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Tissue levels of albendazole after *in vivo* intestinal and gastric absorption in rats*

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Introduction

Hydatidosis or hydatid cyst disease is a parasitic disease which affects an important number of domestic animals and man.

A group of drugs having a broad spectrum of activity against human and animal gastrointestinal nematodes are the benzimidazole derivatives, such as, mebendazole and albendazole. The antihelminthic action of benzimidazole derivatives is caused by the inhibition of glycogen uptake or by the inhibition of reductase fumarate enzyme in the parasite [1, 2].

Their beneficial effect in patients affected by hydatidic cysts has been confirmed by numerous authors [3–6].

The mucous surface in the gastrointestinal tract behaves as a lipid barrier for the absorption of active substances so that absorption depends on their lipid solubility and their percentage ionisation [7, 8]. This study has determined the influence of albendazole concentration and intestinal absorption time has on tissue levels in rats, compared with those obtained after gastric absorption of albendazole solutions.

Experimental

Wistar rats (250 ± 30 g body weight) were used throughout the experiments. The benzimidazole derivative methyl 5-propyliptic-1-H-benzimidazol-2,11-carbonate ($C_{12}H_{15}N_3O_2S$) was supplied by Smith, Kline and French, S.A.E. Its structure and its two main metabolites [9] are shown in Fig. 1.

Two albendazole concentrations, 0.5 and 1.0 mg ml^{-1}, dissolved in dimethylsulfoxide (DMSO), were used in the experiments. This solvent has been considered to be less abrasive than acetic acid. Two types of *in vivo* absorption were developed: intestinal loop perfusion with recirculation [10] and gastric absorption without recirculation [11, 12]. Briefly, the methodology applied for each case was: (a). intestinal absorption was

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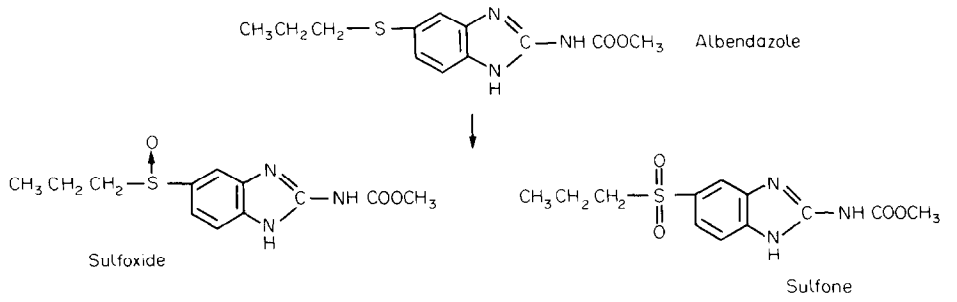

Figure 1

performed by means of a cannula introduced in the bowel just behind the outlet of the bile duct and another, 10 cm distally. These two cannulae were connected to a reservoir (20 ml volume) and a continuous flow of 2 ml min^{-1} through the intestine provided by a perfusion pump. Bile duct was cannulated and bile was accumulated throughout the perfusion time.

(b). Gastric absorption was developed by introducing albendazole solutions through a cannula inserted at the pyloric level of the intestine towards the stomach. The cardiac end of the oesophagus was previously ligated. Bile was collected as in intestinal absorption.

Both intestinal and gastric absorption experiments were maintained for 1 or 2 h. At 15 min intervals, samples of albendazole solutions were taken from the reservoir or inside the stomach, to evaluate the apparent absorption.

Albendazole extraction from tissues was carried out when the experimental time had finished. Blood (through cardiac puncture) and several organs (liver, kidneys, bladder and intestine) were obtained. Blood was centrifuged for 30 min at 3000 rpm to obtain the plasma which was used for further analysis. Organ samples were weighed and homogenised in a double volume of 0.05 M phosphate buffer pH = 7.4. The homogenate was used for albendazole analysis. The intestine and the stomach were rinsed with 0.154 M NaCl and then dried on filter paper, prior to homogenisation.

Albendazole extraction was performed as follows: 500 μl of 2N NaOH and 20 ml of methylene chloride were added to 1 ml of plasma, homogenate supernatant (except for bladder) or bile and vigorously mixed for 1 min. The mixture was then centrifuged at 4500 rpm for 20 min at 4°C and organic fractions were filtered through 0.45 μm mesh filters (Millipore), dried under nitrogen atmosphere at 45°C and finally resuspended in 100 μl of DMSO for HPLC analysis.

For bladder analysis 200 μl of homogenate supernatant were mixed with 1 ml of methanol. After filtering as above, it was directly analysed by HPLC.

Under these conditions, at *ca* 5×10^{-2} mg ml^{-1} albendazole recovery was always higher than 80%. Albendazole levels were measured, from tissue extracts or from intestinal or gastric absorption solutions, by reversed-phase HPLC [13] using a 5-μm Novapack C18 column (Waters). The mobile phase was acetonitrile–triethylamine–water, (60:15:35%, v/v/v) and phosphoric acid was used to obtain a final pH = 3.0. The flow rate was 0.3 ml min^{-1} giving a retention volume of 1.0 ml.

The chromatograph used a Rheodyne injector with a 20 μl loop and a variable wavelength detector (Model 730 SLC. Kontron) adjusted to 292 nm and a sensitivity of 0.004 a.u.f.s. The recorder was set to 0.25 cm min^{-1}.

Albendazole concentrations follow the Beer's law between 1×10^{-3} and 1.25×10^{-1} mg ml^{-1}. All samples were suitably diluted to be in this range and two replicates for each sample were applied. Differences between replicates were lower than 1%. Albendazole detection limit in tissues was 2.5 μg albendazole per ml homogenate.

A $2 \times 2 \times 2 \times 6 \times 2$ class of multiple analysis of variance [14] was performed to establish differences between perfusion type (gastric or intestinal), perfusion time (60 or 120 min), albendazole concentration in the perfusion fluid (0.5 or 1.0 mg ml^{-1}), tissues analysed (absorptive wall, liver, kidneys, urine bladder, bile and blood) and differences between replicates.

Results and Discussion

The results obtained are shown in Figs 2 and 3. Statistical analysis of the main factors involved in the study indicates the existence of significant differences ($P < 0.001$) between gastric and intestinal absorption (Factor I), between the two perfusion times used (Factor II), and in the albendazole tissue levels (Factor IV).

No differences were found between the two concentrations used (Factor III), or between sample replicates (Table 1).

A further study of the interactions between the main factors cited above indicates: (a). interactions between Factors I and IV: albendazole and/or metabolites retained in the absorption wall were significantly higher when the intestinal perfusion was used.

Hepatic and renal albendazole levels were not statistically different, although the levels found in the liver following intestinal absorption were slightly higher.

The difference in albendazole levels in the bladder following the two types of perfusion were statistically significant and these differences were the highest found between tissues. In this way, differences in bile and blood levels were also found, with blood differences more important than those found in bile. Bile and blood levels were higher

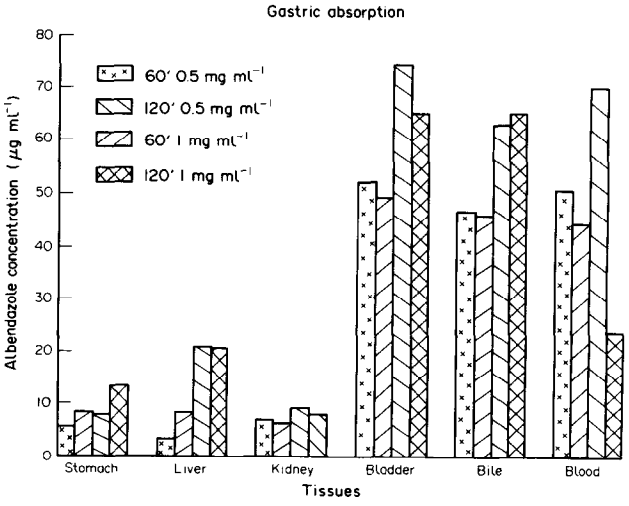

Figure 2
Albendazole tissue levels following gastric absorption of 0.5 and 1.0 mg ml^{-1} for 60 and 120 min. Ordinates are albendazole and/or metabolite concentrations found in tissue homogenates performed as indicated in Materials and Methods.

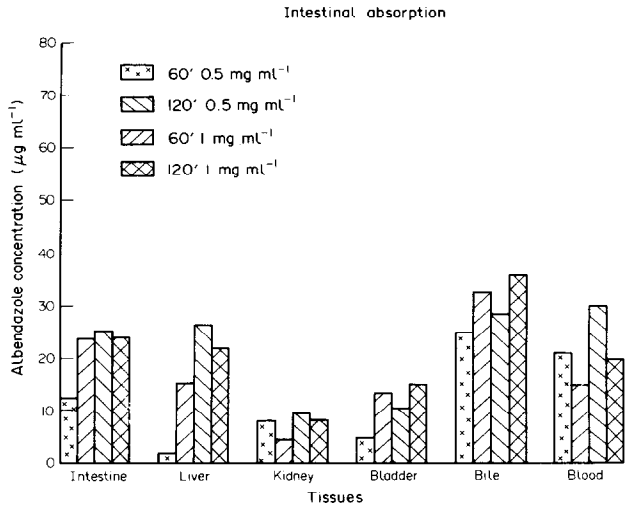

Figure 3
Albendazole tissue levels following intestinal absorption of 0.5 and 1.0 mg ml^{-1} for 60 and 120 min. Ordinates are albendazole and/or metabolite concentrations found in tissue homogenates performed as indicated in Materials and Methods.

Table 1
Analysis of variance for albendazole

Source of variation	Sum of squares	d.f.	Mean square	*F*-ratio	Significance level
Main effects	22778.718	9	2530.9686	79.657	0.0000
Factor V	19.711	1	19.7109	0.620	0.4424
Factor IV	16438.673	5	3287.7346	103.475	0.0000
Factor III	31.694	1	31.6940	0.998	0.3326
Factor II	1518.610	1	1518.6095	47.795	0.0000
Factor I	4770.030	1	4770.0301	150.127	0.0000
2-Factor interactions	13361.403	26	513.9001	16.174	0.0000
Factor V × Factor IV	19.570	5	3.9141	0.123	0.9867
Factor V × Factor III	1.127	1	1.1267	0.135	0.8533
Factor IV × Factor III	1376.521	5	275.3042	8.665	0.0000
Factor V × Factor II	3.398	1	3.3975	0.107	0.7483
Factor IV × Factor II	538.929	5	107.7857	3.392	0.0092
Factor III × Factor II	277.712	1	277.7121	8.740	0.0044
Factor V × Factor I	8.108	1	8.1084	0.255	0.6207
Factor IV × Factor I	10821.709	5	2164.3417	68.118	0.0000
Factor III × Factor I	263.609	1	263.6088	8.297	0.0055
Factor II × Factor I	50.721	1	50.7213	1.596	0.2113
Residual	1906.4013	60	31.773355		
Total (corr.)	38046.522	95			

0, Missing values have been excluded.

following gastric absorption than intestinal absorption; (b). Other interactions between main factors. Significant differences between perfusion time (60 and 120 min) were found mainly for intestinal absorption.

The relationship between the concentrations and perfusion time showed significant differences for the 0.5 mg ml^{-1} solution; (c). Albendazole and/or metabolite levels obtained in tissues versus albendazole concentrations in the perfusion fluid show only significant differences in the kidneys and blood, being the lowest values those found for the 1.0 mg ml^{-1}. The absence of significant differences related to albendazole concentrations may be due to a saturating effect on the absorption process.

In conclusion, since tissue levels were highest following gastric absorption, then this may be the main absorption route of albendazole. It is possible that a secondary step of intestinal absorption exists from albendazole and/or metabolite excreted in the bile fluid.

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