Evaluation of suramin, ivermectin and CGP 20376 in a new macrofilaricidal drug screen, Onchocerca ochengi in African cattle

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Abstract

To aid the development of a macrofilaricidal agent for *Onchocerca volvulus*, the African bovine parasite, *O. ochengi*, was evaluated as a drug screen by testing three known filaricidal drugs. Groups of five Zebu cattle, naturally infected with more than 15 palpable *O. ochengi* nodules in the ventral skin, were treated with either suramin (10 mg/kg/day i.v. for 6 days), ivermectin (200 µg/kg, s.c.), CGP 20376 (20 mg/kg orally) or left untreated as controls and examined at intervals up to 137 days post-treatment (d.p.t.). After ivermectin treatment, microfilarial densities in the skin decreased within one week to virtually zero and remained at a very low level. A similar rapid and profound reduction was seen after CGP 20376 treatment, but by 137 d.p.t. microfilarial skin densities were approaching pre-treatment levels. With suramin, skin microfilarial densities fell to very low levels after 12 weeks but rose slightly by 137 d.p.t. Effects on the macrofilariae were assessed by sequential nodulectomies at −3 and 28, 84 and 137 d.p.t.. By 137 d.p.t. embryogenesis was almost completely interrupted in the CGP 20376 and ivermectin treated animals, although not in the suramin treated group, but in all three groups the majority of remaining intrauterine microfilariae were pathologically altered. Degenerating intrauterine microfilariae accumulated in the ivermectin and in the CGP 20376, but not in the suramin treated worms. The motility of male and female worms was not reduced by any treatment except for female worms at 84 d.p.t. with CGP 20376. Viability of the worms as indicated by the MTT-formazan reduction assay was not reduced in any of the treatment groups. This trial has shown the feasibility and potential value of this new model. Effects on micro- and macrofilariae were similar to those reported at the same dosages and times post-treatment for *O. volvulus* in man in the case of ivermectin and suramin whilst CGP 20376 is not yet tested in man.

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Introduction

Control of human onchocerciasis is increasingly based on large-scale distribution of the new microfilaricidal drug ivermectin (Greene, 1992). However, this drug does not kill the adult worm if given in an annual dosage regimen (Duke et al., 1991, Schulz-Key et al., 1992) and although it may substantially reduce transmission, it will not interrupt it (Remme et al., 1989, Trpis et al., 1990). The development of a safe macrofilaricide, effective preferably by a single oral dose, is therefore the major objective of the recently created Macrofil Chemotherapy Project of WHO/OCP and TDR (Ginger, 1991).

At present, there is only one nodule-forming *Onchocerca* species, namely *O. gibsoni* in Australian cattle available for in vivo tests of potential new macrofilaricide compounds (Copeman, 1979). To aid in the development of a macrofilaricidal agent for *O. volvulus*, the African bovine parasite, *O. ochengi*, was therefore evaluated as a tertiary drug screen by testing three known filaricidal drugs – ivermectin, suramin and CGP 20376 – in naturally infected cattle. In particular, advantage was taken of the special features of the *O. ochengi* model, i.e. that multiple nodule infection is common and that it is possible to remove nodules from the same animal before and at varying intervals post-treatment since nodules are intradermal in the ventral skin, with usually one female and, on average, one male worm per nodule (Trees et al., 1992). Apart from this fact, *O. ochengi* is phylogenetically very closely related to *O. volvulus* (Bain, 1982), with which it shares the same vector fly, *Simulium damnosum* s.l. (Omar et al., 1979). Comparison of the mode of action of the same drugs against *O. volvulus* in man and against *O. gibsoni* in Australian cattle would enable estimates to be made of the predictive value of this new drug-screening model.

Materials and methods

Nine herds of 20 to 34 animals each (female cattle only and calves) were selected to examination. They had been kept for all their life near *S. damnosum* s.l. infested rivers at the Ranch Amao near Ngaoundéré, Northern Cameroon where there is a high prevalence of *O. ochengi* infection (Wahl et al., 1994, Trees et al., 1992). In a crush, the animals were examined for the presence of palpable nodules in the udder and inguinal region. Animals with more than five nodules were then cast and

held with ropes while the ventral skin was thoroughly palpated. The position and number of individual nodules was recorded. Twenty female animals, each with more than 15 nodules, were selected and divided into 4 cohorts of 5 animals. To assign cattle to groups, they were ranked by nodule count, then assigned alternately to give similar ranges and means for nodule count for each group (mean 33 nodules per animal, SD ± 19). These groups also had similar ranges and means for age (mean age 5.1 years, SD ± 0.7), weight (325 kg, SD ± 45) and microfilarial density in the ventral skin (see Table **1**). The weight of cattle was estimated from the girth measurement (at the withers) and the following formula: weight (kg) = $m \times c^3$, where m is a coefficient for Zebu type cattle (for cows m = 77, bulls 78, steer 73) and c is the girth in metres. Treatment and observations were made blind. A random code number (1 to 20) was given to each animal and this number was noted on all samples (skin biopsies, nodules and serum samples) gained from the same animal. The assignment of groups to treatments was at random. In the laboratory, the excised nodules were given new random code numbers to avoid the person reading the embryogrammes being aware that a given lot of nodules came from the same animal.

CGP 20376, N-(2-(1,1-dimethylethyl)-5-methoxybenzothiazol-6-yl)-S-(2-carboxyethyl)-dithiocarbamate was provided by Ciba-Geigy as a micronised powder. For each animal, the pre-weighed dose (20 mg/kg) was dissolved in acidified water (5 % acetic acid) with 0.05 % Tween, at a concentration of 0.5 %. The total volume per animal varied between 1160–1520 ml. Animals were dosed by stomach tube with 1 litre acidified water, followed by the drug suspension followed by a further litre of acidified water. Suramin ('Germanin', suramin sodium powder, Bayer, Germany) was made up as a 10 % solution in sterile water for injection immediately before use and administered by slow injection into the jugular vein at 10 mg/kg daily for 6 days. Volumes injected per animal per day varied from 26 to 38 ml. A commercial 1 % solution of ivermectin ('Ivomec', MSD, UK) was injected subcutaneously at 200 µg/kg in a single dose.

Animals were examined clinically daily throughout the treatment period. At frequent intervals, blood samples were withdrawn into evacuated sterile tubes (Vacutainer, Becton Dickinson, Meylan Cedex, France) with anticoagulant (EDTA) for total and differential leucocyte counts done by standard methods, or with anticoagulant for serum.

As an aid to assessing the efficacy of drug administration, serum concentrations of suramin were assayed by high-performance liquid chromatography (Edwards et al., 1985) predose and at 4, 24, 48, 96 and 168 h.

Three superficial skin slivers ('skin biopsies') were taken, from the shaved skin, with a scalpel from the ventral mid-line of the belly, one just posterior to the umbilicus, one mid-way between umbilicus and udder and one closely anterior to the udder. The skin samples were weighed (mean weight 54 mg SD ± 13), and had a mean area of 60 mm^2. The skin biopsies were incubated in 1 ml RPMI supplemented with 200 U/ml penicillin and 200 µl/ml streptomycin in sterile polystyrene tubes at 37 °C. After 24 hours, the microfilariae were identified and counted under a dissecting microscope at 50 times magnification. Then the skin biopsy was fixed in ethanol 70 % for collagenase digestion in order to assess the proportion of remaining microfilariae (Schulz-Key and Karam, 1984).

Nodules (usually 3 per animal per time point) were excised under local anaesthesia from cast cattle held in lateral recumbency with ropes. The nodules were kept individually in small polystyrene tubes in a cool box and examined within 10 hours. After trimming the nodule, the capsule was carefully opened with a small incision using a scalpel. The adult worms could then be squeezed out into 200 µl PBS on a depression slide and the males picked up with forceps. Usually, the worms were recovered with little or no damage. Male worms and the first 5 mm of the female anterior end (total length of female worms is about 10–20 cm) were transferred (after measuring their motility at room temperature) individually into the wells of a microtitre plate with 200 µl RPMI. They were incubated at 37 °C, 1 hour, then their motility was scored again (0 = immobile; 1 = few slow movements; 2 = continuous contortions). Worms were then assessed individually for viability by the MTT/formazan reduction assay (Comley et al. 1989), using an incubation time of 1 h in MTT. The coloured reduction product, formazan, was leached out of worms by 1/2 h incubation in DMSO and assayed at 492 nm in a portable spectrophotometer (CLS 962 microplate photometer, Cambridge Life Science, Cambridge, UK). At day 137 post treatment (d.p.t.), all animals were carefully repalpated for *O. ochengi* nodules in order to monitor any possible change in nodule size, consistency or number between the different treatment groups.

Each female worm, without the anterior end, was finely cut with scissors on a depression slide, washed into a mortar with PBS to a final volume of 1 ml, then crushed by gentle movements of a piston (Schulz-Key, 1988). The piston was rinsed with another 1 ml PBS to give a final volume of 2 ml suspension. After thorough mixing a sample of the embryonic suspension was transferred to a Fuchs-Rosenthal chamber (0.2 mm deep) and a differential count of the various embryonic stages was made. Degenerating or pathologically altered forms were distinguished from normal embryonic stages. The remaining embryonic suspension (~ 1.9 ml) was fixed by adding one drop of 40 % formalin and stored for later re-examination.

A Friedman-type ranked ANOVA was used to test the variation within and between the treatment groups for the data of skin snipping and nodulectomies (Wittkowski, 1988). Effects of treatments on adult worm motility and proportions of female worms without male partner were tested for homogenicity by χ^2 tests, followed by Ryan's procedure for the comparison of single pairs of observation (Sachs, 1990). A meaningful effect at any examination post treatment was considered if $p < 0.05$ for the comparison both between the control group at the same date and the pre-treatment value of the homologous trial group.

Results

Effects of treatment on microfilariae

Microfilarial densities in skin biopsies of individual animals and their group geometric means are shown in Table **1**. Pre-treatment group geometric means were similar, but individual microfilarial loads varied greatly within groups. Control group mean microfilarial density rose, then fell during the course of the experiment but at all times remained within one order of magnitude of pre-treatment levels. After ivermectin treatment, skin microfilarial densities fell to zero and remained at or near zero for the duration of the experiment.

Suramin had a more delayed action, gradually eliminating most microfilariae by day + 84 d.p.t. In four animals the microfilarial density went slightly up again at + 137 d.p.t., indicating that at least some of the worms had survived and resumed microfilarial production. The fifth animal, which also had the highest pretreatment microfilarial density, showed only a delayed and slight reduction. Drug assays from this animal showed this was not due to drug maladministration. Indeed there was little difference of serum blood levels between animals at any one time point (Fig. **1**).

Table 1 *O. ochengi* skin microfilarial load before and after treatment.

Treatment	Arithmetic mean no. of microfilariae/100 mg skin biopsy* and group geometric means d.p.t.									
	−3		**+7**		**+28**		**+84**		**+137**	
Control	72 1638 305 70 60	**173**	190 2036 528 137 266	**376** (66 %)	159 1921 416 278 512	**448** (67 %)	30 1838 105 12 27	**75** (71 %)	16 185 124 53 297	**90**
Suramin	245 1395 49 35 82	**138**	506 1265 119 73 556	**315** (54 %)	11 578 59 34 271	**82** (63 %)	1 968 6 0 0	**6** (87 %)	2 158 38 5 5	**15**
Ivermectin	703 165 54 12 264	**116**	0 0 0 0 0	**0** (0/20)	0 0 0 0 0	**0** (0/9)	0.5 0 0 0 0	**0.1** (5/21)	5 0 0 0 0	**0.4**
CGP 20376	191 436 45 304 279	**201**	87 0 0 0 0	**1.4** (69 %)	0.7 31 0 0 2	**2** (58 %)	8 577 19 15 14	**29** (87 %)	22 1598 51 10 83	**70**

*: Three biopsies taken from the ventral skin of each animal, 5 animals per group. The figures in brackets give the proportion of microfilariae emerged during incubtation as a percentage (or in actual numbers) of the total of microfilariae counted following collagenase-digestion of the skin biopsies.

After CGP 20376 treatment microfilarial densities fell to zero in 4 of 5 animals within 7 days whilst the remaining animal took 28 days to become (almost) free from microfilariae. By 84 days after the treatment all animals showed increasing densities in the skin and 137 d.p.t., 2 of 5 animals had reached or even surpassed pre-treatment levels.

The proportions of microfilariae emerged after 24 h incubation in RPMI in relation to the total number recovered after collagenase digestion are shown in Table **1**. In untreated animals, on average 68 % of the total dermal microfilarial load emerged from the skin biopsy during the 24 hours of incubation in RPMI. This proportion did not change significantly in those animals treated with suramin or CGP 20376, but was significantly reduced following ivermectin treatment.

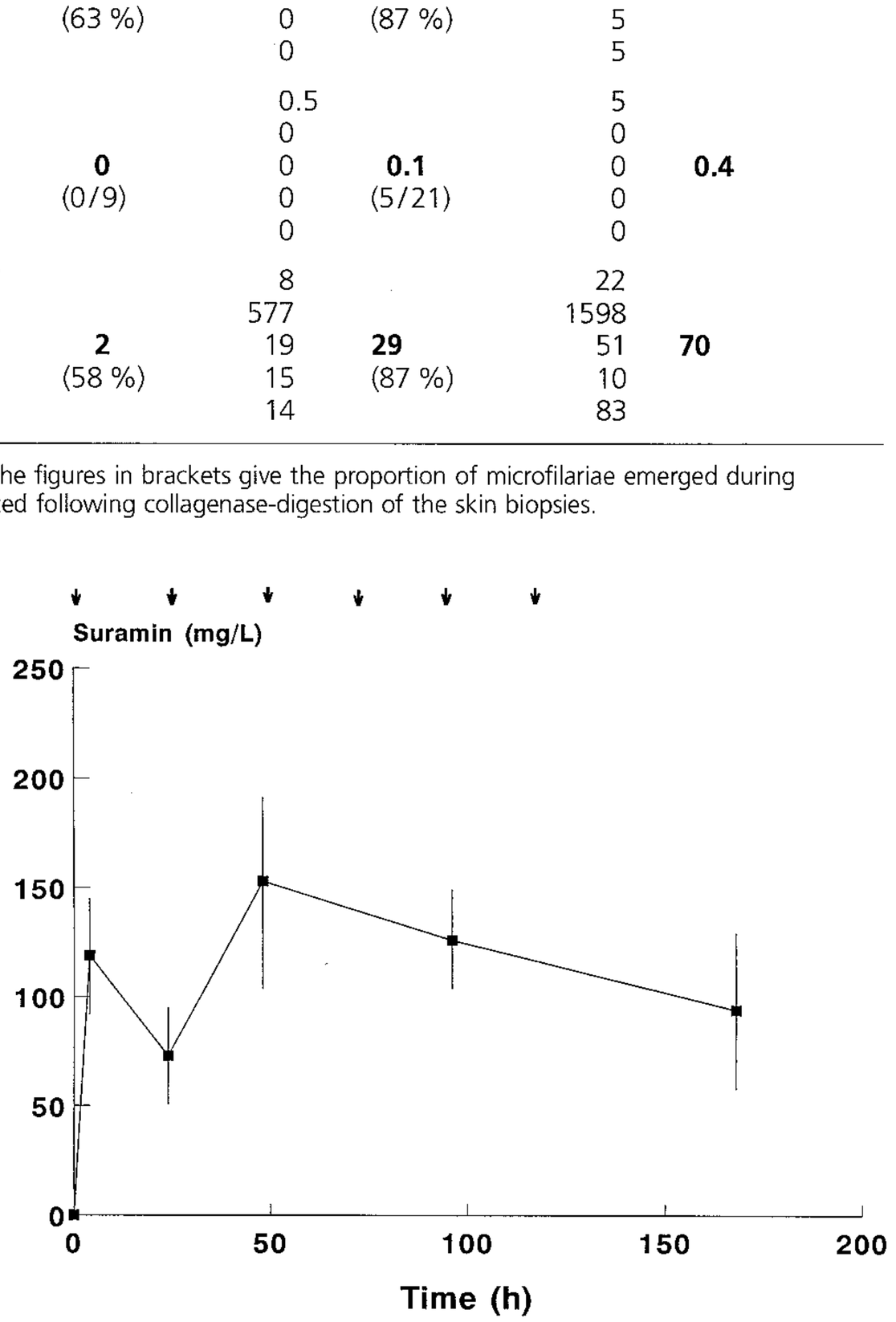

Fig. 1 Concentration (mg/l, $m_a \pm SD$) of suramin in the serum of 5 cattle, treated with 10 mg / kg daily for 6 days.

Effects on macrofilariae and embryogenesis

For each treatment and at each time point an average number of 15.8 female worms (SD ± 2.0, range 11–19) and 14.0 male worms (SD ± 5.5, range 2–23) were examined. In total, 244 nodules were excised, of which 228 contained viable adult *O. ochengi*, 250 female and 223 male worms. One nodule contained a completely calcified and another a very young female. The remaining 14 'nodules' proved to be *Demodex* cysts (7), tick bites (6) and one was a scar.

The mean number of nodules palpated at 137 d.p.t. was the same as the number counted on cattle at the start of the experiment fewer those removed for the control animals (39 nodules/animal). It increased slightly, but statistically insignificant in the suramin and ivermectin treated animals (30 to 34 and 31 to 32 nodules, resp.) and it decreased in the CGP 20376 group from 33 (range 16–62) to 28 (range 21–38) nodules.

None of the drugs apparently killed a significant number of adult male or female worms at the concentrations used within the period of observation (Table **2**). However, there were significantly less male worms in the nodules of CGP 20376 treated animals at day 28 d.p.t. than in the control and in the two other treatment groups ($p = 0.04$). In the same group, 12 of 16 female and 2 of 12 male worms were immotile at room

Table 2 Effects of treatments on *O. ochengi* adult worms: Motility and formazan formation of male and female worms; proportion of female worms without male partner before and at 28, 84 and 137 d.p.t.

Treatment group	d.p.t.	Control	Suramin	CGP 20376	Ivermectin
		Female worms without male partner in nodule			
without male/total (percent)	− 3	2/16 = 13 %	5/15 = 33 %	8/16 = 50 %	8/15 = 53 %
	+ 28	4/19 = 21 %	6/14 = 43 %	7/10 = 70 %*	3/13 = 23 %
	+ 84	4/15 = 27 %	8/18 = 44 %	11/17 = 65 %	5/16 = 31 %
	+137	5/16 = 31 %	7/15 = 47 %	7/16 = 44 %	5/19 = 26 %
		Motility at 37 °C (no. worms examined/immobile/m_a of score)			
females	− 3	11/1/1.6	10/1/1.7	10/0/1.6	13/0/1.8
		23/0/2.0	7/0/2.0	3/0/2.0	10/0/2.0
males	+ 28	17/0/1.8	12/1/1.5	12/0/1.8	13/1/1.8
		18/0/2.0	10/1/1.8	8/0/1.9	13/1/1.8
	+ 84	14/1/1.5	18/1/1.8	16/4/1.0	14/0/1.6
		21/0/2.0	21/2#/1.8	12/0/1.9	17/0/2.0
	+137	16/0/1.7	14/0/1.9	15/1/1.5	19/0/1.8
		16/0/2.0	13/0/2.0	12/0/1.9	12/0/2.0
		Formazan formation (m_a of optic densities measured)			
female/male worms	− 3**	0.12/0.40	0.17/0.47	0.22/0.39	0.21/0.43
	+ 28**	0.20/0.38	0.23/0.46	0.17/0.40	0.22/0.53
	+ 84**	0.15/0.36	0.15/0.36	0.13/0.31	0.14/0.36
	+137***	0.04/0.25	0.06/0.25	0.07/0.18	0.06/0.26

*: $p < 0.05$; #: 1 male calcified at mid-body; **: optic density measured at 492 nm; ***: at 520 nm; m_a: arithmetic mean

temperature at + 84 d.p.t. (motility data at room temperature not shown in Table **2**). The motility of worms after 1 hr incubation at 37 °C remained at control levels (group means between 1.5–2.0) for both male and female worms at all time points for all treatments, except for a transient reduction ($p = 0.1$) at 84 d.p.t. in the CGP 20376 group when mean motility of female worms was 1.0. Similarly, the MTT-formazan assay of male and female worms showed no significant difference from control worms. The mean absorbence of control male worms at 492 nm on days −3, +28 and 84 d.p.t. ranged from 0.33–0.40 whilst that of the anterior of females ranged from 0.12–0.20.

Very obvious effects of treatments however were seen in the embryograms (Table **3**). CGP 20376 stopped the production of new embryonic stages, whilst those already beyond the early morula stage continued their development finally leading to obviously damaged, vacuolised microfilariae, which accumulated in the uteri. Thus, at days 28 and 84 d.p.t., the number of apparently undamaged intra-uterine microfilariae was only slightly lower than in the untreated control, whereas the number of damaged uterine microfilariae steadily increased until most of the intra-uterine microfilariae appeared damaged at 137 d.p.t. Ivermectin at first had little effect on the developing embryonic stages which, at least until 28 d.p.t., developed to stretched microfilariae, but these were not released. Instead they accumulated in the uteri, where they finally disintegrated. Thereafter the delivery of new stages ceased and almost all intra-uterine developing stages appeared degenerate. Suramin slightly reduced the production of young embryonic stages and had a toxic effect on stretched microfilariae, which however did not accumulate in the uteri.

Clinical observations

There were no ill effects observed attributable to systemic side effects of the drug treatments or their anti-filarial activity. A number of the cattle gave birth to healthy calves at full term at various times after treatment.

Eosinophil counts were variable both within animals between time points and between animals within groups. In both treatment groups in which profound and acute anti-microfilarial effects occurred (i.e. ivermectin and CGP 20376), there was a slight eosinopenia at 24 h post treatment. This eosinopenia was transient and eosinophil levels were rapidly restored to pre-treatment levels.

Discussion

This first trial has shown the feasibility of using the cattle parasite *O. ochengi* for drug screening and has demonstrated some of the inherent merits of this model system. It has been possible to show sequential changes over a period of time post-treatment in adult worms. The wounds caused by the nodulectomies were superficial and healed quickly. The time needed to examine one animal, i.e. taking three skin biopsies and removing three nodules was less than 20 minutes. It was possible to carry out three different assays for anti-macrofilarial activity (motility, MTT/formazan reduction and embryogenesis) within 10 h of sampling cattle. Using multiple criteria for drug effects enables a thorough interpretation of treatment efficacy.

Table 3 Effects of treatments on *O. ochengi* embryograms: Total number and percentage of pathological or degenerating stages per female worm examined (oozytes) or per gravid female worm only (developing stages and microfilariae).

	Control				Suramin				CGP 20376				Ivermectin			
Day post treatment	−3	+28	+84	+137	−3	+28	+84	+137	−3	+28	+84	+137	−3	+28	+84	+137
	Oocytes															
nodules/fem. ex.[1]	14/16	11/19	15/15	14/16	14/15	12/14	18/18	15/15	16/16	19/19	16/17	14/16	15/15	11/13	16/16	16/19
10^3 oocytes/fem.	220	106	147	154	194	49	137	100	176	129	197	154	188	118	237	147
% path. degen.	47	49	60	51	28	62	60	54	38	56	61	55	24	50	62	52
	Developing embryonic stages															
gravid fem. ex.[2]	10	8	10	11	5	8	7	7	6	3	6	7	8	8	9	10
10^3stages/fem.[2]	50	39	79	67	59	38	82	47	48	58	35	4*	83	81	38	9*
% path. degen.	11	3	22*	4	13	34*	40*	9	8	4	40*	10	7	12	75*	92*
	Intrauterine microfilariae															
10^3 mff/fem.[2]	14	9	21	20	12	22	20	24	13	36	37	26	20	64*	75*	75*
% path. degen.	12	15	40*	8	10	34	55	63*	16	47	66*	85*	14	37*	94*	96*

[1]: embryograms examined: number of nodules (total gravid and non-gravid females) examined; some nodules contained more than one females, which could not be separated and were examined together
[2]: gravid female worms only (i.e. females containing more than 10 000 embryonic stages) *: $p < 0.05$, see text.

By cutting off the head end of female worms, which is the most active part of the otherwise rather immotile worms and which continues moving for several hours, it was possible to use the MTT/formazan viability assay without compromising embryogenesis data. Comley et al. (1989) showed that it was possible to utilise parts of worms for the MTT/formazan assay and that the anterior end of female filarial worms was probably the most metabolically active part. Considering the difference in size between *Onchocerca* spp. and the fact that our assay was read at 492 nm compared with 510 nm, the mean absorbances observed with *O. ochengi* were similar to those reported for *O. gibsoni*, *O. gutturosa* and *O. volvulus* (Comley et al., 1989).

Nodules were of similar size (5 mm ± SD 0.8) and contained in almost all cases one live female worm together with an average number of one male worm per nodule. Multiple infections with two or more female worms were very rare (as previously found, Trees et al., 1992), but occasionally one 'nodule' excised consisted of two or more separate capsules, each containing one female worm. It was possible to mechanically extract adult worms from the nodule. This is preferable to collagenase-digestion, being quicker, cheaper and less liable to compromise the viability of the worm.

The effects of ivermectin on embryogenesis and uterine microfilariae were very similar to those described for *O. volvulus* (Schulz-Key, 1990, Awadzi et al., 1986, Albiez et al., 1988, Duke et al., 1991, Chavasse et al., 1992) in which an accumulation and subsequent degeneration of mature microfilariae in the uteri was observed. From experience in the treatment of human onchocerciasis, it is unlikely that a single dose of ivermectin, at the (low) concentration used in this trial will permanently reduce the microfilarial density in the skin due to killing of the worms. With *O. volvulus* the microfilarial density in the skin remained low for up to 6 months and reached only 10% of its pre-treatment level after 12 months and 25–30% two years later (Schulz-Key et al., 1992). In our experiment with *O. ochengi*, the total dermal load of microfilariae in the skin was reduced to less than 1% of pretreatment levels and 4 of 5 animals showed no emerging microfilariae at 137 d.p.t. As in human onchocerciasis, some microfilariae were immobilized in the skin (Mössinger et al., 1988), but the majority rapidly disappeared from the skin.

The persisting low microfilarial density in the skin is presumably due to sustained factors preventing the release of uterine microfilariae. From the increasing number of stretched microfilariae (normal and degenerating, 20,000 per fertile female worm before, 64,000 at 28 days, 75,000 at 84 days and 75,000 at 137 days after the treatment) it may be concluded that embryogenesis continued for some weeks post-treatment but that all microfilariae produced remained in the uteri. Very few young embryonic stages were seen at 137 d.p.t. It has been suggested that blockage of the lower uterus by degenerating microfilariae prevents sperm moving up the uterus to fertilise oocytes (Albiez et al., 1988) and recently, evidence based on sperm content in seminal receptacles in *O. volvulus* after repeated ivermectin treatment, has confirmed that suggestion (Chavasse et al., 1993). Ivermectin-facilitated immunity (Schulz-Key et al., 1992) might also be involved in this long-lasting effect. Evidence for a naturally occurring acquired resistance against microfilariae of *O. ochengi* has already been described in old cattle (Trees et al., 1992). *O. ochengi* infected cattle would therefore provide a suitable model for investigating such drug-induced resistance phenomena.

Suramin had a delayed action against microfilariae in the skin and therefore the steady and slow decrease in the skin might also partially reflect the normal rate of clearance of microfilariae from the skin. In 4 of the 5 cattle, skin microfilariae had virtually disappeared at 84 d.p.t. In human onchocerciasis, at the dosage used in our experiment (60 mg/kg) most *O. volvulus* worms survived for up to 7 months, but died later (Schulz-Key et al., 1985, Duke, 1991). Thus, the failure to kill *O. ochengi* in this experiment by 137 d.p.t. is not surprising.

With CGP 20376, the results from the examination of adult worms, which indicated that embryogenesis was almost completely interrupted at 137 d.p.t., appear to contradict the observed increase in skin microfilarial densities after 4 months. One explanation would be that microfilariae in the skin were all killed by the treatment, whilst the release of viable uterine microfilariae continued for some time, at least until day 84 d.p.t. This would be supported by the observation that although the proportion of degenerating microfilariae increased in the uteri, the number of apparently viable ones was similar to untreated controls until 84 d.p.t.

Although microfilarial densities are very variable between individuals, there is evidence of a decrease in microfilarial density in the control group towards the end of the experiment. This is unlikely to be due to the removal of nodules since, if the average life span of a microfilaria is assumed to be around 100 to 200 days, then the first two nodulectomies (6 nodules = 18 % of palpable nodules. The number of palpable nodules corresponds to only about 50 % of the total nodule load) would entail at most a reduction of the same order of magnitude until day 137 d.p.t. Thus it appears more likely to be due to a variation of skin microfilarial densities related to seasonal transmission (also described for *O. volvulus* in man, Fuglsang et al., 1976 and *O. gutturosa* and *O. lienalis* in European cattle, Eichler, 1973, Zahner and Schulz-Key, 1990). During the hot dry season, when the trial started, transmission of *O. ochengi* is highest and it is lowest during the rainy season, i.e. at 137 d.p.t. (Wahl, 1991).

No statistically significant changes in eosinophil counts were observed associated with treatment but the considerable individual variation in eosinophil counts may have masked a slight and transitory eosinopenia in the ivermectin and CGP 20376 groups. An acute, transitory eosinopenia has been observed in *O. volvulus* infected humans after DEC treatment (Ackerman et al., 1990), followed by a more sustained eosinophilia, which we did not observe. There was no evidence of pruritis or a Mazzotti reaction in treated animals. A comparison of cellular events following microfilarial treatment in *O. ochengi* cattle compared to *O. volvulus* infected man might aid an understanding of the pathogenesis of the Mazzotti reaction.

In comparing the action of the same drugs against *O. volvulus* in man and *O. gibsoni* in cattle, the effects on the microfilariae of the three *Onchocerca* species seem very comparable. However, adult worms of both *O. ochengi* and *O. volvulus* appear to be less affected by suramin than *O. gibsoni* and also CGP 20376 is apparently more active against *O. gibsoni* than against *O. ochengi* (Striebel, 1988, and pers. comm.). The possibility that drugs may less easily penetrate the intradermal nodule of *O. ochengi* than that of other *Onchocerca* spp. seems unlikely in view of the profound effects on embryogenesis demonstrated with ivermectin, CGP 20376 and to a lesser extent suramin. Moreover, we have subsequently investigated the penetration of suramin into the *O. ochengi* nodule by using radio-labelled drug, and have demonstrated substantial levels within the nodule (Trees, Wahl, Achu-Kwi and Renz, in preparation).

One present hypothesis therefore is that the reproductive biology of *O. gibsoni*, *O. ochengi* and *O. volvulus* might explain the different mode of action against adults of these three *Onchocerca* species: *O. gibsoni* female worms are short lived (2 years) and produce constantly high numbers of microfilariae (Kläger et al., 1987, Vankan and Copeman, 1988), whilst both *O. ochengi* and *O. volvulus* live longer (about 10 years) and have intermittent periods of non-reproductivity (30 % of the female worms show empty uteri for *O. volvulus*, Schulz-Key, 1988 and 52 % of *O. ochengi*, cf. Table **3**), during which they may be less active and less vulnerable to drug action (uptake). Since the microfilariae of all three species are skin-dwelling and constantly active, the activity of drugs against them would be expected to be similar. Another important consideration is that drug activity is assessed in the *O. gibsoni* system largely on histopathological evaluation of fixed material. Thus direct comparisons with the *O. ochengi* results, in which dynamic evaluations are made on fresh preparations, should be made with caution. What is apparent is that the effects on *O. ochengi* of the drugs examined at the doses used are not widely at variance with their known effects against *O. volvulus*. Further research is required to demonstrate that *O. ochengi* adults can be killed by drug regimens which are known to kill *O. volvulus* adult worms.

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References

Ackerman, S. J., G. M. Kephart, H. I. Francis, K. Awadzi, G. J. Gleich, E. A. Ottesen: Eosinophil degranulation – an immunologic determinant in the pathogenesis of the Mazzotti reaction in human onchocerciasis. J. Immunol. 144 (1990) 3961–3969

Albiez, E. J., G. Walter, A. Kaiser, P. Ranque, H. S. Newland, A. T. White, B. M. Greene, H. R. Taylor, D. W. Büttner: Histological examination of onchocercomata after therapy with ivermectin. Trop. Med. Parasitol. 39 (1988) 93–99

Awadzi, K., K. Y. Dadzie, H. Schulz-Key, H. M. Gilles, A. J. Fulford, M. A. Aziz: The chemotherapy of onchocerciasis XI. A double blind comparative study of ivermectin and diethylcarbamazine and placebo in human onchocerciasis in northern Ghana. Ann. Trop. Med. Parasit. 80 (1986) 433–442

Bain, O.: Le genre *Onchocerca*: Hypothèses sur son évolution et clé dichotomique des espèces. Ann. Parasitol. (Paris) 56 (1981) 503–526

Chavasse, D. C., R. J. Post, P. A. Lemoh, J. A. G. Whitworth: The effect of repeated doses of ivermectin on adult female *Onchocerca volvulus* in Sierra Leone. Trop. Med. Parasitol. 43 (1992) 256–262

Chavasse, D. C., R. J. Post, J. B. Davies, J. A. G. Whitworth: Absence of sperm from the seminal receptacle of female *Onchocerca volvulus* following multiple doses of ivermectin. Trop. Med. Parasitol. 44 (1993) 155–158

Comley, J. C. W., S. Townson, M. J. Rees, A. Dobinson: The further application of MTT-formazan colorimetry to studies on filarial worm viability. Trop. Med. Parasitol. 40 (1989) 311–316

Copeman, D. B.: An evaluation of the bovine *Onchocerca gibsoni, Onchocerca gutturosa* model as tertiary screen for drugs against *Onchocerca volvulus* in man. Trop. Med. Parasitol. 30 (1979) 469–474

Duke, B. O. L.: Suramin and the time it takes to kill *Onchocerca volvulus*. Trop. Med. Parasitol. 42 (1991) 346–350

Duke, B. O. L., G. Zea-Flores, B. Munoz: The embryogenesis of *Onchocerca volvulus* over the first year after a single dose of ivermectin. Trop. Med. Parasitol. 42 (1991) 175–180

Edwards, G., C. L. Rodick, S. A. Ward, K. Awadzi, M. L'E. Orme, A. M. Breckenridge: Determination of suramin in plasma by high performance liquid chromatography. J. Chromatography 343 (1985) 224–228

Eichler, D. A.: Studies on *Onchocerca gutturosa* (Newmann 1910) and its development in *Simulium ornatum* (Meigen) 3. Factors affecting the development of the parasite in its vector. J. Helminthol. 47 (1973) 73–88

Fuglsang, H., J. Anderson, T. F. De C. Marshall, S. Ayonge, C. Fisiy: Seasonal variation in the concentration of *Onchocerca volvulus* microfilariae in the skin? Trop. Med. Parasitol. 27 (1976) 365–369

Ginger, C.: Filarial worms: Targets for drugs. Parasitology Today 7 (1991) 262–264

Greene, B. M.: Modern medicine versus an ancient scourge: progress toward control of onchocerciasis. J. Inf. Dis. 166 (1992) 15–21

Kläger, S., D. Vankan, R. Höflacher, H. Schulz-Key: Untersuchungen zur Reproduktivität von *Onchocerca gibsoni*, einer Filarie des Rindes. Mitt. Österreich. Ges. Tropenmed. Parasitol. 9 (1987) 195–210

Mössinger, J., H. Schulz-Key, K. Dietz: Emergence of *Onchocerca volvulus* microfilariae from skin snips before and after treatment of patients with ivermectin. Trop. Med. Parasitol. 39 (1988) 313–316

Omar, M.S., A. M. Denke, J. N. Raybould: The development of *Onchocerca ochengi* (Nematoda: Filarioidea) to the infective stage in *Simulium damnosum* s.l. with a note on the histochemical staining of the parasite. Trop. Med. Parasitol. 30 (1979) 157–162

Remme, J., R. H. Baker, G. De Sole, K. Y. Dadzie, J. F. Walsh, M. A. Adams, F. S. Alley, H. S. Avissey: A community trial of ivermectin in the onchocerciasis focus of Asubende, Ghana. I. Effect on the microfilarial reservoir and the transmission of *Onchocerca volvulus*. Trop. Med. Parasitol. 40 (1989) 367–374

Sachs, L.: Statistische Methoden 2, Springer Verlag Berlin (1990), 274 pg.

Schulz-Key, H.: The collagenase technique: how to isolate and examine adult *Onchocerca volvulus* for the evaluation of drug effects. Trop. Med. Parasitol. 39 (1988) 423–440

Schulz-Key, H.: Observations on the reproductive biology of *Onchocerca volvulus*. Acta Leiden. 59 (1990) 27–43

Schulz-Key, H., M. Karam: Quantitative assessment of microfilariae and adults of *Onchocerca volvulus* in ethanol-fixed biopsies and nodules. Trans. roy. Soc. Trop. Med. Hyg. 78 (1984) 157–159

Schulz-Key, H., M. Karam, A. Prost: Suramin in the treatment of onchocerciasis: the efficacy of low doses on the parasite in an area with vector control. Trop. Med. Parasitol. 36 (1985) 244–248

Schulz-Key, H., P. T. Soboslay, W. H. Hoffmann: Ivermectin-facilitated immunity. Parasitology Today 8 (1992) 152–153

Striebel, H. P.: Proposed form to evaluate some histological aspects of macrofilarial morphology, its age dependent alterations and drug related changes in nodules of *Onchocerca volvulus* and *O. gibsoni*. Trop. Med. Parasitol. 39 (1988) 367–389

Trees, A. J., G. Wahl, S. Kläger, A. Renz: Age-related differences in parasitosis may indicate acquired immunity against microfilariae in cattle naturally infected with *Onchocerca ochengi*. Parasitology 104 (1992) 247–252

Trpis, M., J. E. Childs, D. J. Fryauff, B. M. Greene, D. N. Williams, B. E. Munoz, M. C. Pacque, H. R. Taylor: Effect of mass treatment of a human population with ivermectin on transmission of *Onchocerca volvulus* by *Simulium yahense* in Liberia, West Africa. Am. J. Trop. Med. Hyg. 42 (1990) 148–156

Vankan, D. M., D. B. Copeman: Reproduction in female *Onchocerca gibsoni*. Trop. Med. Parasitol. 39 (1988) 469–471

Wahl, G.: Die *Onchocerca* Arten der Rinder in Nord-Kamerun und ihre Bedeutung für die Epidemiologie der menschlichen Onchozerkose. Dissertation, Univ. of Tübingen (1991), 103 pg.

Wahl, G., M. D. Achu-Kwi, D. Mbah, O. Dawa, A. Renz: Bovine onchocercosis in North-Cameroon. Vet. Parasit. 52 (1994) 297–311.

Wittkowsky, K. M.: Friedman-type statistics and consistent multiple comparisons for unbalanced designs. J. Amer. Statist. Assoc. 83 (1988) 1363–1170

Zahner, S., H. Schulz-Key: Rinderonchozerkose in Süddeutschland. Verteilung der Mikrofilarien in ihrem Wirtshabitat. Mitt. Österreich. Ges. Tropenmed. Parasitol. 12 (1990) 87–94

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