Praziquantel ve Dimetil Sülfoksit Solusyonlarının Hepatobilyer Sistem Üzerine Etkileri (Deneysel Çalışma)

Praziquantel-Dymethylsuifoxide Solution: The Side Effect on Hepato-biliary System (An Experimental Study)

Praziquantel ve Dimetil Sülfoksit Solusyonları / Praziquantel-Dymethylsuifoxide Solution

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Özet

Amaç

Bu deneysel çalışmada Praziquantel ve Dimetilsulfoksidin hepatobilier sistem üzerine yan etkileri araştırılmış ve alkol, hipertonik ve normal salin ile karşılaştırılmıştır.

Gereç ve Yöntemler

Bu çalışmada toplam 5 grup olup, her grup 10 rat içermektedir. Tüm gruplarda transdoudenal koledok kanulasyonu yapılmıştır ve Dimetilsulfoksid ile Praziquantel solusyonu, % 0.9 Nacl,%20 Nacl veya %98 alkolden herhangi biri enjekte edilmiştir. Cerrahi öncesinde kan örnekleri alınmıştır ve ALT,AST,ALP,GGT düzeyleri ölçülmüştür. Bu ölçümler her ay tekrarlanmış ve 3 ay boyunca devam edilmiştir.

Bulgular

Biyokimyasal tetkik ve kan örneklerinde çalışma başlangıcında anlamlı sayısal farklılıklar yoktu. Skolasidal enjeksiyonu sonrasında ortak hepatik kanal içinde ALT,AST ve ALP düzeyleri hipertonik salin grubunda, kontrol grubuna göre önemli derecede yüksek olarak bulundu. Hipertonik salin grubunda, GGT düzeyleri kontrol grubuna benzerdi. Aynı zamanda tüm biyokimyasal sonuçlar, Dimetilsulfoksidi, Dimetilsulfoksid- Praziquantel ve Alkol gruplarında, Hipertonik salin ve kontrol grublarına göre anlamlı olarak yüksekti ($p< 0.05$). ALT,AST ve ALP düzeyleri Hipertonik salin grubunda en yüksek olarak bulunmuş, GGT düzeyleri Dimetilsulfoksid grubunda en yüksekti ($p< 0.05$). Histopatolojik incelemeler hepatoselüler değişiklerin Hipertonik salin grubunda, kontrol grubuna göre önemli derecede daha yüksek olduğunu gösterdi. Aynı zamanda Alkol, ve Dimetilsulfoksidi, Dimetilsulfoksid- Praziquantel grublarında, diğer gruplara göre bu değerler daha yüksekti. Dimetilsulfoksid ve Hipertonik salin gruplarında, safra kanalları üzerine benzer yan etkiler izlenmiş; Dimetilsulfoksid- Praziquantel solusyonu diğer gruplardan, daha güçlü yan etkilere sahipti. Alkol grubunda, safra kanalları üzerine en kuvvetli yan etkilere sahiptir.

Sonuç

Dimetilsulfoksid- Praziquantel solusyonunun Alkol ile benzer riske sahip olduğu düşünülmekte ve hepatik hidatidoziste hepatobilier kanallar üzerine operasyonlarda Hipertonik saline göre daha fazla kullanılmaktadır..

Anahtar Kelimeler

Hydatik Kist, Alkol, Hipertonik Salin, Praziquantel, Dimetilsulfoksid.

Abstract

Aim

In this experimental study, the side effects of praziquantel and dymethylsulfoxide on the hepatobiliary system has been investigated comparing with alcohol , hypertonic and normal saline.

Material and Methods

This study contains five groups of ten rats each. In all groups, transduodenal choledochal cannulation was done and either dymethylsulfoxide, praziquantel in dymethylsulfoxide solution ,0.9 % NaCl, 20 % NaCl or 98 % alcohol were injected. Before surgery, blood samples were taken for measuring AST, ALT, ALP, GGT levels, and repeated every months for three months period. At the end of study, all rats were sacrificed; hepatobiliary excision was done.

Results

Biochemical content and blood samples have not statistically significant difference at the beginning of the study. After protoscolocidals injection into common bile duct, ALT, AST and ALP levels were siginificantly found higher at hypertonic saline group than control's ($p<0.05$). GGT level in hypertonic saline group was similar to control's. Also allthe biochemical results were siginificantly higher at dymethylsulfoxide, praziquantel in dymethylsulfoxide solution and alcohol groups than hypertonic saline and control groups ($p<0.05$). Although ALT, AST and ALP levels were found highest in hypertonic saline group; GGT level were was highest in dymethylsulfoxide group, ($p<0.05$) (Figure 1, 2, 3, 4). Histopathological research has shown that hepatoceluler changes were siginificantly higher in hypertonic saline group than control group, ($p<0.05$) Also it was higher in alcohol , dymethylsulfoxide and praziquantel in dymethylsulfoxide solution groups than the others, ($p<0.05$) Although dymethylsulfoxide and hypertonic saline have similar side effects on biliary tract; praziquantel in dymethylsulfoxide solution solution has stronger side effect then them, ($p<0.05$) alcohol has strongest side effect on biliary tract, ($p<0.05$).

Conclusions

As a conclusion, it is thought that praziquantel in dymethylsulfoxide solution solution has similar risk to alcohol and more than hypertonic saline on hepatobiliary tract in intraoperative use for hepatic hydatidosis.

Keywords

Hydatid Cyst, Alcohol, Hypertonic Saline, Praziquantel, Dymethylsulfoxide.

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Introduction

Protoscolicidal solutions have great importance in the treatment of hydatid disease. They solutions must be nontoxic and of no side effect. When they pass into the biliary tract, they may cause inflammatory reactions result in caustic sclerosing cholangitis. Praziquantel has been used commonly in hepatic hydatidosis. Because of slightly water-solubility, dymethylsulfoxide is the solvent of choice in parenteral application. In this experimental study, the side effects of Praziquantel and dymethylsulfoxide on the hepatobiliary system has been investigated comparing with alcohol ,hypertonic and normal saline.

Material and Methods

This study contains five groups of ten rats each. In all groups, transduodenal choledochal cannulation was done and either dymethylsulfoxide, praziquantel in dymethylsulfoxide solution, 0.9 % NaCl, 20 % NaCl or 98 % alcohol were injected. Before surgery, blood samples were taken for measuring AST, ALT, ALP, GGT levels, and repeated every months for three months period. At the end of study, all rats were sacrificed; hepatobiliary excision was done After fixation in 10% buffered neutral formaline, sections from the common duct, porta hepatis and liver parenchyma were processed and embedded in paraffin. Tissue sections. 4-6 µm-thick, were stained by hematoxylin and eosin and evaluated under the iight microscope by a pathologist blind to the experimental procedure. Microscopic changes were assessed according to the scoring system presented in table 1 and 2.

The GLM Repeated Measures analysis of variance was performed, because of the same measurement is made several times (on admission, after 1st, 2nd and 3th month) on each subject. The effects of both the between-subjects factors and the within-subjects factors were tested with overall F test. Additionally, after an overall F test, the post hoc tests, the Bonferroni and Tukey's honestly significant difference tests, were used for multiple comparisons to evaluate differences among specific group means. The profile plots (interaction plots) of the group means and measurement times obtained to visualize some of the relationships easily.

The GLM univariate analyses of variance were also performed for each mesasurement by the groups at each time point, after an overall F test, the post hoc tests (The Bonferroni and Tukey tests) were also performed to evaluate differences among specific group means, the Levene's test for homogeneity of variance were also done to see if the variances unequal, when the variances were unequal, The Tamhane's T2 test (conservative pairwise comparisons test) were used. Non-normally distributed measurements were analysed by Kruskal-Wallis one-way analysis of variance and then group comparisons were performed by Mann-Whitney U test. In all evaluations statistical significance were accepted for $p<0.05$.

Table 1. Histopathologic liver evaluation criteria.

Histopathologic findings	Score
Hidropic changes: None Focal (zonal and restricted to few lobules) Marked (zonal but in all lobules) Widespread (all lobules and all zones)	**0 1 2 3**
Fatty change: None Focal (zonal and restricted to few lobules) Marked (zonal but in all lobules) Widespread (all lobules and all zones)	**0 1 2 3**
Drop out necrosis: None Few (few lobules) Widespread (all lobules)	**0 1 2**
Abcess formation: Absent Present	**0 1**
Heamorrhage: Absent Present	**0 1**
Hepatocellular necrosis: None Focal (single focus in single lobule) Marked (single focus in all lobules) Widespread (ponlobular or several foci per lobule in all lobules)	**0 1 2 3**
Portal inflammation: None Rare (involves 1 -2 portal area) Marked (involves >2 portal area)	**0 1 2**
Fibrosis: None Mild (fibrous portal expansion) Severe (bridging fibrosis)	**0 1 2**

Table 2. Scoring system for the extra- and intrahepatic biliary tree

Histopatholojic findings	Score (Intrahepatic)	Score (Extrahepatic)
Epithelial changes: No change Reversible injury Focal changes Epithelial sloughing Necrosis	0 1 2 3 4	0 1 2 3 4
Ductal wall changes: Oedema: Absent Present **Inflammatory infiltration:** None Mild Medium Severe **Fibrosis:** Absent Present	0 1 0 1 2 3 0 **1**	0 1 0 1 2 3 0 1
Total score		

Results

Biochemical content and blood samples have not statistically siginificant difference at the beginning of the study. After protoscolocidals' injection into common bile duct, ALT, AST and ALP levels were found higher at hypertonic saline group than C's ($p<0.05$). GGT level in hypertonic saline group was similar to C's. Also ailthe biochemical results were higher at dymethylsulfoxide, praziquantel in dymethylsulfoxide solution and alcohol groups than hypertonic saline and C groups ($p<0.05$). Although ALT, AST and ALP levels were found highest in hypertonic saline group; GGT level were was highest in dymethylsulfoxide group ($p<0.05$) ,(Figure 1, 2, 3, 4).

In histopathological research no abscess formation, heamorrhagea or fibrosis were found in all groups' hepatic specimens. The other changes, including hydropic and fatty changes, drop out necrosis, portal inflammation

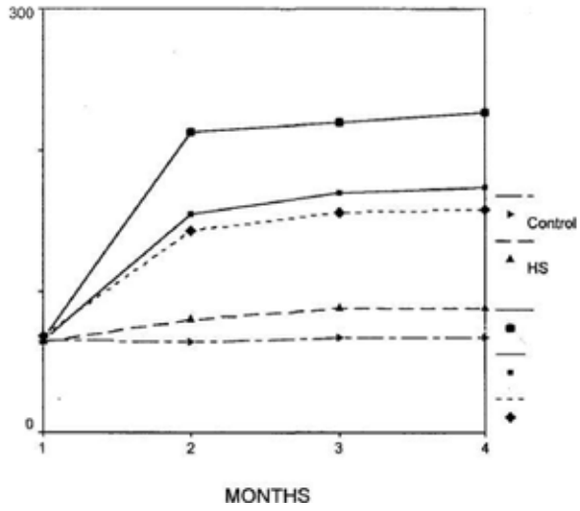

Figure 1: ALT values of the three groups during the study period

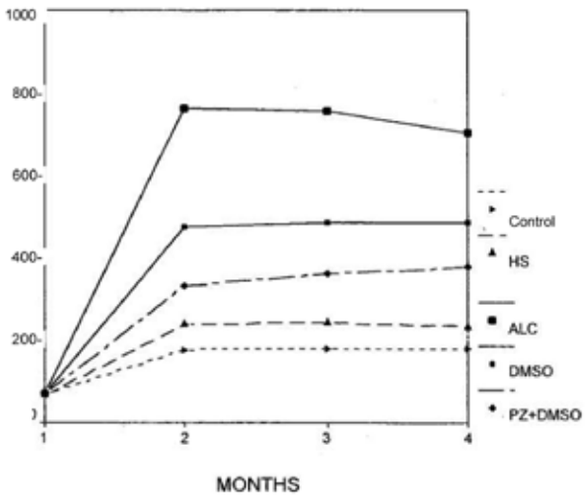

Figure 2: AST values of the three groups during the study period

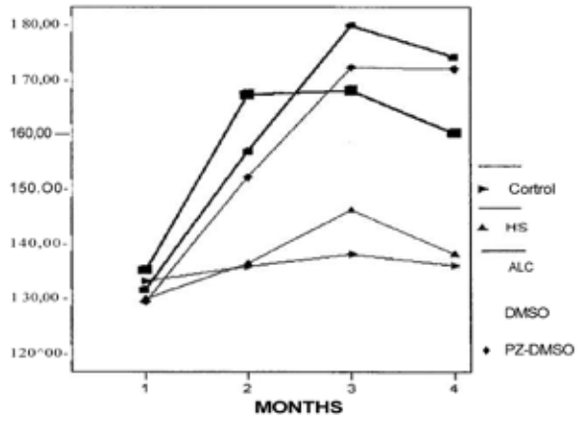

Figure 3: GGT values of the three groups during the study period

were found higher in hypertonic saline group than C group. (p<0.05) The results ofalcoho, dymethylsulfoxide and praziquantel in dymethylsulfoxide groups' were higher than C and hypertonic saline groups'. It was found highest in praziquantel in dymethylsulfoxide group (p<0.05), (Table 3), (Figure 5).

Histopathological changes in biliary tract were similar in hypertonic saline and dymethylsulfoxide groups, but siginificantly higher than C's (p<0.05). The results of praziquantel in dymethylsulfoxide group was siginificantly higher than hypertonic saline, dymethylsulfoxide and C groups (p<0.05). Alcohol was found strongest side effect on biliary tree (p<0.05) ,(Table 4),(Figure 6).

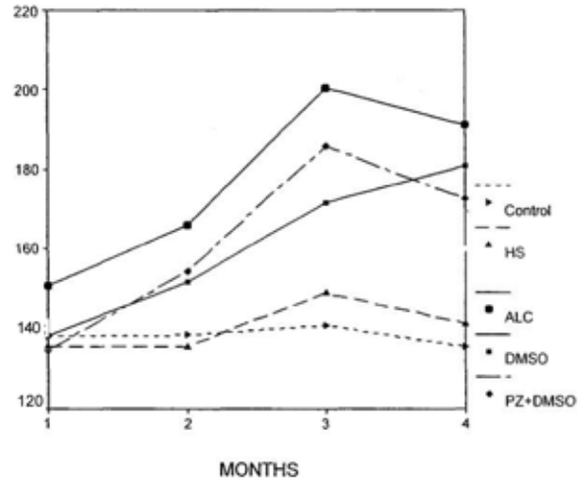

Figure 4: ALP values of the three groups during the study period

Table 3. Histopathologic score of hepatoceluler changes

groups	n	mean±sd (*)		
		1	2	3
control	10	1,1000±0,99443		
HS	10		2,8000±1,13529	
ALC	10			6,0000±0,94281
DMSO	10			6,4000±2,06559
PZ+DMSO	10			6,8000+1,81353

(*): p<0.05 significantly different subgroups displayed in different columns (Tukey's multiple comparison test)

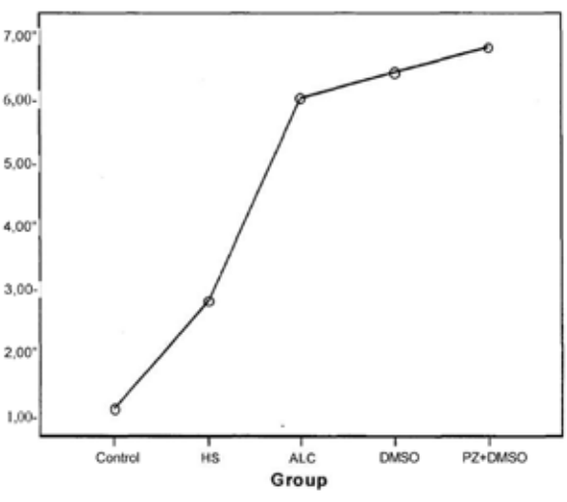

Figure 5: Histopathological changes in liver

Table 4.

groups	n	mean+sd (*)			
		1	2	3	4
control	10	3,400±1,776			
DMSO	10		7,100±2,282		
HS	10		7,600±2,716		
PZ+DMSO	10			10,400±1,776	
ALC	10				13,000+2,211

(*): p<0.05 significantly different subgroups displayed in different columns (Tukey's multiple comparison test)

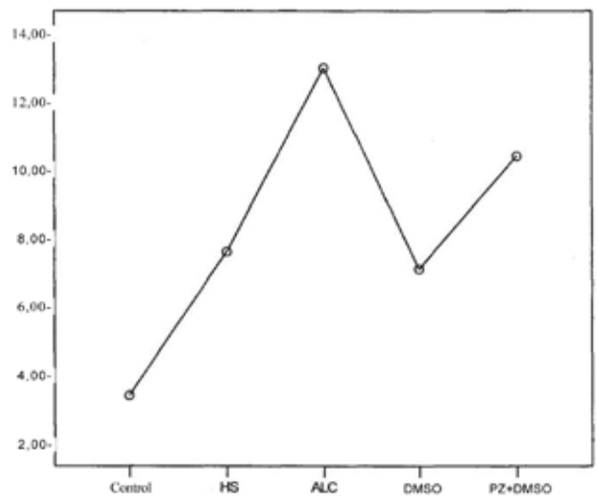

Figure 6: Histopathological changes in biliary tract (Both intra- and extrahepatics)

Discussion

In the conventional or minimally invasive surgery for hydatid disease, protoscolicidal solutions remain indispensable for the dysinfection of the cyst cavity. This necessity justifies the usage of scolocidal solutions routinely. In the presence of cysto-biliary communications, the passage of these solutions may cause tissue damage and relevant histopatoiogical changes in the hepatic tissue as well as in the biliary tree. So, inflamation of common bile duct wall may result in Caustic sclerosing cholangitis [8-13].

Properties of an ideal protoscolocidal solution would be the absence of local and systemic adverse effects together with a complete and rapid scolex killing. Although the side effects of protoscolicidal solutions such as formalin, ethyl alcohol, hypertonic saline, cetrimide, Ag NO3, polyvinyl pyrolidone iodure, and hydrogene peroxide, have been commonly investigated and shown, there is not a concensus for ideal protoscolicidal solutions [8,11,12,14]. We have shown that albendazole solution is one of the most effective protoscolocidal with no side effect on hepatobiliary system [14,15].

At present the most commonly used protoscolicidal agents are 95-98 % ethyl alcohol and 20 % NaCl. Most of the reports noted that hepatic enzymes have been found high including ALT, AST and ALP by using formaline, alcohol and hypertonic saline [1,8]. In contrast some authors noted no changes at hepatic enzymes [11].These studies showed that formaline is the most toxic agent used commonly in history. Amongst the agents used today, ethyl alcohol is the most toxic agent; hypertonic saline is the second. All experimental studies about the side effects of protoscolicidal solutions, report severe stasis, widespread hepatocellular necrosis, flattening of the biliary epithelium, ductal proliferation, cholangitis and pericholangitis. These findings, of a milder degree have been reported after alcohol and hypertonic NaCl solutions. Hydrogen peroxide and cetrimide have similar but lesser side effects [8-10,12,14,16-19].

After alcohol injection, minimal conjestion, mild hydropic degeneration, mild subepithelial biliary fibrosis, proliferation of the bile canaliculi, periductal inflammation and focal necrosis have been reported [11]. After hypertonic NaCl use (10-.20 %) concentric fibrosis around the major bile ducts was additionally reported [1,11]. In this study, enzyme levels showed that direct application of hypertonic NaCl and alcohol not the biliary tract have side effect on hepatobiliary system. But it is stronger for alcohol than hypertonic NaCl. Histopathological findings have supported this decision.

On the other hand, Praziquantel is drug commonly used in the medical treatment of hydatidosis. It is less toxic and better absorbabl than albendazole. After oral administration of praziquantel is rapidly absorbed (80%), subjected to a first pass effect, metabolized and eliminated by the kidneys. It is easily soluble in chloroform and dimethyl sulfoxide, soluble in ethanol and very slightly soluble in water. dymethylsulfoxide is the solvent of choice for parenteral form of praziquantel.

Since 1963, dymethylsulfoxide has been first reported in medical literature by Jacobs, it has been used not only as a solvent but also in the medical treatment of Interstitial Cystitis, Scleroderma , Raynaud's Phenomenon , Lupus , Arthritis , Ulcerative Colitis, Diabetic Ulcerations [20]. One of our studies has shown that praziquantel in dymethylsulfoxide solution complete protscolocidal effect in concentration of 1mgr %[6]. But the results of topical application of praziquantel in dymethylsulfoxide ,on hepatobiliary systeme is not known. In systemic usage of , praziquantel, minimal increases in liver enzymes have been reported in 3.31 to 27% of patients. So, praziquantel should not be given to patients who previously have shown hypersensitivity to the drug or high hepatic enzymes levels [5,7].

Although praziquantel in dymethylsulfoxide solution has been used for a long time in medical treatment of hydatid cyst, intraoperative use has not been reported. In this study we searched the possibility of intraoperative usage of praziquantel in dymethylsulfoxide solution. In this study, we found statistically significant elevations of hepatic enzymes after choledochal injection of praziquantel in dymethylsulfoxide. They were lesser

than alcohol group's but higher than hypertonic saline group's. The results showed that it has strong side effect on hepatobiliary system. These results are stronger than hypertonic saline, but comparable with alcohol . Also dymethylsulfoxide alone was found have similar effect. Histopathological researchs supported the biochemical findings. As a conclusion, it is thought that praziquantel in dymethylsulfoxide solution has similar risk to alcohol and more than hypertonic NaCl on hepatobiliary tract in intraoperative use for hepatic hydatidosis.

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