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Comparison of the Protoscolocidal Effectiveness of Hypertonic Saline, Povidone–Iodine and Albendazole Solutions in an Experimental Lung Hydatid Cyst Model

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Secondary hydatidosis is an important problem encountered during the surgical treatment of hydatid cysts. This study describes an experimental model of secondary hydatidosis by cyst inoculation, used to explore whether simultaneous inoculation of protoscolocidal agents could prevent secondary hydatidosis. Fertile cyst fluid was injected into the pleural space of rabbits alone (group 1, *n* = 8), and in combination with 2% albendazole solution (group 2, *n* = 8), 20% hypertonic saline (group 3, *n* = 8) or 10% povidone–iodine (group 4, *n* = 8). Computed tomography imaging of the thorax, indirect haemagglutination (IHA) titres and eosinophil counts were used to determine cyst development. After 16 months, three control rabbits had pneumothorax, seven had cysts and four had parenchymal nodules. Histopathological investigation of nodules revealed 87.5% cyst formation. Pleural thickening was observed in rabbits from all groups. Cyst formation rates, IHA titres and eosinophilia counts were higher in group 1 than in groups 2 – 4. This study demonstrated the experimental formation of secondary hydatidosis and found that topical protoscolocidal agents were beneficial in preventing cyst recurrence.

KEY WORDS: Albendazole; Povidone–Iodine; Hypertonic saline; Protoscolocidal agents; Lung hydatidosis; Experimental model

Introduction

Hydatid cysts are seen in all societies that deal with agriculture and animal breeding in which measures of environmental health and preventative medicine are inadequate.[1] Hydatid cysts are mainly treated with surgery, although secondary hydatidosis is a major problem with this approach. This complication can be prevented by using different surgical techniques and by using scolocidal substances[2 – 9] such as albendazole, praziquantel, hypertonic saline

and povidone–iodine, which are applied topically after aspiration of the cyst fluid.[3,10,11]

Secondary hydatidosis has been induced experimentally in rats after liver hydatid cyst development by intraperitoneal application of protoscolex and inoculation of daughter vesicles.[12,13] The present study investigated whether cyst inoculation could be achieved in a new experimental model, and whether cyst formation could be prevented with the topical application of scolocidal agents.

Materials and methods

STUDY SITES

All parts of this study were performed in the Faculty of Medicine, Ondokuz Mayis University, Samsun, Turkey. The experimental work was completed in the Surgical Research Laboratory; preparation of the cyst fluid was done in the Departments of Microbiology and Parasitology; immunological investigations were carried out in the Central Laboratory; radiological evaluations were carried out in the Department of Radiology; statistical analyses were carried out in the Department of Biostatistics; and histopathological examinations were carried out in the Department of Pathology.

The study was approved by the Medical Ethics Committee of Ondokuz Mayis University (approval No. GC/42), and was conducted according to national protocols and guidelines for animal welfare.

CYST FLUID PREPARATION

Fresh, fertile cyst fluid was obtained from cattle lungs in a slaughterhouse. The viability of the protoscolex in the material obtained from the cyst inclusions was evaluated using 0.1% eosin staining and an Olympus BX50 light microscope (Olympus Optical Co., Tokyo, Japan). Protoscolices with viability > 95% were washed with isotonic saline before use, and used immediately after isolation.[14 – 17]

ANIMALS AND TREATMENT

New Zealand adult male rabbits weighing 2550 – 3350 g (mean ± SD 2950 ± 150 g) were divided into four groups of eight. Rabbits were kept under normal laboratory conditions, fed with standard diet and had free access to water. Feeding was stopped 12 h before operation. Stomach skin was shaved and disinfected with topical application of povidone–iodine. Ketamine hydrochloride 15 mg/kg (Ketalar; Eczacıbaşı İlaç Sanayi, Istanbul, Turkey) was injected intramuscularly, for general anaesthesia. A 2-cm incision was made through the skin and the subcutaneous layer of the hemithorax into the intercostal space. A No. 18 Shiba needle was then used to inject 1 ml of prepared fertile cyst fluid into the thoracic cavity, through the parietal pleura in all rabbits (Fig. 1). In groups 2 – 4, intrathoracic injections of either 2% albendazole (group 2), 20% hypertonic saline (group 3), or 10% povidone–iodine (group 4) were also administered.

All animals were followed for 16 months. Venous blood samples (3 ml) were taken immediately prior to the operation and at 3, 6, 9 and 16 months following surgery. These underwent indirect haemagglutination (IHA) testing, to check for serum antibodies to *Echinococcus granulosus* using a commercially available assay (Echinococcosis Fumouze®; Laboratoires Fumouze Division Diagnostics, Levallois-Peret, France).[18,19] Blood from these samples also underwent full blood count analysis, to check for eosinophilia.

Cyst development was evaluated by recording computed tomography scans of the thorax after surgery and at 6 and 16

FIGURE 1: Image showing the injection of prepared cyst fluid, with or without protoscolocidal agent, into the thoracic cavity through the parietal pleura of a New Zealand rabbit to stimulate the development of secondary hydatidosis

months following surgery. Images were evaluated for evidence of pneumothorax, cyst formation, pleural thickening and the presence of nodules.

After 16 months, the rabbits were anaesthetized with ketamine (15 mg/kg) and sacrificed by cardiac puncture. Three rabbits died of pneumothorax within 12 months of surgery. Their lungs, and the lungs of the rabbits sacrificed at month 16, were kept in 10% neutral buffered formalin, pH 7.0, for 24 h. After fixation, a section passing through the lung parenchyma and any cyst was taken and processed for routine paraffin embedding and histological examination. Sections of 4 – 6 µm were stained with haematoxylin and eosin, and histopathological examination was performed using an Olympus BX50 light microscope.

STATISTICAL ANALYSES

Data were analysed using one-way analysis of variance and multiple comparisons of the group mean values were corrected with Bonferonni and Tukey tests. The results of the blood tests taken before and after surgery from both the control and the study groups were tested using the repeated-measures analysis of variance. A non-parametric one-way analysis of variance (Kruskal–Wallis) test was applied to data that were not evenly distributed. Pairwise comparison of the importance of the difference between the groups was performed with the paired-samples *t*-test. Pairwise comparison of the groups was performed with a Mann–Whitney *U* test. In all evaluations, a *P*-value < 0.01 was considered statistically significant.

Results

SEROLOGY

In general, IHA blood tests showed an increase in the presence of *E. granulosus*, indicating lung hydatid cyst formation. Measurements at 3, 6, 9 and 16 months after surgery in the rabbits receiving scolocidal

agents (groups 2, 3 and 4) showed no difference in mean IHA titres compared with baseline levels (data not shown). Rabbits receiving cyst injection only (group 1) had a significantly higher mean IHA titre 16 months after surgery, compared with the other three groups ($P < 0.001$; Table 1).

EOSINOPHILIA

Mean eosinophilia counts after 16 months were significantly higher in group 1 rabbits compared with those in groups 2, 3 and 4 ($P < 0.001$), but there was no significant difference between groups 2, 3 and 4 (Table 2).

COMPUTED TOMOGRAPHY OF THORAX

Pneumothorax, parenchymal nodules and cyst formation were only observed in group 1 rabbits 16 months after surgery. In group 1, pneumothorax was detected in three rabbits whose general condition deteriorated within 12 months. Computed tomography revealed parenchymal nodules in four group 1 rabbits. When these nodules were studied to determine cyst development, cysts developed in all four of these rabbits. Seven rabbits from group 1 developed cysts (Fig. 2A, 2B), compared with no cyst development in groups 2, 3 and 4 ($P = 0.001$). In total, five rabbits died during the study, all in group 1. Three died from pneumothorax and no exact cause of death was detected in the other two rabbits, although both had parenchymal nodule formation.

HISTOPATHOLOGY

In group 1, pneumothorax and cyst formation were detected in three rabbits whose general condition deteriorated within 12 months postsurgery. Histopathological examination of lung tissue from animals

TABLE 1:
Indirect haemagglutination test (IHA) data for detection of *Echinococcus granulosus* in New Zealand rabbits 16 months after receiving interpleural injection of fertile hydatid cyst fluid, or fertile hydatid cyst fluid plus 2% albendazole, 20% hypertonic saline or 10% povidone–iodine ($n = 8$ rabbits per group)

Group	IHA titres for *Echinococcus granulosus*
1	1024 ± 312.32***
2	98 ± 29.21
3	64 ± 23.65
4	64 ± 21.87

Data presented as mean ± SD.
***$P < 0.001$ versus groups 2, 3 and 4; data were analysed using one-way analysis of variance, and multiple comparisons of the group mean values corrected with Bonferonni and Tukey tests.
Group 1, interpleural injection of fertile hydatid cyst fluid; group 2, interpleural injection of fertile hydatid cyst fluid containing 2% albendazole; group 3, interpleural injection of fertile hydatid cyst fluid containing 20% hypertonic saline; group 4, interpleural injection of fertile hydatid cyst fluid containing 10% povidone–iodine.

TABLE 2:
Eosinophil counts in New Zealand rabbits 16 months after receiving interpleural injection of fertile hydatid cyst fluid, or fertile hydatid cyst fluid plus 2% albendazole, 20% hypertonic saline or 10% povidone–iodine ($n = 8$ rabbits per group)

Group	Eosinophil count (cells/mm^3)
1	13.284 ± 1.375***
2	5.609 ± 1.155
3	6.422 ± 0.332
4	7.647 ± 0.602

Data presented as mean ± SD.
***$P < 0.001$ versus groups 2, 3 and 4; data were analysed using one-way analysis of variance, and multiple comparisons of the group mean values corrected with Bonferonni and Tukey tests.
Group 1, interpleural injection of fertile hydatid cyst fluid; group 2, interpleural injection of fertile hydatid cyst fluid containing 2% albendazole; group 3, interpleural injection of fertile hydatid cyst fluid containing 20% hypertonic saline; group 4, interpleural injection of fertile hydatid cyst fluid containing 10% povidone–iodine.

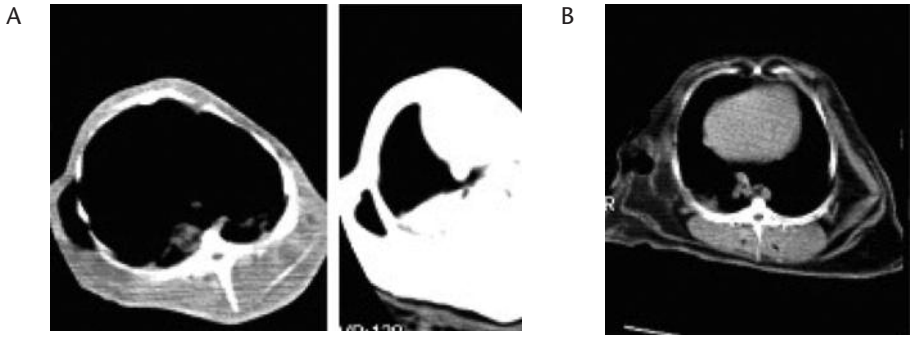

FIGURE 2: Computed tomography of the thorax of rabbits that received an interpleural injection of fertile hydatid cyst fluid and went on to develop hydatid cysts: (A) mediastinal window (6 months); (B) parenchymal window (16 months)

with pneumothorax revealed cyst formation (Fig. 3A, 3B). Pleural thickening was seen in the lungs of seven rabbits (at least one from each group; Table 3, Fig. 4A, 4B).

Discussion

Hydatid cysts, which are very common in Turkey and other countries, are infections caused by the larval form of *E granulosus*. Such infections are mainly located in the liver or lungs but may be seen anywhere in the human body. They are more frequently encountered in countries where animal breeding techniques are developing, animal slaughter is not controlled adequately, and where there are stray dogs.[20,21] Hydatid cysts present an important economic problem for such countries.[2,8,22,23]

Imaging methods that detect space-occupying lesions are important in the diagnosis of hydatid cysts, and serological tests, such as the IHA test, are used for confirmation when radiology and the clinical picture suggest these infections.

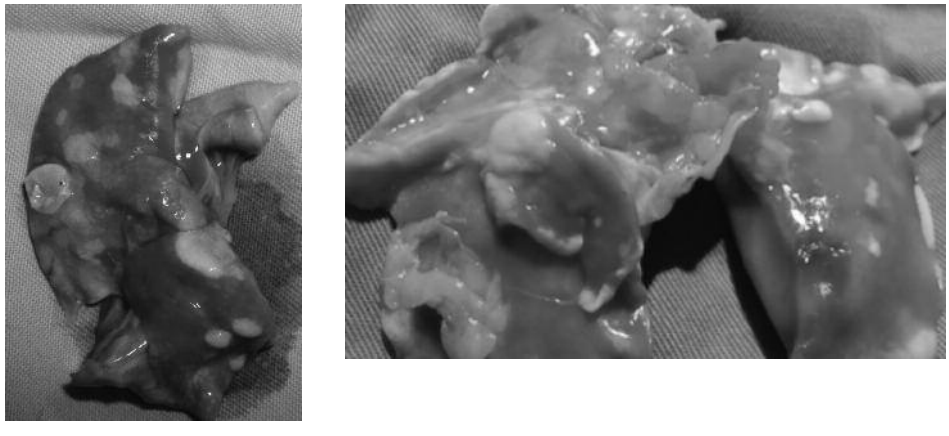

FIGURE 3: Lung tissue from rabbits, excised 12 – 16 months after interpleural injection of fertile hydatid cyst fluid; pneumothorax and cyst formation was observed on histopathological examination: (A) hydatid cyst in the lung; (B) pleural thickening

TABLE 3:
Results for computed tomography scans of the thorax in New Zealand rabbits 16 months after receiving interpleural injection of fertile hydatid cyst fluid, or fertile hydatid cyst fluid plus 2% albendazole, 20% hypertonic saline or 10% povidone–iodine (*n* = 8 rabbits per group)

Group	Pneumothorax	Pleural thickening	Parenchymal nodules	Cyst development
1	3 (37.5)	1 (12.5)	4 (25.0)	7 (87.5)
2	0	2 (25.0)	0	0
3	0	1 (12.5)	0	0
4	0	3 (37.5)	0	0

Data presented as *n* (%).
Group 1, interpleural injection of fertile hydatid cyst fluid; group 2, interpleural injection of fertile hydatid cyst fluid containing 2% albendazole; group 3, interpleural injection of fertile hydatid cyst fluid containing 20% hypertonic saline; group 4, interpleural injection of fertile hydatid cyst fluid containing 10% povidone–iodine

Thus, radiology and serology both contribute to making a diagnosis of hydatid cysts. Furthermore, since the results of serological tests are valuable in treatment follow-up, it is important to know the sensitivity of the tests and the factors that affect them. Some factors that can affect serological testing are other parasitic diseases, cirrhosis and liver or lung malignancies. In cases where IHA tests are normal or slightly abnormal, difficulties in diagnosis can be overcome with imaging.[24,25]

Hydatid cyst development was demonstrated in the present study by the use of both radiology and serology. The increase in IHA titres seen in blood samples from group 1 rabbits indicated that the IHA test is useful for the diagnosis of secondary echinococcus. Cyst development was determined with computed tomography of the thorax at month 16. This imaging technique is important in the diagnosis of small cysts and in the differentiation of ruptured cysts from other pulmonary

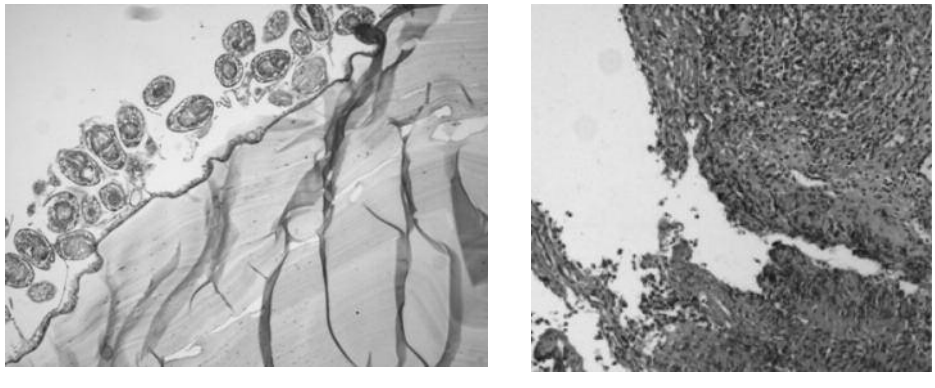

FIGURE 4: Rabbit lung tissue sections 12 – 16 months after interpleural injection of fertile hydatid cyst fluid and protoscolocidal agents show pleural thickening (light microscope; haematoxylin and eosin stain; original magnification ×40): (A) hydatid cyst in the lung; (B) pleural thickening

diseases. If there is a suspicious clinical picture and if suspicious lesions are observed in the lung X-ray, serology should be considered for the diagnosis of hydatid cysts, to obtain information while the infection is at an early stage of disease.

The basic steps in the treatment of hydatid cysts are to empty the cavity (preventing the spread of cysts during the emptying process), and to sterilize and to close the cyst cavity. The crucial part of cyst treatment is the termination of live scolices present in the cyst fluid.[6,17] Various treatment methods and protoscolocidal agents have been recommended for this purpose.[10,26] Studies to determine scolocidal agents that can be used safely for the disinfection of the cyst cavity have increased, and many studies have shown that systemic use of albendazole before and after surgery reduces the rate of hydatid cyst recurrence.[8,27] Albendazole has not, however, been used as a local scolocidal agent during lung hydatid cyst surgery.[14]

To date, no experimental models have been designed that develop secondary hydatidosis in the lung. This could be due to the technical difficulties of the process, including the development of conditions such as pneumothorax that are life threatening.[3,28]

In addition to reports of cysts that develop easily and grow to large dimensions, there have been reports in the literature of cysts that are small and that preserve their stationary position without developing further.[12] Growth rates of cysts can vary depending on their locations in the lung: peripheral cysts grow more rapidly and are larger; central cysts generally grow over a longer time period and are smaller.[29,30]

Artificial hydatid cyst development was achieved in seven rabbits (87.5%) in the present study through injection of cyst fluid containing live protoscolex into the interpleural space without treatment with a scolocidal agent. With this method, over time, secondary hydatidosis developed in the pleura and adjacent tissues.

Cyst inoculation that was treated simultaneously with 20% hypertonic saline resulted in no cyst development, but demonstrated pleural thickening in one rabbit (12.5%). This demonstrated that 20% saline was effective in the prevention of secondary hydatidosis.

No cyst development was observed in the group 4 rabbits in the present study (cyst inoculation plus povidone–iodine), but there was pleural thickening in three (37.5%) animals. Although povidone–iodine was as effective as hypertonic saline in preventing secondary cyst formation, rates of pleural reaction were higher with povidone–iodine than with saline.

In *in vitro* studies, it has been found that all protoscolices lose their viability within 15 min of exposure to 10% albendazole sulphoxide solution.[14,15,21,31] No cyst development was observed in the group treated with albendazole in the present study, demonstrating that interpleural albendazole prevents secondary lung cyst formation. No papers were found in the literature describing the use of albendazole solution as a local scolocidal agent for lung hydatid cyst. Pleural adhesions can develop due to the intrapleural use of talc, tetracycline or bleomycin,[32] but no such data, either in clinical practice or in experimental studies, could be found that related to the scolocidal agents used in the present study. Pleural thickening was observed in two (25%) of the rabbits injected with albendazole in the pleural space.

There were limitations to the present study: it was conducted in an animal model, and the animal numbers used were low. Further, larger studies are needed to support these data.

This is the first report of injecting live protoscolex into the pleural space for experimental cyst development. Secondary hydatidosis development was demonstrated. Preventing the spread of cyst fluid during surgery and the use of topical protoscolicidal agents can prevent secondary hydatidosis developing.

Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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