

RAPID COMMUNICATION

## Effects of long term hydrophilic bile acid therapy on *in vitro* contraction of gallbladder muscle strips in patients with cholesterol gallstones

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### Abstract

**AIM:** To evaluate ursodeoxycholic acid (UDCA) therapy on the *in vitro* contraction of gallbladder smooth muscle strips from cholesterol gallstone patients.

**METHODS:** The contraction forces of gallbladder smooth muscle strips from 28 patients with cholesterol gallstones treated with UDCA were compared with contraction forces from 14 untreated patients. The strips were stimulated with increasing concentrations of cholecystokinin-8 (CCK-8).

**RESULTS:** Although the contraction forces that developed in response to CCK-8 were higher in strips from specimens of UDCA treated patients compared to untreated patients, longer treatment periods (6-wk) caused more contraction responses than the short treatment period of 3-wk ( $F = 19.297$ ,  $1.85 \pm 0.22$  g vs  $1.70 \pm 0.10$  g,  $P < 0.01$ ). Contraction forces developed with maximal stimulation with KCl in the 6-wk treatment group were also higher than contraction forces in the untreated group ( $F = 4.274$ ,  $3.77 \pm 0.45$  g vs  $3.30 \pm 0.30$  g,  $P < 0.05$ ).

**CONCLUSION:** Six-week UDCA treatment caused an increase in contractions of muscle strips from patients with cholesterol gallstones when compared to shorter treatment administration or controls. We suggest that extending UDCA treatment periods may cause more effective contractions in the gallbladder, and thereby increase the rate of response to treatment.

### INTRODUCTION

Surgery is the standard treatment for symptomatic patients with gallstone disease. However, since surgical procedures have the risk of morbidity and mortality, noninvasive treatments are still under investigation, especially for use in high-risk patients. Ursodeoxycholic acid (3 alpha, 7 beta-dihydroxy-5 beta-cholanoic acid-UDCA) is a safe and efficacious drug that has been widely used for oral gallstone dissolution therapy<sup>[1]</sup>.

At least three pathologies must occur together for the formation of cholesterol gallstones; (1) high cholesterol saturation in the gallbladder, (2) decreased motility of the gallbladder, and (3) acceleration of nidus formation.

There are controversial results concerning the effects of UDCA on gallbladder contraction. While some authors have reported impaired gallbladder emptying in patients treated with UDCA<sup>[2,3]</sup> this was not confirmed by others<sup>[4]</sup>. However, these studies have used indirect methods, such as ultrasonography and Tc-HIDA scans for measuring gallbladder emptying. We, therefore, searched for a clinical *in vitro* model to determine whether UDCA treatment increases gallbladder contractions in muscle strips from patients with cholesterol gallstones. Although a similar effect was previously reported<sup>[5]</sup>, our main purpose was to establish a time-response rate relationship.

### MATERIALS AND METHODS

Forty-two patients with cholesterol gallstones who were selected for elective cholecystectomy were enrolled in the study. The inclusion criteria were: (1) presence of gallstones and/or sludge by gallbladder ultrasonography, patent cystic bile duct, absence of choledocholithiasis,

and < 4 mm gallbladder wall thickness, (2) absence of calcification on the plain film of the abdomen, and (3) visualization of the gallbladder by oral cholecystography.

The patients were randomized into three groups at the time of enrollment. All three groups had similar age ( $66.4 \pm 4.3$ ,  $67.4 \pm 3.9$ , and  $67.5 \pm 4.1$ ) and gender (male/female ratio: 6/8, 5/9, and 5/9, respectively) distributions. All patients and controls gave informed consent to participate in the study and the Ethics Committee of Gulhane School of Medicine approved the protocol.

Group I ( $n = 14$ ) was treated with UDCA (10 mg/kg/day) with a single dose at bedtime starting three weeks before surgery. Group II ( $n = 14$ ) received the same treatment but for six weeks prior to surgery. The patients in Group III ( $n = 14$ ) who served as controls received no treatment and underwent surgery after enrollment (Group III). In the treatment groups, the last dose of UDCA was given on the day before surgery. No patient had clinical or laboratory signs of acute cholecystitis when they underwent surgery. Ultrasonographic examination just before the surgery indicated no difference from the initial ultrasonography in terms of size and number of gallstones, sludge, gallbladder wall thickness, and absence of choledocholithiasis.

After cholecystectomy, for *in vitro* analysis, all gallbladder tissues were subjected to a series of manipulations as described by Behar *et al.*<sup>[6]</sup> with modifications. A 10 mm  $\times$  20 mm specimen parallel to the long axis of the gallbladder was put into cold Krebs solution (NaCl: 118.4 mmol/L, KCl: 4.7 mmol/L, CaCl<sub>2</sub>: 1.9 mmol/L, NaHCO<sub>3</sub>: 25 mmol/L, MgSO<sub>4</sub>: 1.2 mmol/L, Glucose: 11.7 mmol/L). The specimens were washed with Krebs solution again and 3 mm  $\times$  10 mm strips were prepared. The serous membrane on the outer surfaces of the strips was cleaned and the gallbladder strips were hung with a 6.0 silk suture into a 10 mm tissue bath (pH: 7.4 and 37°C). The other ends of the strips were tied to an isometric force transducer (Force Transducer, Type 45196 A Nec San-ei Instruments Ltd, Japan). The force transducer was connected to a polygraph writer (360 Polygraph Model 2 G. 66 Nec San-ei Instruments Ltd, Japan).

The tissue bath was perfused with 95% oxygen and 5% carbon dioxide throughout the study. An equilibrium period of two hours was used prior to the start of the experiment. During this period the Krebs solution in the tissue bath was changed regularly every 15 min. After a 2 h equilibrium period, tension in the strip was changed into a basal situation. At this time periodic contractions of 1-2 min occurred. Later, by studying one agonist in each of the gallbladder strips, contraction responses to cholecystokinin octapeptide (CCK-8;  $10^{-10}$ ,  $10^{-9}$ ,  $10^{-8}$ ,  $10^{-7}$ ,  $10^{-6}$  concentrations) and KCl (0.25 mol/L) were recorded.

The differences between basal and CCK-8 induced tensions of the strips were calculated ( $\Delta$  tension) and the mean  $\pm$  SD were obtained. Later, the  $\Delta$  tensions as a percentage of the maximum tension created by KCl were calculated.

Results were expressed as mean  $\pm$  SD. Oneway ANOVA and Tukey HSD tests were used for statistical analysis. A *P* value of less than 5% was accepted as

**Table 1** Response of gallbladder strips against KCl and increasing CCK-8 concentrations (mean  $\pm$  SD)

Agent	Group I ( <i>n</i> = 14)	Group II ( <i>n</i> = 14)	Group III ( <i>n</i> = 14)	<i>P</i>
CCK-8				
$10^{-10}$ M	0.21 $\pm$ 0.02 g	0.25 $\pm$ 0.02 g	0.20 $\pm$ 0.01 g	< 0.001
$10^{-9}$ M	0.32 $\pm$ 0.02 g	0.37 $\pm$ 0.04 g	0.26 $\pm$ 0.02 g	< 0.001
$10^{-8}$ M	0.42 $\pm$ 0.04 g	0.48 $\pm$ 0.05 g	0.36 $\pm$ 0.04 g	< 0.001
$10^{-7}$ M	1.20 $\pm$ 0.04 g	1.35 $\pm$ 0.10 g	1.04 $\pm$ 0.06 g	< 0.001
$10^{-6}$ M	1.70 $\pm$ 0.010 g	1.85 $\pm$ 0.22 g	1.50 $\pm$ 0.10 g	< 0.001
KCl (0.25 mol/L)	3.50 $\pm$ 0.50 g	3.77 $\pm$ 0.45 g	3.30 $\pm$ 0.30 g	< 0.03
Basal	0.13 $\pm$ 0.03 g	0.14 $\pm$ 0.02 g	0.12 $\pm$ 0.05 g	> 0.05

G: gram; KCl: potassium chloride; CCK: cholecystokinin.

significant. All statistical measurements were made by using SPSS PC ver. 11.05 (SPSS Inc. USA).

## RESULTS

Strips prepared from gallbladder tissue showed two contraction patterns in the equilibrium period: (1) contractions as fibrillation, which caused no significant increase in tension, and (2) rhythmic contractions, which had a frequency of 1/min and caused 0.10-0.17 g  $\Delta$  tension. Basal active tensions were  $0.13 \pm 0.03$  g in Group I,  $0.14 \pm 0.02$  g in Group II, and  $0.12 \pm 0.05$  g in Group III (*P* > 0.05); no difference was observed between treatment and control groups regarding basal active tensions. Responses of the treatment and the control groups as  $\Delta$  tension to increasing concentration of CCK-8 and KCl are shown in Table 1. Contraction responses of gallbladder strips were higher with increasing concentrations of CCK-8. UDCA treatment before the cholecystectomy improved *in vitro* contraction responses of gallbladder strips to CCK-8. Contraction responses to different CCK-8 concentrations were significantly higher in the 6-wk treatment group when compared to the 3-wk treatment or the control group. Moreover, except for CCK-8<sup>-10</sup>, contraction responses to different CCK-8 concentrations in the 3-wk treatment group were significantly higher than the controls.

Potassium provokes extracellular calcium entrance through the voltage dependent calcium channels and causes smooth muscle contraction<sup>[5]</sup>. In this study, KCl was used to induce maximum strip contraction. Contraction responses to maximal stimulation with KCl in the 6-wk treatment group were also higher than the 3-wk group, but the difference was not statistically significant. In the 3-wk treatment group, contraction responses were also higher, but not statistically significant than the controls. The only significant difference in maximal stimulation with KCl was obtained for the 6-wk treatment group when compared with the untreated controls ( $3.77 \pm 0.45$  g *vs*  $3.30 \pm 0.30$  g, *P* < 0.05).

## DISCUSSION

The results of the present study indicate that UDCA treatment increases gallbladder contractions in response to

CCK-8, and this effect is more pronounced as the duration of treatment increases. Moreover, UDCA results in a net increase in the maximal contraction capacity of the gallbladder strips.

The results confirm previous findings on gallbladder muscle strips from patients with cholesterol gallstone disease, which is a 3-wk UDCA treatment prior to the operation increases contractility<sup>[5]</sup>. Moreover, in the present investigation, we used KCl for maximal stress, and maximal contraction response in muscle strips from the 6-wk treatment group was significantly higher than the control group. On the other hand, in a previous report, this effect was not observed in the 3-wk treatment group<sup>[5]</sup>. These results suggest that long term treatment with UDCA may be more beneficial in terms of improving gallbladder contractile functions.

Though some clinical studies investigating the effect of UDCA on gallbladder contraction failed to show a significant improvement in gallbladder contractions, this may be associated with the methods used to measure contraction responses of the gallbladder, such as scintigraphy or ultrasonography<sup>[2,3,7]</sup>. For instance, in one of these investigations, gallbladder emptying was found to be improved in patients with successful litholysis, whereas no improvement was observed in patients with unsuccessful litholysis, which might be explained by gallbladder dysfunction at baseline<sup>[2]</sup>. Accordingly, some authors reported significant improvements in gallbladder emptying function after UDCA treatment with similar methods<sup>[4,8]</sup>, which is supported by the *in vitro* data of the present work. In addition, van Der Werf *et al*<sup>[9]</sup> found a negative correlation between the cholesterol saturation index (CSI) and gallbladder emptying function suggesting that a decrease in gallbladder contractility may not be possible after UDCA treatment, which decreases CSI.

It was previously reported that CCK-8 induced muscle strip contractions in patients with cholesterol gallstones is less than that in patients with pigment gallstones<sup>[6]</sup>. It was also observed that spontaneous cyclic contractions were less than in muscle strips of patients with pigment gallstones, which may be caused by the myotoxic effect of cholesterol in the gallbladder. This may be explained, at least in part, by the mechanism of action of UDCA on muscle strips in our study in that UDCA alters the composition of the bile, which results in improvement of the contraction response. Also, excess cholesterol has been suggested to reduce membrane fluidity and deteriorate subsequent cellular functions in smooth muscle cells in the gallbladder wall<sup>[10]</sup>.

In a recent study by Xiao *et al*<sup>[11]</sup>, UDCA treatment in guinea pigs with acute cholecystitis prevented gallbladder muscle dysfunction and improved oxidative stress markers when compared with chenodeoxycholic acid treated guinea pigs. These results indicate that UDCA treatment may improve contraction even in a gallbladder with inflammation but without stone.

In conclusion, in the present investigation, UDCA caused an increase in contraction in gallbladder smooth muscle strips from patients with cholesterol gallstones when compared to untreated controls, not only with a 3-wk but also with a 6-wk treatment period prior to

colecystectomy. Extension of the UDCA treatment period appears to cause more effective contractions in the gallbladder, and thereby may increase the rate of response to treatment.

## COMMENTS

### Background

There are discrepancies between the results of the studies searching the effects of ursodeoxycholic acid on gallbladder contractions. Frequently, indirect methods have been used to measure contractile responses to date. Pretreatment with ursodeoxycholic acid for 3 wk was shown to improve contractility of the muscle strips prepared after cholecystectomy in humans. The present study address the effect of ursodeoxycholic acid in a more extended period as well as the maximal contractility capacity in response to a different inducer.

### Research frontiers

Ursodeoxycholic acid is a naturally occurring hydrophilic bile acid. It has been used for gallstone dissolution clinically with favorable or unfavorable effects. Though it is known to change the composition of the bile, gallbladder emptying has been reported to be impaired in patients treated with ursodeoxycholic acid.

### Innovations and breakthroughs

Strips from gallbladders extracted from the patients treated with ursodeoxycholic acid for 3- or 6-wk prior to cholecystectomy displayed marked improvements in contractile responses. More beneficial results as well as increased maximal contractility capacity were obtained in the 6-wk treatment groups. Ursodeoxycholic acid may be an agent having no adverse action on gallbladder emptying.

### Applications

Response to ursodeoxycholic acid treatment may be delayed in the clinical setting, and treatment should be kept on as long as possible especially for the patients who are not eligible for surgery.

### Terminology

Ursodeoxycholic acid: a hydrophilic bile acid which is the drug of choice for the treatment of primary biliary cirrhosis. It has also been used for other cholestatic disorders, hepatostetosis as well as gallstone dissolution in selected cases. Muscle strip: gallbladder tissue sample prepared for *in vitro* test. Cholecystochinin-8: an enzyme causing contraction of the smooth muscle cells in the gallbladder wall. Potassium chloride (KCl): used to measure maximal contractility capacity in the muscle strips.

### Peer review

The study is interesting and aims to answer a relevant issue in the pathogenesis and treatment of impaired gall bladder contractions in gallstone disease.

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