Experimental Parasitology 127 (2011) 14–17

Contents lists available at ScienceDirect

Experimental Parasitology

journal homepage: www.elsevier.com/locate/yexpr

Echinococcus granulosus: *In vitro* effectiveness of warm water on protoscolices

Mohammad Moazeni *, Mohammad-Reza Alipour-Chaharmahali

Department of Pathobiology, School of Veterinary Medicine, Shiraz University, Shiraz 71345-1731, Iran

ARTICLE INFO

Article history:
Received 15 November 2009
Received in revised form 27 May 2010
Accepted 15 June 2010
Available online 22 June 2010

Keywords:
Echinococcus granulosus
Protoscolices
Hydatid cyst
Scolicidal
Warm water

ABSTRACT

Hydatid disease is one of the most important helminthic diseases worldwide. Hydatid cysts may be found anywhere in the body. The most effective treatment of hydatid cyst is surgical operation. Spillage of live protoscolices during the operation is the major cause of recurrence. Instillation of scolicidal agent into hydatid cyst is the most commonly employed measure to prevent this complication. To date, many scolicidal agents have been used for inactivation of the hydatid cyst content, however, most common scolicidal agents may cause unacceptable side-effects, limiting their use. In this study the scolicidal effect of warm water (45, 50, 55, and 60 °C) at different exposure times (1, 2, 3, 4, 5, 6, 8, 10, 12, and 15 min) is investigated. Protoscolices were collected aseptically from sheep livers containing hydatid cyst. Viability of protoscolices was determined by 0.1% eosin staining. Even though the highest scolicidal activity of warm water at 45 °C was 40.4% at the end of 15 min, the best scolicidal effect (100%) of warm water at 50, 55, and 60 °C was obtained after 5, 2, and 1 min, respectively. The results of this *in vitro* study showed that warm water at 50–60 °C can be regarded as an effective scolicidal agent. Warm water is commonly available, easily prepared, and inexpensive. *In vivo* scolicidal activity of warm water and also the possible side effects need further investigation.

1. 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 systems (Budke et al., 2006). Hydatid disease due to *E. granulosus* remains an important, challenging medical problem. It continues to be endemic in developing nations such as Mediterranean countries, the Middle and Far East, and South America because of their low socioeconomic conditions, but it has a worldwide distribution because of travel and migration. Although most hydatid disease is found in liver and lung, it can arise anywhere in the body (Topcu et al., 2009). 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, and is seen in approximately 10% of postoperative cases. Operative spillage can sometimes also lead to secondary disseminated intraperitoneal hydatidosis (Rajabi, 2009). Avoiding spillage of the cyst contents and the use of effective scolicidal agents are essential to lower the recurrence rate (Topcu et al., 2009). Scolicidal solutions remain indispensable in the treatment of hydatid cyst disease and surgeons need less harmful but more effective drugs in hydatid disease (Adas et al., 2009). The aim of this study was to evaluate the scolicidal effect of warm water at various temperatures and at different exposure times.

2. Materials and methods

2.1. Collection of protoscolices

For this *in vitro* study, we used scolex solutions collected from the cyst containing livers of sheep slaughtered at the Shiraz slaughterhouse, in the south of Iran. The aspirated portion of the hydatid liquid was allowed to settle in a sterile bottle and the supernatant was discarded. The remaining sediment contained thousands of protoscolices. The viability of protoscolices was confirmed prior to the experiments. The viability of protoscolices throughout this study was determined from their motility characteristics and with 0.1% eosin staining under light microscopy.

2.2. Effectiveness of warm water on scolices

In each experiment a drop of protoscolex rich sediment containing at least 1500 protoscolices was added to a test tube containing 10 ml of warm water (45, 50, 55, and 60 °C). The water temperature was monitored using a temperature-controlled water bath.

* Corresponding author. Fax: +98 711 2286940.
E-mail address: moazeni@shirazu.ac.ir (M. Moazeni).

0014-4894/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2010.06.021

Following 1, 2, 3, 4, 5, 6, 8, 10, 12, and 15 min of exposure, the tubes were immediately transferred into a glass beaker containing cold water to prevent the continuation of heat on protoscolices. Then 9 ml of the upper parts of the solutions were removed with a pipette avoiding settled protoscolices. One milliliter of 0.1% eosin solution (25 °C) was added to the tube, mixed gently and after 15 min 1.5 ml of supernatant was removed. Then remaining settled protoscolices were placed on slides and examined microscopically for viability. Each protoscolex that did not take the dye in was accepted as potentially viable (Figs. 1 and 2). At least 1500 protoscolices with no exposure to warm water was considered as the control group in each experiment. The experiments were carried out at room temperature (21–25 °C) .The percentages of dead protoscolices were determined by counting a minimum of 1000 protoscolices.

2.3. Statistical analysis

Differences between the test and control groups were analyzed with Chi-square test. Statistical analysis was performed with GraphPad InStat software. *P* values less than 0.05 were considered to be significant.

3. Results

The scolicidal effect of warm water at different temperatures and with various exposure times was shown in Table 1. While the death rate in the control group was 11%, when protoscolices were plunged into warm water at 45 °C for 1, 2, 3, 4, 5, 6, 8, 10, 12, and 15 min the mortality rates were 11.4%, 13.9%, 14.7%, 18.3%, 21.2%, 24%, 30.4%, 31.4%, 32%, and 40.4%, respectively. Except for 1 min, the difference between the scolicidal effect of warm water at 45 °C was statistically significant comparing to the control group ($P < 0.01$). While the death rate in the control group was 19.6%, all protoscolices were killed after 5 min, when plunged into warm water at 50 °C. The scolicidal effect of such water after 1, 2, 3, and 4 min were 24%, 41.9%, 39.1%, and 47.7%, respectively. The death rate for the relating control groups were 13.8%, 19.6%, 13.8%, and 13.8%, respectively. The difference between the scolicidal effect of warm water at 50 °C in 1–5 min of exposure time was statistically significant in comparison to the control groups ($P < 0.0001$). While the death rate in the control group was 15.57%, when protoscolices were plunged into warm water at 55 °C for 1 and 2 min, the mortality rates were 78.9% and 100%, respectively. Warm water at 60 °C killed all protoscolices after 1 min of exposure (the death rate in control group was 19.6%).

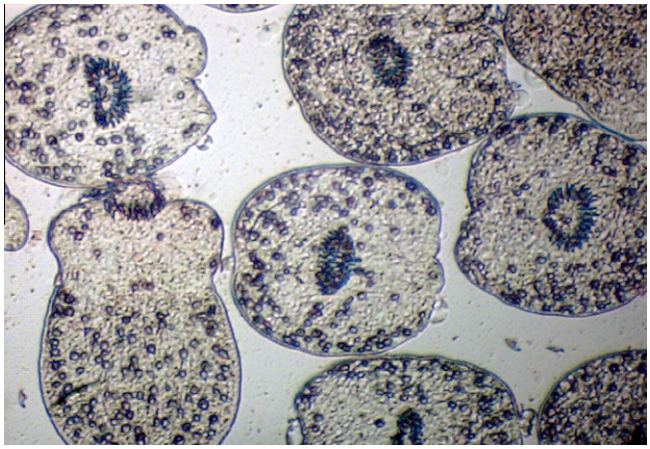

Fig. 1. Live protoscolices of *Echinococcus granulosus* after staining with 0.1% eosin.

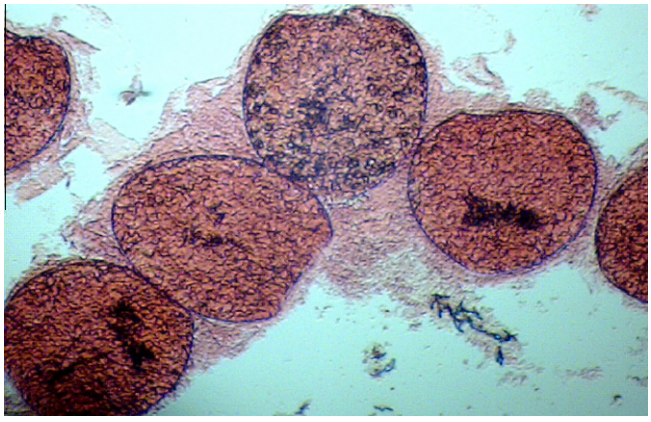

Fig. 2. Dead protoscolices of *Echinococcus granulosus* after application of warm water and staining with 0.1% eosin.

4. Discussion

Up to date, many scolicidal agents have been used for inactivation of the hydatid cyst content. Many of these scolicidal agents may cause unacceptable side-effects, limiting their use. Hypertonic saline (20%) has been found to be 100% effective on scolices of hydatid cyst at the end of 15 min (Erzurumlu et al., 1998), but acute hypernatremia, leading to convulsions, intracranial bleeding, necrosis, and myelinolysis, has been reported for this agent. In addition, adding it to the cyst cavity without evacuation can cause dilution of the hypertonic saline. Therefore, it was concluded that hypertonic saline should not be used as an agent for treating intraperitoneal disease or hydatid cysts (Kayaalp et al., 2001). Caglar et al. (2009) showed that 20% silver nitrate had complete scolicidal effects at the end of 20 min, but lethal reactions may have resulted from the absorption of silver nitrate through the pleura, cyst walls, extensive raw surfaces and the peritoneal membrane (Rajabi, 2009). Cetrimide (0.5–1%) has been found to be 100% effective on scolices of the hydatid cyst at the end of 10 min (Frayha et al., 1981). Unfortunately, because of the toxicity and side effects of cetrimide, such as methemoglobinemia, chemically induced peritonitis, increasing metabolic acidosis, convulsions, and coma, it has limited use in clinical applications (Puryan et al., 2005). Although ethyl alcohol is the scolicidal agent that is usually preferred for ultrasonic-guided percutaneous aspiration, injection and reaspiration (PAIR) of hydatid cysts, it can cause caustic damage to the epithelium of communicating bile ducts leading to sclerosing cholangitis and it is strongly concentration dependent (Besim et al., 1998). Albendazole sulfoxide (20 μg/ml) killed 5% of the scolices in 15 min, scolicidal activity was 50% with a 50 μg/ml solution, and 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 albendazole can lead to the same changes (Deger et al., 2000; Gil-Grande et al., 1993; Yetim et al., 2005). Its harder obtainability, harder preparation, and high cost are some of the main disadvantages. For this reason, it cannot be seen as an ideal scolicidal agent (Adas et al., 2009). Chlorhexidine gluconate (0.04%) demonstrated a scolicidal effect in a short period of time *in vitro* (5 min) and was 100% effective *in vivo*. It is also a non-toxic agent. Thus, chlorhexidine gluconate (0.04%) can be used safely in the treatment of hydatid cysts (Puryan et al., 2005). Honey concentrations of 10% or greater kills all protoscolices and could be used as scolicidal agent safely with no systemic side effects, such as anaphylactic reaction or hypergly-

Table 1
Scolicidal effect of warm water (45, 50, 55, and 60 °C) in different exposure times.

Temperature			Time (min)									
			1	2	3	4	5	6	8	10	12	15
45 °C	Control	Total	1950	1950	1950	1950	1950	1950	1950	1950	1950	1950
		Dead	216	216	216	216	216	216	216	216	216	216
		Dead (%)	11	11	11	11	11	11	11	11	11	11
	Test	Total	1962	2148	1888	1918	1818	2088	2023	1923	1844	1738
		Dead	224	300	278	352	386	502	615	604	590	703
		Dead (%)	11.4	13.9	14.7	18.3	21.2	24	30.4	31.4	32	40.4
50 °C	Control	Total	1895	1680	1895	1895	1680					
		Dead	263	330	263	263	330					
		Dead (%)	13.8	19.6	13.8	13.8	19.6					
	Test	Total	1951	1157	1830	2040	1748					
		Dead	649	778	716	974	1748					
		Dead (%)	24	41.9	39.1	47.7	100					
55 °C	Control	Total	2966	1680								
		Dead	462	330								
		Dead (%)	15.57	19.6								
	Test	Total	1967	1691								
		Dead	1552	1691								
		Dead (%)	78.9	100								
60 °C	Control	Total	1680									
		Dead	330									
		Dead (%)	19.6									
	Test	Total	1709									
		Dead	1709									
		Dead (%)	100									

cemia, and no local side effects on the peritoneal surface or hepatobiliary system (Kilicoglu et al., 2006, 2008). Scolicidal effect of acidic solutions with pH 1, 2, 3, and 4 after 5 min of application were 100%, 99.6%, 98.6%, and 15.5%, respectively. However, these values for alkaline solutions with pH 14, 13, 12, and 11 were 100%, 97.5%, 29.5%, and 24.5%, respectively (Moazeni and Larki, 2010). An ideal scolicidal agent is defined as being potent in low concentrations, acting in a short period of time, being stable in cyst fluid, unaffected 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 (Puryan et al., 2005; WHO, 1996). Dalimi et al. (2005) used low voltage direct electric current to inactivate the scolices of hydatid cyst. They concluded that current densities of 62.5 mA/cm^2 (11 V), 53.71 mA/cm^2 (10 V), and 18.18 mA/cm^2 (5 V), can kill all protoscolices in the hydatid fluid after 1, 2, and 3 min, respectively.

In this study we investigated the effectiveness of warm water on the scolices of hydatid cyst. The results of our study showed that warm water of 50, 55, and 60 °C can kill all protoscolices after 5, 2, and 1 min of application, respectively. The longest survival times of *E. granulosus* protoscolices have been reported 3 days at −10 °C, 36 days at 0 °C, 28 days at 10 °C, 12 days at 20 °C, 4 days at 30 °C, and 3 days at 40 °C (Diker et al., 2007). According to the results of our study, the scolicidal activity of warm water of 50 °C (5 min), 55 °C (2 min), and 60 °C (1 min) was comparable with scolicidal power of 20% hypertonic saline (15 min), 20% silver (20 min), 0.5–1% cetrimide (10 min), and 95% ethyl alcohol (15 min). This *in vitro* study showed that warm water is an effective scolicidal agent. In addition, it is commonly available, easily prepared, and inexpensive. Therefore, it may be used during hydatid cyst surgery. However, *in vivo* efficacy of warm water and also the possible side effects need further investigation.

Acknowledgments

The authors are thankful to Shiraz University for providing financial assistance (Grant No. 87-GR-VT-24). The authors kindly thank Mr. A. Motabi Alavi for his kind assistance in performing this study.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.exppara.2010.06.021.

References

Adas, G., Arikan, S., Kemik, O., Oner, A., Sahip, N., Karatepe, O., 2009. Use of albendazole sulfoxide, albendazole sulfone, and combined solutions as scolicidal agents on hydatid cysts (in vitro study). World Journal of Gastroenterology 15, 112–116.

Besim, H., Karayalçin, K., Hamamci, O., Güngör, C., Korkmaz, A., 1998. Scolicidal agents in hydatid cyst surgery. Hepatopancreatobiliary Surgery 10, 347–351.

Budke, C.M., Deplazes, P., Torgerson, P.R., 2006. Global socioeconomic impact of cystic echinococcosis. Emerging Infectious Diseases 12, 296–303.

Caglar, R., Yuzbasioglu, M.F., Bulbuloglu, E., Gul, M., Ezberci, F., Kale, I.T., 2009. In vitro effectiveness of different chemical agents on scolices of hydatid cyst. Journal of Investigative Surgery 21, 71–75.

Dalimi, A., Ghasemikhah, R., Malayeri, B.H., 2005. *Echinococcus granulosus*: lethal effect of low voltage direct electric current on hydatid cyst protoscoleces. Experimental Parasitology 109, 237–240.

Deger, E., Hokelek, M., Deger, B.A., Tutar, E., Asil, M., Pakdemirli, E., 2000. A new therapeutic approach for the treatment of cystic echinococcosis: percutaneous albendazole sulphoxide injection without reaspiration. The American Journal of Gastroenterology 95, 248–254.

Diker, A.I., Tinar, R., Senlik, B., 2007. Viability of *Echinococcus granulosus* protoscolices at different conditions. Veterinary Parasitology 150, 84–87.

Erzurumlu, K., Hokelek, M., Baris, S., Sahin, M., Birinci, A., Amanvermez, R., Tac, K., 1998. Effect of albendazole sulfoxide solution on the scolices and the hepatobiliary system. European Surgical Research 30, 433–438.

Frayha, G.J., Bikhazi, K.J., Kachachi, T.A., 1981. Treatment of hydatid cysts (*Echinococcus granulosus*) by cetrimide (R). Transactions of the Royal Society of Tropical Medicine and Hygiene 75, 447–450.

Gil-Grande, L.A., Sánchez-Ruano, J.J., García-Hoz, F., Bárcena, R., Rodriguez-Caabeiro, F., Brasa, C., Casado, N., Prieto, J.G., Alvarez, A.I., Aguilar, L., Dal-Ré, R., 1993. Randomised controlled trial of efficacy of albendazole in intra-abdominal hydatid disease. The Lancet 342, 1269–1272.

Kayaalp, C., Balkan, M., Aydin, C., Ozgurtas, T., Tanyuksel, M., Kirimlioglu, V., Akoglu, M., Oner, K., Pekcan, M., 2001. Hypertonic saline in hydatid disease. World Journal of Surgery 25, 975–979.

Kilicoglu, B., Kismet, K., Kilicoglu, S.S., Erel, S., Gencay, O., Sorkun, K., Erdemli, E., Akhan, O., Akkus, M.A., Sayek, I., 2008. Effects of honey as a scolicidal agent on the hepatobiliary system. World Journal of Gastroenterology 14, 2085–2088.

Kilicoglu, B., Kismet, K., Koru, O., Tanyuksel, M., Oruc, M., Sorkun, K., Akkus, M., 2006. The scolicidal effects of honey. Advances in Therapy 23, 1077–1083.

Moazeni, M., Larki, S., 2010. In vitro effectiveness of acidic and alkaline solutions on scolices of hydatid cyst. Parasitology Research 106, 853–856.

Puryan, K., Karadayi, K., Topcu, O., Canbay, E., Sumer, Z., Turan, M., Karayalcin, K., Sen, M., 2005. Chlorhexidine gluconate: an ideal scolicidal agent in the

treatment of intraperitoneal hydatidosis? World Journal of Surgery 29, 227–230.
Rajabi, M.A., 2009. Fatal reactions and methaemoglobinaemia after silver nitrate irrigation of hydatid cyst. Surgical Practice 13, 2–7.
Topcu, O., Sumer, Z., Tuncer, E., Aydin, C., Koyuncu, A., 2009. Efficacy of chlorhexidine gluconate during surgery for hydatid cyst. World Journal of Surgery 33, 1274–1280.
WHO, 1996. WHO Informal Working Group on Echinococcosis: guidelines for treatment of cystic and alveolar echinococcosis in humans. Bulletin of the World Health Organization 74, 231–242.
Yetim, I., Erzurumlu, K., Hokelek, M., Baris, S., Dervisoglu, A., Polat, C., Belet, U., Buyukkarabacak, Y., Guvenli, A., 2005. Results of alcohol and albendazole injections in hepatic hydatidosis: experimental study. Journal of Gastroenterology and Hepatology 20, 1442–1447.