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# A novel formulation of albendazole solution: oral bioavailability and efficacy evaluation

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**Abstract**

To improve the oral bioavailability of albendazole, a poorly water-soluble drug, a new liquid formulation was investigated in mice. The liquid formulation studied was a solvent system of Transcutol® (40% w/w) in 1.2 pH buffer. A significant ($p < 0.05$) increased relative bioavailability was obtained with this albendazole solution as compared with an albendazole suspension. A *Trichinella*/mouse model was used to evaluate the efficacy of the formulation against the different stages of the parasite. The higher concentration–time plasma profile of the albendazole solution would explain the greater therapeutic effect obtained with this new formulation against the systemic phases of the *Trichinella* model studied. This albendazole solution presented an efficacy against the migrating larvae phase twice that of the albendazole suspension. The efficacy of the solution (95.5%) in the encysted phase was significantly ($p < 0.05$) higher than the efficacy obtained with the albendazole suspension (2.4%). © 1997 Elsevier Science B.V.

*Keywords:* Albendazole; Solvent system; Therapeutic effect; Bioavailability

## 1. Introduction

Albendazole (ABZ), methyl[5-(propylthio)-1-*H*-benzimidazol-2yl]carbamate, is a broad-spectrum anthelmintic used against intestinal helminth infections (Cook, 1990). ABZ is a poorly water-soluble drug and is consequently barely absorbed from the gastrointestinal tract. This property is ideal for its use against the geohelminths and other intestinal parasites but is a major disadvantage for use in the treatment of systemic helminthiasis such as echinococcosis (Gil-Grande et al., 1993; Yasawy et al., 1993; Van-Nieuwkerk et al., 1993).

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As the albendazole therapy is specially important in systemic cestode infections, particularly in inoperable or disseminated cases of hydatidosis (Wen et al., 1993) and neurocysticercosis (del Brutto et al., 1993), different efforts have been made to improve ABZ solubility (Estal et al., 1993; Torrado et al., 1996a).

The use of solvent systems in order to raise the solubility and bioavailability of poorly soluble drugs has been studied extensively (MacKellar et al., 1994; Poelma et al., 1990).

Transcutol® (diethylene glycol monoethyl ether), which is a powerful solubilizing agent, and which has also been employed as an absorption promoter of different drugs (Touitou et al., 1994; Sheen et al., 1995) may be an interesting cosolvent.

The main objective of this study was to evaluate the bioavailability and therapeutic effect of an ABZ liquid formulation, developed in a previous work (Torrado et al., 1996b), using Transcutol® as a cosolvent in order to increase ABZ bioavailability. This study includes oral bioavailability and efficacy evaluation of the albendazole solution versus a reference albendazole suspension formulation in mice.

## 2. Materials and methods

### 2.1. Materials

Albendazole (ABZ) was supplied by Chemo-Iberica (Madrid, Spain). Albendazole sulphoxide (ABZ:SO) was supplied by Robert Young and Co. (England). Transcutol® (diethylene glycol monoethyl ether), an oily water-soluble liquid, was provided by Gattefosse Corp. (Madrid, Spain). All other ingredients were of pharmaceutical grade.

### 2.2. Formulations

#### 2.2.1. Albendazole suspension

ABZ was formulated as a methylcellulose 0.5% (w/v) aqueous suspension. The ABZ concentration was 8 mg/ml. The different ABZ doses administered were 2.5, 5, 10, 12.5, 25 and 50 mg/kg.

#### 2.2.2. Albendazole solution

A solvent system of 40% (w/w) of Transcutol® in 1.2 pH buffer (KCl/HCl) was used to dissolve the ABZ (8 mg/ml) (Torrado et al., 1996b). The different ABZ doses administered were 2.5, 5, 10, 12.5, 25 and 50 mg/kg.

### 2.3. Solubility determination

Solubility (w/w) at 24°C was determined using the shaker method (Yu et al., 1994). An excess of the compound was placed in solvent in a screw-capped glass tube, connected to a rotating sample, immersed in a water bath maintained at the required temperature, and agitated continuously for $>24$ h. Albendazole samples were analyzed in a Beckman DU6 spectrophotometer at 291 nm in a 1.2 pH buffer solution.

### 2.4. Acute toxicity study

Female Swiss CD-1 mice (Charles River, Spain) weighing 30–35 g were acclimated to the animal care facility for several days. Animals were maintained on a 12-h light/dark cycle with food and water available ad libitum and divided into groups of eight animals.

Two albendazole formulations (suspension and solution) prepared as described before, were administered orally by bucco-gastric tube. The ABZ doses administered of each formulation were of 12.5, 25 and 50 mg/kg.

Survivals were monitored for 45 days and possible severe adverse effects were examined.

### 2.5. Oral bioavailability

Swiss CD-1 mice weighing 30–35 g were employed. Food and water were supplied ad libitum. Formulations were administered orally via bucco-gastric tube. Each ABZ dosage form (solution or suspension), was administered by a single dose of 50 mg/kg. After drug administration groups of three mice were sacrificed and blood samples were collected at the following times (0.25, 0.5, 0.75, 1.5, 2.0, 3.0, 4.5 and 6.0 h). Blood samples were heparinized and centrifuged individually after the extraction. Plasma samples were freezed until the HPLC analysis.

### 2.6. Plasma samples analysis method

Plasma aliquots of 400 μl were mixed with 2 ml of methanol in a vortex mixer during 15 s and then centrifuged 5 min (2000 rpm). The supernatant phase was filtered through a Millipore HVLP filter of 45 μm, diluted when necessary, and assayed by HPLC. Classical high-performance liquid chromatography separation and quantification of albendazole sulphoxide (ABZ:SO) was performed (Galtier et al., 1991). A Gilson chromatographic system was used. This consisted of an Isochrom solvent delivery system with a Rheodyne valve injector (mod. 231 XL), a variable-wavelength UV absorbance detector (mod. 116) and a SP 4270 integrator. Chromatography was obtained using a Partisil, 5 μm (Whatman S.A., Paris, France) 250 × 4.6 mm column. The column was eluted with a solvent system containing hexane/ethanol 89:11 (v/v). The eluent was run at a rate of 1.0 ml/min and monitored at 291 nm following injected volumes of 20 μl of ABZ:SO standard solutions and samples. The calibration curve was found to be linear in the range of 0.05–2.5 μg/ml.

### 2.7. Efficacy evaluation of ABZ dosage forms in a Trichinella spiralis model.

The GM-1 isolate of *T. spiralis* was used. The isolate was isoenzymatically identified at the *Trichinella* Reference Center (Istituto Superiore di Santá, Roma) and kept under the code MFEL/ES/S2 GM-1-ISS48 (La Rosa et al., 1992). To evaluate the anthelmintic activity of the formulations, groups of eight mice per dose treatment were orally infected with 300 ± 50 L1 muscle larvae isolated from infected carcasses of mice kept for maintenance, by artificial digestion, following the method described by Martinez-Fernández (1978). The ABZ formulations (suspension or solution) were administered orally by buccogastric tube at identical doses. Groups of eight mice per formulation treatment and parasite stage were kept as controls and were given the vehicle alone. Treatments were applied at three different stages (preadult, migrating larvae and encysted larvae) of the parasite. Against preadults, the formulations were administered 24-h post-infection (p.i.) at 2.5, 5 and 10 mg/kg.

To treat migrating larvae it was necessary to firstly remove the adults remaining in the gut without affecting the migratory new born larvae (Denham and Martinez, 1970). This was achieved by treating both the controls and experimental groups, on Day 9 p.i., with trichophon (Neguvon, Bayer S.A., Spain) at 70 mg/kg given orally plus one intramuscular injection of atropine sulphate (Bayer, S.A., Spain), at 1 mg/kg. Thereafter, the ABZ formulations were administered at 25 and 50 mg/kg on Days 13, 14 and 15 p.i. Treatment against encysted larvae was given at 25 and 50 mg/kg, on Days 34, 35 and 36 p.i.

The effectiveness of the treatment against preadult stages was assessed on Day 6 p.i. after killing the mice (previously anaesthetized with ether) by cervical dislocation. The numbers of adult worms remaining in the gut were isolated and counted following the method described by Denham and Martinez (1970).

To measure the effect of the compounds against migrating larvae the mice were killed on Day 30 p.i.; the animals were skinned and eviscerated and their carcasses processed to free the muscle larvae according to Martinez-Fernández (1978). A similar procedure was followed to estimate the effectiveness of drugs against encysted larvae except that killing and larval counting was carried out on Day 46 p.i.

## 3. Results and discussion

A previous screening was performed to determine the solubility of ABZ in different vehicles. The best results were obtained with Transcutol® (Torrado et al., 1996b).

The $LD_{50}$ of Transcutol® administered orally is 8.69 g/kg in rat (The Merck Index, 1989). These toxicity problem led us to look for and select mixtures of Transcutol® with 1.2 pH buffer solution in order to obtain the higher solubility with as little an amount of Transcutol® as possible. A solvent system of Transcutol® (40% w/w) in 1.2 pH buffer was chosen as the best solvent for ABZ. With this 1.2 pH liquid formulation the

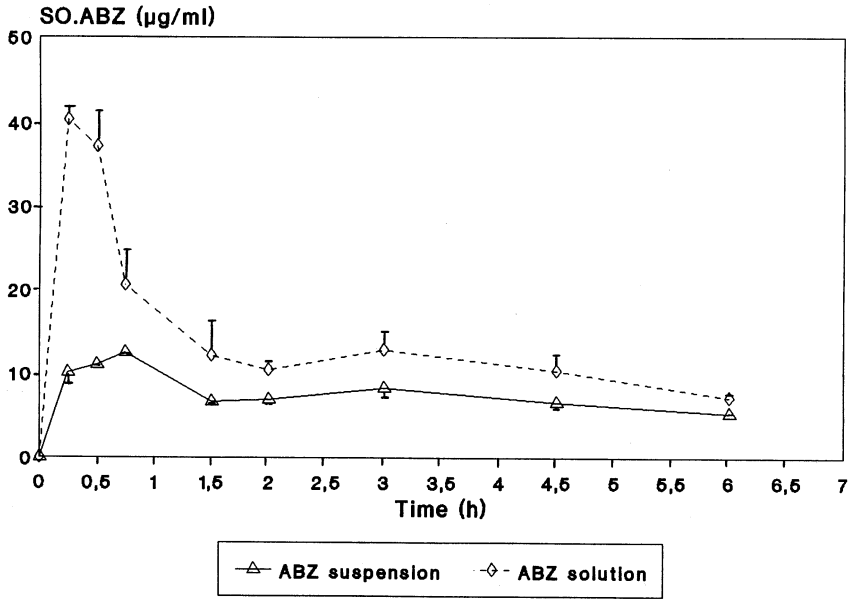


Fig. 1. Plasma levels ($\mu$g/ml) of ABZ:SO versus time (h) after a single dose (50 mg/ml) of ABZ suspension (△) and ABZ solution (◇). Mean and standard deviation for three animals.

maximum dose of Transcutol® administered was 2.5 g/kg, corresponding to the administration of 50 mg/kg of liquid formulation containing a percentage of Transcutol® of 40% w/w. The solubility of ABZ in a liquid formulation of Transcutol® (40% w/w) in a 1.2 pH buffer solution was 10.034 mg/ml.

The results of the acute toxicity study showed a survival rate (at 45 days) of 100% at each dose tested of both formulations. These results of toxicity led us to use these formulations for studies of oral bioavailability and efficacy evaluation.

In our experimental conditions in mouse plasma, unmodified ABZ was never detected due to a strong liver first-passage effect. This result is similar to those obtained by other authors in different animal species (rodents, dogs, ruminants and people) (Delatour et al., 1991; Gyurik et al., 1981). The main two metabolites are the S-oxidation compounds, namely the sulphoxide (ABZ:SO) and sulphone (ABZ:$SO_2$). In our bioavailability studies instead of albendazole, the active metabolite ABZ:SO was evaluated.

Fig. 1 shows the plasma levels of the ABZ:SO after a single dose (50 mg/kg) of ABZ liquid solution and ABZ suspension.

The $T_{max}$ for the ABZ:SO was less than 1 h. This $T_{max}$ value found is smaller than the ones observed in others animal species (Delatour et al., 1990, 1991).

The $T_{max}$ values obtained for both formulations (Table 1) present a statistically significant difference ($p < 0.05$) (one-way ANOVA comparative statistical study).

Fig. 2 shows the antiparasitic efficacy results of both formulations, ABZ solution and ABZ suspension, in the three different *T. spiralis* stages: adult worms, migrating larvae and encysted larvae.

For the albendazole liquid solution, the $C_{max}$ was 45.27 $\mu$g/ml, which showed a significant ($p < 0.05$) improvement versus ABZ aqueous suspension $C_{max}$. The highest plasma concentrations obtained with the ABZ suspension, 12.8 $\mu$g/ml, may be smaller than the minimum active concentration against encysted larvae, so only 2.38% parasite reduction at a 50 mg/kg dose was obtained. Meanwhile, the highest plasma concentrations for the ABZ solution led to a higher activity in encysted larvae (95.50% parasite reduction).

Table 1
Oral bioavailability of ABZ:SO in the mouse following treatment with ABZ solution or suspension at a dose of 50 mg/kg[a]

| Formulations | $C_{max}$ ($\mu$g/ml) | $T_{max}$ (h) | AUC ($\mu$g/h/ml$^{-1}$) | Relative bioavailability (%) |
|---|---|---|---|---|
| ABZ solution | $45.27 \pm 8.1$ | $0.33 \pm 0.2$ | $82.21 \pm 14.1$ | 182.20 |
| ABZ suspension | $12.80 \pm 0.8$ | $0.67 \pm 0.2$ | $45.12 \pm 8.8$ | 100.00 |

[a] Mean and standard deviation for three animals.

The oral bioavailability was determined as the AUC of ABZ:SO concentration versus time curve. The ABZ solution formulation has a higher AUC than the ABZ aqueous 0.5% methylcellulose suspension (see Table 1). Statistically significant differences were obtained with a one-way ANOVA comparative statistical study between both formulations in AUC ($p < 0.05$) as well as in $C_{max}$ ($p < 0.01$).

The increase of the relative bioavailability from solution in relation to suspension may be due to the increase in ABZ solubility due to the effects of Transcutol®. Another explanation would be the possible absorption promoter effect of Transcutol (Touitou et al., 1994).

The treatment against early adult stages of *Trichinella spiralis* by ABZ liquid solution was compared with an ABZ suspension at equal dosages of 2.5, 5 and 10 mg/kg following an infection with an inoculum infestation of 300 larvae per mouse. For all the infected mice treated with ABZ suspension a higher efficacy was obtained as the dose was increased, achieving a 62.75% of worm reduction at the highest dose studied (10 mg/kg) (Fig. 2). However, the solution presented 100% worm reduction in relation to untreated control for all doses studied (2.5, 5 and 10 mg/kg). At this parasitic stage, and for a dose of 2.5 mg/kg, the antiparasitic effect of the ABZ solution became 25-fold greater than the ABZ suspension. Furthermore, the treatment against early adult stages of *Trichinella spiralis* by ABZ liquid solution obtained better results (100% worm reduction) at a dose of 2.5 mg/kg than the ABZ suspension (62.75% worm reduction) at a dose of 10 mg/kg. These results can be explained by the soluble state of the orally administered albendazole.

The antiparasitic activities of the solution at the doses of 25 and 50 mg/kg against migrating larvae were compared with the ABZ suspension results. Both ABZ formulations presented a high efficacy in relation to untreated control, and this efficacy was higher as the dose was increased. The ABZ liquid solution (dose of 50 mg/kg) is significantly ($p < 0.05$) more active (60.00% reduction) than ABZ suspension (29.50% reduction) against migrating larvae probably due to the higher oral bioavailability of the solution.

Against encysted larvae, the ABZ solution was compared to ABZ suspension at equal dosages of 25 and 50 mg/kg. The ABZ suspension had almost no activity at dosages of 25 and 50 mg/kg in relation to untreated control. The ABZ solution formulation was significantly ($p < 0.05$) more active at dosages of 25 and 50 mg/kg (31.72 and 95.5% reduction, respectively) as compared with ABZ suspension formulation. The ABZ solution presents a very high efficacy (95.5% reduction at 50 mg/kg dose) which may be specially important for inoperable or disseminated cases of other systemic cestode infections such as hydatidosis or neurocysticercosis.

Overall, the results of these experiments show that *Trichinella* is a good model for the evaluation of new ABZ formulations. Significant differences can be observed in the efficacy of the ABZ preparations throughout the parasite life cycle.

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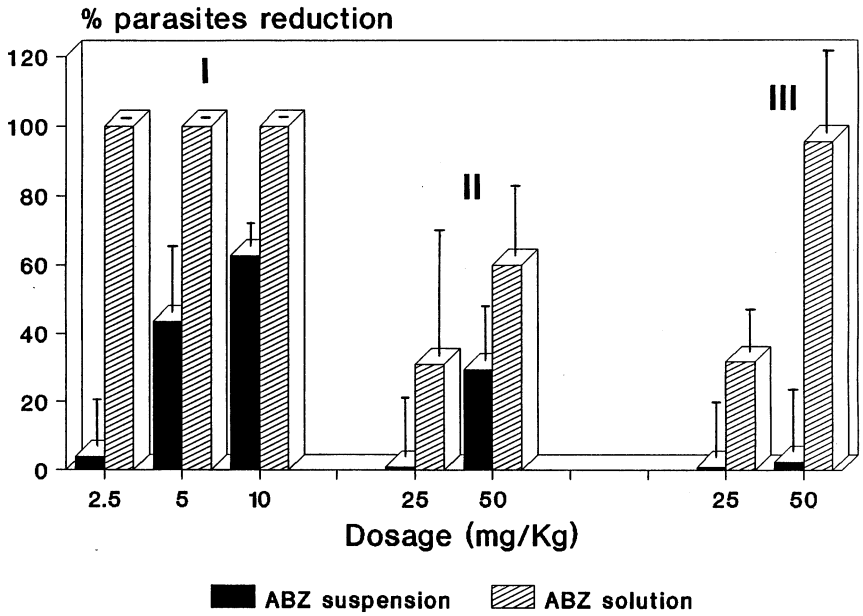


Fig. 2. Antiparasitic efficacy results expressed as the percent reduction of parasites obtained with both formulations: ABZ solution and ABZ suspension, at different doses on the three *T. spiralis* stages: adult worms (I), migrating larvae (II) and encysted larvae (III). Mean and standard deviation for eight animals.

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