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In vitro efficacy of different chemical substances on hydatid cyst components

Ömer Faruk AKINCI[1], Mustafa KARAOĞLANOĞLU[2], Mustafa Sedat BOZKURT[3], Leyla GÖZAYDIN[4], Salih Zeki ZİYLAN[5]

Aim: Cyst membrane, daughter cyst, and pericyst are the focal point of some problems related to the percutaneous treatment of hydatid disease. This study was planned to investigate the in vitro efficacy of sodium hypochlorite, hydrogen peroxide, some widely used scolicidal agents, and some chemical solvents on hydatid cyst components.

Materials and methods: To investigate the effects of various pharmacological and chemical agents on hydatid cyst components, 5 mL each of 15 different agents was put into bottles. Tissue measuring 2 × 1 cm was cut from the pericyst and the cyst membrane was put into each bottle. Two undamaged daughter cysts obtained from the same cyst were added to the bottles. Evaluation was made by visual observation at different intervals over 20 days. Any change in the cyst membranes, daughter cysts, and pericysts including perforation, translucency, destruction, swelling, and melting were recorded and scored.

Results: Hydrogen peroxide was the only agent with maximal effect on the 3 components. One percent sodium hypochlorite solution was also effective and the fastest acting agent on the cyst membranes and daughter cysts. Alcohol had no effect on any of the 3 components. Hypertonic saline had a minimal effect on the cyst membrane and no effect on the pericyst and daughter cysts. Ursodeoxycholic acid and silver nitrate were moderately effective on the pericyst. No chemical solvent was effective on the hydatid cyst contents.

Conclusion: Hydrogen peroxide had the maximal effect on all 3 components of the cyst. Sodium hypochlorite was effective on daughter cysts and cyst membranes and slightly effective on pericysts and it appears to be a good agent to be used in percutaneous drainage. However, further studies on the effect of sodium hypochlorite should be carried out in vitro and then its in vivo efficacy should be investigated.

Key words: Cyst membrane, sodium hypochlorite, daughter cyst, hydatid cyst, percutaneous drainage, scolicidal agent

Hidatik kist komponentleri üzerine değişik kimyasal ajanların in vitro etkileri

Amaç: Hidatik kistin perkütan tedavisi ile ilişkili problemlerin odak noktasında kist membranı, kız veziküller ve perikist bulunmaktadır. Bu çalışma sodyum hipoklorit, hidrojen peroksit, diğer yaygın olarak kullanılan skolisidal ajanlar ve bazı kimyasal çözücülerin hidatik kist komponentleri üzerindeki etkilerinin in vitro ortamda incelenmesi amacıyla planlanmıştır.

Yöntem ve gereç: Değişik farmakolojik ve kimyasal ajanların kist hidatik komponentleri üzerine etkisini araştırmak için 15 farklı ajana ait 5 mLlik solüsyonlar şişelere yerleştirildi. Perikist ve kist membranından elde olunan 2 × 1 cmlik doku parçaları şişelere eklendi. Aynı kistten elde edilen hasarsız iki adet kız vezikül de bu şişelere ilave edildi. Değerlendirme 20 gün boyunca farklı zaman dilimlerinde gözlemlenerek yapıldı. Kist membranı, kız vezikül ve perikistteki perforasyon, translusensi, destrüksiyon, şişme, erime gibi tüm değişiklikler kaydedildi ve skorlandı.

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[1] Department of General Surgery, Sema Hospital, İstanbul - TURKEY
[2] Department of Radiology, Atatürk Educational and Research Hospital, Ankara - TURKEY
[3] Department of Radiology, OSM Middle East Hospital, Şanlıurfa - TURKEY
[4] Department of Radiology, Kahta State Hospital, Adıyaman - TURKEY
[5] Department of Radiology, Faculty of Medicine, Harran University, Şanlıurfa - TURKEY
Correspondence: *Mustafa KARAOĞLANOĞLU, Department of Radiology, Atatürk Educational and Research Hospital, Ankara - TURKEY*
E-mail: mustafakaraoglanoglu@hotmail.com

Bulgular: Hidrojen peroksit her üç komponent üzerinde de en çok etkiyi gösteren tek ajandı. % 1 sodyum hipoklorit solusyonu da kist membranı ve kız vezikül üzerine en hızlı etki gösteren ajandı. Alkol her üç komponente de etki göstermedi. Hipertonik salin kist membranı üzerine minimal etki göstermiş olup, perikist ve kız vezikül üzerine etki göstermemiştir. Ursodeoksikolik asit ve gümüş nitrat perikist üzerine az etkilidir. Kimyasal çözücüler hidatik kist komponentleri üzerine etkili olmamıştır.

Sonuç: Hidrojen peroksit her üç komponent üzerine en çok etkili olmuştur. Sodyum hipoklorit kız vezikül ve kist membranı üzerine etkili olup, perikist üzerinde az etkisi bulunmaktadır, bu da perkütan drenaj sırasında iyi bir ajan olabileceğini göstermektedir. Ancak sodyum hipokloritin in vitro ve in vivo etkinliğinin araştırılması için daha fazla çalışmaya ihtiyaç vardır.

Anahtar sözcükler: Kist membranı, sodyum hipoklorit, kız vezikül, kist hidatik, perkütan drenaj, skolisidal ajan

Introduction

Percutaneous drainage has been increasingly used in the treatment of hydatid cysts (1-4). Some authors perform surgical treatment only in cases not suitable for percutaneous treatment (5). To inactivate hydatid cyst in percutaneous drainage and surgical procedures, some materials such as alcohol, hypertonic saline, and silver nitrate have been used as scolicidal agents. In spite of the fact that percutaneous drainage has been increasingly performed, the effect of scolicidal agents on hydatid cyst contents has not been sufficiently studied. In a recent study conducted by our research group, it has been demonstrated that scolicidal agents such as alcohol and hypertonic saline had little or no effect on hydatid cyst membranes. On the other hand, sodium hypochlorite, which is not used in clinical practice for this purpose, dissolved hydatid cyst membranes in a few minutes (6). This led us to search for a new scolicidal agent that might inactivate the parasites and dissolve the cyst contents. It is known that daughter cysts are important components of type III and IV hydatid cysts, which are fluid collection with daughter cysts and solid collection, respectively (1,7,8). Pericyst is an important barrier between the cyst and the host tissue. An ideal scolicidal agent used in percutaneous drainage should inactivate vital components of the cyst and dissolve cyst membranes and daughter cysts but at the same time should have no harmful effects on pericysts because it is the last protective barrier between the parasite and the host tissue. The aim of this study was to test the in vitro macroscopic efficacy of different scolicidal and chemical solvents on *Echinococcus granulosus* cyst components.

Materials and methods

To investigate the in vitro efficacy of different pharmacolological and chemical agents on daughter cysts, cyst membranes and pericysts 5 mL solution of each in-study pharmacological agent (Table 1) was put into 20 mL bottles. Only crystal clear water was put into a control bottle. Tissue measuring 2 × 1 cm was cut from the pericyst and the cyst membrane obtained from a hydatid cyst of a case that underwent total cystectomy located in the liver put into each bottle. Two undamaged daughter cysts obtained from the same cyst were added to each bottle, which were assigned numbers from 1 to 15. The observations were performed by researchers who did not know the content of the bottles. All the bottles were maintained in the same place and under the same environmental conditions. The observations were recorded at 15, 30, and 60 min; at 2, 3, 6, 12, and 24 h; and on 2, 3, 6, 10, and 20 days. Observed changes in the cyst membrane and pericyst were scored as follows: Level 1, the integrity of hydatid cyst membrane was preserved without melting, destruction, swelling, or translucency; Level 2, there was "moderate change" in membrane swelling or destruction; Level 3, severe change in membrane, destruction or swelling, or having translucency; Level 4, hydatid cyst membrane was fragmented into pieces less than 2 mm in size; Level 5, hydatid cyst membrane melted completely.

Observed changes in the daughter cyst were scored as follows: Level 1, nonperforated daughter cyst; Level 2, perforated daughter cyst; Level 3, the membrane of daughter cyst was melted.

All the bottles were kept closed at room temperature. The final evaluation was performed on the 20th day.

Table 1. In-study agents or substances.

Bottle Number	Preparation	Content	Manufacturer
1	NaOCl 0.05%	Sodium hypochlorite	Sigma 23930-5
2	NaOCl 0.02%	Sodium hypochlorite	Sigma 23930-5
3	NaOCl 0.1%	Sodium hypochlorite	Sigma 23930-5
4	NaOCl 1%	Sodium hypochlorite	Sigma 23930-5
5	$AgNO_3$ 1%	Silver nitrate	Sigma S8157
6	Ursodeoxycholic acid 10%	Ursodeoxycholic acid	Sigma U 5127
7	5-Sulfosalicylic acid 10%	5-Sulfosalicylic acid	Sigma S 7422
8	Triton X 100 10%	t-acetylphenoxypolyethoxyethanol	Sigma X 100
9	H_2O_2 30%	Hydrogen peroxide	Sigma H1009
10	TCA 10%	Trichloroacetic acid	Sigma T 9159
11	Potassium permanganate 1%	Potassium permanganate	Sigma P 9810
12	Brij 35 Solution 10%	23 Lauryl ether	Sigma P 1254
13	NaCl 15%	Sodium chloride 15%	Eczacıbaşı Baxter
14	Absolute Ethyl alcohol	Ethanol	Sigma E 7023

Results

The effects of pharmacological and chemical agents on cyst membranes, daughter cysts, and pericysts are given in Tables 2, 3, and 4, respectively.

Hydrogen peroxide was observed to be the most effective agent on cyst membranes, and the membrane in the sample melted completely on the third day. Sodium hypochlorite solutions were also effective on cyst membranes. The membranes in the sodium hypochlorite either thinned considerably or fragmented into small pieces (Level 4). The ursodeoxycholic acid and alcohol had no effective on the cyst membrane (Table 2).

Hydrogen peroxide was the only agent in which the daughter cyst membranes melted completely during the 20th day control. One percent sodium hypochlorite perforated the daughter cysts at 15 min. Perforations were also observed in the daughter cysts in other sodium hypochlorite solutions, trichloroacetic acid, lauryl ether, and sulfosalicylic acid at different times. At the end of the 20th day, the daughter cysts were undamaged in the potassium permanganate, hypertonic saline, alcohol, silver nitrate, ursodeoxycholic acid, and trichloroacetic acid (Table 3).

Hydrogen peroxide was the most effective agent on the pericyst. While ursodeoxycholic acid and silver nitrate were moderately effective on pericysts, the other agents had little or no effect on the pericysts (Table 4).

Hydrogen peroxide was the only agent with the maximal effect; this means that all the 3 cyst components melted completely in the sample. The lid of the bottle containing hydrogen peroxide flew off because of the production of gas pressure in a few minutes. Therefore, a needle was inserted into the lid of that sample to reduce the pressure. On the other hand, 1% sodium hypochlorite solution was the fastest acting agent on cyst membranes and daughter cysts.

Alcohol, which is a widely used sclerosing agent, had no effect on any of the 3 cyst components.

Discussion

The present study focused on the macroscopic efficacy of the agents on the hydatid cyst components, and therefore the in vitro effect of agents were investigated only by macroscopic observations and not supported by microscopy. Although some agents

Table 2. Observed changes across the study period in the cyst membrane samples.

Bottle Number	Agents	Minute			Hour					Day				
		15	30	60	2	3	6	12	24	2	3	6	10	20
1	NaOCl 0.05%	1	1	2	2	2	2	3	3	3	3	3	3	3
2	NaOCl 0.02%	1	2	2	2	3	3	3	3	4	4	4	4	4
3	NaOCl 0.1%	1	2	2	3	3	3	4	4	4	4	4	4	4
4	NaOCl 1%	2	2	3	3	4	4	4	4	4	4	4	4	4
5	$AgNO_3$	1	1	2	2	2	3	3	3	3	3	3	3	3
6	Ursodeoxycholic acid	1	1	1	1	1	1	1	1	1	1	1	1	1
7	5-Sulfosalicylic acid	1	1	1	1	2	2	2	2	2	2	2	2	2
8	Triton X 100	1	1	1	1	1	1	1	1	2	2	2	2	2
9	H_2O_2 30%	1	2	3	3	3	3	3	4	4	5	5	5	5
10	TCA	1	1	1	1	1	1	1	2	2	2	2	2	2
11	Potassium permanganate	1	1	2	2	2	2	2	2	2	2	2	2	2
12	Brij 35 Solution	1	1	1	1	2	2	2	2	2	2	2	2	2
13	NaCl 15%	1	1	1	1	1	1	1	1	1	2	2	2	2
14	Absolute Ethyl alcohol	1	1	1	1	1	1	1	1	1	1	1	1	1
15	Control	1	1	1	1	1	1	1	1	1	1	1	1	1

$AgNO_3$: Silver nitrate, H_2O_2: Hydrogen peroxide, TCA: Trichloroacetic acidNaOCl: Sodium hypochlorite, NaCl: Sodium chloride
1: Integrity of samples was preserved. No melting, fragmentation, swelling, or translucency. 2: Moderate fragmentation or swelling, 3: Severe swelling or destruction of samples or translucency. 4: Samples were scattered into small pieces. 5: Hydatid cyst membrane melted completely.

Table 3. Observed changes across the study period in the daughter cysts samples.

Bottle Number	Agents	Minute			Hour					Day				
		15	30	60	2	3	6	12	24	2	3	6	10	20
1	NaOCl 0.05%	1	1	1	1	1	2	2	2	2	2	2	2	2
2	NaOCl 0.02%	1	1	1	1	1	2	2	2	2	2	2	2	2
3	NaOCl 0.1%	1	1	1	1	2	2	2	2	2	2	2	2	2
4	NaOCl 1%	2	2	2	2	2	2	2	2	2	2	2	2	2
5	$AgNO_3$	1	1	1	1	1	1	1	1	1	1	1	1	1
6	Ursodeoxycholic acid	1	1	1	1	1	1	1	1	1	1	1	1	1
7	5-Sulfosalicylic acid	1	1	1	1	1	1	1	1	1	1	1	1	2
8	Triton X 100	1	1	1	1	1	2	2	2	2	2	2	2	2
9	H_2O_2 30%	1	1	1	1	1	1	1	1	2	2	2	2	3
10	TCA	1	1	1	1	1	1	1	1	1	1	1	1	1
11	Potassium permanganate	1	1	1	1	1	1	1	1	1	1	1	1	1
12	Brij 35 Solution	1	1	1	1	1	1	2	2	2	2	2	2	2
13	NaCl 15%	1	1	1	1	1	1	1	1	1	1	1	1	1
14	Absolute Ethyl alcohol	1	1	1	1	1	1	1	1	1	1	1	1	1
15	Control	1	1	1	1	1	1	1	1	1	1	1	1	1

$AgNO_3$: Silver nitrate, H_2O_2: Hydrogen peroxide,
TCA: Trichloroacetic acid NaOCl: Sodium hypochlorite,
NaCl: Sodium chloride
1: Nonperforated samples, 2: Perforated samples; 3: Membrane of daughter cyst was melted.

Table 4. Observed changes across the study period in the pericyst samples.

Bottle Number	Agents	Minute			Hour					Day				
		15	30	60	2	3	6	12	24	2	3	6	10	20
1	NaOCl 0.05%	1	1	1	1	1	1	1	1	1	1	1	1	2
2	NaOCl 0.02%	1	1	1	1	1	1	1	1	1	1	1	2	2
3	NaOCl 0.1%	1	1	1	1	1	1	1	1	1	1	2	2	2
4	NaOCl 1%	1	1	1	1	1	1	1	1	1	1	2	2	2
5	$AgNO_3$	1	1	1	1	1	1	1	1	1	1	2	2	3
6	Ursodeoxycholic acid	1	1	1	1	1	1	1	1	1	2	2	3	3
7	5-Sulfosalicylic acid	1	1	1	1	1	1	1	1	1	1	1	1	1
8	Triton X 100	1	1	1	1	1	1	1	1	1	1	1	2	2
9	H_2O_2 30%	1	1	1	1	1	1	1	2	2	3	4	4	5
10	TCA	1	1	1	1	1	1	1	1	1	1	1	1	1
11	Potassium permanganate	1	1	1	1	1	1	1	1	1	1	1	1	1
12	Brij 35 Solution	1	1	1	1	1	1	1	1	1	1	2	2	2
13	NaCl 15%	1	1	1	1	1	1	1	1	1	1	1	1	1
14	Absolute Ethyl alcohol	1	1	1	1	1	1	1	1	1	1	1	1	1
15	Control	1	1	1	1	1	1	1	1	1	1	1	1	1

$AgNO_3$: Silver nitrate, H_2O_2: Hydrogen peroxide, TCA: Trichloroacetic acid, NaOCl: Sodium hypochlorite, NaCl: Sodium chloride 1: Integrity of samples was preserved. No melting, fragmentation, swelling, or translucency. 2: Moderate fragmentation or swelling, 3: Severe swelling or destruction of samples or translucency. 4: samples were scattered into small pieces. 5: Samples melted completely.

used in this study had sclerosing and scolicidal effect, we only aimed to discuss the problem of limitations of percutaneous drainage of hydatid cysts.

Hydatid cyst caused by *Echinococcus granulosus* has a special structure. The outermost layer is the pericyst, which is formed by the host tissue reaction. The laminated membrane is a thick and non-living layer and surrounds the inside of the pericyst. The germinative membrane is an active and living part of the cyst, which is made up of single layer cells and covers the inner portion of laminated membrane. Laminated and germinative membranes are attached to each other and both of these are called the cyst membrane. The cyst interior consists of crystal clear fluid, hydatid sand, and occasionally of daughter cysts, which are formed by intrusions from the germinative layer.

Some important problems are encountered in the treatment of liver hydatid cysts. Medical treatment alone is not sufficient (1,5,7,9-11). Since the 1970s, mebendazole and albendazole have been used for the treatment of hydatid disease. In one study, even after 4 months of treatment with albendazole, 15% of cysts showed no change, with a limited improvement recorded in 24% (12). Studies indicate that albendazole treatment is not highly successful and needs prolonged therapy. Moreover, it has been proven that this agent is teratogenic and hepatotoxic (13-15). Although medical treatment is not effective alone, benzimidazole derivates are commonly used for disseminated systemic disease in inoperable cases, and in prophylaxis during surgery and percutaneous drainage (9,10).

The traditional treatment of liver hydatid cysts is surgical. However, the surgical results indicate a high rate of mortality, morbidity, and recurrence (1,5,8). Surgical procedures range from simple punctures and aspirations of the cyst, to liver resections or even transplantation, but the most common technique is total or partial cystectomy (10). The mortality rate ranges from 0% to 6.3% and the complication rate of surgery varies between 12.5% and 80% in accordance with the surgical technique performed and the nature of the cysts (1,16).

The percutaneous aspiration of hydatid cysts was considered to be contraindicated due to two main potential risks: anaphylactic shock and dissemination of crystal clear fluid into the abdomen (1). However, in the early 1980s several reports of accidental punctures of abdominal hydatid cysts with no severe complications contributed to the application of deliberate puncture of abdominal cysts followed by the introduction of a scolicidal agent. WHO consultants recommended this method as an alternative method to surgery (8).

Percutaneous drainage, previously accepted as contraindicated, has been recently considered as a popular treatment method but it has some limitations as well. To begin with, it is not possible to remove all of the components of the cyst, to destroy the cavity completely, and to drain the cyst which contains solid material. Moreover, the cases treated with percutaneous drainage need a long follow-up period up to 5-10 years (1-3,17).

One of the remarkable findings in this study was to observe that alcohol, commonly used as a scolicidal agent, did not produce the desired effect on any of the 3 components of the hydatid cyst. Although alcohol may be an effective scolicidal agent, it is thought that its use alone in percutaneous drainage may not be suitable, because it would not macroscopically affect the daughter cysts. It is known that the daughter cysts, containing living scolexes, are active components of hydatid cysts and so should be inactivated for effective treatment and for preventing recurrences.

The second important finding of this study was that hydrogen peroxide had maximal effect on all of the 3 components. Hydrogen peroxide is a potent chemical oxidant agent. It oxidizes most biological molecules. Hydrogen peroxide was desired to affect the daughter cyst and the cyst membrane, but its effect on the pericyst was not desired. Because the pericyst is the last barrier between the parasite and the host tissue, the pericyct protects the tissue against the harmful effects of the parasite. The use of hydrogen peroxide as a scolicidal agent is limited. Garcia et al. reported that hydrogen peroxide and povidone iodine show a greater protoscolicidal effect than physiological saline, hypertonic saline, and praziquantel (18). It has been reported that applying of hydrogen peroxide to the cyst cavity caused a gas embolism, which is a life-threatening complication (19). In another study, intraoperative collapse in one patient and an immediate postoperative death occurred after injection of 10% hydrogen peroxide into the cyst. The author stated that the dramatic increasing volume of the cyst may cause a fissure in the cyst wall and allow passage into the circulation (20). In fact, the gas producing effect of hydrogen peroxide is well known (21-23), as proven in our study by the frequent flying off of the lid of the bottle containing hydrogen peroxide due to gas formation and increasing pressure.

The third finding noted in this study was the resistance of the daughter cysts to most agents. Daughter cysts generally were affected later than the cyst membranes. Except for sodium hypochlorite, all of the agents affected the daughter cysts days later. As is already known, daughter cysts exist in some patients undergoing percutaneous drainage. Alcohol and hypertonic saline, widely used as scolicidal agents, were determined not to have any macroscopic effect on the daughter cysts, which means that the cyst activity might continue for a long time. This problem may be overcome by using a scolicidal agent together with another effective agent on the daughter cysts.

An interesting result is that sodium hypochlorite had more effect on the daughter cyst and the cyst membrane than on the pericyst. The higher the concentrations of sodium hypochlorite solutions, the higher its effect on all 3 components. Despite the fact that sodium hypochlorite melted the cyst membranes completely in our previous study (6) it had a moderate effect on the cyst membranes in the present study. These observations may be related to the mechanism of sodium hypochlorite. Sodium hypochlorite is a most potent oxidant agent. It oxidizes all the biological molecules and destroys their structures. Because sodium hypochlorite is a chemical oxidant agent, the dilution leads to a weakening effect. Therefore, the amount of tissue to be affected increased with the amount of sodium hypochlorite. It is thought that sodium hypochlorite might be more effective than the scolicidal agents used today, provided that the likely toxic effects on living tissues could be eliminated. Furthermore, we know that this is an observational study, based on a single experiment. For strong results, similar studies should be performed, also on cysts of different age.

In conclusion, many substances used as chemical solvents were determined in this study to be ineffective on the hydatid cyst contents. Hydrogen peroxide was the most effective agent on all 3 components of the cyst. The more effective hydrogen peroxide is on the pericyst, the more harmful effect it may have on the host tissue. Being considerably effective on daughter cysts and cyst membranes, and slightly effective on pericysts, sodium hypochlorite seems to be a good agent to be used in percutaneous drainage. However, further studies on the effect of sodium hypochlorite should be carried out in vitro, and then its in vivo efficacy should be investigated.

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