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Article *in* Journal of Pharmacy and Pharmacology · January 1998

DOI: 10.1111/j.2042-7158.1998.tb03303.x · Source: PubMed

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J. Pharm. Pharmacol. 1998, 50: 43–48
Received June 9, 1997
Accepted September 26, 1997

Absorption Studies of Albendazole and Some Physicochemical Properties of the Drug and Its Metabolite Albendazole Sulphoxide

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Abstract

In several studies of patients with neurocysticercosis under treatment with albendazole the pharmacokinetic data were difficult to interpret, probably because of slow and erratic drug dissolution response and absorption problems in-vivo. Because there is no information available about the physicochemical properties of the drug, the aim of this work was to explain this erratic behaviour by fully characterizing the solution behaviour of the drug and its metabolite. To accomplish this, the physicochemical properties, pK_a and solubility, and in-vitro plasma binding of albendazole and its main metabolite, albendazole sulphoxide, were studied by conventional methods. The intestinal and gastric absorption and dissolution behaviour of albendazole were also studied.

The solubility of both compounds is very low. Both are amphoteric molecules with two ionization steps, with pK_a values of 10·26 and 2·80 for albendazole and 9·79 and 0·20 for albendazole sulphoxide; low pK_a values were obtained by performing linear free energy relationship calculations. On the other hand, protein binding studies showed that albendazole is 89–92% bound to plasma proteins whereas for albendazole sulphoxide the figure is 62–67%. This metabolite is bound by albumin and to α_1-glycoprotein. Absorption of albendazole occurs along the gastrointestinal tract and is limited by its solubility. Good dissolution profiles were observed when 0·1 M HCl was used as dissolution medium.

The results show that 0·1 M HCl enables discrimination between the drug-release characteristics of different products.

Albendazole is a broad-spectrum antihelmintic drug, effective in the treatment of echinococcosis, hydatid cysts and neurocysticercosis (Morris et al 1983; Sotelo et al 1988). In man it is metabolized rapidly to albendazole sulphoxide which also has antihelmintic activity and possibly also cysticidal activity (Marriner et al 1986). It has been shown that albendazole sulphoxide has better tissue penetration than the parent compound and diffuses across the blood–brain barrier (Jung et al 1990). Pharmacokinetic studies have shown large intra- and inter-individual variations in plasma levels after oral doses. This is because of the low solubility of the drug (Lange et al 1988; Jung et al 1992), among other factors. Despite its wide use in Mexico for the treatment of neurocysticercosis since 1987, little information is available on the characteristics of this drug.

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In studies performed on adults and children with neurocysticercosis, a double peak was observed in some patients (Jung et al 1992, 1997). Because plausible explanations are that in-vivo dissolution of albendazole from the dosage form is slow and erratic or that albendazole is absorbed at different sites of the gastrointestinal tract, it was considered desirable to study the physicochemical properties and protein binding of albendazole and its main metabolite, albendazole sulphoxide, and to determine the sites of absorption of the drug.

Materials and Methods

Albendazole and its sulphoxide were provided by SmithKline Beecham, México. All analytical grade reagents (hydrochloric acid, formic acid, succinic acid, citric acid, *N*-ethylmorpholine, boric acid, sodium bicarbonate, butylamine and sodium hydroxide) were used without further purification. Bovine serum albumin, α_1-acid glycoprotein and

globulin were obtained from Sigma. All solutions were prepared with ion-exchanged doubly distilled water (once from $KMnO_4$). Ionic strength was adjusted to 0·15 M with NaCl.

Equipment and measurements

All volumetric equipment was calibrated by weight. Temperature was maintained constant at 25 ± 0·1°C with a thermostatted bath. All pK_a measurements were made in a sealed thermostatted 1-cm^3 cell always against the same solution (recently distilled water). Readings were taken in quintuplicate at 292, 295, 300, 302, 305 and 307 nm using a Beckman DU-68 spectrophotometer. The potentiometer was a Corning 7 with a combined glass electrode. Dissolution studies were performed in a Hanson Research apparatus. Protein binding was determined in a dialysis cell (Spectrum Medical Industries).

Solutions

Stock 0·1 M buffer solutions were prepared from hydrochloric acid, formic acid, succinic acid, citric acid, *N*-ethylmorpholine, boric acid, sodium bicarbonate, butylamine and sodium hydroxide. Stock $1·8 \times 10^{-4}$ M solutions of albendazole and albendazole sulphoxide were prepared, and stored at 4°C. Measurements were made on solutions prepared by mixing 0·1 M buffer (1 mL) with albendazole or sulphoxide solution (1 mL) in a 10-mL volumetric flask and adjusting the ionic strength with NaCl. Before volume adjustment the pH was measured and adjusted as necessary with dilute acid or alkali. After volume adjustment pH was measured again and this value was used in calculations.

pK_a Studies

Because of the low solubilities of albendazole and its sulphoxide, their pK_a values are extremely difficult to determine by conventional potentiometric methods. We performed a spectrophotometric study in the ultraviolet region to determine the pK_a values of both; because albendazole and its sulphoxide have high molar extinction coefficients they are suitable for such a study. The pK_a values of both compounds in the low-pH region were also predicted by calculations using the Hammett equation (Charton 1965a, b; Perrin et al 1981).

Solubility studies

The solubilities of albendazole and albendazole sulphoxide were measured by adding excess amounts of the compounds to the solvents in screw-capped test tubes; the solvents studied were methanol, ethanol, dimethylsulphoxide, chloroform, acetone, Tween 80, acetic acid and 0·1 M, 1 M and 0·3% HCl. The tubes were shaken at 100 strokes min^{-1} at room temperature for 48 h and a sample was collected after filtration. The concentration of drug in the filtrate was measured by HPLC.

In-vitro absorption studies

Wistar rats, 300–400 g, were used throughout the experiment. The animals were fasted for 20 h before the study. All in-vitro rat intestine preparations were obtained by the technique described by Chowan & Amaro (1977). Samples were taken at 8, 16, 24, 32 and 40 min, centrifuged immediately, and the supernatant was separated for further analysis by HPLC. Assays were performed at least six times each using the different regions of the intestine: duodenum, jejunum, ileum and colon.

Gastric absorption studies were performed by means of a cannula inserted at the pyloric level, the cardiac end of the oesophagus having previously been ligated. A solution of albendazole (5 $\mu g\,mL^{-1}$; 6 mL) was introduced and samples were taken at 5, 10, 15, 20, 25 and 30 min. The samples were centrifuged immediately and the supernatant was separated and stored under refrigeration until analysis by HPLC. The zero-order absorption rate-constant was calculated by linear regression using the equation:

$$C = C_0 - kt \qquad (1)$$

where C and C_0 are the observed concentrations at times t and 0, respectively.

Young male Wistar rats, 300–400 g, were used to determine if albendazole might be absorbed by specific processes. Four concentrations of albendazole were used: 1·5, 3, 4 and 5 $\mu g\,mL^{-1}$. Samples from the serosal and mucosal side were assayed by HPLC. All samples were analysed using the method described below.

Krebs–Ringer solution (0·025 mL) containing mebendazole (6 $\mu g\,mL^{-1}$) as internal standard was mixed with the sample (0·5 mL) and chloroform (0·5 mL) was added. Each sample was centrifuged, and the chloroform phase was separated and evaporated at 40°C under a stream of nitrogen. All samples were reconstituted with 0·5 mL mobile phase (methanol–phosphate buffer, 70 : 30 v/v, pH 5·7) and injected into a Waters HPLC apparatus equipped with a 250 mm × 4·6 mm i.d. C_{18} column. The flow rate was kept at 1 mL min^{-1} and a variable-wavelength detector was operated at 292 nm. Response was linearly dependent on concentration from 0·1–5 $\mu g\,mL^{-1}$ with a coefficient of variation of less than 1·6%.

Extent of binding of albendazole and its metabolite to plasma from man, bovine serum albumin and α_1-acid glycoprotein

Plasma, bovine serum albumin or α_1-acid glycoprotein (1 mL) were dialysed against 1·0 mL buffer in the dialysis cell. Albendazole or albendazole sulphoxide at concentrations of 0·5, 1·0, 2·0, 3·0 and 4·0 $\mu g\,mL^{-1}$ were added to the plasma compartment. After 3-h incubation, samples (0·5 mL) were removed from both compartments and analysed the same day by an HPLC method reported elsewhere (Hurtado et al 1989).

The extent of binding, $E_b(\%)$ was calculated using the equation:

$$E_b(\%) = [(C_p - C_F)/C_p] \times 100 \qquad (2)$$

where C_P and C_F are, respectively, the drug concentrations in the protein and buffer compartments after dialysis.

Dissolution studies

Three products from Mexican manufacturers of 200-mg albendazole tablets were used to select the proper medium for tests of the dissolution of albendazole. The study was performed using US Pharmacopeia apparatus (paddles) and four dissolution media: 0·1 M HCl at 100 rev min^{-1}; 1 M HCl at 50 rev min^{-1}; 0·3% HCl at 50 rev min^{-1}; and 0·3% HCl at 75 rev min^{-1}, using a Hanson Research apparatus. Samples (2 mL) were taken at 5, 10, 20, 30, 45, 60, 90 and 120 min, filtered and assayed spectrophotometrically at 295 nm. Media was replaced at 37 ± 0·5°C. Calibration curves for the media were constructed over the concentration range 2–20 $\mu g\,mL^{-1}$.

Results

pK_a

Spectrophotometric determination of the pK_a values of albendazole and its sulphoxide was performed in the pH range 6 to 12. The data were fitted to the equation:

$$A_{obs} = [A_I + A_M(10^{pH-pK_a})]/(1 + 10^{pH-pK_a}) \qquad (3)$$

where A_{obs}, A_I and A_M are the observed absorbance, the absorbance of the neutral species and the absorbance of the anionic form respectively from which pK_a was calculated. The pK_a values obtained for albendazole and its sulphoxide were 1026 ± 0·17 and 9·79 ± 0·16, respectively. That both albendazole and its sulphoxide are more soluble when the pH is approximately 3 indicates there is another pK_a. Although we were unable to measure these pK_a values, they were calculated by use of the Hammett equation; the values obtained were approximately 2·8 for albendazole and 0·3 for albendazole sulphoxide.

Table 1. Solubility data (mg mL^{-1}) for albendazole and albendazole sulphoxide.

Solvent	Albendazole	Albendazole sulphoxide
Methanol	0·25	0·230
Ethanol	0·35	0·020
Dimethylsulphoxide	5·00	0·167
Chloroform	2·00	0·238
Acetone	0·83	0·040
Acetic acid	5·00	2·000
HCl, 0·1 M	0·04	0·900
HCl, 1 M	1·25	10·000

Solubility

Solubility data (mg mL^{-1}) obtained for albendazole and albendazole sulphoxide are listed in Table 1.

In-vitro absorption studies

Plots of the cumulative amount of albendazole transported from the mucosal to the serosal sides of everted rat intestinal sacs, prepared from each region of the intestine, are given in Figure 1. The same figure shows the absorption kinetics in the stomach. Plots were linear, indicating that drug-transport followed zero-order kinetics. Rate-constants were: duodenum 45 (± 11) ng mL^{-1} min, jejunum 115 (± 25) ng mL^{-1} min, ileum 132 (± 28) ng mL^{-1} min, colon 89 (± 15) ng mL^{-1} min. Gastric absorption studies resulted in an observed rate-constant of 752·6 (± 91) ng mL^{-1} min.

Extent of binding

Binding of albendazole to plasma proteins from man was high (89–91%) and constant in the range 0·5–4·0 $\mu g\,mL^{-1}$. Albendazole was bound to a lesser extent (63–65%) and binding was again constant in the same concentration range. When studies were performed with bovine serum albumin it was found that albendazole was 82% bound whereas albendazole sulphoxide was 34% bound. Studies with α_1-acid glycoprotein showed that albendazole was 11% bound and albendazole sulphoxide was 32% bound.

Dissolution

Figure 2 shows the dissolution profiles of three commercial albendazole products in the dissolution

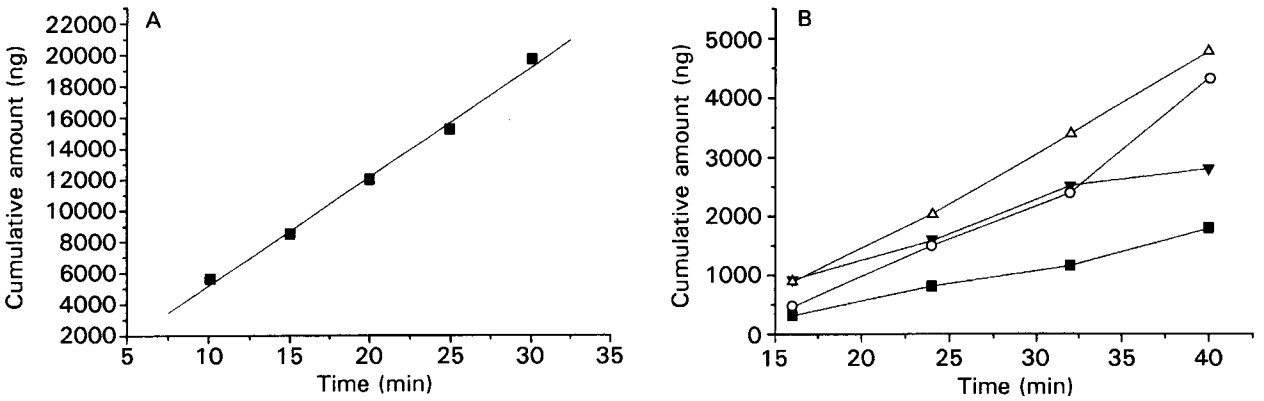

Figure 1. Absorption of albendazole along the gastrointestinal tract. A. Gastric absorption; B. ■, Duodenum; ○, jejunum; △, ileum; and ▼, colon.

media investigated. In 0·3% HCl at 50 rev min^{-1} dissolution of all the products was slow and incomplete—none of the products dissolved by more than 70% in 2 h. When the speed was increased to 75 rev min^{-1}, dissolution improved only slightly. However, when 1 M HCl was used, the profiles were indicative of faster dissolution and dissolution was almost complete.

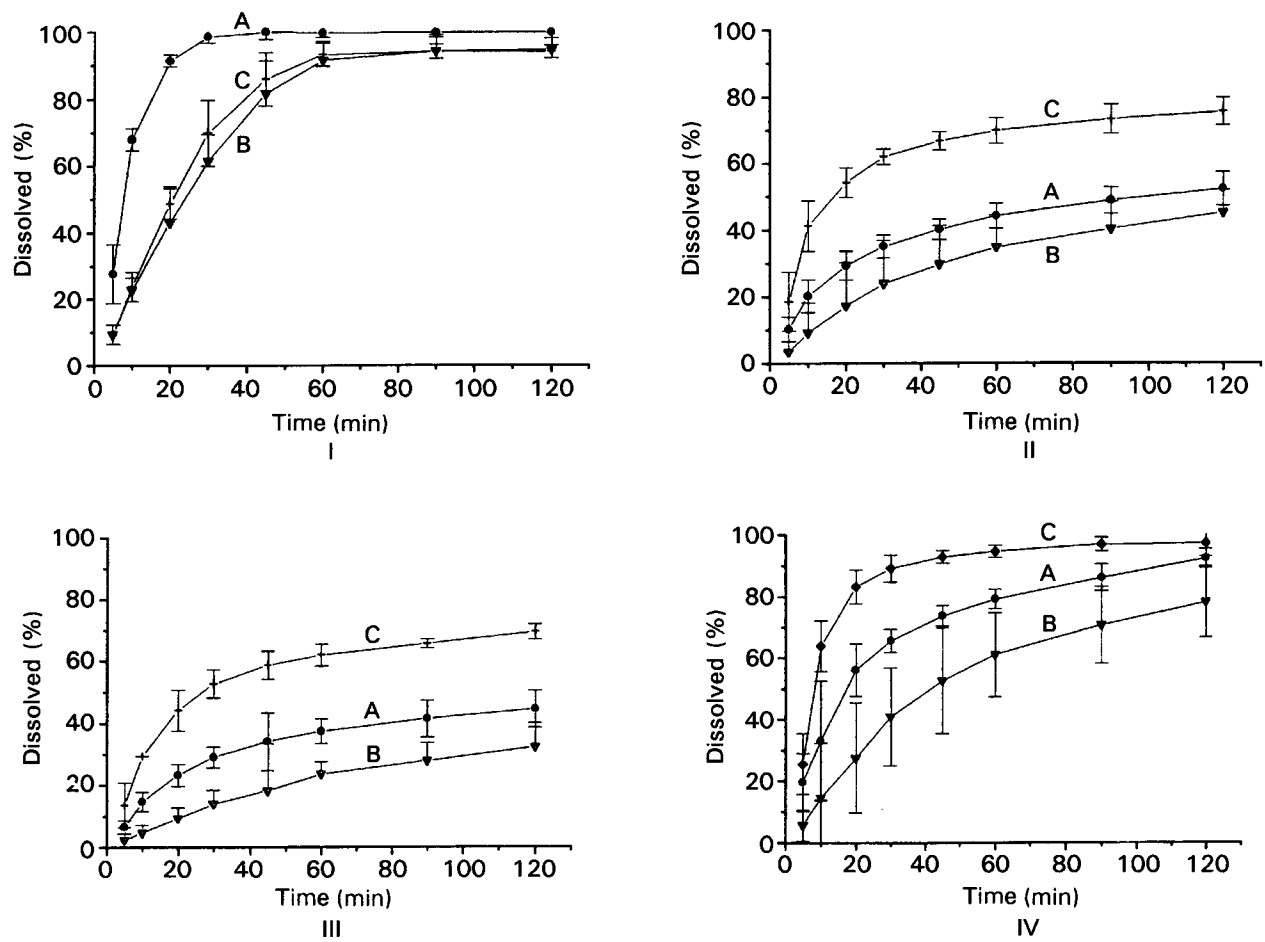

Figure 2. Mean amount (%) of three different Mexican commercial albendazole products (A, B, C) dissolving in different dissolution media: I, 1 M HCl, 50 rev min^{-1}; II, 0·3% HCl, 75 rev min^{-1}; III, 0·3% HCl, 50 rev min^{-1}; IV, 0·1 M HCl, 100 rev min^{-1}. A. Eskazole, Armstrong Laboratories, B. Gascot, Valdecasas Laboratories, C. Zentel, SmithKilne Beecham Laboratories.

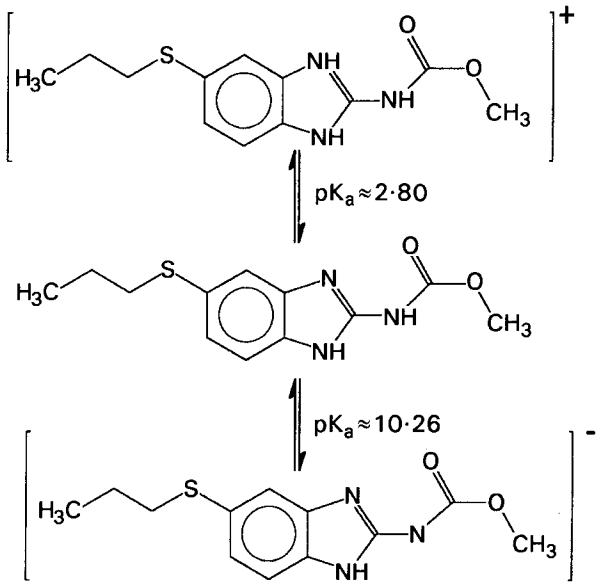

Figure 3. Effect of pH on the ionization of albendazole.

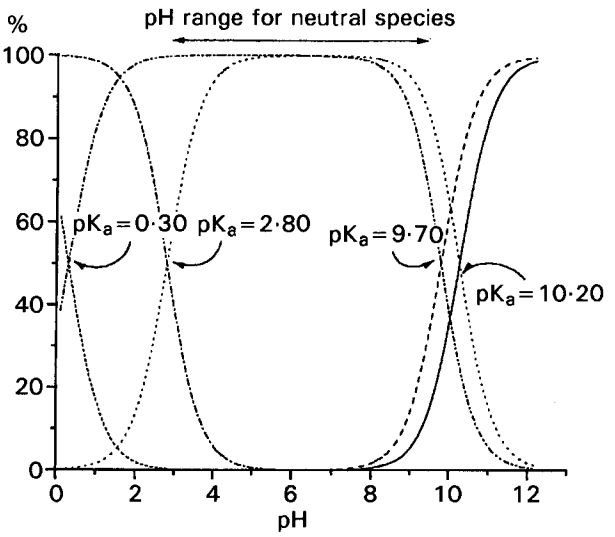

Figure 4. Relationship between charged species of albendazole (Alb) and albendazole sulphoxide (S-Alb) and pH: ——Alb^-, - - - -S-Alb^-, · · · · ·Alb, ·-·-·-Alb^+, ·-·-·-·-S-Alb, ····· S-Alb^+.

Discussion

These results imply that both albendazole and its sulphoxide are amphiprotic compounds. The pK_a values of 10·47 and 9·79 are attributable to the loss of the proton of the amino group at position 1. The solubility data suggest a second pK_a; these and theoretical grounds suggest pK_a values of 2·90 for albendazole and 0·30 for albendazole sulphoxide. These values are attributable to the protonation of the nitrogen at position 3 of the imidazole ring (Figure 3).

The solubilities of the compounds were low in most of the solvents. Solubility was highest in dimethylsulphoxide, chloroform and acetic acid. The solubility of the sulphoxide was less than that of albendazole except in HCl.

From Figure 1 it is apparent that albendazole is absorbed along the intestine. This could be because albendazole is almost un-ionized between pH 6 and 9. Figure 4 shows how the charged species are affected by pH and implies that between these pH values albendazole occurs as a neutral molecule, which explains its high absorption. That albendazole sulphoxide is found as its un-ionized form at pH 7·4 also explains the passage of sulphoxide into cerebrospinal fluid. Absorption decreased in the order: ileum > jejunum > colon > duodenum; absorption kinetics were always zero-order. Because precipitation of the drug was observed in all our studies, the zero-order absorption rate-constant can be attributed to the existence of a saturated solution that is producing an apparent zero-order input, with drug being dissolved as it is absorbed.

Few studies have been performed to determine absorption kinetics. De Estal et al (1993) measured a first-order rate of absorption after intestinal perfusion of albendazole in Tween 80 and taurocholate. This could be because of improved solubility of albendazole in these surfactants.

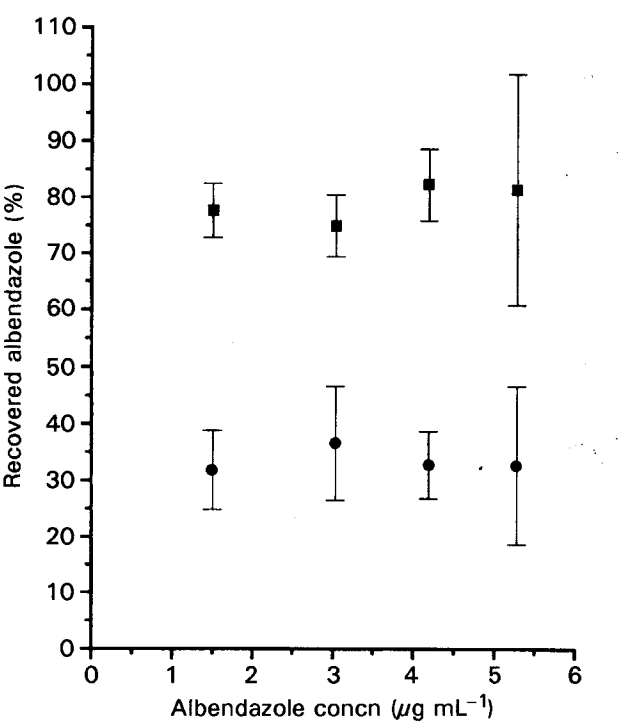

Figure 5. Relationship suggesting the passive transport of albendazole across the everted intestine of the rat. ●, Mucosal; ■ Serosal.

Absorption in the stomach was higher than in the intestine. The rate-constant was again zero-order and was larger than that in the intestine. Our results agree with those obtained by Prieto et al (1988) who found tissue levels to be highest after gastric absorption of albendazole. That absorption is higher in the stomach than in other regions of the gastrointestinal tract could be attributed to the greater solubility of the drug in acidic media. Therefore, even when the concentration of the protonated ion is high, it is feasible that when albendazole is in contact with the cell membrane it loses the proton and is absorbed. This can be explained in terms of the high lipid-solubility of the dissolved ionized form enabling partitioning into the cell membrane.

Because of the low solubility of albendazole in Krebs–Ringer solution we could not use concentrations higher than 5 $\mu g\,mL^{-1}$ to determine the absorption process.

Data given in Figure 5 suggest that absorption takes place by a non-saturable process such as passive diffusion. Prieto et al (1991) found that the kinetics of albendazole absorption in the stomach follow a passive-diffusion process. From our results we conclude that the same process occurs in the intestine. Studies in man have revealed great inter-individual variation after oral administration of albendazole. Results obtained in the current study show that solubility and not absorption is the rate-limiting step in the absorption of the drug.

In-vitro binding studies showed a great difference between the extents of binding of the drug and its metabolite. These differences could be because albendazole sulphoxide is less hydrophobic and therefore less bound than the parent compound. Considering that in man only albendazole sulphoxide is found in plasma, the extent of binding has no clinical significance.

Presently there are no compendial requirements for dissolution studies of albendazole dosage forms. We have determined the release characteristics of different brands of this drug. Dissolution media were selected on the basis of the solubility of the drug and that the main site of absorption is the stomach. From Figure 2 it is apparent that solubility and the rate of dissolution are highest in 1 M HCl. In 0·3% HCl saturation of the medium is avoided and so sink conditions are not obtained; therefore, an increase in stirring rate is not reflected in the dissolution profile. From our results it is apparent that 0·1 M HCl as dissolution medium enables discrimination between the drug-release characteristics between products. However it is important to determine the impact of these differences on in-vivo absorption.

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