	82	
NO. AND (%) OF FEMALE WORMS				
Recovered	106	127	101	
Dead or moribund	18 (17.0)	23 (18.1)	9 (8.9)	0.12
Dead and calcified*	8 (7.5)	10 (7.9)	2 (2.0)	0.126
Live	88 (83.0)	104 (81.9)	92 (91.1)	–
NO. AND (%) OF LIVE FEMALE WORMS				
Producing embryos	58 (65.9)	42 (40.4)	64 (69.6)	<0.0001
With degenerate embryos†	22 (37.9)	35 (83.3)	28 (43.8)	<0.0001
Not producing embryos	30 (34.1)	62 (59.6)	28 (30.4)	–
With relict/degenerate embryos‡	5 (16.7)	15 (24.2)	2 (7.1)	0.148
NO. AND (%) OF MALE WORMS				
Recovered	47	57	52	
Dead or moribund	0 (0)	1 (1.8)	2 (3.8)	–
Live	47 (100.0)	56 (98.2)	50 (96.2)	–
No. and (%) of live male worms with normal spermatogenesis	46 (97.9)	53 (94.6)	48 (96.00)	0.702
No. and (%) of nodules with microfilariae in the capsule	46 (54.8)	26 (23.2)	54 (65.8)	<0.0001

*The percentages shown are of the total population of adult female worms.
†The percentages shown are of the live females producing embryos.
‡The percentages shown are of the live females not producing embryos.

TABLE 5. *Changes in the levels of microfilaridermia seen in the year following treatment with levamisole only, levamisole plus ivermectin or levamisole plus albendazole, showing the percentage reductions compared with the day-0 values*

	Treatment arm					
	Levamisole only ($N=22$)		Levamisole–ivermectin ($N=22$)		Levamisole–albendazole ($N=22$)	
Time	GMC and (CI) (microfilariae/4 mg skin)	Reduction (%)	GMC and (CI) (microfilariae/4 mg skin)	Reduction (%)	GMC and (CI) (microfilariae/4 mg skin)	Reduction (%)
Day 0	82.1 (56.90–118.30)	–	71.9 (45.10–114.23)	–	88.5 (66.60–117.50)	–
Day 4	75.0 (51.21–109.73)	8.6	5.0 (2.99–8.07)	93.0	63.3 (44.67–89.46)	28.5
Day 8	56.0 (34.64–90.24)	31.8	1.2 (0.62–2.00)	98.3	76.4 (51.19–113.72)	13.7
Day 30	60.7 (37.19–98.64)	26.1	0.05 (−0.02–0.14)	99.9	86.2 (60.74–122.25)	2.6
Day 180	75.6 (49.69–114.69)	7.9	1.4 (0.63–2.42)	98.1	69.9 (48.64–100.13)	21.0
Day 270	71.6 (45.30–112.81)	12.8	3.7 (1.80–6.79)	94.9	72.7 (49.82–105.94)	17.9
Day 365	60.0 (36.71–97.58)	26.9	4.0 (1.89–7.46)	94.4	67.3 (46.58–97.09)	24.0

GMC, The geometric mean of the sum of densities (in microfilariae/mg) found in the snips from both iliac crests and both calves; CI, 95% confidence interval.

compare the LI values with those in the other two treatment arms. In the LI treatment arm, the counts of mff were already much reduced by day 4 and were even lower on days 8 and 30. Between day 30 and day 270 they appeared to increase slightly but then they appeared to remain unchanged between day 270 and the final check on day 365 (Table 5).

About 30% of all the subjects of Trial 2 each had mff in the anterior chamber of at least one eye; the counts were highly variable and the initial values differed between treatment arms. The means and (ranges) of the counts for both eyes were 1.1 (one to eight) for the LO treatment arm, 3.6 (one to 68) for the LI and 7.7 (one to 95) for the LA. A meaningful assessment of the effect of each treatment was thus limited to a description of the pattern of response within each treatment arm. There was no significant reduction in the ocular parasite counts in the LO or LA treatment arms over the period of observation. The day-180 counts in the LI treatment arm were, however, significantly lower than the pre-treatment counts, although two of the four LI subjects who had ocular mff pre-treatment still had one or eight mff in their eyes on day 365 (data not shown).

DISCUSSION

In both trials, the levamisole alone or in combination with ivermectin or albendazole was well tolerated, although there was an unexpectedly high incidence of itching and rash. The co-administration of levamisole with ivermectin or albendazole resulted in clinically significant drug–drug interactions. Treatment with levamisole alone or levamisole–albendazole led to AE of similar frequency and severity and, in the subjects with onchocerciasis, had minimal effects on the mff and adult worms. The combination of levamisole with ivermectin was not macrofilaricidal, and did not augment the potent effect against skin and intra-uterine mff that has been observed with ivermectin alone (Awadzi *et al.*, 1995*a*, 2003).

The combinations of levamisole with albendazole or ivermectin have never been considered previously for the treatment of filarial infections. Thus, in any development of such drug regimens for human use, their safety and pharmacokinetic interactions must first be explored in healthy volunteers. Only if the results of these first trials are encouraging should the efficacy and tolerability of the regimens, in subjects infected with the target parasite, be investigated. Initially in Trial 1, only two healthy volunteers were exposed to each of the treatments (LO, LA and LI), the size of each successive group investigated increasing by three subjects to a maximum of five subjects/regimen in the final group. The subjects were closely observed in hospital, were visited in their villages after discharge, and were seen in hospital at trial termination on day 30. In Trial 2, only subjects with light infections with *O. volvulus* were initially treated, before the responses to treatment of heavily infected individuals were explored. The drug-related AE recorded in the healthy volunteers investigated in Trial 1 and the relatively high frequencies of the AE in the LO and LA treatment arms of Trial 2 were unexpected. The explanation for these findings is best found by considering some of the features of untreated onchocerciasis, and the pathogenesis of the various AE that occur during the treatment of the disease. The key elements are summarized in Figure 5.

The tissue damage produced by the death of mff is responsible for much of the pathology in onchocerciasis. A competent immune system determines the human host's ability to kill mff and defines the clinical spectrum of a disease that includes: (1) endemic normals or the putatively immune — individuals who have lived in endemic areas for many years, have been exposed to infection, but who are seemingly free of infection; (2) individuals with microfilaridermias associated with generalized or no skin manifestations; and (3) individuals with

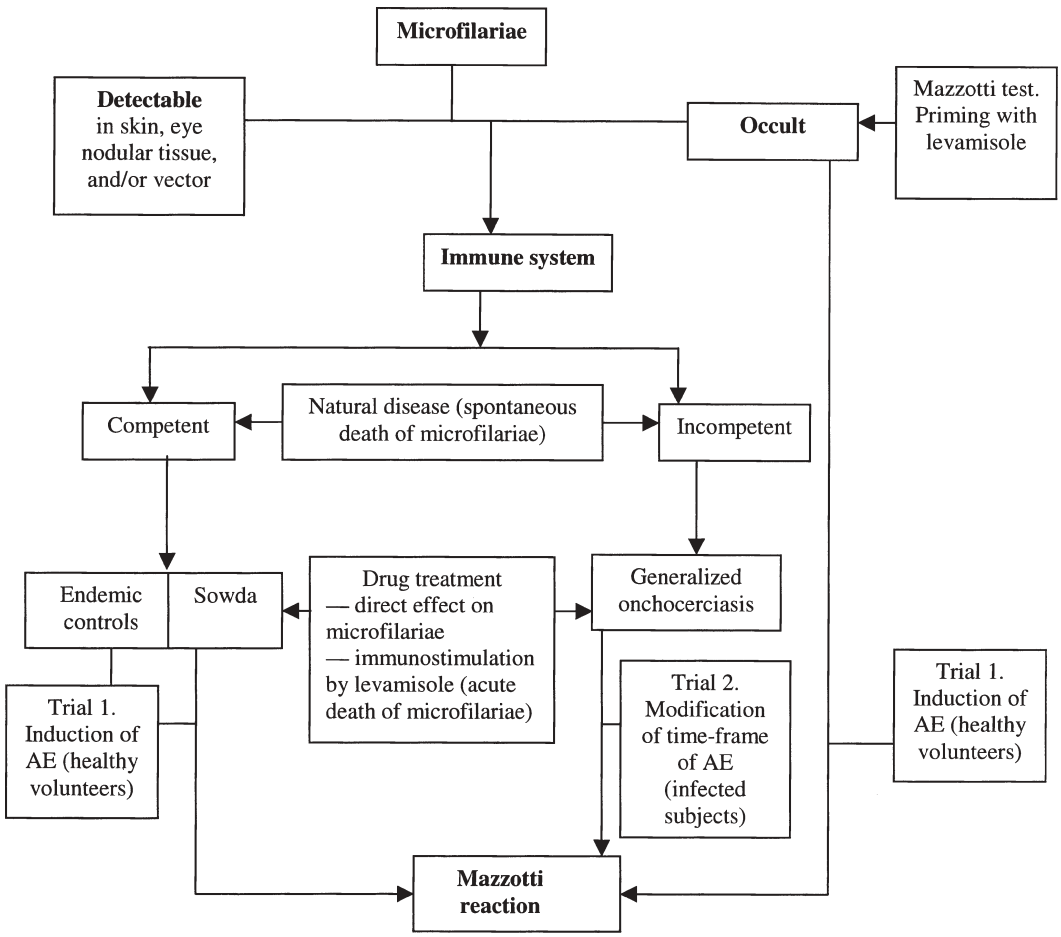

FIG. 5. The interaction of the microfilariae and the immune system in the genesis of the pathology of onchocerciasis, and the influence of levamisole on the Mazzotti reaction in subjects (healthy volunteers and infected individuals) treated with levamisole only, levamisole plus ivermectin, or levamisole plus albendazole. AE, Adverse events.

hyper-reactive onchodermatitis (or Sowda) with severe, asymmetric skin lesions and light microfilaridermias (Ottesen, 1995; Ali *et al.*, 2002). A cellular (lymphocyte) immune hypo-responsiveness to onchocercal antigens is characteristic of those with generalized onchocerciasis; endemic normals have higher cellular immune responses but lower specific antibody responsiveness, whereas those with the hyper-reactive form have the highest titres of specific antibodies (WHO, 1995; Ali *et al.*, 2003). In the present study, the subjects of Trial 1 were equivalent to endemic normals, whereas those of Trial 2 represented subjects with generalized onchocerciasis.

The death of mff also evokes a series of events (symptoms, signs and laboratory events) known collectively as the Mazzotti reaction. In areas where onchocerciasis is endemic, subjects undergo a chronic reaction even in the absence of any intervention. This is the result of the death of mff at the end of their normal life-span, or of the immune hyper-reactivity that is characteristic of the localized form of the disease. The mff that contribute to the Mazzotti reaction may be readily demonstrable in skin-snips or the eyes and nodular tissue of the human residents and in the *Simulium* vector, or may be occult, being undetectable even by sensitive parasitological methods. Their

presence in humans can be inferred, however, from the observation of a Mazzotti reaction in response to diethylcarbamazine treatment (WHO, 1987).

The AE that follow the treatment of *O. volvulus* infections result from the intrinsic (pharmacological) properties of the drug, the death of the mff (Mazzotti reaction), progressive disability from pre-existing lesions, coincidental illness occurring during or after the drug administration, or unknown factors. In the present study, the main factors that are relevant are the intrinsic drug effects, the Mazzotti reaction, and the unknown. The series of drug-related AE described in Trials 1 and 2, taken as a whole, are consistent with those that occur in the Mazzotti reaction (WHO, 1987). As they are not characteristic of the AE associated with any of the three drugs when they are administered to healthy volunteers, they are unlikely to be intrinsic drug effects. The marked difference in the frequency and severity of the AE, between the presumably uninfected volunteers of Trial 1 and the subjects with microfilaridermias investigated in Trial 2, support a relationship with the intensity of infection with *O. volvulus*.

Levamisole acts as an immunostimulant and restores cell-mediated immune mechanisms that have become depressed in peripheral T-lymphocytes, although the drug has only marginal effects in the immunocompetent (Renoux, 1980). The itching and rash seen in six of the 42 healthy volunteers (endemic controls) in Trial 1 probably resulted from the enhancement of the immune response by levamisole, such that these six individuals were capable of killing the mff that were present in them. Although no mff had been found in skin-snips taken from seven sites (right outer canthus, both scapulae, both iliac crests and both calves) on each of the volunteers of Trial 1, the presence of occult mff cannot be excluded. Thus, the interaction of an enhanced immune response with the occult mff present in a few subjects resulted in a Mazzotti reaction. The drug-related AE in Trial 2 were probably the result of the putative effect of levamisole on the immune system, the presence of (demonstrable) mff in the skin, and the potent effect of ivermectin on the mff. The major AE in this trial were itching and rash. For the data analysis, the onset of itching was divided into early ($\leqslant$10 h post-treatment) and late ($>$10 h). Early itching occurred in all eight subjects in the LO treatment arm who reported pruritus, nearly all (eight out of 10) such subjects in the LA treatment arm and in half (nine out of 18) of those in the LI treatment arm. Since early itching is uncharacteristic of ivermectin treatment, and occurred at higher frequencies after LO or LA than after LI, it may represent the priming effects of levamisole on the immune system. Late itching that occurred from 22 h post-treatment was virtually limited to the subjects treated with ivermectin, and is consistent with the expected onset of itching caused by ivermectin alone (Awadzi *et al.*, 1990*a*). As well as the more frequent and more severe itching, ivermectin treatment was associated with arthralgia, other body aches, lymphadenitis, fever and swellings (Table 2) — AE that appear to result from the direct effect of ivermectin on the mff. Thus, in Trial 2, the subjects given LI experienced a 'biphasic' Mazzotti reaction, with early and late phases attributable to the effects of levamisole and ivermectin, respectively. Since levamisole and albendazole have little effect on the mff, the AE in the LO and LA treatment arms would be expected to result predominantly from the putative, immunostimulatory effects of levamisole (as occurred in Trial 2).

The proposed mechanisms of the Mazzotti-type reactions seen in Trial 1 and of the alteration in the time frame of the AE to ivermectin treatment in Trial 2 cannot yet be supported by any immunological findings. Although similar phenomena were not observed in previous studies with levamisole, in a different population (Awadzi *et al.*, 1982, 1990*b*; Taylor *et al.*, 1985), the mechanisms proposed above appear to be

the only plausible explanation for the events that were observed in the present study.

The successful implementation of combination chemotherapy demands that no serious AE occur, including any that are attributable to drug–drug interactions. Pharmacokinetic studies elucidate those drug–drug interactions that may contribute to an improved response to multiple-drug therapy. The present assessment of drug–drug interactions drew on data from a previous investigation in which albendazole and ivermectin had been administered as single agents (Awadzi *et al.*, 2003). The use of these 'historical' data seems justified on several grounds. Firstly, the studies providing the historical data were, like the present study, randomized, double-blind and placebo-controlled. Secondly, the older data were obtained from a group of patients whose demographic characteristics were essentially identical to those of the present subjects. Thirdly, the methods used by Awadzi *et al.* (2003), for sample collection, handling, storage at the trial site and shipment, were the same as those used in the present study. Finally, in the earlier study, the relevant plasma drug concentrations were determined by the same laboratory personnel, using the same analytical procedures, as in the present study.

Taken together, the present results provide no evidence indicating that systemic exposure to levamisole is altered substantially by concomitant administration of either albendazole or ivermectin. Comparison of the pharmacokinetic parameters for albendazole sulphoxide and ivermectin, as determined in the present study, with those obtained previously (Awadzi *et al.*, 2003) indicates that co-administered levamisole elicits a decrease in the 'area under the curve' of albendazole sulphoxide and an increase in the same parameter for ivermectin. The reasons for these effects and their clinical consequences are not immediately apparent. Albendazole and ivermectin are both substrates of cytochrome P450 (CYP) 3A4, the most widely distributed human P450, which is expressed both in the liver and small intestine. There is, therefore, the potential for drug–drug interactions at each of these sites. The information provided by Rawden (1999) indicates that albendazole may be converted to albendazole sulphoxide, the major plasma metabolite in man, by microsomes prepared from human intestinal tissue. Moreover, ivermectin (Kwei *et al.*, 1999), but not albendazole (Merino *et al.*, 2002), is a substrate for p-glycoprotein. This transport protein is expressed in a variety of tissues, and interactions at the cellular level could conceivably occur through direct competition for binding. While conversion of levamisole to a 4-hydroxy metabolite has been demonstrated (Kouassi *et al.*, 1986), the anthelmintic properties of this metabolite and its contribution to the pharmacological profile of levamisole are unknown. Furthermore, there is no information available regarding the site of the metabolism, the enzymes involved, or the probability that levamisole is a substrate for p-glycoprotein. Such information is needed if the LA and LI interactions are to be explained and their clinical importance is to be established.

Levamisole had little effect on the efficacy of ivermectin or albendazole against *O. volvulus*. The increase in the bio-availability of ivermectin seen when levamisole was co-administered was unlikely to alter the efficacy of the ivermectin, since doses much higher than the standard dose used in Trials 1 and 2 are no more effective against the mff and adult worms (Awadzi *et al.*, 1995*b*, 1999; Gardon *et al.*, 2002). The reduction in the bio-availability of albendazole (measured as the sulphoxide) seen when levamisole was co-administered was probably not a factor in the observed lack of efficacy of the albendazole, since a similar dose of albendazole (400 mg) also showed little activity when given as a single agent (Awadzi *et al.*, 2003). These findings, taken together with the results of previous studies with albendazole in which higher doses were used (Awadzi *et al.*, 1991, 1994), indicate that the

minimum dose of albendazole for the treatment of onchocerciasis should be 800 mg. In the earlier study of mebendazole (Awadzi *et al.*, 1982), two doses of levamisole were given 48 h apart and the mebendazole was started at least 24 h after the second dose. At the time the mebendazole treatment was initiated, the plasma concentrations of levamisole would have been too low to affect the kinetics of mebendazole. The augmented efficacy of mebendazole observed was therefore attributed to the immunomodulating properties of levamisole, and formed the basis of the current studies. It is uncertain whether the use of the same dose regimen of levamisole with ivermectin or albendazole would yield more favorable results.

The combination of levamisole with ivermectin or albendazole induced unexpected adverse events, altered the time-frame of reactions to ivermectin, and resulted in significant drug–drug interactions. These findings emphasise the need to study drug combinations cautiously, as in the present studies, prior to their use in large groups of subjects. This requirement is independent of whether or not the drugs are already in use for other indications.

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