

Effect of Different Concentrations of Hypertonic Saline at Different Times on Protoscolecocytes of Hydatid Cyst Isolated From Liver and Lung

Karaciğer ve Akciğerden İzole Edilen Hidatik Kist Protoskoleksleri Üzerine Hipertonik Tuzun Farklı Zaman ve Farklı Konsantrasyonlardaki Etkisi

Khosrow Hazreti Tappeh¹, Ali Einshaei², Rahim Mahmudloo², Habib Mohammadzadeh¹, Mansoor Tahermaram¹, Seyed Javad Mousavi²

¹Department of Parasitology and Mycology, Urmia University of Medical Sciences, Urmia, Iran

²Department of Surgery, Urmia University of Medical Sciences, Urmia, Iran

ABSTRACT

Objective: Most surgeons inject scoloidal materials into the cyst before or after its removal, since any contamination to normal sites will cause re-growth of the same cyst. The aim of this study was to determine the lethal effect of hypertonic saline at different doses and different times on protoscolecocytes of lung and liver.

Methods: The livers and lungs of killed animals with hydatid cyst disease were gathered from Urmia Industrial Abattoirs. They were transferred to the university parasitological lab immediately. The hydatid cyst fluid was aspirated with a 10 mm syringe and poured into a 15cc tubes. The movement of protoscolecocytes and staining with 0.1% eosin was the test to determine viability of protoscolecocytes. Those with color absorption were those which were not viable. Different concentrations of hypertonic saline were given at different time.

Results: The results showed that in 20% of hypertonic saline in the 4th minute, 80% of protoscolecocytes were alive while in the 5th minute 50% were alive, in the 7th minute 20% and 8th minute 5%, 9th minute all of them were dead. In the 10% concentration, at up to 9 minutes 50% were alive, in the 18th minute 20% and in 30 minutes 10% of protoscolecocytes were alive. In the 5% concentration at up to 10 minutes 90% were alive while in the 22nd minute 80% and in 30 minutes 70% of protoscolecocytes were alive.

Conclusion: When we inject 20% hypertonic saline into the cyst cavity there is a probability that the cyst contaminates the bile duct and liver through the small hole we made. This material may cause widespread necrosis of the liver. We should use 10% hypertonic saline minimally for 45 minute before surgery and after cyst removal, since the hypertonic saline itself may cause injury to the biliary system.

(*Turkiye Parazitol Derg* 2011; 35: 148-50)

Key Words: Hydatid cyst, hypertonic saline, *Echinococcus granulosus*

Received: 09.05.2011

Accepted: 20.07.2011

ÖZET

Amaç: Birçok cerrah hidatik kisti çıkarmadan önce veya sonra yeni bir kist gelişimine engel olmak amacıyla kistin içine skolisidal materyal enjekte etmektedir. Bu çalışmanın amacı, akciğer ve karaciğerdeki hidatik kistlerde bulunan protoskoleksler üzerine farklı zaman ve dozlarda uygulanan hipertonik tuzun öldürücü etkisini belirlemektir.

Yöntemler: Hidatik kist saptanan akciğer ve karaciğerler, Urmia şehri mezbahasındaki kesimlerden elde edilmiştir. Bu kistler hemen üniversite parazitoloji laboratuvarına getirilmiştir. Hidatik kist sıvısı 10 ml'lik şırınga ile aspire edilerek 15 ml'lik tüplere aktarılmıştır. Protoskolekslerin hareketleri gözlemlenmiş ve canlılıklarını belirlemek için %0.1'lik eosin ile boyanmıştır. Boya alanlar cansız olarak değerlendirilmiştir. Hipertonik tuzun farklı konsantrasyonları farklı zamanlarda verilmiştir.

Bulgular: Hipertonik tuzun %20 konsantrasyonunda; 4 dk'da protoskolekslerin %80'inin, 5 dk'da %50'sinin, 7 dk'da %20'sinin, 8 dk'da %5'inin canlı olduğu ve 9 dk'da hepsinin öldüğü belirlenmiştir. Hipertonik tuzun %10'luk konsantrasyonunda ise 9 dk'ya kadar protoskolekslerin %50'sinin canlı olduğu, 18 dk'da %20'sinin, 30 dk'da %10'unun canlı olduğu belirlenmiştir. %5 konsantrasyonda ise 10 dk'ya kadar %90'ının canlı olduğu ve 22. dk'da %80'inin, 30 dk'da %70'inin canlı olduğu gözlemlenmiştir.

Address for Correspondence / Yazışma Adresi: Dr. Rahim Mahmudloo, Department of Surgery, Urmia University of Medical Sciences, Urmia, Iran Phone: +984413654928 E-mail: mahmodlou@yahoo.com
doi:10.5152/tpd.2011.37

Sonuç: Hidatik kist boşluğunun içine %20'lik hipertonic tuz enjekte edildiği zaman, kistin safra kanalı ve karaciğeri şırınga tarafından açılan küçük delikten kontamine etme olasılığı bulunmaktadır. Bu materyal karaciğer nekrosisinin yayılımına sebep olabilir. Hipertonic tuzun kendisi de bilier sistemde hasara sebep olabileceğinden operasyondan en az 45 dk önce ve kist çıkarıldıktan sonra %10'luk hipertonic tuz mutlaka kullanılmalıdır. (*Türkiye Parazitoloji Dergisi* 2011; 35: 148-50)

Anahtar Sözcükler: Hidatik kist, hipertonic tuz, *Echinococcus granulosus*

Geliş Tarihi: 09.05.2011

Kabul Tarihi: 20.07.2011

INTRODUCTION

Echinococcosis in humans and animals is an economic and public health concern in many parts of the world (1). The infection is most prevalent in sheep- and cattle-raising regions like Australia, South America, the Middle East, South Africa, Eastern Europe, and the Mediterranean region (2). Cystic hydatid disease affects mainly the liver (50-70% of all cysts) but can also develop in lung (20-30%) and, less frequently, in spleen, kidney, bone, brain, and other organs (3).

There are currently three treatment options for hydatid disease of the liver: surgery, which remains the most efficient treatment, percutaneous aspiration, and medical treatment (4). Surgery is still the preferred method of treatment, although it increases the risk of intraoperative spillage of scolices.

Currently, many scolical agents, which have some complications of their own, have been used for inactivation of the cyst content (5). Various scolical solutions have been used for surgical and precutaneous approaches. 1, 2 Caustic sclerosing cholangitis is a dreadful complication after surgical treatment. Hypertonic saline is one of the most common scolical agents in the world. The rationale for its use is simply that it affects a sufficiently strong osmotic gradient across the outer cuticular membrane of the scolex to cause lysis.

The objective of this study is to determine the scolical effect of hypertonic saline in different concentrations using different exposure times.

METHOD

Protoscolices of *Echinococcus granulosus* were obtained from the infected livers and lung of sheep slaughtered at Urmia city in Northwest of Iran. The hydatid fluid was transferred into glass cylinders and left to set for 30 min.

The protoscolices settled down at the bottom of the cylinders. The supernatant was removed and the yielded protoscolices were washed two times with normal saline. The viability of the protoscolices was confirmed by their motility under a light microscope and protoscolices stained with 0.1% eosin were examined under at room temperature. Each protoscolex that did not take the dye in was accepted as potentially viable.

The live protoscolices were finally transferred into a dark container containing normal saline solution and stored at 4°C for further use.

The mean number of protoscolices in the cyst fluid obtained from different cysts in different periods was found as 1000 protoscolices/mm³ and fourteen samples of 0.5 cc sediment were obtained. Various concentrations of 1 cc sodium chloride solutions were added to each sediment (1%, 2%, 3%, 4%, 5%, 6%,

7%, 8%, 9%, 10%, 20% respectively). We waited 1, 2, 3, 4, 5, 6, 7, 8, 9, 10,29, and 30 minutes for each concentration, following which the upper portions of the solutions were removed. The remaining settled protoscolices were then washed in normal saline and after staining, examined for viability.

RESULTS

The viability ratios of protoscolices after exposure to different concentrations of the sodium chloride in the different times are shown in Table 1.

Hypertonic saline 20% had a strong scolical effect, because killed all of the protoscolices at the end of 9 minutes. The hypertonic saline 10% killed ninety percent of the protoscolices at 30 minute (Table 1).

DISCUSSION

Surgery is still the treatment that has the potential to remove *Echinococcus granulosus* cysts and lead to complete cure. The main principles of surgery are the prevention of spillage of the scolices by a suitable placement of swabs soaked with scolical agents (6). If the liquid content of the cyst penetrate to neighbor organs as the surgeon is trying to expel and/or unload it, there will be always a risk of creation of new cysts and recurrence of the disorder (7). So to prevent (or at least decrement of probability of) this phenomenon, scolical agents were injected to cyst during surgery by surgeon (8). Various materials were already used as scolical agents (9). The use of protoscolical substances for intraoperative killing of protoscolices is questionable, as there is no ideal agent that is both effective and safe (10). Hypertonic saline has been used as a scolical in various concentrations 3-30% (11). It is known that high concentration saline can cause sclerosing cholangitis and bile duct strictures.

Avoiding spillage of the cyst contents and the use of effective scolical agents are essential to lower the recurrence rate (12). Various scolical agents such as 95% alcohol, povidone iodine, hypertonic saline, hydrogen peroxide, 5% formalin, silver nitrate, cetrimide, and albendazole have been evaluated for scolical effects (6, 12). Deliberation on the side effects of some materials like Silver Nitrate, Formalin, Hypertonic Saline, and Cetrimide-C on 24 Guinea pigs was cited in an article published in 1991. These effects were scrutinized in two phases: first phase at the first week and the other after five months. Damages caused by formalin were 45% in first week and 40% after five months. Cetrimide-C made 11% of damage in first week and 18% after five months. Silver Nitrate caused a damage of 4% in first week and 7% after five months, and hypertonic Saline caused 9% and 4% damages in first week and after five months, respectively (13). A good scolical agent might do its lytic effect in lowest concentrations, shortest time, and with the least side effects.

Table 1. Scolicidal effects of different concentrations of hypertonic saline and exposure times

	Scolocidal agents	Time (Minute)												
		2	4	6	8	10	12	14	16	18	20	24	26	30
Toxicity	Salin0.9%	98	98	98	98	98	96	96	96	95	90	90	90	95
	Salin1%	98	98	98	98	98	96	96	96	95	90	90	90	95
	Salin2%	98	98	98	98	98	96	96	96	95	90	90	90	95
	Salin3%	98	98	98	98	75	96	96	96	95	90	90	90	95
	Salin4%	95	90	90	90	90	90	90	90	95	90	80	80	80
	Salin5%	98	90	90	90	90	90	90	90	90	90	70	70	70
	Salin6%	85	85	85	85	85	85	85	80	80	80	80	80	80
	Salin7%	90	90	90	90	90	80	80	80	80	80	80	50	50
	Salin8%	80	80	70	70	70	70	70	70	70	70	40	40	40
	Salin9%	90	90	60	60	60	40	40	40	30	30	30	30	20
	Salin10%	90	80	40	40	40	20	20	20	20	10	10	10	10
	Salin20%	80	80	20	5	0	0	0	0	0	0	0	0	0

Injection of a scolecidal agent into a cyst without unloading its contents could cause its dilution, as lytic effect of a scolecidal agent is relevant to its concentration and not to contact time.

We found different concentrations of hypertonic saline, for example 1%, 2%, 3%, 4% and 5% ineffective even at the end of 30 minutes. Saidi and Kayaalp demonstrated that 5% saline was ineffective in 60 minutes. Ten percent saline was effective at the end of 75 minutes (11), but we found 10% saline to be effective in 30 minutes. Another study had shown that 20% saline was effective in 5 minutes (14), but we shown that 20% saline was effective in 9 minutes.

According to the results of present study, hypertonic saline with concentration 20% killed all protoscoleces of hydatid cyst at the end of 9 minutes. Hypertonic saline did not cause any systemic side effects when it was applied intraperitoneally.

Acknowledgements

We would like to thank Vice Research Chancellor of Urmia University of Medical Sciences for funding this research.

Conflict of Interest

No conflict of interest was declared by the authors.

REFERENCES

- Lahmar S, Lahmar S, Boufana B, Bradshaw H, Craig PS. Screening for *Echinococcus granulosus* in dogs: comparison between arecoline purgation, coproELISA and coproPCR with necropsy in pre-patent infection. *Veterinary Parasitology* 2007; 144: 287-92. [CrossRef]
- Zulfikaroglu B, Koc M, Ozalp N, Ozmen MM. A rare primary location of echinococcal disease: report of a case. *Upsala Journal of Medical Sciences* 2005; 110: 167-71. [CrossRef]
- Ammann R, Eckert J. Cestodes: *Echinococcus*. *Gastroenterol Clin North Am* 1996; 25: 655-89. [CrossRef]

- Adas G, Arıkan S, Kemik O, Oner A, Sahip N, Karatepe O. Use of albendazole sulfoxide, albendazole sulfone and combined solutions as scolecidal agents on hydatid cysts (in vitro study). *World J Gastroenterol* 2009; 15: 112-6. [CrossRef]
- McManus DP, Zhang W, Li J, Bartley PB. Echinococcosis. *Lancet* 2003; 362: 1295-304. [CrossRef]
- Sahin M, Eryılmaz R, Bulbuloglu E. The effect of scolecidal agents on liver and biliary tree (experimental study). *J Invest Surg* 2004; 17: 323-6.
- Barros JIU. Hydatid disease of the liver. *AM J Surg* 1987; 135: 597-600.
- Hazrati tappe KH, Mousavi S J, Barazesh A. In vitro study of scolecidal effect of Methylen Blue in Hydatid cyst. *Iranian South Medical Journal* 2010; 13: 123-8.
- Eyüpoğlu B, Doğanay M, Reis E, et al. The effects of scolecidal agents on hepatopancreaticobiliary system "An experimental study". *Turkish J Gastroenterol* 1999; 10: 280-6.
- Yetim I, Erzurumlu K, Hokelek M, Baris S, Dervisoglu A, Polat C, et al. Results of alcohol and albendazole injections in hepatic hydatosis: Experimental study. *J Gastroenterol Hepatol* 2005; 20: 1442-7. [CrossRef]
- Kayaalp C, Balkan M, Aydin C, Ozgurtas T, Tanyuksel M, Kirimlioglu V, et al. Hypertonic saline in Hydatid Disease. *The world journal of surgery* 2001; 25: 975- 9. [CrossRef]
- Topcu O, Sumer Z, Tuncer E, Aydin C, Koyuncu A. Efficacy of chlorhexidine gluconate during surgery for hydatid cyst. *World J Surg* 2009; 33: 1274-80. [CrossRef]
- Besim H, Karayalçın K, Hamamci O, Güngör C, Korkmaz A. Scolicidal agents in hydatid cyst surgery. *HPB Surgery* 1998; 10: 347-51. [CrossRef]
- Karayalçın K, Besim H, Sonisik M, Erverdi N, Korkmaz A, Aras N. Effect of hypertonic saline and alcohol on viability of daughter cysts in hepatic hydatid disease. *European Journal of Surgery* 1999; 165: 1043-4.