

## In vivo effect of albendazole and mebendazole on hydatid cyst of mice

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### ABSTRACT

In current study, a few sheep cystic livers and lungs were obtained from Mashhad slaughter house and protoscolex were separated aseptically. Thirty 6-week-old Swiss mice were divided into 5 groups and two thousands of live protoscolices were inoculated intraperitoneally in each mouse. In group 1 and 2 (prophylactic groups), mice were given 150 mg/kg of oral albendazole and mebendazole for 10 days. In group 3 and 4 (treated groups), mice were treated orally with 300 mg/kg of albendazole and mebendazole for 22 days with an interval of 2 days after every 4 days of treatment 6 months after inoculation. The control group (group 5), was sham injected with normal saline. Mice were killed after 7 months and internal organs were observed for hydatid cyst. In group 1, 2 cysts were observed in the liver of mice. In this group, although albendazole did not prevent cyst formation, but the number and also the size of the cysts were lower and smaller than the control group. In group 2, 3 cysts were observed in internal organs. In group 3, there was no cyst in internal organs and so in this group it is concluded that albendazole prevented cyst formation. In group 4, 1 cyst was observed on the liver of mice. In control group a lot of cysts were observed in internal organs of mice and the average size of cysts were bigger than prophylactic and also treatment groups.

**Keywords:** Hydatid cyst, Albendazole, Mebendazole, Mouse

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### INTRODUCTION

Cystic echinococcosis (CE) caused by the metacestode (larval) stage of *Echinococcus granulosus* (*E. granulosus*) is still an important public health concern in many countries of the world, such as Mediterranean and South American countries. The disease affects humans as well as domestic livestock including cattle, sheep, camels, pigs, horses and others (Torgerson & Budke 2003). In the early 1970s, benzimidazole

methylcarbamates derivatives (BZD) were proved to be effective against *E. granulosus*, and since then, many investigators have used albendazole (ABZ) and mebendazole (MBZ) for the treatment of human hydatidosis (Teggi *et al* 1993). Albendazole and mebendazole are considered to be equally effective. When evaluated up to 12 months after initiation of benzimidazole treatment, 10%–30% of patients exhibit cyst disappearance, 50%–70% shows degeneration of cysts and 20%–30% show no morphological changes in the cyst appearance. Continuous or intermittent treatment

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with albendazole is recommended for a period of 6 months (Kern 2003). At present time, benzimidazole methylcarbamate compounds such as albendazole and mebendazole have been used to treat CE in humans (El-On. 2003). Mebendazole was the first compound widely used (Bekhti *et al* 1997). Although effective, the main disadvantage was the extremely high doses and prolonged administration period required to achieve a satisfactory clinical efficacy. Albendazole was therefore a major breakthrough because, while absorption of the parent compound remained poor, its metabolite, albendazole-sulphoxide (ABZSO) was shown to be an active anthelmintic agent (Horton. 2003). Both ABZ and ABZSO have been shown to be active against protoscolices of *E. granulosus* in *in vitro* culture systems. However, the *in vitro* protoscolicidal action of ABZ and ABZSO is very slow and requires a longer incubation period compared to that observed for MBZ (Pérez-Serrano *et al.*, 1994). *In vitro* and *in vivo* differences in the protoscolicidal activity of BZD anthelmintics have been observed. The significance of the effects of albendazole, mebendazole has been discussed by a few researchers (Pérez-Serrano *et al* 2001, Rodríguez-Cabeiro *et al* 1989, Stettler *et al* 2004). The aim of the present work was to determine *in vivo* protoscolicidal effect of albendazole and mebendazole against *E. granulosus* in mouse.

## MATERIALS AND METHODS

*E. granulosus* hydatid cysts containing protoscolices were removed under aseptic conditions from infected sheep with hydatid cysts presented for routine slaughter in abattoirs in Mashhad. This study has been conducted in 2008. Briefly, the hydatid cysts (2–5 cm in diameter) were cut open and vesicle fluid (containing protoscolices) was separated from the metacestode tissue and host adventitia. Protoscolices were

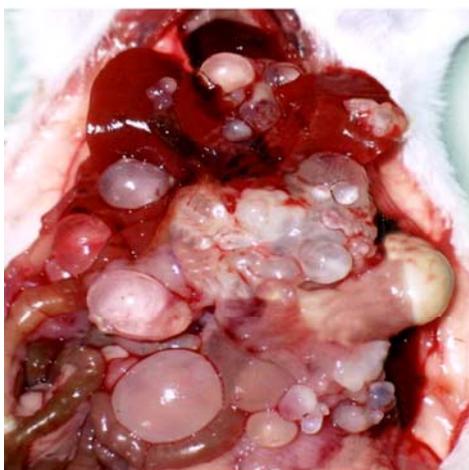
collected aseptically from cysts and were maintained in Hank's Balanced Salt Solution (HBSS) containing penicillin (500 IU/ml), streptomycin (500 IU/ml). Prior to inoculation, the collected protoscolices were rinsed 2-3 times with HBSS containing antibiotics. Viability of the protoscolices was confirmed by visual inspection through inverted microscope. The protoscolices were resuspended in HBSS, and 2000 of protoscolices in 0.5 ml of HBSS were inoculated intraperitoneally in each mouse. Male and female mice weighing 20–22 g were used in these studies.

Thirty mice were inoculated with protoscolices of *E. granulosus* and divided into 5 groups. Two prophylactic groups, just after challenge with protoscolices, received 150 mg/kg BW /day albendazole (group 1) and/or mebendazole (group 2) orally for 10 days. Two other groups, as treatment groups, 6 months after challenge with protoscolices were treated orally with 300 mg/kg of albendazole (group 3) and mebendazole (group 4) every 4 days with an interval of 2 days for totally of 24 treatment days (six times). The fifth group animals were assigned as control and mice were sham injected with normal saline. All mice were sacrificed after 7 months and internal organs were observed for hydatid cyst. For histopathological study, tissue samples were taken from liver of control and test mice. Samples were fixed in buffer formalin 10% and then histopathologic section was prepared by routine method. Samples were stained by hematoxylin and eosin and finally were observed microscopically.

## RESULTS

All the mice were killed 7 months after the inoculation and internal organs were inspected for hydatid cyst(s). In group 1, which received albendazole for 10 days, 2 cysts were observed on liver of mice. The size of cysts of this group was smaller than the size of the cysts in internal organs

of the control group. In group 2, which received mebendazole for 10 days, 3 cysts were observed in internal organs and the size of cysts was bigger than the group 1, but smaller than the control group. In group 3, which treated with albendazole for 22 days, no cyst was found in the internal organs (Figure 1).



**Figure 1.** Treated group with albendazole. None of mice had viable hydatid cysts in internal organs (Above). Hydatid cysts in internal organs of mice in control group (Below).

Group 4, animals which were treated with mebendazole for 24 days, just one cyst was observed on the liver of mice. In control group (Figure 1) a high number of cysts were observed in

the internal organs of all mice and the average size of cysts were about 6.5mm and bigger than prophylactic and treated animals with both drugs. Histopathological study of the cyst showed that typical double layered wall and in some cases with clear fluid inside (Figure 2). Some samples revealed a precystic structure, which consisted of connective tissue and scattered inflammatory cells.

## DISCUSSION

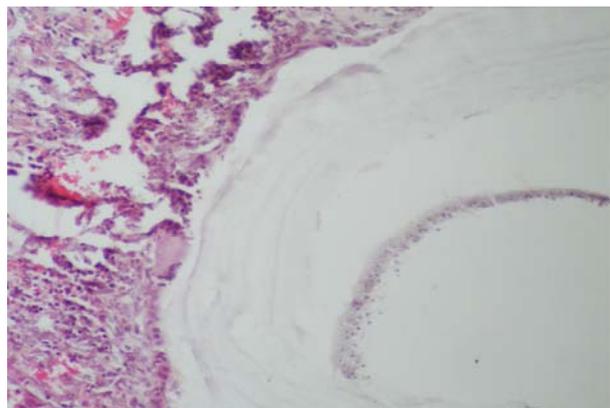
In the present study, the efficacy of albendazole and mebendazole in the prevention of hydatid cyst in mice was conducted. In a study by (Keshmiri *et al.*, 2001) the effect of albendazole on hydatid cysts of human has been evaluated. Twenty-nine patients with 240 cysts received albendazole (400 mg twice a day, in 3 cycles of 6 weeks long with 2 weeks off between cycles). Some patients with liver cysts after treatment showed increasing heterogeneity and density suggestive of inactive cysts. Patients with larger cysts and those with pulmonary involvement were better responders. The observed results are encouraging, showing high efficacy of albendazole for the treatment of hydatidosis and should be offered to patients as an alternative before surgical treatment is considered. In comparison with the results of (Keshmiri *et al* 2001), our results showed that when mice were treated with albendazole, no cysts were found in internal organs and this result indicated that Albendazole, a more recently developed benzimidazole, is more effective than mebendazole as it is considered by (Anadol *et al* 2001, Schipper & Kager 2004, Shaw *et al* 2006). It has been reported that continuous long term albendazole therapy in animal models is parasiticidal against larval stage of *E. multilocularis* especially in the early stages of infection (Liu *et al* 1998). The effectiveness of albendazole (ABZ) and albendazole sulphoxide (ABZ.SO) and ABZ +

ABZ.SO treatment in mice infected with *Echinococcus granulosus* protoscolices has been described. The results indicated that ABZ and ABZ + ABZ.SO had an important effect upon larval growth in *E. granulosus* (Perez-Serrano *et al.*, 1997). The efficacy of Ivermectin (IVM) alone, albendazole (ABZ) alone and a combination of IVM plus ABZ against *Echinococcus granulosus* protoscolices was studied by means of an *in vitro* incubation. The maximum protoscolicidal effect was detected when combination of IVM+ABZ were used (Casado *et al* 2002). It has been reported that protoscolices of *E. granulosus* were incubated *in vitro* with praziquantel (PZ), albendazole (ABZ), or a combination of both (PZ + ABZ). PZ and ABZ displayed slower protoscolicidal activity when applied separately than when used in combination. The PZ + ABZ treatment was effective only against small cysts, which had collapsed at 10 days postinoculation (Urrea-Paris *et al* 2000).

It has been reported by (Dvoroznakova *et al* 2004), that the reduction of cyst growth after treatment with ABZ and liposome.ABZ was similar up to week 4 after last dose, but the parasitostatic effect of liposome. ABZ lasted 4 weeks longer than the effect of free drug. At the present time, albendazole is considered the treatment of choice for echinococcosis in human (Shuhua *et al* 2002). Feng *et al* (1995) have shown that when infected mice were treated with 25 mg/kg/day of mebendazole, for 14 days, the glutathione S-transferase (GST) activity of both collapsed and full cyst walls were inhibited by 30.1% and 26.8%, respectively. Albendazole (300 mg/kg/day) for 14 days had no apparent effect on GST of *E. granulosus* cyst wall and it has been suggested that the inhibition of GST activity in the cyst wall induced by mebendazole might damage the defense system of the parasite.

In another experiment by (Daniel-Mwuambete *et al* 2003), two different preparations, solution and suspension, of three benzimidazole carbamate

drugs (mebendazole, albendazole & ricobendazole) were compared by analyzing their *in vivo* activity against *Echinococcus granulosus* cysts in a mouse model. The effect was more prominent on mebendazole-treated mice, at doses of 25-50 mg/kg. It has been reported by (Anadol *et al* 2001) that although mebendazole, the first benzimidazole used, has some beneficial effects on the disease in selected patients, it has also been associated with treatment failure in some cases, perhaps because of its poor absorption. It has shown by (Nakaya *et al* 1998) that hydatid cysts were severely damaged in mice treated with mebendazole and new vesicles did not develop around the damaged ones.



**Figure 2.** Microscopic cyst in liver of mice. The arrows indicate microscopic cyst and tissue reactions around cyst.

Also, hydatid cysts reappeared after treatment with mebendazole was terminated. In another experiment by (Ammari & Omari 2002), they indicated that the postoperative prophylactic course of mebendazole is reliable, safe and with minor side effects and the recurrence rate of the disease was reduced to the lowest possible levels.

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