

*Liver and biliary***Penetration of albendazole sulphoxide into hydatid cysts**

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**SUMMARY** The penetration of albendazole sulphoxide, the principal metabolite of albendazole into hydatid cysts (*E granulosus*) was measured by means of *in vitro* animal and clinical studies. The drug freely diffuses across the parasitic membranes. Cyst/serum concentrations of 22% were achieved in patients, longer pre-operative therapy produced higher concentrations.

The medical treatment of hydatid disease is a recent and fast developing area. The first drug which was shown to be active was mebendazole<sup>1</sup> but it is probably of limited clinical value.<sup>2,3</sup> Mebendazole is an extremely insoluble drug and the serum and cyst concentrations that can be achieved are of the order of 100 and 1 µg/l respectively.<sup>4,6</sup> Concentrations of the hydroxymetabolite of mebendazole are higher<sup>6</sup> but this is thought to be inactive (personal communication). We have reported encouraging clinical and animal<sup>7-9</sup> results using albendazole—another benzimidazole carbamate. The postulated reason for this is that albendazole achieves much higher concentrations of one of its principal metabolites albendazole sulphoxide<sup>10</sup> which is believed to be the active agent.<sup>11</sup> The aim of this study was to investigate the penetration of albendazole sulphoxide into hydatid cysts *in vitro*, in an animal model and in patients undergoing elective operations. We believe that these data are essential, together with the results of *in vitro* therapy studies<sup>11</sup> in order that the optimal dosage and length of therapy may be designed.

**Methods****CYSTS***In vitro* cyst entry

Three types of cyst were studied—human daughter cysts, small (1 mm) cyst masses from gerbil intraperitoneal infections, and single larger gerbil cysts

(Fig. 1). All these cysts were maintained in tissue culture medium at 4°C and used within one week. They were incubated at 37°C in culture medium (RPMI 1640 with 25% fetal calf serum, 0.42% D-glucose, 0.45% yeast extract) with a known concentration (500, 1250, or 2000 µg/l) of albendazole sulphoxide for four, eight, or 25 hours.

The fluid within the cysts was then removed by aspiration and the concentration of albendazole sulphoxide assayed by HPLC.

*In vivo* ovine cyst entry

In order to study the time course of drug entry into live cysts *in vivo*, two sheep with radiographic evidence of active pulmonary hydatid disease<sup>2</sup> were subjected to thoracotomy under general anaesthesia. In the first sheep the cysts were found to be dead on examination of the cyst contents, but in the second sheep the 10 cm pulmonary cyst was found and live protoscolices were identified on microscopy of aspirated cyst contents. A specially designed catheter/flange device was fixed to the cyst (Fig. 2). A 4 cm wide disc with a central hole and luer lock was cemented to the lung surface using cyanoacrylate adhesive, after the surface of the lung had first been carefully dried and degreased with acetone. When the adhesive had dried an iv catheter with a central needle was thrust through the central hole, adhesive and host tissue until the cyst was entered and the catheter was locked onto the luer hub. An extension tube allowed repeated sampling through this catheter. A single 60 ml dose of 2% albendazole (40 mg/kg) (Valbazen drench SK & F) was given directly into the stomach and samples of 2 ml cyst fluid and 5 ml serum taken at intervals for five

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Fig. 1 Gerbil peritoneal cysts alongside 10p coin, used *in vitro* entry studies.

hours. Aspirated cyst fluid was replaced by stored cyst fluid from other animals to maintain cyst tension. The concentration of albendazole sulphoxide was measured by HPLC.

#### *In vivo* studies in human cysts

Two groups of patients were studied:-

(a) Seven patients undergoing elective surgery for hydatid cyst in Athens (BG) were treated with albendazole 10 mg/kg for 36 hours before the operation (three doses of 5 mg/kg were given at 12 hour intervals, the last being approximately four hours before operation). During the operation at least 5 ml cyst fluid was taken from the cyst before injection of a scolicalid agent. A sample of blood was taken simultaneously and both stored at  $-20^{\circ}\text{C}$  and transported to Nottingham for assay. (b) The second group of 12 patients were from the United Kingdom and nine of these had been treated for four weeks or more before operation with albendazole 10 mg/kg/day in divided dosage. The remaining three patients had been treated for one to five days before cyst/serum sampling.

#### STANDARDS AND REAGENTS

Albendazole sulphoxide (Fig. 3) was dissolved in absolute ethanol, other reagents used in the extraction and assay procedure were acetonitrile, water and sodium carbonate (anhydrous) (BDH Chemicals Ltd, Poole, Dorset), chloroform AR and diethyl ether AR (May and Baker, Dagenham), hydrochloric acid 1.0 M and orthophosphoric acid 88% (Fisons Plc, Loughborough), and sodium hydroxide 5.0 M.

#### SERUM AND CYST FLUID ASSAY

The extraction followed the method described by Brandimarte *et al.*<sup>3</sup> One millilitre of serum or 2 ml cyst fluid samples were placed in glass stoppered test tubes and extracted twice with 5 ml chloroform for 60 seconds. The two chloroform phases from each sample were combined and 9 ml transferred to a clean tube and the solvent removed by evaporation under a stream of air. Each sample residue was redissolved in 5 ml 1.0 M solution hydrochloric acid, and adjusted to pH 7-8 with 400  $\mu\text{l}$  5.0 M solution sodium hydroxide and solid sodium carbonate. The

## Penetration of albendazole sulphoxide into hydatid cysts

neutralised samples were then washed twice with 5 ml diethyl ether for 30 seconds, and these fractions discarded. The aqueous fraction remaining was then extracted twice more with chloroform for 60 seconds, the two extracts being combined and 9 ml of this evaporated to dryness under a stream of air. Each sample residue was resuspended in 200  $\mu$ l absolute ethanol immediately before injection of 20  $\mu$ l aliquots into the HPLC apparatus.

### APPARATUS

Analyses were carried out using an SF 400 spectro-flow isocratic pump equipped with a spectroflow 757 variable wave length absorbance detector (Kratos Analytical Instruments, Urmston, Manchester) and a Bryans 28000 flat bed chart recorder. The stationary phase was octadecyl silica gel RP-18, 5  $\mu$ m (hypersil ODS 2), and the mobile phase was 60% aqueous acetonitrile adjusted to pH 3.5 with orthophosphoric acid.

### OPERATING CONDITIONS

The operating conditions were similar to those used by Bogan and Marriner.<sup>14</sup> A flow rate of 1.5 ml/min and a detector wavelength of 292 nm were used at ambient temperature throughout. The recorder setting was 10 mV full scale deflection.

### QUANTIFICATION OF ALBENDAZOLE SULPHOXIDE

Injections of known concentrations of 500 or 1000  $\mu$ g/l standard solutions of sulphoxide were carried out before, during and after injection of sample extracts, and the known peak heights of standard concentrations used to calculate the concentration of samples. In order to determine the percentage of sulphoxide recovered by the extraction procedure, known amounts of standard were added to serum and cyst fluid, and extracted in the same manner as unknown samples. Two or three standards were included with each batch of samples.

## Results

### CHROMATOGRAPHY

When serum or cyst free of added standards was extracted in the same manner as samples no extraneous peaks were found which might interfere with the analysis of albendazole sulphoxide (Fig. 2). Repetitive ( $n=3$ ) injections of an extract of human serum, which had a concentration of 200  $\mu$ g/l sulphoxide, showed a variation of 7.6% in the measured height of the sulphoxide peak. A similar test using cyst fluid (200  $\mu$ g/l) showed a variation of 2.7% ( $n=3$ ). The limit of detection for albendazole sulphoxide was 10  $\mu$ g/l. Concentrations of 100, 200, and 300  $\mu$ g/l sulphoxide in cyst fluid samples had a

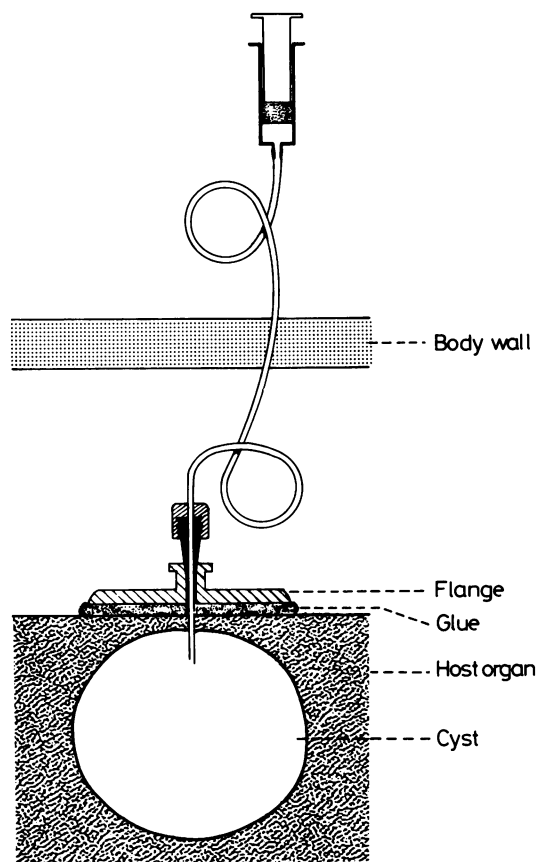


Fig. 2 Cannula/flange device used in *in vivo* study.

mean recovery of 74.4% (SD 11.6%,  $n=44$ ), and in serum samples mean recovery was 67.6% (SD 9.3%,  $n=36$ ).

### IN VITRO CYST ENTRY

The concentrations of albendazole sulphoxide detected within human daughter cysts and gerbil peritoneal cysts incubated *in vitro* are given in Table 1. If the dilution effect of adding the cysts to the

Table 1 *In vitro* cyst entry

	Cyst Diameter (mm)	Incubation (h)	ALBSX concn. in medium ( $\mu$ g/l)	ALBSX concn. in cyst fluid ( $\mu$ g/l)
Human daughter cyst	9	4	1250	1378
	8	8	1250	1693
Gerbil peritoneal cyst (masses)	1	8	500	286
	1	25	500	471
Gerbil peritoneal cysts (single)	20	25	2000	867
	16	25	2000	1342

Table 2 *In vitro* ovine cyst entry

Time (h)	ALBSX concentration $\mu\text{g/l}$	
	Serum	Cyst
0.5	0	0
1	0	0
2	204	0
3	333	0
4	288	0
5	270	0

medium is allowed for, these results constitute equilibration in five of the six experiments and this occurred within four hours in the shortest experiment.

## IN VIVO OVINE CYST ENTRY

Whilst albendazole sulphoxide was detected in the serum of sheep after two hours none was detected within the cyst fluid even at five hours (Table 2).

Table 3 *Cyst and serum concentrations of albendazole sulphoxide in Greek patients treated for 24 hours*

	Patient		Weight (kg)	Cyst		ALBSX concentration		
	Age	Sex		Site	Size (cm)	Serum ( $\mu\text{g/l}$ )	Cyst ( $\mu\text{g/l}$ )	Cyst fluid appearance
HB	52	F	62	Liver	8	246	0*	Turbid
AK	60	F	63	Liver	30	412	82	Turbid
AL	50	M	65	Liver	10	244	26	Clear, DC
AS	72	F	75	Ant abd wall	5	304	14	Clear, DC
OP	42	F	53	Liver	4	214	85	Turbid
AB	30	F	62	Spleen	15	545	69	Clear
DK		M	75	Liver	15	303	32	Clear, DC
					Mean	324	44	Serum/cyst =13%
					SD	117	34	
					Median	303	69	
					Range	(214-545)	(0-85)	

\*=Calcified cyst.

DC=Daughter cyst present.

Table 4 *Cyst and serum concentrations of albendazole sulphoxide in patients from the UK*

	Patient		Cyst		ALBSX concentration		Duration of pre-op treatment (weeks)	Cyst fluid appearance
	Sex	Weight (kg)	Site	Size (cm)	Serum ( $\mu\text{g/l}$ )	Cyst ( $\mu\text{g/l}$ )		
FM	F	59	Liver	20	2350	240	4	Live
EC	F	45	Liver	15	670	100	1	Clear
CE	F	60	Liver	10	240	80	0-15	Clear, DC
PW	F	56	Liver	20	168	40	12	Bile, DC
MS	F	-	Liver	15	1209	111	8	-
CM*	F	-	Liver	10	567	161	0-15	Not E. gran
EP	M	75	Liver	10	518	198	12	Bile, DC
WP	M	-	Liver	15	59	72	8	-
CMcD	F	60	Bone	10	1482	349	4	Turbid, DC
BK	F	55	Liver	20	996	132	8	Turbid, DC
MG	F	62	Liver	15	146	357	4	Turbid, DC
JC	M	70	Ant abd Wall	10	254	52	8	Clear
				Mean	721	157		Serum/cyst =22%
				SD	683	108		
				Median	543	122		
				Range	(59-2350)	(40-357)		

\*Simple cyst, not hydatid.

DC=Daughter cysts present.

## IN VIVO CLINICAL STUDIES

No albendazole (parent compound) was detected in the cyst fluid but the principal metabolite (albendazole sulphoxide) was consistently detected. Results for Greek and British patients are given in Tables 3 and 4 and these differ considerably. Median serum concentrations in patients from the UK were higher 543 µg/l (59–2350) than those of Greek patients 303 µg/l (214–545) though this did not achieve statistical significance. The cyst concentrations were even more different: the median for the Greek patients was 69 µg/l (0–85) compared with 122 µg/l (40–357) in patients from the UK. The cyst concentrations of sulphoxide were 22% of the serum value in British patients and 13% in Greek patients. We found no albendazole sulphoxide in the one heavily calcified cyst in this series. There did not appear to be any relationship between cyst size, site, or viability and cyst concentration (one of the highest concentrations measured (FM) was in a patient in whom live protoscolecocytes were found within the cyst).

Patients treated for only 36 hours before operation are compared with those who were treated for a longer time (usually one month) in Table 5. Both serum and cyst concentrations were higher in the group of patients treated for one month before operation, a median serum concentration of 833 µg/l (59–2350) and cyst fluid concentration of 121 µg/l (53–349) was achieved compared to 303 (214–567) and 69 (0–161) µg/l in the serum and cyst fluid of the patients treated for only 36 hours.

Table 5 Concentration of albendazole sulphoxide in serum and cyst fluid of patients given short or long courses of albendazole before operation

	Short (36 hours)		Long (approx 1 month)	
	Serum	Cyst fluid	Serum	Cyst
	240	80	2350	240
			670	100
	567	161	168	40
	246	0	1209	111
	412	82	518	198
	244	26	59	72
	304	14	1482	349
	214	85	996	132
	545	69	146	357
	303	32	254	52
Median	303	69	833	121
range	214–567	0–161	59–2350	53–349
Mean	341.6	61	785.2	165
(SD) SE	(134) 45	(49.3) 16.4	(733) 231	(116) 36.6

## Discussion

The measurement of albendazole sulphoxide by HPLC<sup>13, 14</sup> has proved a reliable and feasible technique. The *in vitro* cyst entry experiments which showed relatively rapid equilibration of the drug can perhaps be criticised because the viability of these cysts could be suspect. It is very likely, however, that they were alive and were maintained in conditions which are routinely used for *in vitro* culture of protoscolecocytes in our laboratory. The metabolic rate of the parasite in the *in vitro* culture system may be entirely different from that *in vivo*. The *in vivo* sheep entry experiment showed no drug entry at five hours whilst this occurred earlier than this in the *in vitro* experiment. Clearly this experiment needs to be repeated and extended but the results do perhaps indicate that drug penetration of the ectocyst (not present or minimal in the cysts used in the *in vitro* drug studies) may be a more important barrier to drug entry than the laminated or germinal layer of the parasite itself.

*In vivo* human studies have shown the presence of albendazole sulphoxide in the cyst fluid, and have shown higher concentrations in patients treated for longer than 24 hours. These data must be compared with the published results for mebendazole where in animals cyst concentrations of 5–10% of serum levels have been reported.<sup>15</sup> *In vitro* studies<sup>16</sup> have suggested that C<sub>14</sub> labelled mebendazole diffuses readily through the laminated and germinal layer as in our study. Four authors have measured intracyst concentrations of mebendazole.<sup>4–13</sup> Three authors found cyst concentrations of around 10% of serum levels, one found cyst concentrations of nearly equal to those of the serum, and it is likely that blood or bile contaminated the cyst fluid in the two patients in this report.<sup>13</sup> One other group have reported cyst fluid concentration of albendazole in 10 patients<sup>17</sup> and these varied between 60 and 4284 µg/l median 172, it is likely that the patient with the highest concentrations had serum concentration of the cyst fluid at the time of operation.

Until the relative efficacy of albendazole, mebendazole, and their metabolites are known we shall not be able to make any direct comparison but in patients treated with albendazole 10 mg/kg for one month before operation the mean cyst concentration was 165 µg/l which was approximately one fifth of the serum concentration. The serum concentrations of albendazole sulphoxide achieved by albendazole 10 mg/kg are considerably greater than those achieved by mebendazole 50 mg/kg. Both serum and cyst fluid concentration of albendazole sulphoxide were higher in the 'long' treatment group and thus a finite length of time must also be allowed for this



equilibration process when designing the length of any therapeutic regimen.

There is no evidence that live cysts are able to exclude this drug, as in one UK (FM) and two Greek patients (AB, DK) who had live protoscolecies present, no trend to lower concentrations was seen.

Together with a knowledge of the *in vitro* activity of albendazole sulphoxide<sup>11</sup> these data should allow the logical planning of the dosage of albendazole. A dose of 10 mg/kg/day is capable of achieving (in most patients) cyst fluid concentrations in excess of 100 µg/l which is at the lower end of effective concentrations in *in vitro* culture.<sup>11</sup>

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