EFFECT OF TWO FORMULATIONS OF BENZIMIDAZOLE CARBAMATES ON THE VIABILITY OF CYSTS OF *ECHINOCOCCUS GRANULOSUS IN VIVO*

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Summary:

Two different preparations, solution and suspension, of three benzimidazole carbamate drugs, mebendazole, albendazole and ricobendazole, were compared by analyzing their *in vivo* activity against *Echinococcus granulosus* cysts in a mouse model. Polyvinylpyrrolidone was used for the elaboration of drug solutions and these formulations manifested better results in terms of reduction of number of viable hydatid cysts in mice than the reference drug suspensions. The effect was more prominent on mebendazole-treated mice, at doses of 25-50 mg/kg. There was a correlation between ED_{50} and pharmacokinetical parameters of $AUC_{0-\infty}$ and C_{max}, showing that a significant improvement on solubility affects the *in vivo* activity of these drugs.

KEY WORDS : *Echinococcus granulosus*, hydatid cyst, albendazole, ricobendazole, mebendazole, efficacy, solid dispersion, *in vivo*.

Résumé : EFFET DE DEUX FORMULATIONS DE CARBAMATES DE BENZIMIDAZOLE SUR LA VIABILILITÉ DES KYSTES D'*ECHINOCOCCUS GRANULOSUS IN VIVO*

Deux préparations différentes, solution (PVP) et suspension (CMC), de trois carbamates de benzimidazole, mebendazole, albendazole et ricobendazole, ont été comparées en mesurant leur activité in vivo *vis-à-vis des kystes d'*Echinococcus granulosus *dans un modèle murin. La polyvinylpyrrolidone a été employée pour l'élaboration des solutions de principes actifs, et ces formulations ont montré de meilleurs résultats en termes de réduction du nombre de kystes viables chez les souris que les suspensions du médicament de référence. L'effet était plus important sur les souris [PVP-mebendazole] traitées aux doses de 25-50 mg/kg. Il existe une corrélation entre les ED50 et les paramètres pharmacocinétiques d'*$AUC_{0-\infty}$ *et* C_{max}, *prouvant qu'une amélioration significative de la solubilité du principe actif affecte l'activité* in vivo *de ces médicaments.*

MOTS CLÉS : Echinococcus granulosus, *kyste hydatique, albendazole, ricobendazole, mebendazole, efficacité, dispersion pleine,* in vivo.

Unilocular hydatidosis is a cosmopolitan zoonotic disease caused by the metacestode (larval) stage of *Echinococcus granulosus*. There is not a definitive chemotherapy available already (Carpio *et al.*, 1995), and surgery is the standar treatment. The drugs usually used for anti-hydatid cyst treatment are benzimidazole carbamate derivatives such as mebendazole (MBZ), albendazole (ABZ) and its active metabolite commercialized as ricobendazole (RBZ). They have limited solubility and therefore poor absorption following oral administration (Da Silva *et al.*, 1997). Despite this, they are extensively used in hydatid disease, although long-term treatments are usually required and adverse side effects and failed results may occur (El-On, 2003). Several factors may account for the differences observed in therapy reports, including host-dependent factors as drug absorption and residence time in the host body. Therefore, one way of improving the therapeutic effectiveness of bencimidazoles would be by increasing their solubility by formation of drug complexes with polyvinylpyrrolidone. If solubility is the limiting step for their oral absorption (Cotting *et al.*, 1990) the solution formulations should provide better bioavailability than the conventional drug suspensions. Thus, the intention of the present study is to carry out comparative and analytical studies of different formulations of ABZ, RBZ and MBZ in an *in vivo* mouse model.

MATERIAL AND METHODS

Drugs solubilization was performed by complexation with polyvenylpirrolidone K12 PF (PVP) (Basf, USA) in the proportions of 1:20 (w:w) for MBZ (Sigma, USA) and 1:10 for both ABZ (SmithKline Beecham, England) and RBZ (Chemo Ibérica, Spain). The solvent evaporation method was used in preparation of solid complexes (Torrado *et al.*, 1996). Drug concentrations were adjusted to 1 ml/day doses of 5, 25 and 50 mg of drug per kg of body

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weight. PVP-dispersed drugs were dissolved in distilled water. The reference drugs were suspended in 1 % carboxymethylcellulose (CMC).

Twenty five to forty days old female NMRI mice were infected by i.p. injection of 2,000 protoscoleces of sheep origin in 0.5 ml of Hank's Balance Salt Solution. Eight to ten months post infection, mice were divided into 21 groups of 10 mice each: blank control, CMC control, PVP control, and a group for each of the drug-formulation-dose combinations. Except for the blank control group, the preparations (CMC or PVP with/without drugs at the appropriate concentration) were respectively administered by gavage (0.5 ml at 12 hours intervals; 1 ml/day in total) during two regimes of five consecutive days with a two days break in between. Drugs effect was evaluated 30 days post-treatment. Mice were sacrificed by anaesthesia with diethyl-ether followed by cervical dislocation. The developed cysts were removed and counted; their viability was determined on the basis of their macroscopic appearance (turgency, color, hydatid fluid transparence). The ED_{50} for each drug-formulation-dose combination was determined by the extrapolation method from the mean percentages of viable cysts in mice.

For the drug relative bioavailability studies, the PVP-drug complexes were dissolved in deionized water and orally administered to two months old female NMRI mice by a bucco-gastric tube at a dose of 100 mg/kg. The reference formulations were prepared using a 1 % CMC suspension as vehicle. Blood samples were collected by exanguination of at least three mice by heart puncture at 15, 30, 45, 60, 90, 180, 360 and 540 minutes post-administration. The blood samples were individually heparinized and centrifuged. The resulting plasma samples were stored at –20° C until HPLC analysis. The parameters $AUC_{0-\infty}$, T_{max} and C_{max} were calculated as the mean values of time (T_{max}) taken to achieve the maximum plasma concentrations (C_{max}) for the mice, which is the highest drug plasma concentration reached by each administered formulation. $AUC_{0-\infty}$ was calculated as the sum of AUC_{0-9} (determined by the trapezoidal rule method) and $AUC_{9-\infty}$ (estimated as the quotient of C_9 and K_e). K_e was calculated as the slope from the final phase of the log concentration-time curves (Shargel & Yu, 1993).

Differences among the means of various groups were identified by Analysis of uni-and-multivariable variances and significance level was set at $P < 0.05$.

RESULTS

Results for the *in vivo* treatment of infected mice are shown in Figure 1. ABZ and RBZ behaved similar at the different doses and at the two formulations and they only showed a significative activity at the higher dose of 50 mg/kg/day. MBZ showed to be the most effective at 25 and 50 mg/kg/day for

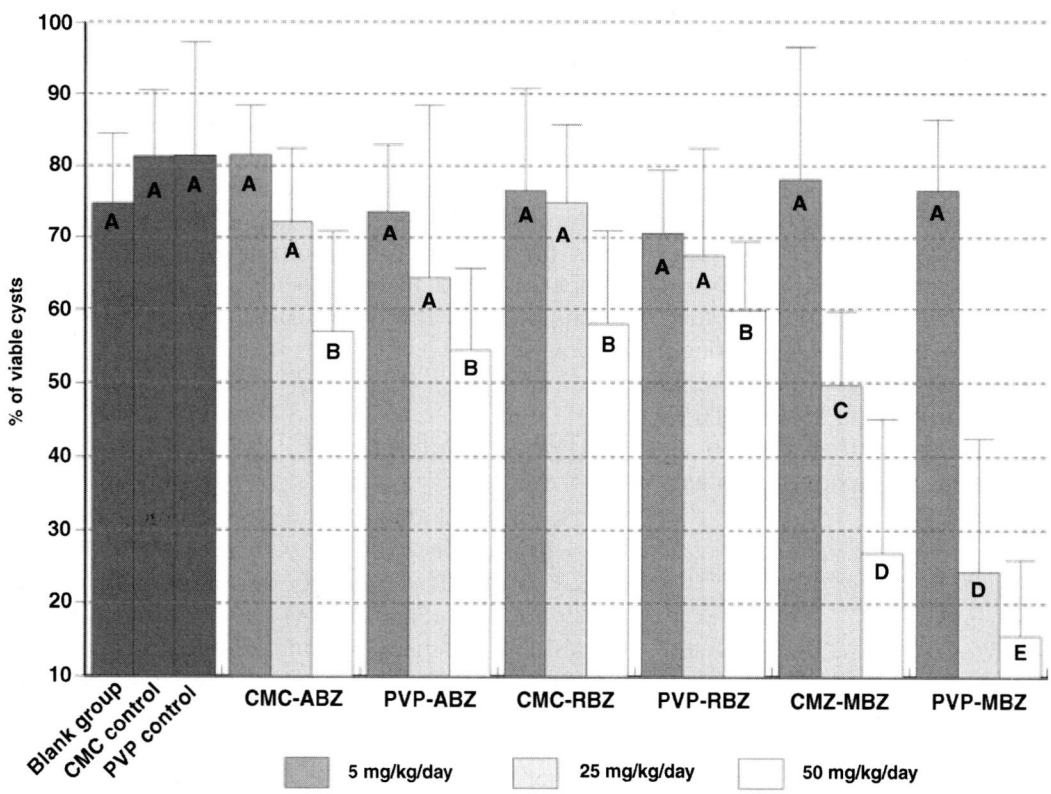

Fig. 1. – *In vitro* effects of carboxymethylcellulose (CMC)- and polyvinylpyrrolidone (PVP) preparations of albendazole (ABZ), ricobendazole (RBZ) and mebendazole (MBZ) on *Echinococcus granulosus* cyst viability in experimentally infected mice. Results are given as mean percentage of viable cysts (bars) ± standard deviation (lines). The same letter within each column denotes that no significant differences ($p < 0.05$) were found.

Drug preparations	ED_{50}	$AUC_{0-\infty}$	C_{max}	T_{max}
CMC-ABZ (suspension)	65	0.5	0.3 ± 0.1	0.4 ± 0.4
PVP-ABZ (solution)	56	0.8*	0.7 ± 0.1*	0.3 ± 0.1*
CMC-RBZ (suspension)	71	216.5	28.5 ± 1.0	1.3 ± 0.3
PVP-RBZ (solution)	79	188.9*	37.1 ± 2.0*	0.3 ± 0.1*
CMC-MBZ (suspension)	31	9.3	1.1 ± 0.1	1.1 ± 0.4
PVP-MBZ (solution)	24	25.9*	6.1 ± 0.4*	0.8 ± 0.3*

Table I. – Correlation between *in vivo* pharmacokinetical parameters and drug activities (ED_{50}) against *Echinococcus granulosus* cysts in mice. The asterisk denotes that correlation is statistically significant ($p < 0.05$).

both formulations, with an increased efficacy when in PVP respect to CMC at both doses.

Table I shows the correlation between drugs activities (ED_{50}) and physicochemical parameter results. Significant negative correlations were found between ED_{50} and $AUC_{0-\infty}$ and C_{max} and positive correlation with T_{max} for the preparations of ABZ and MBZ. On the other hand, from RBZ preparations a negative correlation was observed among ED_{50} and $AUC_{0-\infty}$ and T_{max}, whilst a positive correlation was observed with C_{max}.

DISCUSSION

The presence of the parasite in the host do not affect the pharmacokinetical parameters of bencimidazoles orally administered (García-Llamazares *et al.*, 2001), then it should be valid to apply results obtained from healthy mice to the parasited ones. CMC and PVP have showed no anti-cyst activity, and their importance is only related to their contribution to drug absorption and thus increased bioavailability.

The present study shows that changes in the pharmacokinetical parameters obtained with PVP formulations should increase the *in vivo* efficacy of the bencimizadoles against cystic *E. granulosus*. Although this result should be expected against tissue parasites, they were not observed by López *et al.* (1997) when testing the same PVP formulations of ABZ and RBZ against parenteral *Trichinella spiralis* encysted larvae. The data obtained show that PVP-drug formulations are specially recomended for the less soluble drugs (as MBZ), while for those more soluble (as ABZ and RBZ) the pharmacokinetical differences should be almost negligible and their anti-cyst activity should have no changes.

The anomalous pharmacokinetic results obtained by us in relation to the RBZ formulations are probably due to the small differences found between them. RBZ is the most soluble drug and so the election of a solution dosage form instead of a suspension form is less critical with regard to the pharmacokinetical characteristics than with the other less soluble drugs, MBZ and ABZ.

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Reçu le 27 janvier 2003
Accepté le 9 septembre 2003