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Ivermectin distribution in the plasma and tissues of patients infected with *Onchocerca volvulus*

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Abstract *Objective*: To determine the distribution of ivermectin in plasma and tissues of onchocerciasis patients following a single oral dose of 150 μg kg^{-1}.
Setting: Medical Department at Soba University Hospital, Khartoum.
Patients: Twenty five patients and fourteen healthy volunteers.
Methods: Serial blood samples were obtained from both groups. Tissue samples were removed from various patients as full thickness skin punch biopsies or during nodulectomy. Ivermectin concentration was determined by radioimmunoassay.
Results: The plasma pharmacokinetic variables for patients were; maximum plasma concentration 52.0 ng ml^{-1}; time to achieve maximum concentration, 5.2 h.; elimination half life, 35.0 h; and the area under the plasma concentration curve versus time, 2852 ng · h ml^{-1}. In healthy volunteers, the plasma ivermectin distribution was similar to that in patients, and both groups showed a tendency for a second rise in plasma concentration of the drug suggestive of enterohepatic recirculation. Ivermectin was detected in tissues obtained from patients. Fat showed the highest and most persistent levels, whilst values for skin, nodular tissues, and worms were comparable. Subcutaneous fascia contained the lowest concentrations.
Conclusion: Infection with *O. volvulus* does not affect the pharmacokinetics of ivermectin, and filarial infected tissues and parasites themselves do take up the drug. There may be prolonged retention of ivermectin because of depot formation in fat tissue.

Key words Ivermectin, Onchocerciasis; pharmacokinetics, tissue concentration, enterohepatic

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Introduction

Ivermectin is a macrocyclic lactone with potent antiparasitic activity, widely used in veterinary medicine (Campbell et al. 1983; Campbell 1985). Ivermectin pharmacokinetics and tissue distribution, have been extensively studied in animals (Chiu and Lu 1989).

Following successful clinical trials of ivermectin in human onchocerciasis (Aziz et al. 1982; Taylor and Greene 1989), it has become the drug of choice for symptomatic treatment, with a potential for strategic use in controlling the transmission of *Onchocerca volvulus* infection (Greene 1992). However, despite its wide popularity, knowledge of the pharmacokinetics of the drug in man is rudimentary. There are only a few published reports about oral bioavailability in healthy volunteers (Edwards and Breckenridge 1988; Goa et al. 1991; Edwards et al. 1988), or in patients (Okonkwo et al. 1993). Ivermectin concentration is determined by HPLC (Downing 1989; Chiou et al. 1987). This method although specific, generally requires large samples when dealing with tissue samples, extensive instrumentation, and sample preparation is time consuming.

The development of a radioimmunoassay (RIA) for ivermectin by Marschke (1989), employing antibodies with high avidity and specificity for the parent compound, has made it possible to measure the drug in very small volumes of plasma and tissue. The procedure is sensitive down to 1 ng ml^{-1}, the antibodies show only 19–23% cross reactivity with avermectin, and the amount required for 50% displacement of the label (ED50) is 3 ng ml^{-1}. This development has made possible the study of ivermectin distribution in patients, especially in organs with a high filarial density, and the

results should shed light on some important issues in drug/parasite/disease interactions.

In this study the distribution of ivermectin in onchocerciasis patients was determined in samples of plasma, skin, subcutaneous fascia, fat, onchocercal nodules and worms, collected at various time points after a single oral dose of 150 μg kg^{-1} in patients and in volunteers.

Materials and methods

Subjects and sampling procedure

Twenty five patients with onchocerciasis and microfilaria-positive skin snip biopsies participated in the study. Microfilarial loads ranged from 1.2 to 164 mf mg^{-1}. All were admitted to hospital. Fourteen healthy volunteers were recruited for the study of ivermectin plasma pharmacokinetics. Oral and written consent were obtained from each subject. Ethical clearance for the study was obtained from the University of Khartoum, Faculty of Medicine Research Committee. All subjects were non smokers, did not consume alcohol, and were not on medication. The females were non-pregnant and not lactating. Each subject received a single oral dose of ivermectin 150 μg kg^{-1} body weight after an overnight fast. Subjects were allowed only water for 2 h following treatment. Heparinised venous blood samples 5 ml were taken from an indwelling cannula in the antecubital fossa at 0, 1, 2, 3, 4, 6, 8, 12, 24, and 48 h post treatment no blood samples were obtained at 48 h from the volunteers. The plasma was immediately separated and stored at −20 °C until the assay.

Tissue samples were obtained from the patients at various times taking care not to subject any individual to more than one surgical intervention. Tissues were obtained either as full thickness 3 mm skin punch biopsies or during surgical nodulectomy. Samples were immediately frozen in liquid nitrogen and kept until assayed.

Analysis of plasma samples

Ivermectin concentrations in plasma samples were determined without extraction by the RIA technique of Marschke (1989). One hundred μl from each sample or standard (chromatographically purified ivermectin), tritiated ivermectin (Amersham, 22.4 Ci/mmol^{-1}, 6000 dpm per assay-tube), and antiserum (diluted 1/1000) were added to 12 × 75 mm glass tube and mixed. The tube was kept at 4 °C for 16 h. Stirred charcoal suspension, 0.7 ml, (0.75% Sigma activated C-4386) was added to all tubes except the Total Count tube to which was added 0.7 ml RIA buffer. The tube contents were mixed immediately and at 7.5 and 15 min. Following centrifugation, the supernatant decanted into 10 ml scintillation cocktail. Tritium was determined with a liquid scintillation counter.

Ivermectin extraction from tissues

The tissue samples were dissected into their different components to obtain skin, fascia, subcutaneous fat and nodules. Worm fragments were obtained from onchocercal nodules by the collagenase technique (Schultz-Key et al. 1980). Individual tissue samples were weighed and assayed in duplicate. The tissue fragments were repeatedly homogenised in acetone using an electrical homogeniser with vortex mixing. After centrifugation, the acetone layer was transferred to a new tube and evaporated. Acetonitrile was added to the residue, which was repeatedly extracted into hexane. The acetonitrile layer was collected in 75 × 15 mm glass tubes and evaporated to dryness. The residue was dissolved in 100 μl of RIA buffer. The amount of ivermectin extracted from each tissue sample was then determined by RIA.

To assess the efficacy of extraction, lean beef muscle and fat samples were spiked with known amounts of ivermectin. About an 85% extraction rate was obtained.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using GraphPAD GPIP Inplot and the Medusa software package. The following were determined; maximum plasma concentration (C_{max}); the time to the maximum concentration of the drug (t_{max}); elimination half life ($t_{1/2}$); and the area under the plasma concentration curve time (AUC).

Statistical analysis

The unpaired t-test was applied. $P < 0.05$ was considered significant.

Results

Plasma ivermectin concentrations

No significant differences were observed between pharmacokinetic parameters in patients with onchocerciasis and healthy volunteers (Table 1). Ivermectin appeared in plasma within 1 h after the oral dose (5.7 to 38.8 ng ml^{-1}), and was detected for up to 48 h (10.8–62.6 ng ml^{-1}). Some of the individual plasma concentration profiles (6 patients and 5 volunteers) showed a second rise in plasma concentration following an initial decrease. The secondary peak mostly occurred between 6 and 12 h after the dose. The curves of the mean plasma concentrations of the patients and the volunteers showed that the elimination phase was similar and slow, and that it exhibits linear decay (Fig. 1).

Tissue ivermectin concentrations

Ivermectin concentrations in different tissues of patients with onchocerciasis are shown in Table 2. The drug was detected in all tissue sampled. Fat showed the highest concentrations. Values for the skin, nodules, and worms from the same patient were comparable. The lowest concentrations were consistently seen in subcutaneous fasciae.

Table 1 Ivermectin pharmacokinetics mean (SD) in 14 healthy volunteers, and 14 patients infected with *O. volvulus* following oral administration of 150 μg·kg

	C_{max} (ng ml^{-1})	t_{max} (h)	$t_{1/2}$ (h)	AUC (μg·h ml^{-1})
Healthy subjects	54.4 (12.2)	4.9 (1.5)	36.6 (10.2)	3.18 (1.39)
Patients	52.0 (12.0)	5.2 (1.9)	35.0 (9.2)	2.850 (0.841)

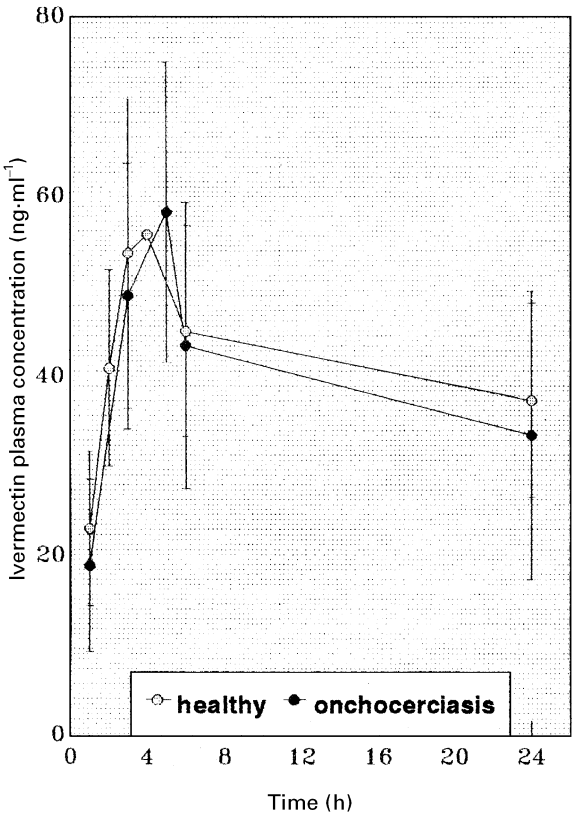

Fig. 1 Plasma Ivermectin concentrations [Mean (SEM)] in healthy volunteers ($n = 14$) and onchocerciasis patients ($n = 14$) after 150 µg/kg single oral dose

Table 2 Ivermectin concentration in tissues (ng g^{-1} of tissue) of 10 patients with onchocerciasis, after a single oral dose of 150 µg kg^{-1} *NA* sample not available; *NB* repetition of time points represents analysis of tissues from different patients.

Time (h)	Skin	Fascia	Fat	Nodule	Worm
4[a]	90.9	NA	141	62.4	NA
6	70.5	31.8	NA	70.8	79.4
24[a]	NA	26.2	NA	31.6	NA
30	71.7	38.2	NA	54.7	NA
48[a]	66.6	NA	NA	101.5	NA
72	NA	42.5	NA	56.4	NA
72	64.9	NA	NA	NA	44.2
72	41.4	18.5	NA	37.1	59.5
4 Days	NA	58.9	117.6	NA	NA
5 Days	15.6	NA	94.1	NA	NA

[a] Corresponding plasma values for these tissue samples were 46, 28.8 and 24 ng ml^{-1}, respectively

Ivermectin administration to healthy volunteers did not cause any post-treatment reactions. All the patient's skin snips obtained on D7 were negative for microfilariae.

Discussion

Our results show that infection with *O. volvulus* does not affect the distribution of ivermectin in plasma of patients compared to healthy volunteers. In both groups there was a common tendency for a secondary peak to appear, suggestive of enterohepatic circulation. Accumulation of the parent drug in fat probably contributes to prolonged retention of ivermectin in the body.

The ivermectin plasma half-life in healthy volunteers was reported to be 12, 22, and 28 h (Fink and Porras 1989; Edwards 1987; Edwards and Breckenridge 1988), and 56 h in onchocercal patients (Okonkwo et al. 1993). In comparison our results were 36.6, and 35 h, respectively. The AUC we obtained was higher than the reported values of 885 (389) and 1545.3 (190.5) ng·h ml^{-1}, (Edwards 1987; Edwards and Breckenridge 1988; Okonkwo et al. 1993). The difference in the systemic availability of ivermectin can be attributed to several factors. In previous reports it was not stated if subjects had taken food during the 2 hours immediately after dosing and we now know that food intake can result in a significant reduction in the amount of ivermectin absorbed (submitted). Ivermectin consist of a mixture of ≥ 80% dihydroavermectin B_{1a}(H_2B_{1a}) and ≤ 20% dihydroavermectin B_{1b}(H_2B_{1b}). The HPLC method used measured only the H_2B_{1a} fraction of the compound. Details of the specific recovery rates were not given and values as low as 60% by extraction from plasma have generally been accepted (Downing 1989). The sensitivity of the RIA we used may have been superior, since the antibody is expected to react with the whole compound and no extraction procedure was applied during analysis of plasma samples. However, we are aware of the fact that cross reactivity of the antibody with ivermectin metabolites could be an added factor. The sensitivity of the RIA was demonstrated by its ability to show secondary peaks similar to those observed in a study of ivermectin disposition in four healthy volunteers given the tritium-labelled drug (Fink and Porras, 1989).

Ivermectin is mainly excreted in bile (Fink and Porras 1989) and is undetectable in urine (Okonkwo et al. 1993). The excretion of ivermectin in bile was expected since it has a high molecular weight and is very lipid soluble (Fisher and Morzik 1989). Hence, enterohepatic circulation of the drug is to be expected. Similar secondary peaks are frequently seen with compounds that undergo hepatic recycling (Miller 1984; Terhaag and Hermann 1986).

Ivermectin depletion appears very slow in most tissues. The pattern of differential distribution of ivermectin concentrations obtained in our human tissue samples was comparable to that seen in animals (Chiu and Lu 1989). The high concentration of ivermectin in fat is a function of the lipid solubility of the drug, and fat acts as a reservoir for ivermectin. This could explain previous observations that ivermectin was detected in human milk for 12 days following a single oral dose (Chiou et al. 1987). Ivermectin concentrations in areas of high filarial density, the nodule, worm and skin were comparable. This supports our previous observation

that, *O. volvulus* is accessible to blood-borne agents (Mahmoud et al. 1991).

The sustained reduction of *O. volvulus* microfilariae in skin tissues has been explained by the effect of the drug on the gravid uterus of the female worm (Albiez et al. 1988). The sustainability of the effect may also be attributable in part to prolonged retention of the drug in the body. However, even allowing for prolonged persistence of the drug, the relation between ivermectin pharmacokinetics and its antiparasitic effect is still far from being understood. Since ivermectin does not directly affect target organisms (microfilariae) *invitro*, nor is there evidence of bioconversion *invivo* to metabolites with direct activity (Soboslay et al. 1987). The likelihood is that suitable host responses act in concert with ivermectin to bring about the antiparasitic effects (Baraka et al. 1995). Defining these effector mechanisms awaits further studies.

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