In Vitro Evaluation of the Scolicidal Activity of Cyclosporin A against the Protoscolices of Human Echinococcus Granulosus

Samer Noori Hashim, Ibrahim Adham Majeed, Kassim Jalil Al-Shmma

ABSTRACT:

BACKGROUND :

Hydatid disease, also known as echinococcosis or hydatidosis, is caused by infection with larva of Echinococcus granulosus, which causes cystic hydatid disease. Surgery is the recommended treatment for hydatid cysts; however, drug therapy and percutaneous drainage have recently been introduced as alternative treatments. The scolicidal agents, including 3% hydrogen peroxide, 10% chlorhexidine, 20% hypertonic saline have been used mainly during surgical manipulation of the cysts. Recently cyclosporin A has been found to be lethal for E. granulosus protoscolices in vitro using cultured sheep hydatid cyst.

METHOD:

The present work was designed to evaluate the effectiveness of different concentrations of cyclosporin A as a scolicidal agent on protoscolices of Echinococcus granulosus in vitro. Hydatid cysts were collected from 42 patients (10 males and 32 females with age range from 12–61 years) that have hydatosis during the surgical operation for the removal of these cysts. Eeosin exclusion test was utilized to examine the scolicidal activity of different concentrations of cyclosporin A compared to different concentrations of hydrogen peroxide, in addition to the effects on the integrity of the germinal layer compared to that produced by sodium hypochlorite.

RESULTS AND DISCUSSION:

The results indicated that cyclosporin A, when used in a concentration of 25 µg/ml, is more effective and safer than 3% solution of hydrogen peroxide; it can be used with sodium hypochlorite for complete scolicidal effect and melting of the germinal layer. In conclusion, cyclosporin A can be a good candidate as a scolicidal agent during surgical removal of hydatid cysts.

CONCLUSION:

The present work indicated that cyclosporin A has a good scolicidal effect when used in a concentration of 25 µg/ml, and it is more effective and safer than 3% solution of hydrogen peroxide, it can be used in combination with sodium hypochlorite for complete scolicidal effect and melting of the germinal layer of the hydatid cyst. This may give an opportunity for using this compound in percutaneous drainage

KEY WORDS: Cyclosporin A, Protoscolices, hydatidosis, scolicidal activity

INTRODUCTION:

Echinococcus granulosus is the causative agent of hydatid disease in man and many other mammals [(1)]. Theoretically, echinococcosis can involve many organs, but liver is the most common organ involved, followed by the lungs, and the two organs account for 90% of the cases [(2)]. In the liver, the pressure effect of the cyst can produce symptoms of obstructive jaundice and abdominal pain with biliary rupture, jaundice and articaria [(3)]. A rupture into the biliary tree can lead to obstruction by the daughter cysts, producing cholangitis [(4)]. Secondary infections follow cyst rupture, and scolices can grow in the peritoneum, pleura, bronchial tree and bile ducts, or be carried via blood to distant organs [(5)]. Although surgery is the recommended treatment for hydatid cysts [(6, 7)], drug therapy and percutaneous drainage were introduced as alternative treatments [(8)]. Recurrence of the disease is much more common among patients who have evidence of spread of the protoscolices at the time of primary operation, and can result from spillage of cyst fluid during operation [(9)]. Scolicidal agents, including 3% hydrogen peroxide [(10)], chlorhexidine [(11)], formalin [(12)], hypertonic saline [(13)] and absolute ethanol [(14)] are used mainly during surgical removal of the cysts for the aim of avoiding relapses and peritoneal dissemination.

Department of Clinical Pharmacy, College of Pharmacy, University of Baghdad, Baghdad, Iraq

Cyclosporin A is a cyclic peptide of 11 amino acids produced by the fungus Hypocladium inflatum, it is an effective immunosuppressive agent mainly used to prevent tissue rejection during organ transplantation [15]. Cyclosporin A has anti-parasitic effects against variety of helminthes and protozoan parasites mediated through interaction with cyclophyllin [16]. It has also found to be lethal in vitro for the protoscolices of E. granulosus [17]. The present study was designed to evaluate the efficacy of different concentrations of cyclosporin A as a scolicidal agent against human E. granulosus protoscolices in vitro.

MATERIALS AND METHODS:

Two hundred specimens of hydatid cysts were collected fro 42 patients with liver hydatidisis during surgical operation for the removal of cysts, conducted in Al-Najaf Teaching Hospital during the period from February to June 2006; the specimens were stored refrigerated at 4ºC for about 24 hours before starting experiments. After wiping the surface of the cysts with 95% ethanol, the hydatid fluid was aspirated, and the hydatid sand was collected after opening the cyst under aseptic technique [18]. Eeosin exclusion test was used for the estimation of viability of the protoscolices [19] in the samples. The hydatid fluid and sand centrifuged at 2000 rpm for 10 min at 4ºC, the precipitate was re-suspended in phosphate buffer saline, and used for the evaluation process after determining the number of viable protoscolices in each sample, samples with less than 90% viability are discarded [20].

The effect of different concentrations (5, 10, 15, 20 and 25 µg/ml) of cyclosporin A (LC Labs, USA) on the viability of the protoscolices, utilizing eosin exclusion test was evaluated, and compared with that produced by different concentrations (3%, 6%, 9% and 12%) of hydrogen peroxide (GCC Company, UK) as a standard scolicidal agent; the results were expressed as mean ± SE. The effects of the same concentrations of cyclosporin A used before on the integrity of the hydatid membrane was performed [21], and compared with that produced by different dilutions (0.1%, 0.55, 2.5%, and 5%) sodium hypochlorite (CCS Labs, UK), appearance of the germinal membrane that include translucency, destruction, swelling and melting was recorded.

Statistical analysis of data was performed utilizing paired Student's t-test and ANOVA, values with $P<0.05$ were considered significantly different.

RESULT:

The data presented in figure 1 showed that the viability of protoscolices decrease from 97% ± 0.95 to 75.2% ± 3.62 after 60 min in control ; viability decreases significantly from 96.2% ± 1.94 to 5.5% ± 0.54 after 20 min after using 5 µg/ml cyclosporin A. Further decrease in viability was reported (97.9% ± 2.89 to 7.5% ± 0.67, $P< 0.05$) after 15 min of using 10 µg/ml cyclosporin A. Meanwhile, increasing the concentration of cyclosporin A to 15, 20 and 25 µg/ml was associated with decrease in the time required to show significant differences in percent viability of protoscolices (97% ± 3.81 to 22.4 % ± 0.65 after 10 min, 95.1% ± 0.92 to 20.2% ± 1.61 after 5 min and 97.6% ± 2.8 to zero after 5 min respectively) compared to control.

The results presented in figure 2, which reveal exposure time and percent viability of protoscolices after using different concentrations of hydrogen peroxide, showed that viability of protoscolices decreases from 96% ± 1.23 to 74% ± 0.83 after 60 min in control; while viability decreases significantly from 95% ± 0.98 to 4% ± 0.51 after 25 min, when 3% hydrogen peroxide solution was used, and from 99% ± 2.11 to 5% ± 0.43 after 20 min, when 6% hydrogen peroxide was added to the incubation medium. Increasing the concentration of hydrogen peroxide in the incubation medium up to 10% and 12% results in decreasing the time required to produce significant differences in viability (96% ± 1.82 to 21% ± 0.94 after 10 min and 92% ± 1.75 to 23% ± 0.78 after 10 min respectively) compared to control.

The data presented in table 1 showed the integrity of hydatid membrane of daughter cysts when using different concentrations of cyclosporin A. From this table it is clear that the integrity of hydatid membranes do not change when exposed during 72 hrs to all concentrations of cyclosporin A. Table 2 showed the time necessary for melting of the germinal membrane of daughter cysts when using different concentrations of sodium hypochlorite. The hydatid cyst membrane was completely melted in 1.83 ± 0.16 min in a 5% solution of sodium hypochlorite, in 5.16 ± 0.3 minutes by a 2.5% sodium hypochlorite and in 21 ± 0.81 min by 0.5% sodium hypochlorite. However, 53.66 ± 1.68 min was needed for 0.1% sodium hypochlorite solution to melt the hydatid membrane.

DISCUSSION:

During surgical operation to remove the hydatid cyst from the body, the standard procedure for treating most cysts in soft tissue of any organ is evacuation of the cyst, obliteration of the residual cavity and the addition of scolicidal solutions [19]. The usual scolicidal used in Iraqi hospitals is hydrogen peroxide solution. Recently, it has been found that cyclosporin A is employed as scolicidal agent in vitro in hydatid cyst obtained from the sheep [17]. In this work, the effects of different concentrations of cyclosporin A on the percentage viability of protoscolices at different time of exposure have been studied in vitro (figure 1). From the results, it is obvious that the concentration of 25 µg/ml is effective in killing all the protoscolices found in the hydatid cyst fluid after 5 minutes. While the lower concentrations required longer time to produce the same effect; which was different from the results of Colebrook et al (2005), who found that 10 µg/ml of cyclosporin A was effective in killing all protoscolices cultured in vitro after 7 days of treatment [17]. Regarding the use of hydrogen peroxide, the standard scolicidal solution used in Iraq, higher concentration of hydrogen peroxide is expected to be caustic [22], the scolicidal effect of the 3% solution of hydrogen peroxide in this work indicated that the viability of protoscolices is zero after 30 min (figure 2), which is a longer than that observed with cyclosporin A. However, the time required by the surgeon in hospitals is 10 minutes, which is not sufficient to kill all protoscolices when using hydrogen peroxide; at that time, 50% of the protoscolices were still viable. Increasing the concentrations of hydrogen peroxide produce zero viability after 15 min (figure 2). However, these concentrations are toxic to the tissue [23]. It has been demonstrated that protoscolices of Echinococcus granulosus can metabolize hydrogen peroxide in vitro, and Echinococcus granulosus thioredoxin peroxidase may play a role in protecting the parasite from oxidative damage [24]. Sodium hypochlorite was ineffective in killing all the protoscolices at any tested concentrations and it is a toxic compound to the tissue [25]. This is in agreement with the results of others [26] who found that sodium hypochlorite is ineffective as a scolicidal agent and it is toxic to the tissue when used during surgery. Percutaneous drainage has been used as a method for treating hydatid cyst [27]. In Iraq, this technique is used in treating hepatic hydatid cyst using hydrogen peroxide as a scolicidal agent. However, the hydatid membrane of the cyst is highly resistant to such scolicidal agent. The unsuccessful percutaneous drainage of hydatid cysts may result in problems associated with the cyst membrane [28]. In order to clarify the effect of different scolicidals on hydatid cyst membrane the effect of different concentrations of cyclosporin A and sodium hypochlorite on the integrity of the cyst membrane was evaluated. The results showed that different concentrations of cyclosporin A had no effect on the cyst membrane after 72 hrs of exposure (table 1). However, 5% solution of sodium hypochlorite melted the cyst membrane completely at the first few min; whereas 0.1% concentration melted the membrane within 54 min (table 2). Because of the use of this agent on living tissue is limited [25], further study is needed to investigate its clinical usefulness.

CONCLUSION:

The present work indicated that cyclosporin A has a good scolicidal effect when used in a concentration of 25 µg/ml, and it is more effective and safer than 3% solution of hydrogen peroxide, it can be used in combination with sodium hypochlorite for complete scolicidal effect and melting of the germinal layer of the hydatid cyst. This may give an opportunity for using this compound in percutaneous drainage.

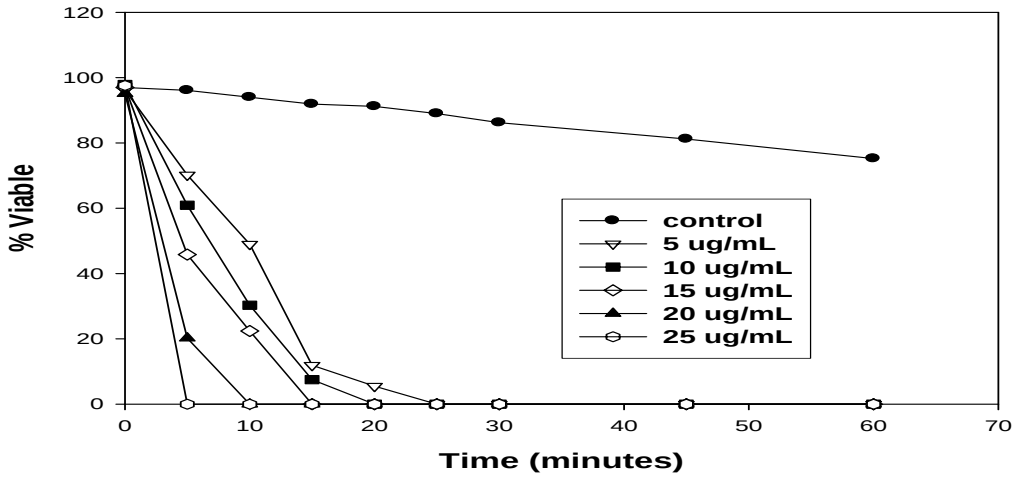

Figure 1.

The effect of different concentrations of cyclosporin A on the percent viability of protoscolices at different time of exposure in vitro.

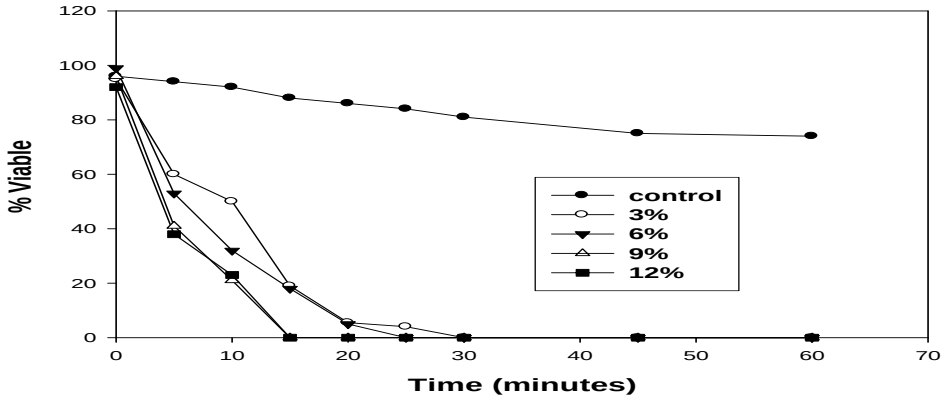

Figure (2):- The effect of different concentrations of hydrogen peroxide on the percent viability of protoscolicies at different time of exposure in vitro.

Table 1. The effect of different concentrations of Cyclosporin A solution on integrity of hydatid membranes of daughter cysts.

Concentration of Cyclosporin A solution used in the test (µg/mL)*	integrity of hydatid membrane
5	No changes appear on membrane after 72 hours.
10	No changes appear on membrane after 72 hours.
15	No changes appear on membrane after 72 hours.
20	No changes appear on membrane after 72 hours.
25	No changes appear on membrane after 72 hours.
* Number of samples = 6 for each test	

Table 2. The effect of different concentration of Sodium hypochlorite on the integrity of hydatid membrane of daughter cysts.

Concentration of Sodium hypochlorite solution %v/v *	Mean time required for melting of the membrane
5.0	1.83 ± 0.16
2.5	5.16 ± 0.3
0.5	21 ± 0.81
0.1	53.66 ± 1.68
* Number of samples = 6 for each test; results were expressed as mean ± SE.	

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