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*Echinococcus granulosus*: EVALUATION OF  
PROTOSCOLICIDAL ACTIVITY OF SEVERAL  
SUBSTANCES

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ABSTRACT

Surgical treatment of hydatid disease requires the use of protoscolicidal substances to prevent secondary infection of the patients. The most commonly used substance in Chile is Na Cl (20%) due to its low frequency of adverse effects. We tested the protoscolicidal activity of two benzimidazole drugs, albendazole and mebendazole, currently used for chemotherapy of hydatidosis disease in humans. Bovine and human protoscolices were used in the test. Vitality of protoscolices was evaluated by Evans blue vital staining. Isolated protoscolices were exposed to several concentrations of albendazole and mebendazole for 10 min. Protoscolices were also exposed to hypertonic NaCl (20%) solution for the same time. In protoscolices obtained from cattle, mebendazole at 300 µg/ml produced the highest mortality (72.5%), followed by albendazole at 120 µg/ml with a mortality of 69.5%. Hypertonic NaCl produced a 3.8% mortality. Protoscolices obtained from two patients were less susceptible to the treatments. Mebendazole at 600 µg/ml produced a 12% mortality, and albendazole at 120 µg/ml produced the highest mortality (14%). Untreated protoscolices remained viable during the test (98 to 100%). Under these conditions, the benzimidazolic drugs were better protoscolicidal agents than Na Cl 20% and they were also more effective on the bovine cysts protoscolex than on those isolated from humans. Further evaluation of benzimidazoles and other protoscolicidal substances should be carried out on parasites obtained from human patients.

**KEYWORDS:** *Echinococcus granulosus*. Hydatid disease. Protoscolicidal, albendazole, mebendazole.

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## INTRODUCTION

Hydatid disease is a serious parasitic zoonosis produced by the larval stage of *Echinococcus granulosus*, when humans become an accidental intermediate host. The most affected countries in the world are those with economies based on livestock farming coupled with less than adequate sanitary infrastructure (Sapunar, 1998). The life cycle of the parasite depends directly on the close relationship between dogs, ruminants and people, which exists in the southern regions of South America, Australia, extended regions in Asia and several Mediterranean countries (Tamames, 1997).

Chile is one of the affected countries in South America. Human hydatidosis prevalence is 1.9 per 100,000 with a mortality rate of 0.3 per 100,000 (Serra et al., 1996). The only highly effective treatment is surgery; when this is not possible, patients receive chemotherapy with benzimidazole drugs. Surgery is carried out after the infection has been confirmed and the cysts localized, usually in liver or lungs. Surgery is performed only in patients in good physical conditions. The surgical procedure requires the use of substances to neutralize protoscolices that may contaminate the surgical area during the cyst removal and prevent secondary infections. Several products have been used, which show different levels of effectiveness. Some are toxic for the patient. Sapunar et al (1989) proposed the use of 30% NaCl solution for 10 min. Other authors (Ben Amor et al, 1986; Gargouri et al, 1990; Khuroo et al, 1991) recommended the application of a 20% solution for 7 to 10 min. Other chemicals used include solution of 2% formalin for 5 min, silver nitrate and 95% ethanol injected inside the cyst (Belghiti et al, 1986; Houry et al, 1990; Filice et al, 1991, Morris and Richards, 1992; Pawlowski, 1993, WHO 1996). Hydrogen peroxide, teacridine lactate, 0.5% cetrimethyl ammonium bromide (Cetrimid<sup>®</sup>) for 1 min, and 10% iodine povidone have also been reported to be effective in neutralizing the parasite (Morris and Richards, 1992; Pawlowsky, 1993; Tamames, 1997; Landa et al, 1997). For the treatment of the hydatidic cyst the technique of Punction Aspiration Instillation Reaspiration (PAIR) has been used (WHO, 1996) that includes ultrasound guided percutaneous punction of the cyst, aspiration of a substantial quantity of the liquid in the cyst, injection of proescolicidal substances as ethanol 95% or Na Cl 20% for 15 minutes at least and subsequent aspiration of the content of the cyst. Nevertheless, this surgical technique is not in use in our country.

In Chile, 20% NaCl solution is currently preferred as protoscolicidal treatment during hydatid disease surgery. The product is recommended because it is safe and inexpensive. The solution is injected in the visible portion of the cyst, estimating the volume to be injected by its size, usually 5 to 30 ml. The cyst is removed from the organ after a 10 minute interval. However, it has been reported (Landa J et al., 1997) that a significant number

of protoscolices removed from the treated cysts are viable, representing a risk for the patient.

For this reason, we examined the protoscolicidal activity of two benzimidazole derivatives, albendazole and mebendazole. These drugs are utilized in chemotherapy of hydatid disease patients who are weakened by the infection preventing surgical intervention. The study compared the effectiveness of albendazole and mebendazole solutions against 20% NaCl solution on isolated protoscolices obtained from cattle and two patients.

## MATERIAL AND METHODS

The study was conducted from March to December 1998. Protoscolices from bovines were isolated from livers and lungs removed from cattle in the local slaughterhouse (Lo Valledor, Santiago, Chile).

Protoescolices from the patients were obtained from The Salvador Hospital and Thorax National Institute of Santiago, Chile.

The animal organs were washed with tap water to remove the blood. The cysts were removed from the host's tissue and examined in the laboratory. The fertility of the cysts was determined by microscopic examination (25X) to determine the presence of protoscolices in the liquid phase or on the germinative membrane.

The vitality of the protoscolices was measured using vital staining method; 1/1.000 Evans blue was diluted in Phosphate Buffer Saline (PBS) pH 7.2 (Pauluzzi et al, 1964). The cyst fluid was collected and centrifugated at 2,000 rpm for 5 min using 50 ml plastic tubes. The pellet was washed three times with 9‰ NaCl and weighed. Untreated protoscolices were re-suspended in sterile saline, stained with Evans blue solution (1:1) and observed under the microscope at 40X. To evaluate the vitality, 100 protoscolices were counted in triplicate. Dead parasites stained blue. Vitality was expressed as the mean of three counts (% live protoscolices).

The protoscolicidal activity of the products was evaluated by exposing 100 mg of pelleted parasites to mebendazole, albendazole and NaCl 20% for 10 min and the procedure was conducted in triplicate including the following concentrations of benzimidazole compounds: albendazole at 3, 15, 30, 60, 120 µg/ml and mebendazole at 100, 200, 300, 600, 1,200, 3,000, 6,000 and 60,000 µg/ml.

These concentrations were chosen according to the useful plasmatic concentrations described for each one of the drugs. The counting of dead or living protoscolices were recorded after the 10 min time of exposure. The pH of each solution was measured at the beginning of the test and at 10 min to evaluate possible changes during the incubation with the drugs or sodium chloride solution.

The exact test of Fischer was used for statistical analysis for differences of percentages in pairs of samples and was considered significant when a probability of less of 5% of occurrence was obtained.

## RESULTS AND DISCUSSION

Evans blue staining differentiated dead from viable protoscolices. The dye stained the structures located inside the parasite and live parasites will remain colorless. The results obtained (Table 1-2) are summarized in Figure 1.

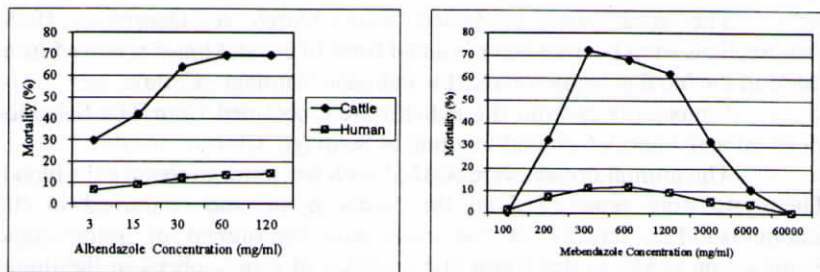


Figure 1. Mortality *in vitro* of *Echinococcus granulosus* after exposure to albendazole and mebendazole

During the observation period, control (untreated) protoscolices showed a viability ranging from 98 to 100%. Albendazole at 3 to 120  $\mu\text{g/ml}$  reduced the vitality of the bovine treated protoscolices ranging from 29.7 to 69.5%, and 6.5 to 14.3% in human treated protoescolices respectively. Mebendazole at 100 to 300  $\mu\text{g/ml}$  showed effectiveness ranging from 0.2 to 72.5% in bovines and 1.5 to 11.7% in humans respectively. Higher concentrations (600 to 60,000  $\mu\text{g/ml}$ ) of this drug produced a reduced effectiveness ranging from 68 to 0% in bovines and 9.1 to 0% in human respectively.

NaCl (20%) effectiveness in bovine parasites was low (3.8%) as well as on those of human origin (3%). This minimal effectiveness is a serious concern since this solution is used routinely for this type of surgery in Chile. The test was conducted following the same protocol applied in the operating room and if the time of exposure is reduced for any reason, the effectiveness may decrease to nearly zero. In addition, adverse reactions have occurred due to exposure of the patient to NaCl. It is also necessary to recommend a close follow-up of the patients after surgery to determine possible secondary echinococcosis.

Table I. Mortality *in vitro* of *Echinococcus granulosus* protoscolices obtained from cattle after exposure to 20% NaCl and various concentrations of albendazole and mebendazole using Evans blue viability test.

Tube	Treatment	Mean Viability Control (%)	Mean Live (%)	Mean Dead (%)
1	20% NaCl	100	96,2	3,8
	Albendazole µg/ml			
2	3	100	70,3	29,7
3	15	100	58,2	41,8
4	30	99	36,0	64,0
5	60	98	30,8	69,2
6	120	100	30,5	69,5
	Mebendazole µg/ml			
7	100	100	99,8	0,2
8	200	100	67,4	32,6
9	300	100	27,6	72,5
10	600	100	32,0	68,0
11	1,200	100	38,0	62,0
12	3,000	100	68,1	31,9
13	6,000	100	89,6	10,4
14	60,000	100	100	0

p ≥ 0.01

Table II. Mortality *in vitro* of *Echinococcus granulosus* protoscolices obtained from two patients after exposure to 20% NaCl and various concentrations of albendazole and mebendazole using Evans blue viability test.

Tube	Treatment	Mean Viability Control (%)	Mean Live (%)	Mean Dead (%)
1	20% NaCl	100	97,0	3,0
	Albendazole µg/ml			
2	3	100	93,5	6,5
3	15	100	91,2	8,8
4	30	100	87,9	12,1
5	60	100	86,5	13,5
6	120	100	85,7	14,3
	Mebendazole µg/ml			
7	100	100	98,5	1,5
8	200	100	93,0	7,0
9	300	100	88,9	11,1
10	600	100	88,3	11,7
11	1,200	100	90,9	9,1
12	3,000	100	94,9	5,1
13	6,000	100	96,2	3,8
14	60,000	100	100	0

p ≥ 0.01

In humans mebendazole at 600 µg/ml produced a 12% mortality, and albendazole at 120 µg/ml the highest protoscolices mortality (14%). Untreated protoscolices remained viable during the test (100%). The difference was statistically significant to the Fischer test.

Benzimidazole treatment of the protoscolices resulted in significantly higher mortality of the protoscolices, without reaching 100%. Mebendazole at a concentration higher than 600 µg/ml showed a reduced effect against the protoscolices.

These studies should be continued in order to identify other substances that can achieve 100% mortality of the protoscolices, to prevent reinfection of the patient during surgery.

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