



## Preoperative Albendazole and Scolices Viability in Patients with Hepatic Echinococcosis

Carlos Manterola, M.D., Ph.D.,<sup>1,2</sup> Juan A. Mansilla, M.D.,<sup>1</sup> Flery Fonseca, M.Sc.<sup>3</sup>

<sup>1</sup>Department of Surgery, Faculty of Medicine, Universidad de La Frontera, Casilla 54-D, Temuco, Chile

<sup>2</sup>CIGES (Training, Research and Management for Health based on Evidence), Faculty of Medicine, Universidad de La Frontera, Casilla 54-D, Temuco, Chile

<sup>3</sup>Department of Pre-clinical Sciences Faculty of Medicine, Universidad de La Frontera, Casilla 54-D, Temuco, Chile

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**Abstract.** To determine the plasmatic and intracystal concentrations of albendazole sulfoxide (AS) and correlate them with the viability of the scolices in patients surgically treated for hepatic hydatid cysts (HHC) that received albendazole preoperatively, as an indirect way of evaluating the scolicide efficacy of the drug. A non-consecutive series of patients with uncomplicated HHC, underwent operation at the Department of Surgery, Regional Hospital of Temuco, Chile, between 2001 and 2002. The patients were given 10 mg/kg/day of albendazole for 4 days prior to the surgery. Intraoperative samples of venous blood and hydatid fluid were taken, in which the plasmatic concentration (PIC) and intracystal concentration (ICC) of AS were measured by means of high-performance liquid chromatography. With the remaining hydatid fluid, the viability of the scolices was examined. The following variables were taken into consideration: diameter, type of cyst, number of cysts, and development of cyst–biliary communications. Descriptive statistics were used in the calculation of medians, averages, and standard deviations, and analytical statistics were used for the comparison of continuous variables applying the *t*-test and the Mann-Whitney *U*-test. A total of 26 patients with HHC, with a median age of 39.5 years (range: 16–80 years); 16 were women (61.5%). Nineteen patients presented only one cyst (73.1%), and the diametric median of the cysts was 14.5 cm (range: 7–30). Fourteen patients presented univesicular cysts (53.9%), and the remaining 12 had multivesicular cysts (46.1%). The average PIC of AS in the whole series was  $1.88 \pm 0.5 \mu\text{g/ml}$  and the ICC was  $0.26 \pm 0.2 \mu\text{g/ml}$ . The ICC in viable hydatid cysts was  $0.25 \mu\text{g/ml}$  versus  $0.28 \mu\text{g/ml}$  in non-viable cysts ( $p = 0.7556$ ). The absence of association between intracystal levels of AS and the viability of the scolices allows one to posit indirectly that albendazole is ineffective as a scolicidal agent administered preoperatively for 4 days.

Echinococcosis is a zoonosis of great importance in Chile because it generates public health, economic, and social problems. It is estimated that the national prevalence is 6.3 per 100,000 inhabitants [1], however, in the foothill areas of the Andes mountains in the south of the country, the prevalence has reached 48 per 100,000 inhabitants [2, 3].

The treatment of choice for hepatic hydatid cysts is surgery, using any of various techniques. The contribution of anthelmintic drugs such as albendazole [3, 4] and c/s/ benzimidazo carbamate, has been described. These block the capture of glucose on the part of the susceptible parasites in both the larvae and adult stages, exhausting their glycogen reserves and thus diminishing the formation of ATP. As a result, the parasite is immobilized and dies [5].

Albendazole has been used to avoid relapses in cases of both multiple and large cysts [6]. Therapies vary in dosage, duration and administration. It has been used pre operatively and post-operatively for days or months [1, 7, 8]; however, its efficacy and usefulness are not entirely clear.

The object of this study is to determine the plasmatic concentration and intracystal concentration of AS (the active metabolite of albendazole) and to correlate them with the viability of the scolices in patients treated surgically for hepatic hydatid cysts who received albendazole preoperatively, as an indirect way of evaluating the scolicidal efficacy of the drug.

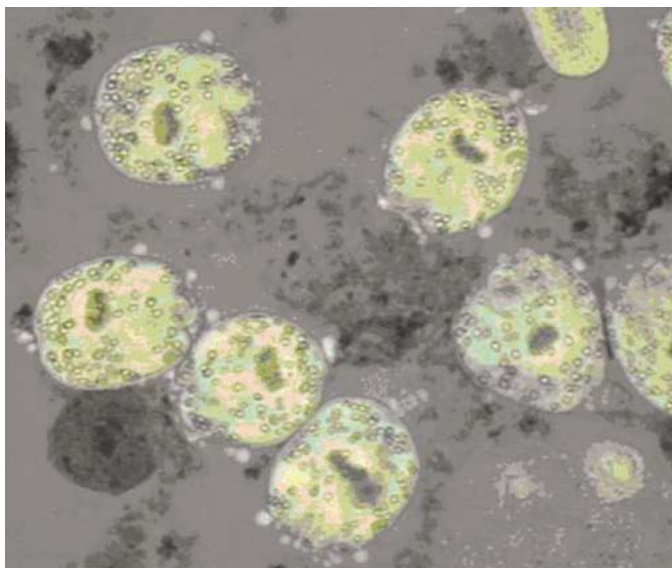
### Materials and Methods

#### Study Design

The study was designed as a prospective and non-consecutive series of cases.

#### Study subjects

Patients with hepatic hydatid cysts, treated preoperatively with albendazole and operated in the Surgery Service at the Regional Hospital of Temuco, Chile, between March 2001 and December 2002 were studied. Subjects excluded were those hypersensitive to albendazole (with the antecedent of having used albendazole previously and having developed problems that could be associated with drug intake) c/s/. Because of the high prevalence of echinococcosis in our region, is not infrequent to treat people who have been treated previously for hydatid disease (including use of



**Fig. 1.** Trypan blue dye at 1.5%. Here viable scoleces of normal morphology can be seen with their intact membrane.

mebendazole or albendazole), especially during childhood, pregnancy, or breast-feeding. This study included patients with calcified hepatic hydatid cysts, those with hepatic abscess of hydatid origin [9], those with an incomplete preoperative albendazole treatment, and those who had received previous treatment with albendazole or other anthelmintic drug.

#### Procedure

All patients in the study were given a dosage of 10 mg/kg/day of albendazole, divided into three doses, for 4 days prior to the surgical intervention.

#### Protocol

During the surgical intervention, samples of 10 cc of peripheral venous blood and 10 cc of hydatid fluid were taken from the center of the cyst before the surgical intervention was completed. The hydatid fluid was stored in a sterile receptacle at room temperature for later analysis of its general characteristics and for viability of the scoleces in each of the specimens. The viability was studied by means of direct observation under a light microscope and Trypan blue dye at 1.5%. A total of 100 scoleces per field were observed under a microscope to this end (Fig. 1). For the purposes of this study, the cyst was considered viable when more than 30% of the scoleces observed were viable [10–12].

#### Determination of AS Concentrations

The blood and hydatid fluid samples were centrifuged and subsequently frozen at  $-20^{\circ}\text{C}$  for a later analysis, which consisted of the determination of SA by high-performance liquid chromatography (HPLC). Prior to this analysis, all samples—the plasma as well as the hydatid fluid—were purified through Sep-Pak C-18 filters. Samples were subsequently passed through  $0.45\text{-}\mu\text{m}$  filters again. For the AS determination by HPLC, a Merck-Hitachi L-

6200 with intelligent pump was used as well as an L-4200 UV/V is detector, using a computational integrator; an acidic water: an acetonitrile mixture 40:60% v/v was eluted to a flow of 1 ml/min. In order to confirm the peak of AS, a reference standard was used in which the calibration curve was performed and determined at 290 nm. The reference standard was obtained from 10,200 mg albendazole tablets that were weighed and ground in a mortar, taking the average of the 10 tablets. Thus 100 mg of albendazole were obtained and then diluted in 2 ml of formic acid. Then 50 ml of this solution was finished with HPLC methanol, and the pertinent dilutions were performed through which a known concentration of the drug at a determined weight could be obtained [13–15].

#### Other Variables Studied

The following variables were also considered: diameter (using the 50-percentile to dichotomize the variable), type of cyst (univesicular and multivesicular), the number of cysts (one and two or more), and the development of cyst-biliary communications (yes or no). Furthermore, an evaluation was carried out on all patients of hematological variables and hepatic function before and after the conclusion of the study.

#### Ethical Aspects

The study was approved by the Ethics Committee of the Faculty of Medicine at the Universidad de La Frontera. The patients consented voluntarily to participate in the study, maintaining their anonymity.

#### Analysis Plan

Descriptive statistics were used in the calculation of medians, averages, and standard deviation, and analytical statistics were used for the comparison of continuous variables applying the *t*-test and the Mann-Whitney *U*-test.

#### Results

Twenty-six patients with hepatic hydatid cysts were studied in this way, with a total of 34 hepatic hydatid cysts. The median age of patients in this series was 39.6 years (range: 16–80 years); 16 (61.5%) were women and 10 (38.5%) were men. Nineteen patients presented only one cyst (73.1%), and the median of the major diameter of all cysts was 14.5 cm (range: 7–30cm). Fourteen patients presented univesicular cysts (53.9%), and the remaining 12 had multivesicular cysts (46.1%).

It was verified that 21 of the 34 cysts studied (61.8%) were viable. The average plasmatic concentration of AS in the entire series was  $1.88 \pm 0.50 \mu\text{g/ml}$ ; and the intracystal concentration of  $0.26 \pm 0.20 \mu\text{g/ml}$ .

Through analysis of the subgroups, it could be established that the average intracystal concentration of AS in viable hydatid cysts versus those considered non-viable was  $0.25 \pm 0.2 \mu\text{g/ml}$  and  $0.28 \pm 0.2 \mu\text{g/ml}$ , respectively ( $p = 0.7556$ ). On the other hand, upon comparing the average intracystal concentration of AS in univesicular cysts and multivesicular cysts, the figures  $0.29 \pm 0.2 \mu\text{g/ml}$  vs.  $0.22 \pm 0.2 \mu\text{g/ml}$ , respectively ( $p = 0.5136$ ) were verified. An association with the variables of diameter, number of

**Table 1.** Association between the intracyst concentration of AS and the variables studied (N = 26) (*t*-test and U of Mann Whitney)

Variable	Albendazole sulfoxide intracyst concentration (ug/ml) Mean $\pm$ SD	<i>P</i>
Type of cyst		0.5136
Univesicular cysts ( <i>n</i> = 14)	0.29 $\pm$ 0.29	
Multivesicular cysts ( <i>n</i> = 12)	0.22 $\pm$ 0.24	
Viability		0.7556
Alive ( <i>n</i> = 16)	0.29 $\pm$ 0.20	
Dead ( <i>n</i> = 10)	0.25 $\pm$ 0.21	
Number of cysts		0.811
One ( <i>n</i> = 19)	0.23 $\pm$ 0.20	
Two or more ( <i>n</i> = 7)	0.36 $\pm$ 0.40	
Biliary communications		0.2628
No ( <i>n</i> = 10)	0.34 $\pm$ 0.30	
Yes ( <i>n</i> = 16)	0.22 $\pm$ 0.21	
Diameter of the cyst		0.1214
7 a 14 cm ( <i>n</i> = 13)	0.35 $\pm$ 0.31	
14.1 a 30 cm ( <i>n</i> = 13)	0.18 $\pm$ 0.20	

**Table 2.** Association between the plasma concentration of AS and the variables studied (*n* = 26; *t*-test)

Variable	Albendazole sulfoxide plasma concentration ( $\mu$ g/ml) Mean $\pm$ S.D.	<i>p</i> value
Type cyst		0.7036
Univesicular cysts ( <i>n</i> = 14)	1.84 $\pm$ 0.49	
Multivesicular cysts ( <i>n</i> = 12)	1.93 $\pm$ 0.59	
Viability		0.8789
Alive ( <i>n</i> = 16)	1.89 $\pm$ 0.61	
Dead ( <i>n</i> = 10)	1.86 $\pm$ 0.40	
Number of cysts		0.4494
One ( <i>n</i> = 19)	1.83 $\pm$ 0.54	
Two or more ( <i>n</i> = 7)	2.01 $\pm$ 0.50	
Biliary communications		0.9798
No ( <i>n</i> = 10)	1.92 $\pm$ 0.53	
Yes ( <i>n</i> = 16)	1.86 $\pm$ 0.54	
Diameter of the cyst		0.8584
7 a 14 cm ( <i>n</i> = 13)	1.86 $\pm$ 0.57	
14.1 a 30cm ( <i>n</i> = 13)	1.90 $\pm$ 0.50	

cysts, or the existence of biliary communication was not verified (Table 1). Nor was any association found between the plasmatic concentrations of AS with those variables previously mentioned in the study (Table 2). Neither adverse reactions to the drug nor any alteration in the hematological or hepatic function variables were reported.

## Discussion

The number of cases studied seems small for the time over which the study was conducted; however, it is important to note that various patients undergoing operation during this time period were not considered because they presented some of the aforementioned exclusion criteria. This selection makes the difference with the majority of studies where treatment with albendazole has been evaluated and those studies that have included patients with hydatid cysts in different anatomical locations (lung, abdominal cavity, and pelvis) [16].

Of the studies that deal with medical therapy for the treatment of hepatic hydatid cysts, two kinds have been published to measure plasma concentration of albendazole: one using HPLC

and another using electrophoresis. The idea of using HPLC arose because it is a simple method of analysis and is not limited by the volatility or instability of the compound of the sample. This method is used primarily for the separation of the sample's components, in which these are distributed in two phases: one stationary, the other mobile [13, 14, 17]. By using this method the AS could be separated, isolated, and quantified.

Another important point in the methodology of the analysis is the purification of the samples prior to performing the HPLC. This was carried out in order to avoid the characteristic contamination of each sample, in particular those from hydatid fluid, as many of those samples presented remains of membrane, figurative elements, and impurities. Without the purification steps, there could have been interference in the measuring, such as not achieving an adequate advance of the sample of the chromatographic column or producing an obstruction of the same.

The absorption albendazole is variable and irregular, although it can improve if it is consumed with fatty foods. From our experiment, we could claim that plasma concentration as well as intracyst concentration were lower than reported elsewhere [18, 19]. Furthermore, the intracyst concentration was a little bit less than 15% of the plasmatic.

With regard to the viability of cysts, the same criteria were used as defined in a previous report [12]. The degree of viability was calculated according to the number of living scolices per field from a total of 100 scolices observed under the microscope. The cut-off point chosen (arbitrarily) for viability (30% living scolices) was chosen with the aim of obtaining the greatest margin of potentially infecting scolices. In spite of this, only 60% of the cysts could be considered viable. This fact concurs with previously reported findings [12]. This finding leads us to conclude that albendazole has limited efficacy as an anthelmintic, at least in its preoperative administration and at the dosage used. Similar studies have used more prolonged schemas, but they did not study the viability of the scolices [8, 16, 19]. Perhaps the efficacy of preoperative albendazole should be evaluated with these types of schemas but using the methodology previously described so as to be able to compare the results in groups of similar people but with different levels of exposure to the drug in such a way that the real usefulness of preoperative albendazole can be better defined. Nevertheless, more prolonged therapies present some inconveniences related to costs, adverse reactions and their consequences (those described in up to 15% of patients) [16]. Therefore, when prolonged schemas are applied (3 or more cycles of 30 days), there must be strict control over the hematological parameters and hepatic function, all of which must be measured at least every 15 days.

Another aspect to consider, and one that causes us to reflect on the usefulness of albendazole, is the absence of an association between the intracyst concentration of AS and the viability of scolices. A logical hypothesis that would support the usefulness of the drug would be a greater intracyst concentration of AS; a lower concentration would be owing to the viability of scolices, a fact that this experiment at least did not show. In addition, of the little that has been done to study the "Viability of cysts" together with "levels of albendazole," the "viability was assessed with echocardiographic or computerized axial tomographic images, a fact that has been neither validated nor takes into account reproducibility studies [6, 20].

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