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The Effects of Albendazole Solution at Scolocidal Concentration in the Rat Brain

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Summary

Intra-operative cyst rupture is a catastrophic event in the intracranial hydatid cyst disease. Dissemination of the cyst contents may lead to severe anaphylactic reactions and an increased risk of recurrence.

Several scolicidal agents have been used to eradicate the infective scolices but recurrences occur and no solution has been evaluated for its adverse effects to the brain tissue. Being a specific scolocidal agent albendazole has been shown to be 100% scolicidal in vitro. In this study, we present the electrophysiological and histopathological effects of intracerebral 2% albendazole injection in the rat brain. Vascular, neuronal and glial as well as inflammatory changes were evaluated in order to detect any adverse pharmacological effects. Electrophysiological and most microscopic parameters showed no significant effects attributable to albendazole but in 25% of the albendazole group cerebral gliosis was detected whereas no gliosis was present in the control group. It is concluded that being a specific scolicidal agent albendazole offers an efficient alternative for ruptured cerebral hydatid disease, but the significance and clinical importance of the gliosis should be further investigated.

Keywords: Albendazole; cranial; cyst hydatid.

Introduction

The treatment of intracranial hydatid cyst disease is problematic. Using the ''dowling technique'', experienced surgeons can remove the cyst unruptured from superficial and noneloquent areas of brain [2, 3, 8, 18]. However, the surgical removal of cysts without rupture is often impossible, and treatment has been difficult when the cyst is closely associated with vital structures or in widely disseminated cases [2, 8, 22, 23].

Cyst rupture can cause systemic anaphylactic reactions which can sometimes be severe, or may lead to dissemination and recurrence of the disease [2, 7, 8, 18, 20, 23]. For the prevention of these complications many surgeons use topical irrigation with 3% NaCl (hypertonic saline), 10% formalin, 0.5% silver nitrate, hydrogen peroxide, 1% aqueous iodine solutions [1, 3, 13, 14, 17, 21]. To the best of our knowledge there have been no reports on the scolocidal effectiveness and safety of these solutions as to their effect on the brain itself.

In this study, the effects of the topical use of albendazole (ALB) solution at a scolocidal concentration in the rat brain was evaluated by electrophysiological (EP) and histopathological (HP) methods.

Methods and Patients

The study was performed at Ondokuz Mayıs University Faculty of Medicine. 44 adult Wistar rats, weighing 250 $\pm$ 50 g were used; the rats were formed into two main groups; 20 in for EP (10 test, 10 control) and 24 in for HP studies (16 test, 8 control). Approval of the ethics committe and surgical animal research laboratories were obtained.

Preparation of the Albendazole Solution

20 mg albendazole (kindly provided by llsan drug corp) was dissolved in one liter of isotonic saline by continuous stirring for 12 hours at room temperature by a magnetic mixer to obtain a final concentration of 2 mg/dl. By high performance liquid chromatomography analysis the final working solution was found to have 1.7 µg/ml ALB. The solution was sterilized by UV.

EP Study Group

The experiments were performed on anaesthetized (urethane 1.25 g/kg, intra peritonally) adult male Wistar rats. The left cerebral cortex was exposed by craniectomy. Ag-AgCl ball electrodes were placed over the somatomotor cortex, the common reference electrode being fixed on the pinna and electrocorticographical (ECoG) activity was recorded monopolarly with a four channel recorder. A polyethlene cannula was introduced into the right femoral artery to monitor blood pressure, which was kept above 100 mmHg All contact and incision points were infiltrated with procain hydrocloride to minimize possible sources of pain. Temperature was maintained between 36.5 and 37.5 °C with a heating pad. 5 µl ALB solution or 5 µl 0,09 NaCl was injected into the left somatomotor cortex using a Hamilton micro syringe.

HP Study Group

24 rats were used (eight for control and 16 for treatment group). The rats anaesthetized with 10 mg/kg ketamine HCl (Ketalar®) and 8 mg/kg xylazine (Rompun®) and prepared for left frontal craniectomy in the prone position, with left precoronal parasagittal incision, a craniectomy of two milimeters diameter was perfomed with a high speed drill. After the exposure of dura using a 25 G micro syringe, 5 µl of isotonic saline in the control group or 5 µl of 2% ALB solution in the treatment group was injected into the frontal cortex at a depth of approximately two milimeters. Skin was closed with 3/0 absorbable sutures. No other treatment was used.

On the third post operative day the animals were anaesthetized and total craniectomy was performed after perfusion-fixation with Hank's balanced salt solution pH 7.4 and 10% formaline and 1.5% glutheraldehyde solutions. All intracranial neural structures were fixed in 20% buffered formalin for 72 hours. With the help of a stereomicroscope injection sites were identified and, three milimeters thick coronal/sagittal brain slices were taken parallel to the injection sites. The slices were prepared according to routine tissue processing protocols and paraffin blocks were sectioned at 4–6 micrometers and stained by H&E. The slides were evaluated by double-blind technique for the presence (+) or absence (−) of signed criteria of neurons, glial matrix and supportive cells as presented below:

For neurons:	1 – Neuronal atrophy (Acute or chronic)
	2 – Neuronophagy
	3 – Chromatolysis
	4 – Vacuolization
	5 – Mineralization
For glial matrix:	1 – Necrosis
	2 – Haemorrhage
	3 – Inflammation
	4 – Oedema
For supportive cells:	1 – Gliosis
	2 – Glial necrosis
	3 – Storage materials
	4 – Microglial nodules

For each animal atrophic neurons were counted in five high-power microscope fields with ×10 ocular and ×40 objective magnifications (Nikon, Optiphot-2) and average numbers for each rat were calculated and recorded. Differences between numerical data from different groups were statistically evaluated by Mann-Whitney U test. p values less than 0.05 were considered significant.

Results

EP Findings

A total of 10 rats were treated with ALB solution (5 µl, intracortically). The ECoG activity was recorded for three hours and there was no change in recording, with respect to the control. Intracortical injection of an identical volume of physiological saline also did not affect ECoG activity (Fig. 1).

HP Findings

The microscopic evaluation with signed criteria for neurons, glial matrix and supportive cells did not show any degenerative findings such as neuronophagia, vacuolization, mineralization, necrosis, oedema and development of microglial nodules either in the control group or in the treatment group. On the other hand, while there was no gliosis in any of the control group animals, in four cases of the ALB group gliosis was observed (Fig. 2).

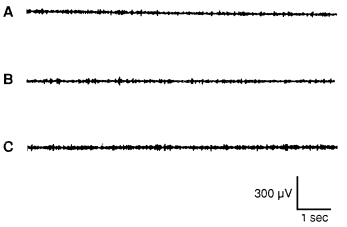

Fig. 1. Typical examples of experimental ECoG recordings before and after ALB (1,7 µg/ml, 5 µl) solution or saline injection. (*A*) Baseline. (*B*) 10 minutes after saline injection (*C*) 10 minutes after ALB solution injection

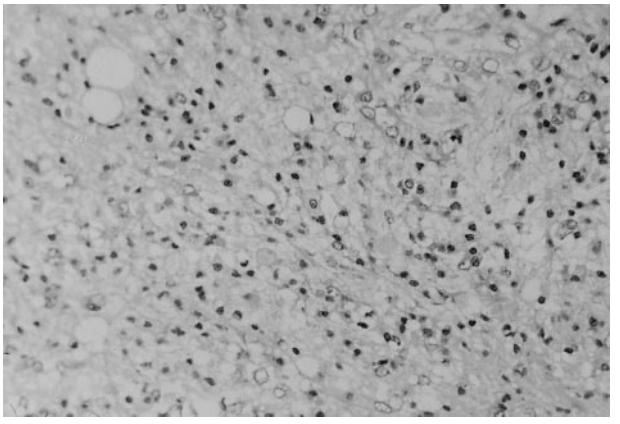

Fig. 2. Gliosis in supportive cells in the treatment group (×400 H&E)

Acute haemorrhage (without vascular damage) and inflammation were found in two cases in the ALB group and in none of the cases of the control group (Fig. 3).

Presence of variable degrees of acute and chronic atrophy in all animals of the ALB and control groups required quantitation of this parameter. The average values of each animal were evaluated statistically between the groups and no significant difference was found ($p > 0.05$).

Chromatolysis was found in 4/8 and 8/16 rats in the control and ALB groups respectively (Fig. 4).

The inspection of the storage material and foreign body showed hair pieces in the glial matrix in one animal of the control group and none in the treatment group. The results are summarized in the Table 1.

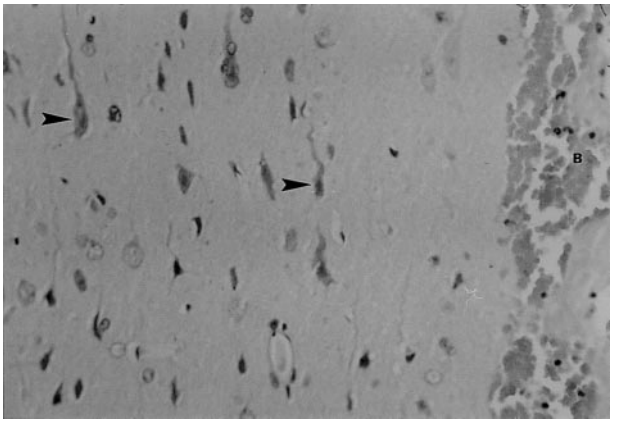

Fig. 3. Acute haemorrhage (without vascular damage) (*B*) and neuronal atrophy (×200 H&E, arrow heads)

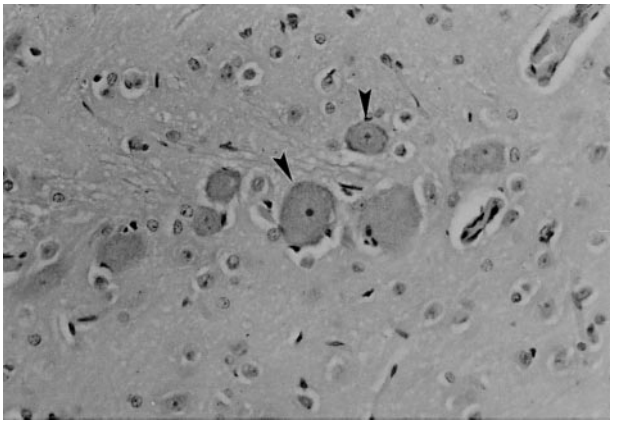

Fig. 4. Central chromatolysis in neuronal cells (×400 H&E) (arrow heads)

Table 1. *The Summarized Histopathological Findings of Experiments*

The histopathological findings		Control (n:8)	Treatment (n:16)
For neurons	neuronal atrophy	8/8	16/16
	neuronophagy	–	–
	cromatolysis	4/8	8/16
	vacualization	–	–
	mineralization	–	–
For glial matrix	necrosis	–	–
	haemorrhage	–	2/16
	inflammation	–	2/16
	oedema	–	–
For supportive cells	gliosis	–	4/16
	glial necrosis	–	–
	storage materials	1/8	–
	micro glial nodules	–	–

Discussion

1–2% of hytatid cyst cases are cerebral. The most preferable treatment is the surgical removal of the cysts without rupture, but this cannot be achieved in all patients. Erşahin *et al.* [8] reported an incidence of 37% intra-operative cyst rupture in pediatric intracranial cases. Furthermore, due to its complicated anatomy, microvesicular and invasive structure as well as obvious limitations of the surgical exposure, rupture and recurrence is a rule in spinal hydatid disease [18, 19, 23, 24]. Cyst rupture may lead to anaphylactic reactions as well as dissemination and/or recurrence of the disease. Although the only way to prevent anaphylaxis is not to rupture the cyst, dissemination and risk of recurrence may be minimalized if the infective scolices can be effectively killed. It is therefore necessary to have reliable therapeutic measures to be applied when a hydatid cyst ruptures. The most rational approach is the application of a scolocidal agent to the contaminated area; in this respect, the ideal solution should be highly effective in eliminating all infective scolices without local or systemic toxicity or adverse effects to the host. As for hydatid disease elsewhere in the body, contaminated surgical area is being treated either with hypertonic saline which probably kills scolices by dehydration or with formaline, silver nitrate, hydrogen peroxide and iodine solutions which are directly toxic although not scolex specific. Although studies on the effectiveness of these solutions are scarce, literature review reveals that no solution is both highly effective and safe. Lunardi *et al.* [15] reported 2 recurrences in 5 accidently ruptured cases which were irrigated with hydrogen peroxide or formalin solution. Furthermore, Çataltepe *et al.* [4] have used hypertonic saline solution in a series of 120 patients and have reported a recurrence rate of 26.6%. It has been argued that application of 10% formalin soaked in large cottonoids may be irritating for neural tissues [18]. ALB is a specific scolocidal agent and its long term systemic administration has been used in widespread, recurrent and inoperable hydatid disease [5, 12, 16]. ALB blocks glucose uptake of the larval and adult stages of the *Eccinoccocus* organisms, thus depletes glycogen stores and decreases ATP production which results in immobilization and death of the parasites [6, 11, 22, 25]. The ALB is poorly absorbed in the gastrointestinal tract. Although it has been reported that absorbtion of ALB from blood to tissues is high, ALB levels in the cyst fluid depends on the thickness of the cyst wall and the

presence of calcification [12, 16]. Long term oral ALB treatment has been reported to cause hepatic damage ranging from slight elevations of the liver enzymes to irreversible hepatic damage as well as fever, gastrointestinal pain and anaphylactic reactions [9, 22]. Being a specific scolocidal agent, it is rational to use ALB topically in ruptured hydatid cysts. In an in vitro study, Erzurumlu *et al.* [10] have shown that 0,5% ALB killed 45% of scolexes whereas 1% ALB solution had a 100% scolocidal effect. Taking the latter value as the minimum effective scolocidal concentration of ALB, we used a twice higher concentration to study the possible effects of the topical ALB solutions in the rat brain.

The present electrophysiological results clearly demonstrate that 5 µl 2% ALB solution did not affect ECoG activity of the rat brain. In the histopathological evaluation acute haemorrhage detected in two animals of the ALB group without accompanying vascular inflammation was attributed to the surgical technique rather than the pharmacological effects of ALB. Similarly the presence of inflammation and micro-abcesses in two animals of the ALB group most probably result from surgical contamination rather than ALB itself as these foci were closely associated with the injection sites. In both the ALB and the control groups, neuronal atrophy was detected but the differences were not statistically significant; these finding most probably reflect the direct mass effect caused by the intra-parenchymal injection and are not due to the pharmacological effects of ALB. It is remarkable that in four animals of the ALB group (25%) gliosis was noted as compared to none in the control group. Although the electrophysiological results show no functional abnormalities attributable to ALB, these tests were performed immediately after injection whereas gliosis was detected in rats sacrified three days after the injection. The clinical significance of this finding is at present obscure and how the degree and extend of the gliosis progresses in the longer term and whether long term changes in the host brain can be acceptable as compared to the scolocidal benefit gained needs further study. However, it should be stressed that a variable amount of gliosis is universally present around a cerebral hydatid cyst [18] and in the proposed surgical management ALB solution should be applied to the contaminated operative field or into the cyst rather than being injected into the brain tissue itself.

In conclusion, topical use of 2% ALB solution in ruptured cerebral hydatid cysts is a promising alternative for control of the recurrence and dissemination of the disease but its long term effects on the brain parenchyma and its clinical significance should be further investigated.

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Comments

Although infection by eccinoccocus organisms is not common in the western world, and therefore nor is cerebral hydatidosis, but the current migratory trend could modify the present situation. This gives an extra point to this manuscript. Intra-operative rupture of a cerebral hydatic cysts can be a serious complication due to, among other aspects, the spreading of scolex. The work by Senel and coworkers offers a critical and well documented laboratory analysis of the neural reactions of an otherwise positive drug in the management of hydatidosis. The neurosurgical recommendations of their findings are sensible.

F. Isamat

The clinico surgical experience in hydatid cyst disease in endemic countries has shown that until now there is no solution which can prevent the recurrence of the brain hydatid cyst in case of rupture during surgery.

The authors are entirely right to say that all other solutions used to irrigate the operative field are not effective.

L'albandazole on the other hand proved effective in its use by systemic administration.

So I believe that it is very interesting to use it as a scolicidal solution during surgery to avoid recurrence.

A. El Khamlichi

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