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ORIGINAL PAPER

In vitro effectiveness of acidic and alkline solutions on scolices of hydatid cyst

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Abstract The most confident way for treatment of hydatid cyst is surgical operation. Spillage of the cyst contents during the operation is the major cause of recurrence after hydatid cyst surgery. Instillation of scolicidal agent into hydatid cyst is the most commonly employed measure to prevent this complication. In the present study, the scolicidal effect of highly acidic and alkaline solutions is investigated. Protoscoleces were collected aseptically from sheep livers containing hydatid cyst. Acidic solutions with pH 1, 2, 3, and 4 and alkaline solutions with pH 11, 12, 13, and 14 were used for 5,10, and 15 min in the experiments. Viability of protoscoleces was assessed by 0.1% eosin staining. Scolicidal effect of acidic solution with pH 1 after 5 min and with pH 2 and 3 after 10 min was 100%. Scolicidal effect of acidic solution with pH 2 and 3 after 5 min was 99.6% and 98.7%, respectively. Acidic solution with pH 4 after 5, 10, and 15 min killed 15.5%, 21.5%, and 22.6% of protoscoleces, respectively. Alkaline solution with pH 14 after 5 min and with pH 13 after 15 min killed all protoscoleces. The scolicidal effect of alkaline solution with pH 13 after 5 and 10 min was also 97.5% and 99.7%, respectively. These values for alkaline solution with pH 12 were 29.33%, 33.44% and 37.09%, respectively. The scolicidal effect of solution with pH 11 was 24.5%, 30.5%, and 31.3%, respectively. Although the *in vitro* scolicidal effect of highly acidic or alkaline solutions was satisfactory in our study, *in vivo* efficacy of these solutions and also possible side effects, remain to be more investigated.

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Introduction

Cystic echinococcosis is a condition of livestock and humans that arises from eating infective eggs of the cestode *Echinococcus granulosus*. Dogs are the primary definitive hosts for this parasite, with livestock acting as intermediate hosts and humans as aberrant intermediate hosts. The outcome of infection in livestock and humans is cyst development in the liver, lungs, or other organ system (Budke et al. 2006). Although reported from several countries, the disease is endemic in South America, in the Mediterranean region, Far East, and Middle East (Bozdag et al. 2000; Barnes and Lillemoe 1997). There are currently three treatment options for hydatid disease of the liver: surgery, which remains the most efficient treatment, percutaneous aspiration, and medical treatment (Adas et al. 2009). Spillage of the cyst contents is very common, despite taking technical precautions. This is the major cause of recurrence, which is seen in approximately 10% of postoperative cases. Operative spillage can sometimes also lead to secondary disseminated intraperitoneal hydatidosis (Rajabi 2009). Instillation of scolicidal agent into a hepatic hydatid cyst is the most commonly employed measure to prevent this serious complication (Tozar et al. 2005). Up to date, many scolicidal agents have been used for inactivation of the cyst content, but there is no ideal agent that is both effective and safe (McManus et al. 2003). Scolicidal solutions remain indispensable in the treatment of hydatid cyst disease and the surgeons need less harmful but more effective drugs in hydatid disease(Adas et al. 2009).

In the present study, the scolicidal effect of highly acidic and alkaline solutions is investigated.

Material and methods

Collection of protoscolices

Livers of sheep naturally infected with hydatid cyst were obtained from local abattoirs. The livers were taken to the laboratory within 2 h. The hydatid fluid of cysts was aseptically transferred into the glass cylinders and left to set for 30 min. The protoscolices were settled down at the bottom of cylinders. The supernatant was then removed and the yielded protoscolices were washed three times using normal saline and were finally transferred into a dark container containing normal saline and stored at 4°C for further use.

Preparation of acidic and alkaline solutions

Different pH ranges of acidic and alkaline solutions were prepared by adding HCl and KOH to distilled water respectively. In this study acidic solutions with pH 1, 2, 3, and 4 and alkaline solutions with pH 11, 12, 13, and 14 were used in experiments.

Effectiveness of acidic and alkaline solutions on scolices

Protoscoleces (at least 500 in each experiment) were plunged to 10 ml of acidic and alkaline solutions (in a test tube) for 5, 10, and 15 min. At least 500 protoscolices with no exposure to acidic or alkaline solution was considered as control group in each experiment. All experiments were performed at room temperature.

Viability test for protoscolices

A 0.1% eosin solution (eosin powder in distilled water) was added to the samples in a ratio of 1:1. After 15 min, the viability of protoscolices was determined by observing the change of color under a light microscope. The live protoscolices did not change in color but the dead ones were stained red (Figs. 1 and 2).

Statistical analysis

Results are presented as percent of dead protoscolices. Comparison of mortality rate between different acidic and alkaline solutions and control groups was performed by Fisher's exact test. All analyses were done by GraphPad InStat software and P-values less than 0.05 was considered statistically significant.

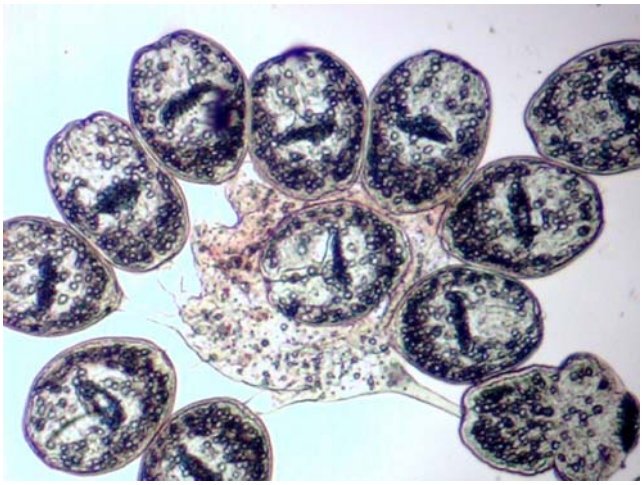

Fig. 1 Alive protoscolices

Results

Scolicidal effect of acidic solution with pH 1 after 5, 10, and 15 min and with pH 2 and 3 after 10, and 15 min was 100%. Scolicidal effects of acidic solution with pH 2 and 3 after 5 min were 99.6% and 98.7%, respectively. Acidic solution with pH 4 after 5, 10, and 15 min killed 15.5%, 21.5%, and 22.6% of protoscoleces, respectively (Table 1). Except for pH 4 at 5 min, the difference between the scolicidal effect of all acidic solutions (pH 1, 2, 3, and 4) at all times (5, 10, and 15 min) was statistically significant comparing to control groups ($P<0.001$). Alkaline solution with pH 14 after 5, 10, and 15 min and with pH 13 after 15 min killed all protoscoleces. The scolicidal effects of alkaline solution with pH 13 after 5 and 10 min were also 97.5% and 99.7%, respectively. These values for alkaline solution with pH 12 were 29.33%, 33.44%, and 37.09%, respectively. The scolicidal effect of solution with pH 11

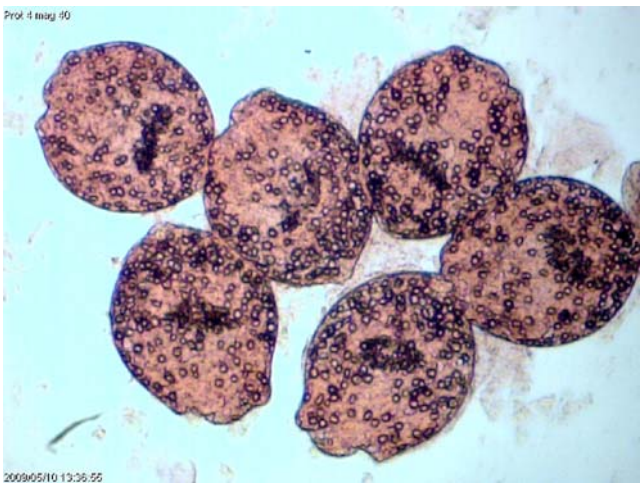

Fig. 2 Dead protoscolices after application of acidic or alkaline solution

Table 1 Scolicidal effect of acidic solutions after 5, 10, and 15 min of application

pH		Time (min)					
		5		10		15	
		Test	Control	Test	Control	Test	Control
1	Total protoscolices	550	775	844	775	627	775
	Dead protoscolices	550	48	844	48	627	48
	Mortality rate (%)	100%	6.2%	100%	6.2%	100%	6.2%
2	Total protoscolices	514	1030	419	1030	427	1030
	Dead protoscolices	512	130	419	130	427	130
	Mortality rate (%)	99.6%	12.6%	100%	12.6%	100%	12.6%
3	Total protoscolices	346	1030	486	1030	443	1030
	Dead protoscolices	341	130	486	130	443	130
	Mortality rate (%)	96.7%	12.6%	100%	12.6%	100	12.6%
4	Total protoscolices	615	647	460%	647	403	647
	Dead protoscolices	95	85	99	85	91	85
	Mortality rate (%)	15.5%	13.1%	21.5%	13.1%	22.6%	13.1

was 24.5%, 30.5%, and 31.3%, respectively (Table 2). Scolicidal effects of all alkaline solutions (pH 11, 12, 13, and 14) at all times were extremely significant compared with control groups ($P<0.0001$).

Discussion

Dissemination of protoscolex-rich fluid during surgery is a major cause of recurrence (Kilicoglu et al. 2008). Preoperative destruction of the cyst's contents and preventing infection of the surrounding area has an important role for success of the operation; also, this procedure helps to prevent the illness from returning (Khuroo et al. 1993). Avoiding spillage of the cyst contents and the use of effective scolicidal agents are essential to lower the recurrence rate (Topcu et al. 2009). Various scolicidal agents such as 95% alcohol, 10% povidone iodine, hypertonic saline, hydrogen peroxide, 5% formalin, silver nitrate, cetrimide, and albendazole have been evaluated for scolicidal effects and hepatobiliary complications in the presence of cystobiliary communications (Landa García et al. 1997; Topcu et al. 2006; Sahin et al. 2004). Even though 95% Ethyl alcohol had been found to be 100% effective at the end of 15 min (Erzurumlu et al. 1998), severe hepatobiliary complications have been reported for alcohol (Yetim et al. 2005). Hypertonic salt water (20%) was found to be 99.5% effective at the end of 10 min (Adas et al. 2009), but severe hepatobiliary complications have been reported for 10%–20% NaCl(Topcu et al.

Table 2 Scolicidal effect of alkaline solution after 5, 10, and 15 min of application

pH		Time (min)					
		5		10		15	
		Test	Control	Test	Control	Test	Control
11	Total protoscolices	620	647	465	647	457	647
	Dead protoscolices	152	85	142	85	143	85
	Mortality rate (%)	24.5%	13.1%	30.5%	13.1%	31.3%	13.1%
12	Total protoscolices	339	668	341	668	480	668
	Dead protoscolices	117	90	114	90	178	90
	Mortality rate (%)	29.3%	13.5%	33.4%	13.5%	37%	13.5%
13	Total protoscolices	325	668	325	668	471	668
	Dead protoscolices	317	90	324	90	471	90
	Mortality rate (%)	97.5%	13.5%	99.7%	13.5%	100%	13.5%
14	Total protoscolices	392	668	354	668	431	668
	Dead protoscolices	392	90	354	90	431	90
	Mortality rate (%)	100%	13.5%	100%	13.5%	100%	13.5%

2006). While 10% H2O2 was defined as a powerful scolicidal agent *in vitro* by Meymerian et al. (1963), fatal air embolism and anaphylactic shock have been reported with this scolicidal agent by Adas et al. (2009). Severe hepatobiliary complications have been reported for formalin when used as a scolicidal agent (Houry et al. 1990; Topcu et al. 2006). The scolicidal power of 20% silver nitrate is 100% after 20 min (Caglar et al. 2008), but intraoperative shock, cardiovascular collapse, renal failure, liver insufficiency, postoperative dyspnoea, cyanosis, and hypotension has been reported after the use of 0.5% silver nitrate as a scolicidal agent in the hydatid surgery (Rajabi 2009). Even though cetrimide has been found to be 86.9% effective at the end of 5 min (Adas et al. 2009), this scolicidal agent has the disadvantages of metabolic acidosis and methemoglobinemia (Urrea-París et al. 2000). Albendazole (ABZ) is the most widely used substance for the medical treatment of hydatid cyst disease. It has a scolicidal effect via its biologically active metabolites sulfone and sulfoxide. ABZ sulfoxide is more effective than ABZ sulfone (Adas et al. 2009).Twenty microgram per milliliter ABZ sulfoxide killed 5% of the scolices in 15 min, scolicidal activity was 50% with a 50 μg/mL solution, and it was 100% for a 100 μg/mL solution (Erzurumlu et al. 1998). It is known that scolicidal solution injection in the cysts or the biliary system leads to a rise in liver enzyme level. It has also been shown that systemically administered ABZ can lead to the same changes (Deger et al. 2000; Yetim et al. 2005; Gil-Grande et al. 1993). An ideal scolicidal agent is define as being potent in low concentrations, acting in a short period time, being stable in cyst fluid, not affected by dilution with the cyst fluid, being able to kill the scolex in the cyst, being non-toxic, having low viscosity, and being readily available and easily prepared, as well as being inexpensive (WHO 1996; Puryan et al. 2005).

According to the results of present study, acidic solution with pH 1 killed all protoscolices of hydatid cyst at the end of 5 min. The same efficacy with pH 2 and 3 was obtained at the end of 10 min of application. One hundred percent scolicidal efficacy was obtained through application of alkaline solutions with pH 14 at 5 min and with ph 13 at 15 min. Although the *in vitro* scolicidal effect of highly acidic or alkaline solutions was satisfactory in our study, *in vivo* efficacy of these solutions and also possible side effects, remain to be more investigated.

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