Liver hydatid disease: morphological changes of protoscoleces after albendazole therapy

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Background. Postoperative recurrence of the liver hydatid disease befalls approximately 10–30% of patients. Preoperative or postoperative therapy with albendazole in single therapeutic protocol (800 mg/d, within 28 days) indicated the need to evaluate the hydatid cyst liquid protoscoleces viability. Morphological changes of protoscoleces following the treatment with drugs are not well known. Aim. To estimate the viability of protoscoleces after preoperative or postoperative albendazole therapy, and their ability for cystic metamorphosis. Methods. A prospective, randomized clinical trial included 30 patients with liver hydatid disease, treated with albendazole and surgically (I group), and 30 patients in the control group treated only surgically (II group). The concentration of albendazole and its active metabolite albendazole sulphoxide in the cysts contents were determined using HPLC. Estimation of protoscoleces viability was based on the established micromorphologic criteria, and compared between the patients treated with albendazole, and the patients treated only surgically. Biological assessment of the viability was performed on protoscoleces with uncertain signs of the disturbed viability (unchanged structure, evaginated, without movements) using intraperitoneal injection of 1 ml of protoscoleces prepared suspension to AO type of rats. Results. The concentration of albendazole in cysts' contents ranged from 0 to 64.9 μg/ml, and of its active metabolite from 0.5 to 40.8 μg/ml. The presence of fully viable protoscoleces in the albendazole treated patients was significantly lower than in the control group. A significant difference was noticed in the presence of disintegrated protoscoleces without movements in the albendazole treated group, compared to the control group. Biological assessment of the viability showed incapability of these protoscoleces for cystic metamorphoses. Conclusion. Low viability of parasites due to medicamentous therapy is very useful and important to surgeons, because the fertility of cysts is lower, and the risk of the disease recurrence is reduced.

Key words: liver diseases, parasitic; echinococcosis, hepatic; albendazole; surgical procedures, operative; microscopy; chromatography, high pressure liquid; cell survival; recurrence.

Introduction

Cystic hydatid disease is a widespread human infection caused by the larval stage of the taeniid tapeworm Echinococcus granulosus. The disease is well known in rural areas of Serbia and Montenegro. Surgery is still the treatment of choice (1, 2). According to the WHO guidelines for the medicamentous treatment of hydatidosis, one of the important indications for chemotherapy is the prevention of secondary echinococcosis (3). Hepatic hydatidosis represents 10–30% of the disease recurrence after the surgery (4–8). Spillage of protoscoleces (PS) during the surgery is apparently an important cause of the recurrence (9). Recurrent disease can be a major clinical problem (10), and the subsequent reoperations are associated with progressive increase in complications and higher mortality rate (11).

The viability of PS in hydatid cysts is unknown before its surgical removal. The use of albendazole (ABZ) to reduce the biological activity of the parasite in the cystic stage, provides new therapeutic perspectives, especially in the cases of the abdominal localisation (12).
The aim of this study was to analyze the percentage of viable PS and PS in various stages of degeneration in the hydatid cysts removed during surgery, as well as their ability to metamorphose into cysts after the ABZ therapy.

**Methods**

The prospective, randomized clinical trial, which included 60 patients with liver hydatid disease, was performed at the Clinic for General and Vascular surgery, Military Medical Academy, Belgrade.

The first group included 30 patients, treated preoperatively with ABZ 400 mg/twice a day, for 28 days. In the second, control group, 30 patients were only surgically treated. The concentrations of the ABZ and albendazole active metabolite albendazole sulphoxide (ABZ-SO) in the cysts' contents were determined using HPLC (13).

The viability of PS was assessed using direct microscopic examination of the contents from the extirpated liver hydatid cysts. The biological assessment of PS viability was performed in the cases with uncertain viability signs (unchanged PS structure, evaginated, without movements). The assessment was not performed on PS with normal structure, and with the signs of movements – viable PS, in the cases of disintegrated PS (edema, destruction of tegmentum, deformation of hooklets' row), or in the cases of cysts with only free hooks of PS. The content of hydatid cysts (PS and germinal layer), from the surgically treated patients was washed and accumulated. One ml of that suspension was intraperitoneally inoculated to the male AO type rats, aged 6–8 weeks, under aseptic conditions. Six months after the inoculation, the animals were sacrificed, and the intraperitoneal sites checked for the secondary cysts of *Echinococcus granulosus*.

Postoperative follow-up demonstrated no recurrence of the disease in the first group, and the patients were considered cured after 24 months.

In the second group, there were recurrences of the disease in 3 patients, who had preoperative multiple cysts or hepatic cysts with irregular cavities that communicated among themselves.

**Table 1**

<table>
<thead>
<tr>
<th>Micromorphological characteristics of protoscoleces</th>
<th>Group II</th>
<th>Group I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopic findings</td>
<td>n = 30</td>
<td>n = 30</td>
</tr>
<tr>
<td>Alive PS*, normal structure, with sign of movements</td>
<td>7</td>
<td>3.3</td>
</tr>
<tr>
<td>Vital, evaginated PS*, unchanged structure, without movements</td>
<td>8</td>
<td>26.6</td>
</tr>
<tr>
<td>Disintegrated PS (edema, destruction of tegmentum, deformation of hooklets' row)</td>
<td>8</td>
<td>26.6</td>
</tr>
<tr>
<td>Free hooks of PS*</td>
<td>7</td>
<td>23.3</td>
</tr>
</tbody>
</table>

* Statistically significant II : I p<0.05

The signs of intraperitoneal parasite development were not found in the animals.

Fig. 1 – Vital PS, a) invaginated, b) evaginated, rich with glicogene and with a row of hooks. PS like this is showing ameboid movements.
The risk of secondary echinococcosis. Thus, testing of hydatid cysts contents viability after the surgical removal was important for subsequent medicamentous treatment, and for the assessment of the risk of the disease recurrence (14).

The patophysiological mechanism of ABZ-SO effect on PS in human hydatid cysts, however, hasn't been revealed yet.

The viability of the cysts can be determined by the presence of PS in various stages of degeneration. This method was frequently used. The absence of parasite growth in the experimental animals should be used as the final criteria. However, the presence of viable PS does not necessarily indicate the eventual development of secondary echinococcosis, which is more likely to appear after the surgery, when it is obligatory to check for them (14).

In order to determine more precisely the ABZ effects on the treated patients, we compared some basic PS viability parameters in the patients treated with ABZ, with parameters in the patients surgically treated. We analyzed the percentage of completely vital PS in both groups, and divided the cases with vital PS and unchanged structure from the minimal signs of disturbed viability. The hydatid contents of PS were assessed using intraperitoneal inoculation of AO rats. We expected that the completely vital PS, intraperitoneally applied by El Mufti et al. (15) would result in cystic metamorphosis but in the case of disintegrated PS we could not expect a positive biological answer.

In our study, all the patients in the first group were found to be with the presence of ABZ-SO in all cysts. The presence of the fully viable PS in the ABZ treated patients was significantly lower than in the the control group. Also, the significant difference between the two groups of patients was found in the presence of disintegrated PS without movements. Biological check of viability using intraperito-
neal inoculation to AO rats showed similar incapability for cystic metamorphosis. The average percentage value of viable PS in the cysts according to Pappalardo et al. in the patients treated with mebendazole was 31.1%, which was significantly lower than in the patients treated only surgically, and was up to 70%. In addition, the tendency of PS to develop cyst in inoculated BALB/c mice is lower than that of PS obtained from the cysts of the patients who were only surgically treated (5).

Rahemtulla et al. (16) also inoculated six experimental animals intraperitoneally 28 days with suspension (0.5 ml) of PS and germinal epithelium. After 11 months, the signs of parasite development were not found. The studies on the efficacy of preoperative ABZ therapy in primary disease indicated the possibility of reducing the number of viable PS in the treated cysts (17). Ayles et al. (18) concluded that the preoperative medical therapy reduced significantly the number of cysts that contain viable PS. It was also possible to make an algorithm for the management of the patients with hydatid disease with chemotherapy and surgery, yet the assessment of the results using indirect techniques was still difficult.

Our findings, as well as the findings of the other authors, suggested that the lower viability of parasite was the result of medicamentous therapy. The results like these are significant for a surgeon, since they allow him to operate smaller fertile cysts, reducing significantly the risk of undesirable rupture of cysts. Under these conditions, when PS were, first treated with drugs, and under unfavorable metabolic conditions, according to Pappalardo et al. (5), they were not capable of metamorphosis. It was identical to our micromorphological research on PS and to the results of biological viability check.

**Conclusion**

Significantly lower PS viability in the group of patients preoperatively treated with ABZ was found, using both microscopic examination of the hydatid cysts contents and biological check of the material. At the same time, the investigation of hydatid cysts contents, in the control group showed a significant number of completely vital PS. In the intraoperatively obtained hydatid cysts contents in patients treated with ABZ, the concentration of ABZ-SO (active metabolite) was 0–40.8 μg/ml. The obtained concentration was below the level of minimal therapeutic concentration, under *in vitro* conditions. Considering the effects of ABZ therapy, it could be speculated that concentrations of this metabolite could also be found under *in vivo* conditions. Based on the obtained results, it could be concluded that the use of ABZ 800 mg/day within 28 days, was sufficient to achieve the therapeutic drug level under *in vivo* conditions.

Low viability of PS due to medicamentous treatment is very useful and important for surgeons, because fertile cysts are smaller the risk of undesirable cysts rupture is lower, and the risk of the disease recurrence is reduced.

**REFERENCES**

Abstract


Ehinokokusno oboljenje jetre: morfološke promene protoskoleksa posle lećenja albendazolom


Ključne reči: jetra, parazitne bolesti; ehinokokoza jetre; albendazol; hirurgija, operativna, procedure; mikroskopija; hromatografija, tečna, pod vp; čelića, preživljavanje; recidiv.

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