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Investigation of cholelitholytic and biologic effects of methyl tertiary butyl ether (MTBE) in dogs with implanted gallstones

Safra taşı implante edilmiş köpeklerde metil tert bütil eter (MTBE)'nin kolelitolitik ve biyolojik etkilerinin değerlendirilmesi

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ÖZET: *Son yıllarda, özellikle operasyon riski taşıyan safra taşlı hastaların tedavisinde, çeşitli cerrahi dışı yaklaşımlar, gün geçtikçe önem kazanmaktadır. bu tekniklerin biri de, safra taşlarının MTBE ile dissolüsyonudur. Her ne kadar, MTBE'ye bağlı minimal anestezik etki, kusma ve hemoliz gibi yan etkiler bildirilmişse de, safra yolları, karaciğer ve duodenumun doku cevapları, henüz tam olarak bilinmemektedir.*

Bu çalışmamızda biz, MTBE'nin klinik uygulamasından önce, hayvan modellerinde, akut biyolojik ve biyokimyasal etkilerini ortaya koymayı amaçladık. 7 sokak köpeği (1 kontrol, 6 safra taşı implante edilmiş denek) çalışma için seçildi.

Eşit sayı ve büyüklükteki safra taşları, köpeklere cerrahi olarak implante edildi ve 5F kateterler, operasyon esnasında safra kesesine yerleştirildi. Operasyondan 7-10 gün sonra 5-10 ml. MTBE, safra kesesine kateter yolu ile, manuel olarak uygulandı. Seri ultrason ve skopilerle, safra taşı boyutları ölçüldü.

Nekropside, köpeklerin safra kesesinde, küçük fragmante taşlara ve rezidüel doku artıklarına rastlandı. Bazı köpeklerde, safra kesesi ve ana safra yolları mukozasında küçük kanama odakları gözlendi. Safra kesesindeki bu nonspesifik inflamatuar değişikliklerin operasyona, katater veya implante edilen safra taşlarının direkt etkilerine veya MTBE'ye bağlı olabileceği düşünüldü.

Sonuç olarak, oldukça zor klinik tolerasyona, biliyer sistemdeki hafif ve orta derecedeki doku yanıtı ve minör biyokimyasal değişikliklere rağmen, MTBE'nin mükemmel bir kolesterol çözücü ajan olduğu ortaya konmuştur.

Anahtar Kelimeler: **Methyl tertiary butyl ether, safra taşı**

SUMMARY: *Recently, several nonsurgical approaches for treatment of gallstones have become more popular, especially for patients that are considered high risk surgical candidates. One of these new techniques is gallstone dissolution with Methyl Tertiary Butyl Ether (MTBE). Although minimal anesthetic side effect have been noted vomiting and hemolysis due to MTBE have been reported. Tissue response of the biliary system, liver and duodenum have not been well-studied.*

In this study, we planned to determine the acute biological and biochemical effects on animal models before clinical application of MTBE. 7 mongrel dogs (one control, 6 with implanted galsstones) were studied. Gallstones of equal number and size were surgically implanted into the dogs and 5F catheters were sutured into the gall bladders. MTBE was infused 7-10 days postoperatively via the catheters in aliquots of 5-10 ml. Stone diameter was measured using serial ultrasonography and radioscopy.

At necropsy, the dogs had fragmented and reduced sized gallstones and residual debris in their gall bladders. In some dogs, the gall bladders and common bile duct mucosas showed small hemorrhages. It was thought that nonspecific inflammatory changes of the gall bladders could have resulted from the surgical placement of the catheters and gallstones, or the MTBE.

To conclude, in spite of poor clinical tolerance, mild to moderate tissue response in the biliary system and minor biochemical changes, MTBE is an excellent cholesterol - solubilizing agent.

Keywords: **Methyl tertiary butyl ether, gallstone**

RECENTLY, several new non-surgical approaches have been improved in treatment of patients with gallstones. These therapy modalities have been increasingly more popular, especially in patients with increased surgical risks. One of these approaches, medical dissolution of cholesterol gallstones, was first undertaken by Walker in 1891 and then improved. Since that time, many attempts have been made to provide the safest and most reliable method of treating cholelithiasis by infusion of other solvents. Many observers have studied methyl tertiary butyl ether (MTBE), one of these dissolution materials, first in animals and then in humans, and have reported their results (1,2,3).

However, some adverse effects of MTBE, such as minimal anesthetic effect, vomiting or hemolysis

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Table 1. Characteristics of animals studied

				Implanted Gallstones	
Dog Number	*Sex*	*Weight (kg)*	*Number*	*Size (mm)*	*Weight (mg)*
1	F	15	1	17 x 11	1050
2	F	18	1	20 x 17	1900
3	F	19	1	17 x 16	1200
4	F	22	1	18 x 18	1350
5	F	21	1	17 x 17	1290
6	M	19	1	18 x 19	1600
Control	M	20	1	20 x 20	1800

have been reported; tissue effects on the biliary system, liver and duodenum are not well known.

The aim of the present study was to evaluate the anatomopathologic and biochemical acute effects of MTBE in animal models before clinical application.

MATERIALS AND METHOD

MTBE is a potent cholesterol solvent with a cholesterol-solubilizing capacity of 13.7 g/100 ml. MTBE has a boiling point of 55.2°C and remains liquid at body temperature. After absorption in the gut, a small percentage of MTBE may be metabolized to methanol, formaldehyde, formic acid, and tert-buthanol. MTBE, methanol and tert -buthanol in sufficient concentrations are potentially toxic compounds and may be stored in tissues (1).

In this study a 97% solution of MTBE was used (Aldrich Chemical Co. Inc., Milwaukee, USA). Seven mongrel dogs (one control, six with implanted gallstones) were selected. Characteristics of the animals were seen in Table I.

Cholesterol gallstones screened by cholecystography and ultrasonography were removed by cholecystectomy from patients with gallstones, then held in 0.9% solution of sodium chloride.

Stones were implanted into the dogs' gallbladders. At the same time, bile volumes were measured. During the operation, 6F pig tail biliary catheters were fitted into the gallbladders, while in dog 1 the catheter was inserted percutaneously into the gallbladder by fluoroscopic guidance. The proximal end of the biliary catheter was buried into the anterior wall of the abdomen. Implanted gallstones were screened by ultrasonography on the seventh postoperative day. MTBE with average perfusion volume of 5 cm3 (range 2-10 cm3) was manually infused into gallbladder with a glass syringe through the inserted catheter. MTBE was changed every 30 minutes and the solvent and bile aspirated each time were collected to determine cholesterol content by calorimetric analysis tihs procedure was continued until no stone was observed by fluoroscopy (Figure 1).

Asportat aminotrancsferase (AST), alanin aminotransferase (ALT), alkalen phosphotec (ALP) and amylase values were measured by Beckman CX5 autoanalyzer before and after MTBE application.

To prevent fluid loss, a buffered Ringer's solution was infused intravenously. To prevent hyperhydration, haematocrit count was obtained every hour. For controlling the level of anesthesia, the animals were monitared by ECG. Body temperature was kept constant at 38°C.

After 24 hours, each dog was killed except the control dog, and necropsies were performed. In the control dog, stone size was determined every weer for a month by ultrasonography. After a one month follow-up, the changes were examined at necropsy.

The gallbladder, common bile duct, liver, duodenum, pancreas; lung and kidney tissues were fixed in a buffered 10% neutral formalin solution. Paraffin sections, cut at 6 micron thickness were stained with haematoxyline-eosin.

RESULTS

Gallbladders of animals were ultrasonographically evaulated immediately after gallstone implantation and before MTBE application. No change was observed in stone diameters and gallbladders. MTBE was applied to the experimental dogs by observing the stones that were surgically implanted to their gallbladders by scopy; the procedu-

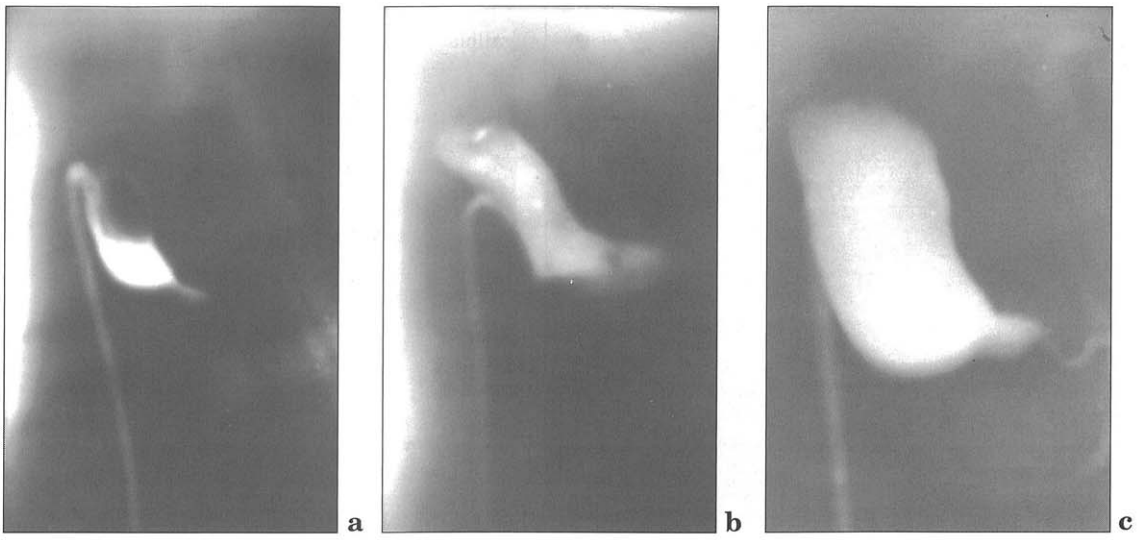


Figure 1. Case 1 Fluoroscopic examination of gallbladder **(a)** Before application of MTBE, **(b)** Three hours, and **(c)** Six hours after application.

re was completed when the stones seemed to disappear radiologically after approximately 5-8 hours. MTBE and bile cnotent, collected during the procedures were measured by calorimetry and cholesterol ratios were found as 87%, 90%, 88%, 86%, 89%, and 86% respectively. Weights of residual stones after necropsy, MTBE application period and cholesterol ratios were shown in Table II.

In the fifth hour of MTBE application, respiratory depression was observed in the first dog. After 24 hours, AST, ALT, and amylase values were found to be increased in the second dog, while increase in ALP and amylase values were determined in the others.

Increase in saliva and intermittent vomiting periods were observed in all of them after a short period of MTBE application. MTBE odor was smelt on the breath of all animals (Table III).

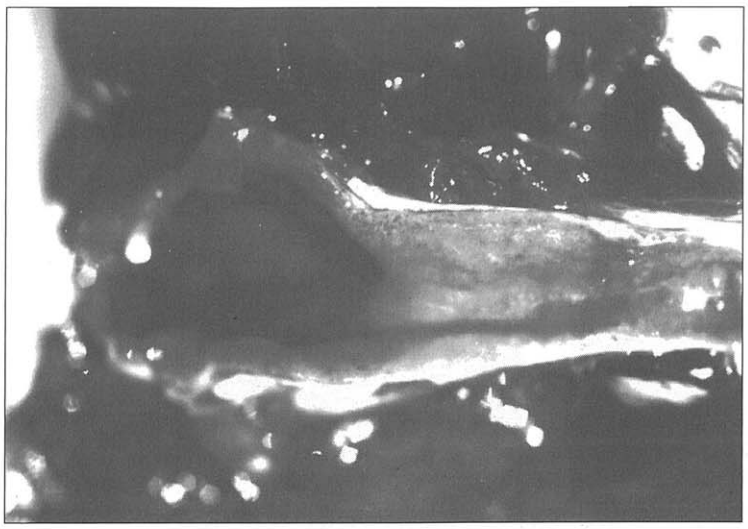
The control animal was monitored for a month by weekly ultrasonographic procedure. It was determined that stone diameters which had been first measured as 20 x 20 cm decreased to 16 x 16 cm. The weight of the stone after the necropsy was shown in Table II.

At necropsy the dogs had fragmented and reduced sized gallstones and residual debris in their gallbladders. In some dogs, the gallbladder and common bile duct mucosa shoused small hemorrhages, the latter ones in dogs 3, 4, 5, and 6 were associated with obstructions due to small stone fragments (Figure 2).

On light microscopy, small areas of necrosis were observed in the gallbladder and the common bile duct in four cases. Mild epithelial hyperplasia was

Table 2. Weights of residual stones after necropsy and cholesterol saturations of bile contents

Animal No.	*Stone weight (mg)*	*MTBE application*	*Sum of weights of period (hour)*	*cholesterol (%) residual stones after necropsy (mg)*
1	1050	5	136.5	87
2	1900	6	190	90
3	1200	7	144	88
4	1350	8	189	86
5	1850	6	185	92
6	1900	7	130	89



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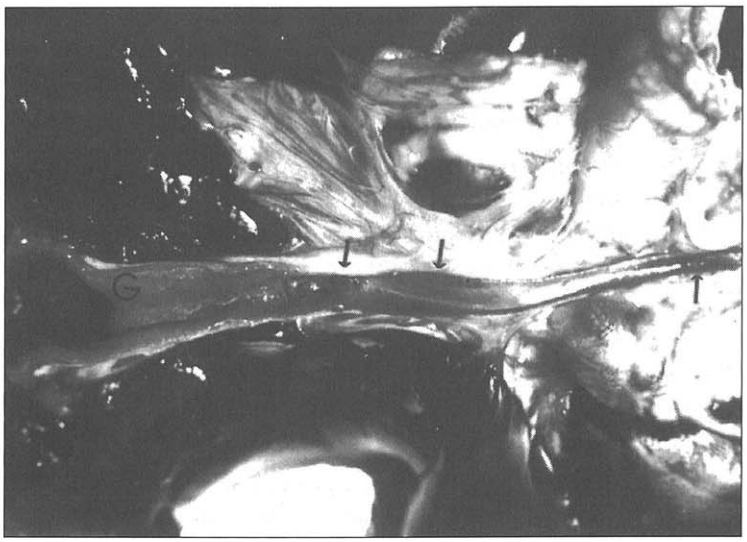

b

Figure 2. (a) Case 4, The gallbladder (G), and the common bile duct showing small hemorrhages (arrows); **(b)** Case 4, the appearance of the gallbladder mucosa.

noted in three cases. There were mild and moderate non-specific inflammatory changes consisting of hyperemia, hemorrhagia, edema, and lymphoid cell infiltrations in the lamina propria and submucosa of the gallbladder (Figure 3, 4). Surrounding liver tissue showed mild passive hyperemia. A mild hyperemia of the duodenal mucosa was also seen in some dogs. No histologic abnormalities were observed in the pancreas, lungs or kidneys.

DISCUSSION

Experimental studies with MTBE were first done by Allen et al, and McGahan et al (1,4,5). In these studies tprevious ones, contradictory results have been reported. The anesthetic effect observed in the first dog during MTBE application was not only due to MTBE, but also due to deep ketamine anesthesia. Although, researchers like Teplich et al and Hellstern et al established an anesthetic effect with MTBE in their study with small animals, this effect was not determined in studies done with dogs by Allen et al. The increase in saliva and intermittent vomiting periods observed in all dogs in our study were similarly determined in some

Table 3. Clinical features of animals

Animal No.	*Increase in saliva and vomiting*	*Sedation*	*MTBE odo±r in the breath of animal*
1	+	+	+
2	+	-	+
3	+	-	+
4	+	-	+
5	+	-	+
6	+	-	+

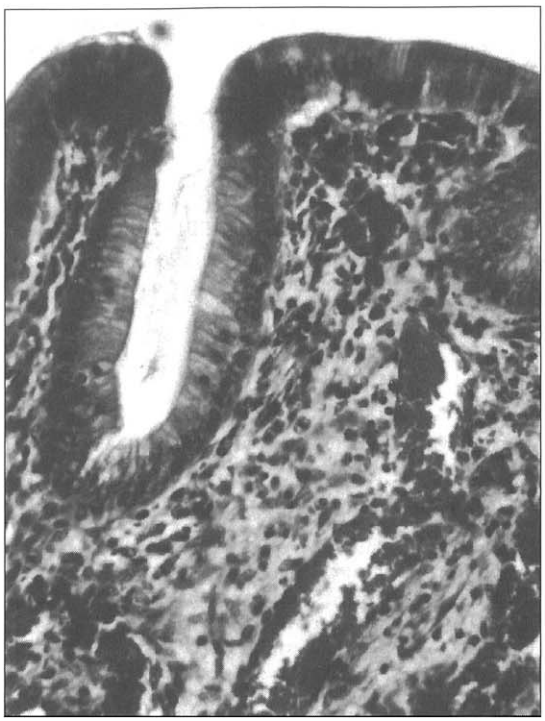

Figure 3. Case 3, Hyperemic capillaries, microscopic hemorrhages and lymphoid cell infiltrations in the gallbladder mucosa. Epithelial cells are intact (H x E x 295).

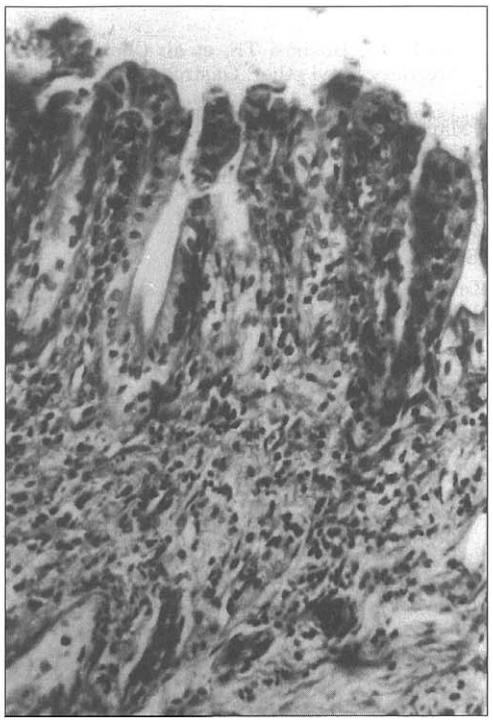

Figure 4. Case 2, Degenerative changes in epithelial cells. Proprial lymphoid cell infiltrations are seen (H x E x 280).

animals studied by Allen et al. This adverse effect was thought to be due to a central effect similar to diethyl ether and distention of the biliary system (1).

Increase in AST, ALT, and amylase values in the second dog after 24 hours of procedure, was thought to be due to traumatic percutaneous application. After necropsy the increase in amylase and ALP values in dogs 3, 4, 5, and 6 were suggested to be the ruslt of smallstones obstructins the common bile duct. Allen et al also observed increase in ALP values in some dogs and suggested it was due to residual stones.

Substances such as pigment, calcium, carbonates, magnesium and copper within the chemical structure of cholesterol gallstones could not be dissolved with dissolving agents. These substances stayed has residues in the biliary trees and could cause secondary biochemical changes (1).

The gallstone of the control animal was determined ultrasonographically to decrease in size at the end of the fourth week, ant this was confirmed at necropsy. In thestudy of Allen et al, it was observed that the stone of the control animal decreased in size.

Because the sterol secretion does not exceed the michele formation capacity of bile salts + lechitin in their gallbladders, dogs do not form fallstones, thus, the cholesterol saturation index tends to be decreased (CI).

The pathologic examination of the gallbladder and the common bile duct demonstrated that tissue reactions after MTBE application were mild to moderate. Similar results have been reported in humans, dogs and pigs (4,5,6,7). However Adam et al described severe acute necrotic changes in their rabbit study (7). It was thought that on-specific inflammatory changes of the gallbladder and common bile duct in our study could have resulted from the surgical placement of the catheter and the gallstones, or MTBE itself.

To conclude in spite of considerably poor clinical tolerance, mild to moderate tissue response in the biliary system and minor biochemical changes, MTBE is an excellent cholesterol solubilizing agent.

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