Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu, India

# Enhancement of bioavailability and anthelmintic efficacy of albendazole by solid dispersion and cyclodextrin complexation techniques

R. KALAISELVAN, G. P. MOHANTA, S. MADHUSUDAN, P. K. MANNA, R. MANAVALAN

*Received November 27, 2006, accepted January 9, 2007*

*R. Kalaiselvan, Research Scientist (NDDS), Ranbaxy Research Labs, R&D-I, sector-18, Plot 20, Gurgaon, Haryana-122 015, India*
*kalaipharmacy@yahoo.com*

*Pharmazie 62: 604–607 (2007)* *doi: 10.1691/ph.2007.8.6274*

The objective of this study was to improve the oral bioavailability and therapeutic efficacy of albendazole (ABZ) employing solid dispersion and cyclodextrin complexation techniques. Solid dispersion (dispersion) was prepared using ABZ and polyvinylpyrrolidone (PVP) polymer (1 : 1 weight ratio). Ternary inclusion complex (ternary complex) was prepared using ABZ, hydroxypropyl β-cyclodextrin (HPβCD) and L-tartaric acid (1 : 1 : 1 molar ratio). In rabbits with high gastric acidity (gastric pH $\approx$ 1), ternary complex and solid dispersion showed a bioavailability enhancement of 3.2 and 2.4 fold respectively, compared to a commercial suspension ($p < 0.05$). The rise in gastric pH (pH $> 5$) caused a 62% reduction in AUC (area under the plasma level curve) for the commercial suspension, whereas the reduction in case of PVP dispersion and ternary complex was only 43% and 37% respectively. The rapid absorption of the drug from solid dispersion and ternary complex was reflected in improved anthelmintic efficacy against the systemic phases of *Trichinella spiralis*. The ternary complex was significantly more efficient than solid dispersion and exhibited the highest larvicidal activity (90%) at a dose of 50 $mg \cdot kg^{-1}$ ($p < 0.05$). These results suggest that the bioavailability and therapeutic efficacy of the ternary complex might be high even if there is a great variation in the gastric pH.

## 1. Introduction

Pulmonary echinococcosis is considered to be the most lethal type of helminthiasis in man (incidences more frequent in children) and number of such cases are reported to be increasing, especially in northern hemisphere countries (Kumar and Chattopadhyay 1992; Keramidas et al. 2004). Albendazole (ABZ) is the drug of choice for the clinical treatment of echinococcosis and other systemic helminthiasis such as trichinellosis and neurocysticercosis (Anonymous 1995; Liu and Boireau 2002; Keramidas et al. 2004). ABZ belongs to BCS (biopharmaceutical classification) system type II (low aqueous solubility with high permeability), thus showing dissolution rate limited absorption (Amidon et al. 1995; Jung et al. 1998). ABZ was reported to form a weak binary complex with hydroxypropyl β-cyclodextrin (HPβCD), and the resultant solid complex was found to increase the relative bioavailability by only 40%, when compared with a suspension dosage form in mice (Castillo et al. 1999). In another bioavailability study, the use of higher quantities of HPβCD (40% w/v) to prepare a solubilized system of ABZ, was found to cause diarrhoea in human volunteers due to the osmotic effects of cyclodextrin (Rigter et al. 2004). Solid dispersions, cyclodextrin binary complexes and surfactants were shown to improve the dissolution of ABZ (del Estal et al. 1994; Torrado et al. 1996; Castillo et al. 1999; Mallick et al. 2003). However, few such preparations have been used clinically.

In our previous studies polyvinylpyrrolidone (PVP) was found to inhibit both isothermal and nonisothermal crystallizations of amorphous ABZ due to an increase in the glass transition temperature of the system (Kalaiselvan et al. 2006a). Drug-cyclodextrin-hydroxy acid ternary complexes have been shown to improve the dissolution of ABZ and other weakly basic drugs compared to the drug-cyclodextrin binary complex due to improved inclusion efficiency and a favorable pH presented in the diffusion layer (Mura and Faucci 2001; Kalaiselvan et al. 2006b).

Gastric pH has been reported to have a profound effect on intestinal absorption of ABZ (Kohri et al. 1998). Because of the poor solubility of the drug in alkaline and neutral solutions, absorption of ABZ decreased at higher gastric pH. The objective of the current study was to achieve a pH-independent bioavailability enhancement using ABZ-PVP solid dispersions and a solid complex of ABZ-HPβCD-tartaric acid (ternary complex).

## 2. Investigations, results and discussion

### *2.1. Bioavailability*

After oral administration, unmodified ABZ was not detectable in plasma samples. This is a consequence of hepatic first-pass metabolism, which is consistent with the results previously obtained in different animal species (Delatour et al. 1991; Kohri et al. 1998; Lopez-Garcia et al. 1998).

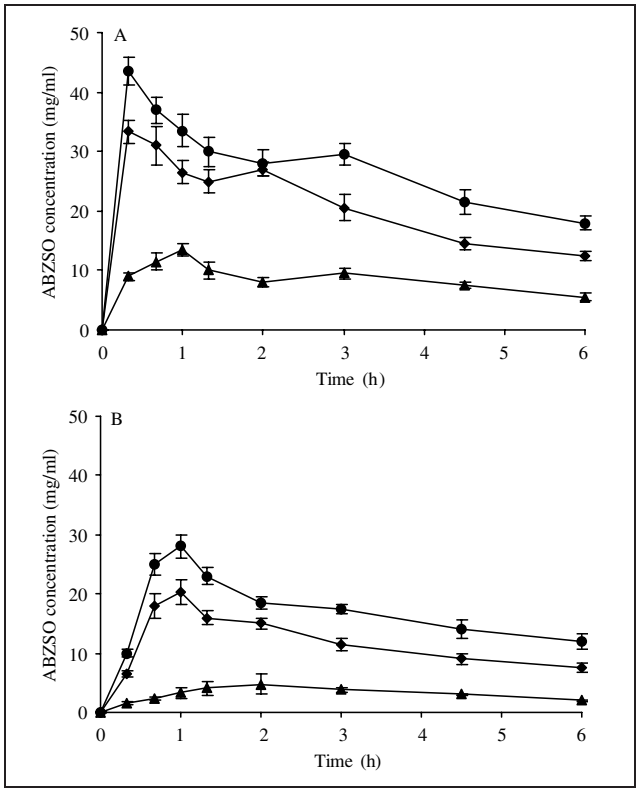


Fig. 1: Mean plasma concentration of ABZSO after single oral administration of commercial suspension (▲), PVP dispersion (◆), ternary complex (●) to normal rabbits (A) and low acidity rabbits (B). Values are mean ± SD (n = 6)

In the present bioavailability studies, the active metabolite, albendazole sulphoxide (ABZSO) was evaluated. A greater anthelmintic efficacy was previously reported against the systemic phases of *Trichinella spiralis* in mouse with a higher plasma level time profile of ABZSO following oral administration of ABZ with methimazole (Lopez-Garcia et al. 1998).

Mean plasma concentration time profiles of ABZSO obtained after a single dose (50 mg/kg) administration of various ABZ formulations to normal rabbits (gastric pH ≈ 1) and low acidity group (gastric pH > 5) are shown in Fig. 1. The different pharmacokinetic the parameters are summarized in Table. An enhanced GI absorption of ABZ from PVP dispersion and ternary complex was evident from the significantly reduced $T_{max}$, and increased $C_{max}$ and AUC values compared to the suspension, for both gastric pH levels ($p < 0.05$) (Table). Relative bioavailability values ($F_r$) of at least 243 and 317% were obtained for the PVP dispersion and ternary complex respectively in both types of rabbits. The ternary complex exhibited the highest bioavailability ($p < 0.05$).

The gastric pH greatly affected the bioavailability of ABZ from the suspension. However, the influence of gastric pH on the absorption of ABZ from the PVP dispersion and ternary complex was relatively weak ($p < 0.05$). For instance, the rise in gastric pH caused a 62% reduction in AUC for the commercial suspension, whereas the reduction in case of PVP dispersion and ternary complex was only 43% and 37% respectively (Table). This might be due to a faster dissolution of the PVP dispersion and ternary complex even at higher gastric pH. The pH of the diffusion layer for the ternary complex might be decreased due to the presence of tartaric acid, resulting in rapid *in vivo* dissolution. Hence, the bioavailability of ABZ from the PVP dispersion and the ternary complex might be improved, even if there is a great variation in the gastric pH of the patients. Clinical studies have previously shown a large inter-subject variability in the bioavailability of ABZ due to poor and pH-dependent solubility of the drug (Jung et al. 1992; Kohri et al. 1998).

Kohri et al. (1999) improved the bioavailability of ABZ in rabbits by oral administration of ABZ-hypromellose-hypromellose phthalate ternary dispersion (1 : 5 : 5), prepared using solvent method. This dispersion exhibited around 50% improvement in AUC of ABZSO at normal gastric acidity, and a 4-fold improvement at low gastric acidity condition, compared to the physical mixture.

### *2.2. Anthelmintic efficacy*

The mean counts of adult worms, migrating larvae and encysted larvae found in control animals were 128.7 ± 13.5, 74322.4 ± 9216.6 and 69448.4 ± 7275.7 respectively. Fig. 2 shows the anthelmintic efficacy of the ternary complex, PVP solid dispersion and reference product (suspension) against *Trichinella spiralis* as a model parasite in mice. As can be seen from Fig. 2, the efficacy of suspension against adult worms improved as the dose was increased from 2.5 to 10 $mg \cdot kg^{-1}$ (51% worm reduction at the highest dose). However, the PVP dispersion and ternary complex resulted in 63% and 78.5% worm reductions respectively, when compared with untreated control even at the lowest dose (2.5 $mg \cdot kg^{-1}$). At this parasitic stage and at the dose of 2.5 $mg \cdot kg^{-1}$, the anthelmintic efficacies of PVP system and the ternary complex were 11 and 14 fold, respectively, greater than the those of the suspension ($p < 0.05$). Furthermore, the treatment with the PVP dispersion and ternary complex obtained the maximum worm reduction (100%) at a dose of 5 $mg \cdot kg^{-1}$, whereas the suspension produced only 31% reduction ($p < 0.05$). The improved efficacy of the solid dispersion and ternary complex may be attributed to faster *in vivo* dissolution.

**Table: Bioavailability parameters of formulations at two different gastric acidity levels**

| Formulations | Type of rabbits | Pharmacokinetic parameters (mean ± s.d.) (n = 6) | | | |
|---|---|---|---|---|---|
| | | $C_{max}$ ($\mu g \cdot ml^{-1}$) | $T_{max}$ (h) | AUC ($\mu g \cdot h \cdot ml^{-1}$) | $F_r$ (%) |
| Commercial suspension | Normal | 13.5 ± 1.1 | 0.94 ± 0.25 | 50.2 ± 4.5 | 100.0 |
| | Low acidity | 4.8 ± 0.6 | 2.22 ± 0.66 | 19.2 ± 2.1 | 100.0 |
| Solid dispersion | Normal | 33.3 ± 2.2* | 0.39 ± 0.14* | 121.8 ± 7.2* | 242.6* |
| | Low acidity | 20.3 ± 1.5* | 0.89 ± 0.17* | 68.9 ± 4.2* | 358.9* |
| Ternary complex | Normal | 43.5 ± 1.9* | 0.39 ± 0.14* | 158.9 ± 11.9** | 316.5** |
| | Low acidity | 28.0 ± 2.0* | 0.84 ± 0.18* | 99.8 ± 8.0** | 519.8** |

Values are mean ± SD (n = 6); * $p < 0.05$ against suspension; ** $p < 0.05$ against suspension and solid dispersion

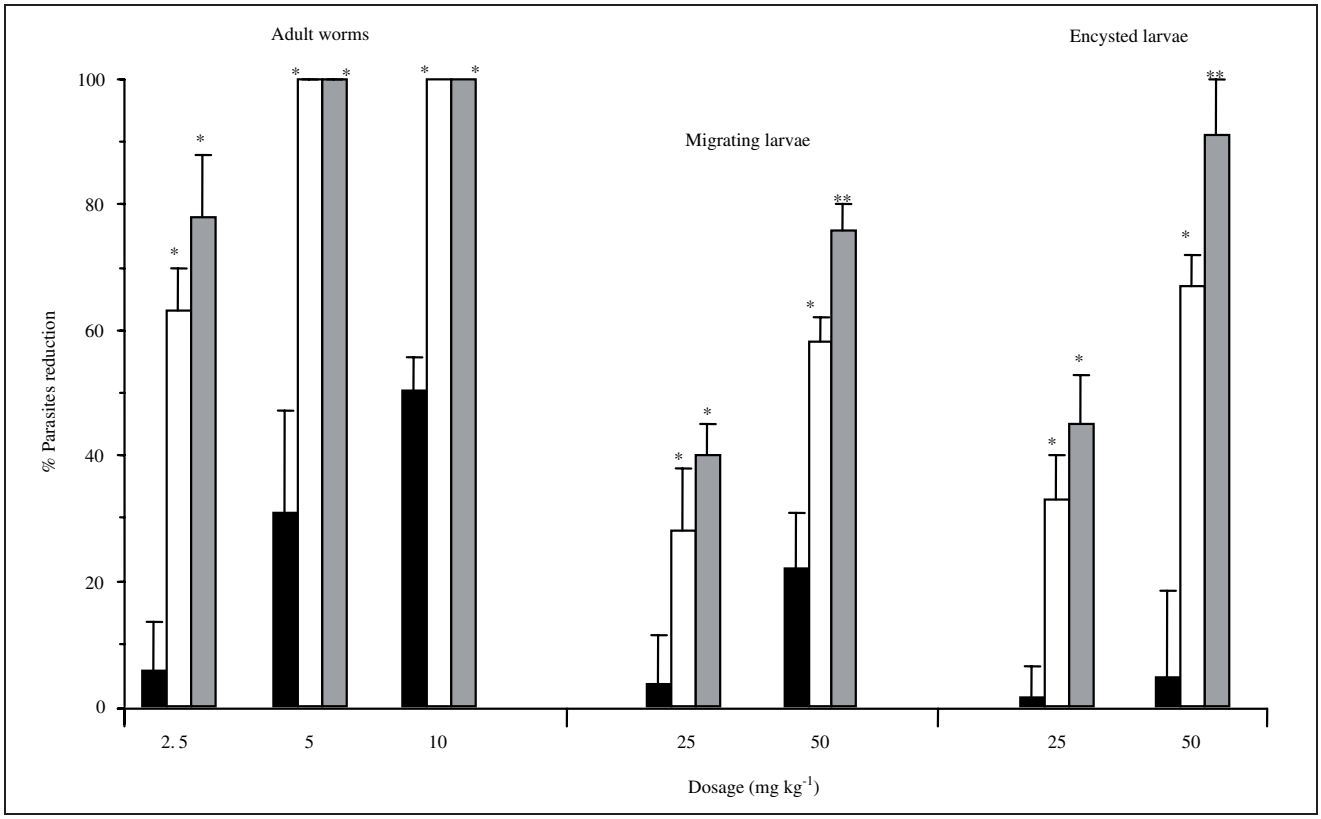


Fig. 2: Anthelmintic efficacy of suspension (■), PVP dispersion (□), ternary complex (▨) against different stages of *T. spiralis* in mice; Values are mean ± SD (n = 3); * $p < 0.05$ against untreated control; ** $p < 0.05$ against untreated control and the group treated with solid dispersion

The larvicidal activities of the PVP system and the ternary complex at the doses of 25 and 50 $mg \cdot kg^{-1}$ against the migrating larvae were compared with ABZ suspension results. All the formulations exhibited a higher efficacy when compared with untreated control in a dose dependent manner. Solid dispersion and the ternary complex were significantly more effective than the suspension at both the doses studied ($p < 0.05$). They were 7 and 10 fold more effective than the suspension at 25 $mg \cdot kg^{-1}$ dose. The ternary complex was also significantly more efficient than PVP dispersion at 50 $mg \cdot kg^{-1}$ dose ($p < 0.05$).

Against the encysted larvae, the suspension had almost no activity at a dose of 25 $mg \cdot kg^{-1}$. On the other hand, the PVP dispersion and ternary complex were significantly more effective at dosages of 25 and 50 $mg \cdot kg^{-1}$ compared to the suspension ($p < 0.05$). Particularly at 25 $mg \cdot kg^{-1}$, PVP dispersion and the ternary complex were 23 and 30 times more efficient than the suspension. At the highest dose level (50 $mg \cdot kg^{-1}$), the ternary complex was significantly more efficient than PVP dispersion ($p < 0.05$).

The improved bioavailability of PVP dispersion and ternary complex was thus reflected in superior anthelmintic efficacy against the systemic phases of *T. spiralis*. Further, the efficacy of the ternary complex was slightly higher than the PVP dispersion only at the highest dose (50 $mg \cdot kg^{-1}$) against this parasite model. An enhanced anthelmintic activity against *T. spiralis* was previously reported for ABZ when coadministered with methimazole, an inhibitor of microsomal oxidases (Lopez-Garcia et al. 1998).

## 3. Experimental

### 3.1. Materials

ABZ, albendazole sulphoxide (Juggat Pharma, Bangalore, India), mebendazole (Cadila Pharmaceuticals Ltd., Ahmedabad, India), PVP K-17 (BASF India Ltd., Chennai, India), and HPβCD (Natco Pharma Ltd., Hyderabad, India) were gift samples. Albendazole suspension (Zentel®, Glaxo SmithKline, Mumbai, India), lansoprazole capsules (Lancus®, Cadila Pharmaceuticals Ltd., Ahmedabad, India), trichlorphon (Dylox®, Sigma-Aldrich Fine Chemicals, Missouri, USA), atropine sulphate injection (Tropine®, Neon Labs, Mumbai, India), methanol, pepsin and L-tartaric acid (SD Fine Chem, Mumbai, India) were purchased from commercial sources. All other materials used were of either analytical or HPLC grade. Experimental protocols for the animal studies were approved by the Institutional Animal Ethics Committee (CPCSEA/160/1999).

### 3.2. Preparation of solid dispersion and ternary cylclodextrin complex

Dispersions were prepared as reported previously (Kalaiselvan et al. 2006a). Drug-PVP physical mixture (PM) (1 : 1 weight ratio) was dissolved in methanol, stirred overnight, and cast on Teflon sheets. The solvent was allowed to evaporate in a partially opened desiccator at room temperature for 3 days. The samples were then placed under vacuum for 2 days and the resulting films were gently ground into powder form with a mortar and pestle for 1 min. The powder obtained was dried under vacuum at room temperature for 24 h and at 40 °C for 12 h. The samples were passed through a 60 mesh sieve and packed in an airtight container.

A ternary complex (drug-HPβCD-tartaric acid) was prepared as described previously at a 1 : 1 : 1 molar ratio (Kalaiselvan et al. 2006b). The aqueous solution of HPβCD was added to methanolic solution of ABZ and L-tartaric acid. The resulting mixture was stirred for 1 h and then the solvent was evaporated at 45 °C with a rotary evaporator. The coprecipitate was passed through a 30 mesh, dried under vacuum at 45 °C (24 h), and passed through a 60 mesh before packing in an airtight container. The PM was prepared by mixing the required components using mortar and pestle for 2 min.

### 3.3. Bioavailability evaluation

The study was performed in Swiss male albino rabbits (normal and low acidity groups). A commercial suspension (Zentel® suspension containing ABZ 40 $mg \cdot ml^{-1}$, Glaxo SmithKline, Mumbai, India) was used as the reference product for comparison. In each case, a sample equivalent to 50 mg ABZ $\cdot kg^{-1}$ of body weight was orally administered with water (20 ml).

In six normal rabbits (rabbits fasted for 12 h and provided only with water), ternary complex, PVP dispersion and the commercial suspension were administered orally in a Latin square crossover fashion (consisting of 3 study periods and a washout period of 14 days). The formulations were also evaluated in low acidity rabbits. Blood samples were withdrawn from the marginal ear vein at 0, 20, 40, 60, 80, 120, 180, 270, and 360 min, heparinized and centrifuged individually. The plasma was separated and freezed until analyzed by HPLC (Kalaiselvan et al. 2006c).

The formulations were also evaluated in low acidity rabbits. A group of six rabbits with low gastric-acidity was obtained as reported previously (Kohri et al. 1998). The method involved oral administration of the granular content of lansoprazole capsules (Lancus capsules, Cadila Pharma Ltd., Ahmedabad, India) equivalent to 5 mg of lansoprazole, 15 and 3 h before the test sample administration. Immediately before the sample administration, water (10 ml) was given orally to all rabbits through a plastic catheter, and a sample of gastric juice was withdrawn by suction for the determination of pH using a pH paper.

### *3.4. Anthelmintic efficacy evaluation*

Anthelmintic efficacy of the formulations was evaluated using the GM-1 isolate of *Trichinella spiralis* (GM-1-ISS03) as a model parasite. Mouse carcass infected with this strain was supplied by the Trichinella Reference Center (Instituto Superiore di Sanita', Rome, Italy). To evaluate the anthelmintic activity of the formulations, a group of three mice (Swiss albino) per dose treatment was orally infected with $300 \pm 50$ L1 muscle larvae released from infected mouse carcass kept for maintenance. The method of artificial digestion (in 0.12 N HCl containing 1% w/v pepsin) was used for this purpose (Lopez-Garcia et al. 1998; Forbes and Gajadhar 1999).

A suspension of ABZ ($4\ mg \cdot ml^{-1}$) formulated using carboxy methyl cellulose (CMC) in water (0.5% w/v) was used as reference product for comparison. The ABZ formulation (formulated suspension, PVP dispersion or ternary complex) was administered orally with water. A group of three infected mice per parasite stage was kept as control (0.5 ml of water was administered).

Treatments were applied at three different stages (adult worms, migrating larvae and encysted larvae) of the parasite. Against the adults, the formulation was administered 24 h post-infection (p.i.) at 2.5, 5, or $10\ mg \cdot kg^{-1}$ dose. To treat migrating larvae it was necessary to first remove the adults from the gut without affecting the migratory newborns. This was achieved by treating both controls and experimental groups, on day 9 p.i., with trichophon (Dylox®, Sigma-Aldrich Fine Chemicals, Missouri, USA) at $70\ mg \cdot kg^{-1}$ given orally plus one intramuscular injection of atropine sulphate (Tropine® injection, Neon Labs, Mumbai, India), at $1\ mg \cdot kg^{-1}$. Thereafter, the ABZ formulation was administered at 25 and $50\ mg \cdot kg^{-1}$ on days 13, 14 and 15 p.i. The treatment against encysted larvae (25 and $50\ mg \cdot kg^{-1}$) was given on days 34, 35 and 36. p.i.

The effectiveness of the treatment against the adult stage was assessed on day 6 p.i. after sacrificing the mice (previously anaesthetized with ether) by cervical dislocation. The adult worms remaining in the gut were isolated and counted as reported previously (Denham and Martinez 1970; Lopez-Garcia et al. 1998). The method involved incubating the gut in saline (15 ml at 37 °C for 2 h), centrifuging the resultant liquid to obtain the worms at the bottom. To measure the effect of formulation against migrating larvae, the mice were sacrificed on day 30 p.i., after which, they were skinned, eviscerated, and the carcasses digested individually in artificial gastric fluid to free the muscle larvae for counting in a microscope. A similar procedure was followed to estimate the effectiveness of the treatment against encysted larvae except that sacrificing and larval counting were carried out on day 46 p.i.

### *3.5. Data analysis and statistics*

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed with SPSS software (version 10.7).

Acknowledgements: The support of the Indian Council of Medical Research (ICMR), New Delhi, by providing Senior Research Fellowship to one of the authors (RK) is gratefully acknowledged. We thank Dr. E. Pozio (Department of Infectious, Parasitic and Immune-mediated Diseases, Instituto Superiore di Sanita', Rome) for providing *T. spiralis* larvae and guiding in anthelmintic efficacy studies.

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