Complex with n-3 polyunsaturated fatty acids and ursodeoxycholic acid dissolves cholesterol gallstones by attenuating cholesterol saturation and suppressing mucin production in mice

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Directed by Professor **Dong Ki Lee**

The Master's Doctoral Dissertation
submitted to the Department of Medicine
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Master of Medical Science

Sung Ill Jang

December 2016

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December 2016

ACKNOWLEDGEMENTS

I am very pleased to have a great opportunity to perform this study and appreciate to professor ***Dong Ki Lee***. He is the supervisor of this study and has made a significant intellectual contribution to this study. He established this novel concept and actually performed this meaningful and notable treatment method for patients suffering from gallbladder stones. I am extremely grateful to his devoted work for this study.

I also appreciate to professor ***Don Haeng Lee and Soon Koo Baik*** for his special academic assistance for acknowledgement of gallstone resolution methods. He has always supported our study by providing recommendations as a collaborator of this study. I specially thanks to professor ***Joon Seong Park and Jae-Joon Chung*** for assistance and recommendation to complement this study.

Despite limitations, complex with n-3 polyunsaturated fatty acids and ursodeoxycholic acid may become new novel

treatment methods for cholesterol gallstone after human study. I hope that this treatment will to be a blessing to the patient because it greatly improves their quality of life.

Dr. Sung Ill Jang

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ABSTRACT

Complex with n-3 polyunsaturated fatty acids and ursodeoxycholic acid dissolves cholesterol gallstones by attenuating cholesterol saturation and suppressing mucin production in mice

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Background and Aim: The increasing prevalence of cholesterol gallstone (CG) disease has become an economic burden to the healthcare system. Ursodeoxycholic acid (UDCA) is the only established medical agent used to dissolve gallstones. In investigating novel therapeutics for CG, we assessed the therapeutic effects of n-3 polyunsaturated fatty acids (PUFA) with ursodeoxycholic acid (UDCA) on CG induced by feeding a lithogenic diet (LD) containing high cholesterol levels to mice.

Methods: Mice were divided into the following seven groups: (A) regular diet (RD); (B) UDC9A; (C) PUFA; (D) PUFA+UDCA; (E)

Complex 0.5; (F) Complex 1.0; (G) Complex 2.0. After LD feeding for 8 weeks, 10 mice in each group were sacrificed in order to quantitate GCs formed, and CG was set as the baseline. Therapeutic agents were administered orally and the RD diet maintained for 12 weeks. The levels of phospholipids, cholesterol and bile acid in bile and serum, CG dissolution, gallbladder wall thickness, MUC gene expression in gallbladder were analyzed.

Results: There was no difference in mean body weight and liver weight between each group, and therefore, therapeutic agents did not cause any systemic side effects in the mice. Mice in the complex with PUFA and UDCA treatment (Groups E-G) showed significantly higher stone dissolution than the control RD group (Groups A). Bile phospholipid and bile acid levels were significantly elevated and cholesterol saturation index was decreased in the complex group. Although hypertrophy of the gallbladder wall was evident in mice fed LD, the wall thickness of gallbladder in mice treated with PUFA with or without UDCA was significantly thinner than in mice fed RD. MUC 2, 5ac, 5b and 6 mRNA expression levels were also significantly decreased in the PUFA-fed groups, and this was suppressed by PUFA with or without UDCA.

Conclusions: Complex with PUFA and UDCA dissolves cholesterol gallstone in mouse through increasing the levels of bile phospholipids and bile acid, decreasing cholesterol saturation and suppressing bile mucin formation. Further human study is required to investigate the therapeutic effects of the complex with PUFA and UDCA in patients with CG.

Key word: Cholesterol gallstone; n3-polyunsaturated fatty acids; Ursodeoxycholic acid, Mucin, Cholesterol saturation index

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I. INTRODUCTION

Cholesterol gallstone (CG) disease is associated with an increased concentration of cholesterol compared to bile salts and phospholipids. When the amount of biliary lipids, inorganic salts, or organic salts forms in the bile acid exceeds the maximum solubility, the acid precipitates into crystals through nucleation and eventually forms gallstones. Westernized diets are high in calories, cholesterol, saturated fatty acids, carbohydrates, and proteins, while being low in dietary fiber, thereby affecting gallstone formation.[1,2] This can be inferred from an epidemiological study in which an increase in the prevalence of complications of CG disease was noted among Canadian Eskimos consuming western diets.[3] Recently, the number of patients with cholesterol or mixed gallstones has rapidly increased in Asia owing to westernization of the Asian diet.

Surgical treatments, such as cholecystectomy, are standard for gallstone

treatment, and medical treatments are restrictive. Although laparoscopic cholecystectomy is safer than open cholecystectomy, there are still problems associated with surgery. The prevalence of laparoscopic cholecystectomy among gallstone patients is 1.6–12%, and the complication rate with the procedure reaches 2%, with bile duct damages being the major cause. Cholecystectomy for gallstones results in a 100% success rate, but 20% of patients continue to complain of pain characteristic after the surgery. For this reason, a diverse spectrum of gallstone patients who are eligible for cholecystectomy may benefit more from observation or oral solubilizer treatments than from surgery.

Gallstones rarely disappear naturally, and about 1% of asymptomatic gallstone patients, annually, become symptomatic or develops complications. Thus, because gallstone patients are always exposed to biliary pain and gallstone-related complications, there is a growing need to develop suitable medical treatments. Since chenodeoxycholid acid (CDCA) and ursodeoxycholic acid (UDCA) were introduced in the 1970s and 1980s, respectively, for the treatment of gallstones, medical treatments with oral solvents have widely replaced high-risk open cholecystectomy [4].

Studies have used a variety of drugs to treat gallstones. An animal study by Wang et al.[5] found that ezetimibe reduced cholesterol absorption in the intestines and decreased biliary cholesterol secretion, thus preventing gallstone formation and conserving gallbladder motility. Ezetimibe was also reported to

increase gallstone dissolution and lower biliary cholesterol saturation, which reduces the formation of cholesterol crystals. Another study showed that myriocin decreased serum and biliary ceramide concentrations in the C57BL/6J mice and further inhibited phosphorylation p38, thereby reducing gallstone formation, but this study could not identify the mechanisms underlying this effect[6]. Capsaicin and curcumin, extracted from spices in singular or combined forms, were also observed to lower biliary cholesterol and increase phospholipid levels, thereby suppressing biliary gallstone formation in mice by increasing the cholesterol: phospholipid ratio[7]. Despite these studies, UDCA remains the only widely accepted medical agent in the treatment of cholesterol gallstones thus far.

A number of epidemiological studies have investigated the potential therapeutic effects of omega-3 polyunsaturated fatty acid for treatment of CG. Eskimos who have high fish oil intake had lower prevalence of CG than Westerners[8]. Furthermore, complications with CG increased among Canadian Eskimos when their diets become westernized.[3] Fish oil is rich in omega-3 fatty acid and consists of two major components, eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22,6n-3)[9]. Ever since Wechsler[10] and colleagues reported reduction in biliary cholesterol and cholesterol saturation index(CSI) in adult men after administering omega-3 fatty acids for 6 weeks in 1989, studies on omega-3 fatty acid, bile, and gallstones have continued. Adding fish oil to food lowered biliary phospholipid concentrations

and reduced the formation of cholesterol monohydrate crystals in Prairie dogs[11]. That is to say, fish oil intake may increase cholesterol solubility by converting arachidonate, a biliary phospholipid, to EPA and DHA, thus reducing biliary cholesterol saturation levels and increasing cholesterol anticrystalizing activity levels[11,12].

Berr et al.[13] reported that omega-3 fatty acids (N-3 polyunsaturated fatty acid) reduced biliary cholesterol saturation in humans, and Mizuguchi et al.[14] reported that administration of EPA (a component of omega-3 fatty acid) alone was still effective in inhibiting cholesterol CG. Thus, EPA and DHA may inhibit HMG CoA reductase activity in the liver and increase bile secretion, thereby significantly reducing the amount of serum and liver cholesterol[15]. Despite increased biliary cholesterol secretion induced by omega-3 fatty acid, the lithogenic index may also decrease, because the simultaneous increase of both bile acid and phospholipid secretion prevents cholesterol oversaturation[15]. Given these results, biliary phospholipid, a major cholesterol solubilizer in the mechanism of gallstone formation, may be used to treat gallstones if its molecular structure can be manipulated to ensure reduction of the amount of biliary cholesterol crystals by elevating biliary phospholipid concentrations or effectively dissolving cholesterol. In this study, PUFA (Omacor®, Pronova Biocare, Sandefjord, Norway), the omega-3 fatty acid concentrate, was administered to a mouse model on a high cholesterol diet, and then, levels of cholesterol, phospholipid and bile acid in serum and bile, as well as gallbladder

and liver tissues were analyzed to investigate the effects of PUFA on CG treatment and its mechanism in vivo. This study also aimed to compare the therapeutic effects of PUFA against UDCA, the only oral gallstone solubilizer, as a conventional treatment method, and aimed to verify the therapeutic effects of co-administration of PUFA and UDCA. This study will help confirm the mechanisms underlying the therapeutic effects of PUFA for gallstones and establish the foundation of clinical trials, ultimately suggesting a noble treatment method for cholesterol gallstone patients.

II. MATERIALS AND METHODS

1. ANIMALS AND METHODS

A. Animals and diets.

C57BL/6J (male, 8 weeks old) mice, purchased from the Central Lab. Animal (Seoul, Korea), were used. Two hundred and forty five mice were fed with a lithogenic diet (LD) for 8 weeks and divided into 7 groups; (A) regular diet (RD) (control group); (B) RD + UDCA; (C) RD +PUFA; (D) RD + PUFA+UDCA; (E) RD + Complex 0.5; (F) RD + Complex 1.0; (G) RD + Complex 2.0. Ten mice in each group were sacrificed to quantitate the gallstones formed to calculate baseline weights of cholesterol gallstone before administration of therapeutic agents. Therapeutic agents are administered to mice for 12 weeks maintaining RD. The LD (DYET#102136, Dyets, Bethlehem, PA, USA) consisted of 15% anhydrous Milkfat, 2.0% corn oil, 1.0% cholesterol,

0.5% cholic acid, and contained 4379.70 kcal/kg. The RD (Laboratory Rodent DIETS, WOOJUNG Co., Korea) consisted of 6.4% of crude fat, and contained 2793 kcal/kg. PUFA (51mg/kg/day, Omacor®, Pronova Biocare, Sandefjord, Norway) and UDCA (12.5mg/kg/day, Ursa®, Daewoong Pharm., Seoul, Korea) and was diluted in 0.75% Tween-80 and administered orally by sonde for 12 weeks. Each one gram capsule of Omacor (omega-3 acid ethyl esters) contains at least 900 mg of the ethyl esters of omega-3 fatty acids. These are predominantly a combination of EPA (approximately 465 mg) and DHA (approximately 375 mg). Complex contains a mixture of the two agents, PUFA and UDCA, which were equally manufactured. A mixture containing PUFA (51mg/kg/day) and UDCA (12.5mg/kg/day) in a 1:1 ratio was set as Complex 1.0. A mixture containing half or double the content of Complex 1.0 was set as Complex 0.5 or Complex 2.0.

Serum and bile were collected and stored at -80°C prior to use. The liver and gallbladder were separated and divided into two samples, one of which was frozen in liquid nitrogen and the other fixed in 10% formalin. The experimental protocol was reviewed by the ethical committee (IACUC No. 2015-0238).

B. Methods

(A) Quantification of body weight, liver weight and gallstones

Body weights of the mice were measured in each group every week during the study period. After 8 weeks of LD treatment, body, liver and gallstones weights

were measured, and set as the base weight. After administration of therapeutic agents, the final body, liver and gallstone weights were measured. Gallstone weight reductions due to the effects of the therapeutic agents were compared between each group. The size of gallstone was assessed into a 6-score system: 0 = clear bile; 1 = little sludge; 2 = wide-spread sludge; 3 = large levels of sludge; 4 = a few small formed stones; 5 = several formed stones; 6 = full of formed stones.[16] Average scores of each group were compared.

(B) Microscopic studies of the gallbladder (Hematoxylin and eosin staining)

Fixed tissue was processed routinely for paraffin sections. Gallbladder tissues were fixed in 10% formalin for 2 days and sliced into six strips. All tissue strips were embedded in paraffin and cut into 4-mm sections. Sections were deparaffinized, rehydrated and stained with hematoxylin and eosin (H&E). Stained sections were dehydrated, cleaned, mounted, and subsequently examined by light microscopy.

(C) Quantification of total phospholipid, cholesterol and bile acid in bile and serum.

Total phospholipid, cholesterol and bile acid levels in bile and serum were determined via a colorimetric assay (BioAssay Systems, Hayward, CA, USA). Briefly, bile samples were diluted 10-fold and treated with working reagent.

The absorbance was measured using a microplate spectrometer (Epoch, BioTek, Winooski, VT, USA) at 570 nm and 340 nm for the quantification of phospholipids and cholesterol, respectively.

(D) Quantitative real-time polymerase chain reaction assay for MUC gene.

From gallbladder tissue, total RNA was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA,USA) and treated with RNase-free DNase (Promega, Madison, WI, USA) for 30 min at 37°C. The RNA was then cleaned using an RNeasy kit (Qiagen, Germantown, MD, USA). A reverse transcription reaction was performed using a commercially available High Capacity cDNA Synthesis Kit (Thermo Scientific, Rockford, IL, USA). MUC 2, MUC 5ac, MUC 5b and MUC 6 mRNA were quantitated using TaqMan polymerase chain reaction (PCR) with 18S ribosomal RNS (Rn18s) as an mRNA internal control. The PCR reaction and analysis were performed using the Applied Biosystems 7300 software (Applied Biosystems). The relative abundance of the target genes was obtained using the comparative threshold cycle (Ct) method. All PCR primers and fluorogenic probes were purchased from Applied Biosystems: Muc2 (Mm00458299_m1), Muc5ac (Mm01276725_g1), Muc5b (Mm00466391_m1), Muc6 (Mm00725165_m1), and Rn18s (Mm03928990_g1).

2. Statistics

All data are expressed as means ± standard error (SE). Statistically significant differences among the groups were assessed using Student's t-test, or the Mann-Whitney U-test. Statistical significance was defined as a P-value ≤ 0.05. Analyses were performed using the SPSS (18.0) software.

III. RESULTS

1. Effects of PUFA on gallstone formation, liver and body weight.

There was no difference in body and liver weight before and after the administration of therapeutic agents between each group. Body weight increased and liver weight decreased over time in all groups, but they were not statistically different from the control group (Group A), and there were no systemic side effects caused by the therapeutic agents (Figure 1). Reductions in gallstone size and weight after the administration of therapeutic agents were compared for each group. The size of CG decreased macroscopically after the administration of therapeutic agents in every group (Figure 2). The size decreased to a statistically significant degree in group B (UDCA) and group F (complex 1.0) (Figure 3). CG weight was decreased in the Complex groups (Figure 4A), with the highest loss in Complex 1.0, but the loss was not dose-dependent (Figure 4B).

2. Quantification of total bile phospholipid, cholesterol and bile acid.

There was no difference in the total cholesterol in bile between each group, but

the amount of phospholipid and bile acid was found to increase in the PUFA-treated groups. As a result, cholesterol saturation index was decreased in these groups (Figure 5). However, there was no difference in the total cholesterol and phospholipid in serum between each group (Figure 6).

3. Microscopic studies of the gallbladder (Hematoxylin and eosin staining)

After measuring GB wall thickening before and after the treatment, it was found that GB wall thickening was reduced in all of the groups that were treated with PUFA, regardless of UDCA administration (Figure 7). The mucosal epithelium of the gallbladder in PUFA with or without UDCA showed transparent and slim changes and the height of gallbladder wall decreased compared with the RD group (Figure 8)

4. Quantitative real-time polymerase chain reaction assay for MUC gene.

Treatment with PUFA resulted in a significantly decreased expression of MUC2, MUC5AC, MUC5B, and MUC6 genes in the gallbladder of mice in the PUFA and UDCA groups. It seems likely that these changes result in a decreased viscosity of bile, which attenuated
the formation of CG.

IV. DISCUSSION

This study investigated the dissolving effects of mixtures containing UDCA and PUFA on gallstones. In our previous study, our research team reported that PUFA inhibited the formation of gallstones by suppressing mucin production.[17] However, the study had a limitation in that it did not identify the mechanism by which PUFA suppresses gallstone formation. The present study focuses on verifying therapeutic effects of PUFA, rather than its prophylactic effects.

Therapeutic effects of PUFA and UDCA mixtures on CG formation caused by a high cholesterol diet were verified, but the effects were not dose-dependent. In Complex 0.5 and Complex 1.0, CG was decreased in a dose-dependent manner, but CG dissolving effect in Complex 2.0 was lower compared to Complex 1.0. The amount of PUFA used in Complex 2.0 was 102mg/kg/day, and this is equivalent to 6000mg in an adult that weighs 60kg, an amount that would be considered excessive. As a result, therapeutic effects were decreased upon excessive administration of PUFA.

Today, UDCA is used as a therapeutic agent for CG. In this study, the amount of gallstone was reduced in UDCA alone group compared to the control group (RD group), but not to a statistically significant degree. The observed lack of therapeutic effects of UDCA is attributed to the short administration period. Since there is a report that the response rate in humans who had taken UDCA for 2 years was 20~30%,[4] 12 weeks is considered rather short. Just like the

UDCA group, therapeutic effects were also observed in the PUFA group, but they were not statistically significant. In our preliminary research, an EPA-treated group experienced therapeutic effects on CG that were statistically significant, but the present study used an EPA-DHA mixture, which resulted in poorer CG dissolving effects. We cannot also exclude the possibility that we might not have been able to observe full effects of the agents due to the short treatment period. However, it appears that the two agents have synergic effect or additive effect in dissolving CG when used as a chemically complex form because there was significant decrease of CG in Complex groups. Because PUFA and UDCA do not share the same mechanism of action, it can be assumed that they would have a beneficial additive effect on treatment of cholesterol gallstones. UDCA acts on multiple proteins and downregulates the CSI,[18] while PUFA seem to stabilize cholesterol-phospholipid vesicles after micellization.[19]

However the group that was treated with UDCA and PUFA that were simply mixed did not show the same results as the Complex groups. This may be because UDCA and PUFA were not evenly mixed, and seeing from this result, it appears that administering agents that are simply mixed together cannot bring about the same effects. Compared to mixture agents like these, complex agents are advantageous in that they can be administered in a fixed amount by evenly mixing UDCA and PUFA, which have different properties, using surfactants. Complex agents showed therapeutic effects on CG not only from quantitative

aspects, but also qualitative aspects. CG stone size was reduced in the UDCA group and Complex 1.0 group. A decrease in the size of CG is a prerequisite for the resolution of CG, and it can be said that complex agents are qualitatively related to CG dissolution.

To investigate the mechanism by which complex agents treat CG, the total cholesterol, phospholipid, and bile acid in the bile and serum were measured, and cholesterol saturation index (CSI) was compared. Precipitation of cholesterol crystals from supersaturated bile is a prerequisite for gallstone formation.[1] Cholesterol is dissolved by mixed micelles that are formed by bile salts and phospholipids in the bile. Therefore, as lithogenic index or percent cholesterol saturation decreases, the probability of CG formation also decreases.[20] CSI is determined by the quantity of cholesterol, bile acid and lecithin in the bile.

Since over 95% of phospholipids are lecithin, phospholipids were measured instead of lecithin in our study. In our study, there was no difference in the total cholesterol in bile between the groups, but in the group treated with PUFA, the amount of bile acid and phospholipid was increased. Accordingly, CSI was decreased in the PUFA-treated group. It is speculated that PUFA decreases the quantity of CG by increasing the level of bile acid and phospholipids within the bile, thereby adjusting CSI within the bile. Since the level of cholesterol and phospholipid did not change in the serum, it is believed that the observed effect

only occurs in the bile.

Biliary mucin provides cytoprotection for the gallbladder epithelial cells. In addition, mucin itself performs important roles in gallstone formation.[21,22] Our study confirmed that PUFA suppresses mucin gene expression that affects the secretion of mucin within the gallbladder. Although mucin overproduction is an important prerequisite for gallstone formation, the mechanism that promotes mucin secretion during the formation of gallstones has not been clearly identified.[23] We suggest that the increased bile phospholipid and bile acid levels following PUFA administration, lower CSI and thus decreases the amount of CG induced high cholesterol diet. And PUFA also decreases the chemical irritation to the mucosa of the gallbladder. This may result in a decreased expression of the MUC gene and lower mucin formation, promoting nucleation in bile.

The limitation of this study is that parameters such as the level of biliary proteins including mucin, nucleation time, and cholesterol monohydrate crystals were not measured, and therefore, there was no analysis on the overall process of gallstone formation. Moreover, the study could not differentiate between endogenic and exogenic bile acid during the analysis on bile acid according to UDCA administration. In addition, the study did not perform an accurate analysis on lecithin as it measured the amount of phospholipids, rather than lecithin. The levels of collected bile were low and, as such, measurement of

these parameters was not possible.

V. CONCLUSION

Complex with PUFA and UDCA dissolves cholesterol gallstone in mouse through increasing the levels of bile phospholipids and bile acid, decreasing cholesterol saturation and suppressing bile mucin formation without systemic side effects. Further human study is required to investigate the therapeutic effects of the complex with PUFA and UDCA in patients with CG.

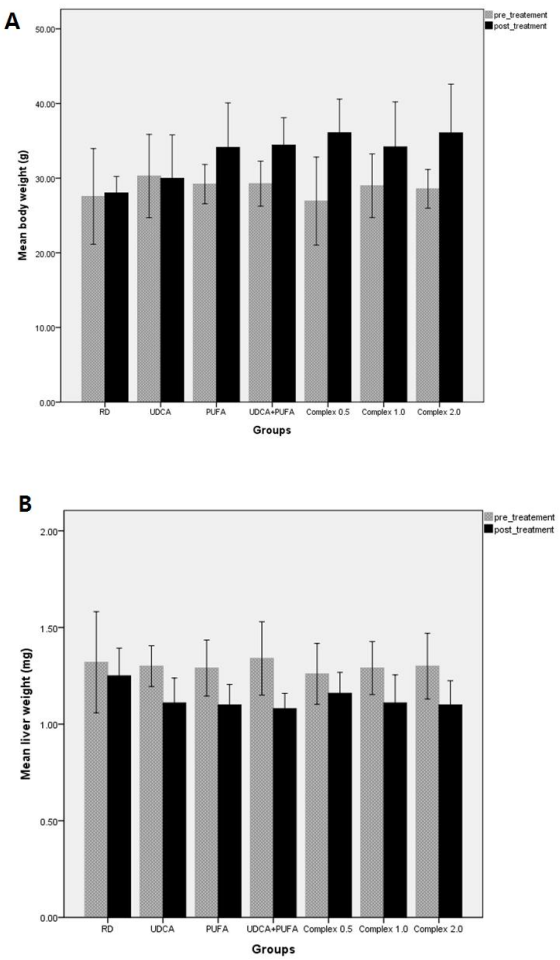

Figure 1. Body weight and liver weight before and after the treatment. In all groups, body weight increased (A), and liver weight decreased (B) over time according to the growth of individual mouse. However, there were no statistical differences between each group, and no systemic side effects caused by the administration of therapeutic agents were observed.

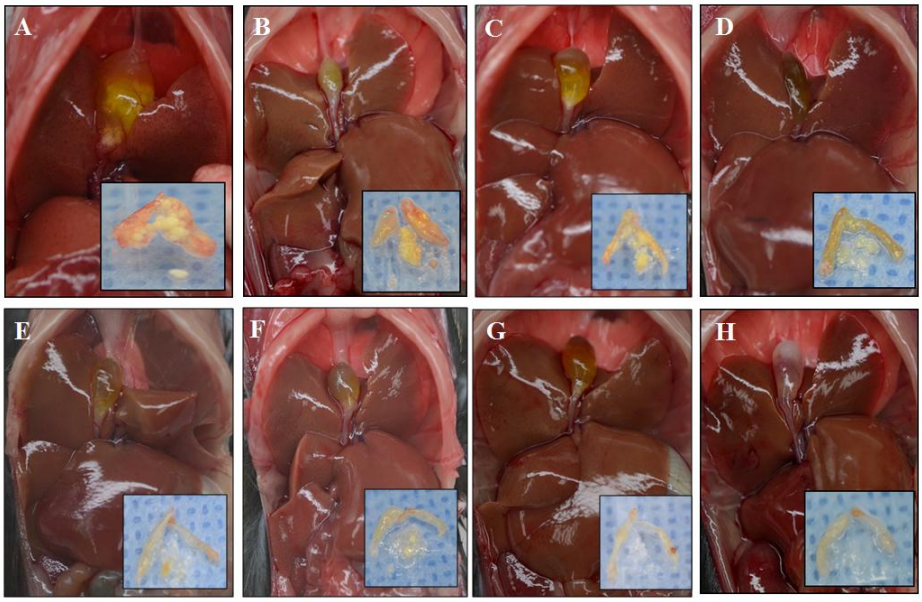

Figure 2. Gross findings of liver, gallbladder and gallstones

(A) gallstone after lithogenic diet ; (B) regular diet (RD); (C) RD+ ursodeoxycholic acid (UDCA); (D) RD + polyunsaturated fatty acids (PUFA); (E) RD + UDCA + PUFA; (F) RD + Complex 0.5; (G) RD + Complex 1.0; (H) RD + Complex 2.0. Mice fed with Complex displayed significantly fewer gallstones. (A) The growth of gallstone in the sacrificed mice before the administration of therapeutic agents was confirmed. The gallstone size and quantity were grossly reduced in all groups compared with the control group (B), after being treated with therapeutic agents.

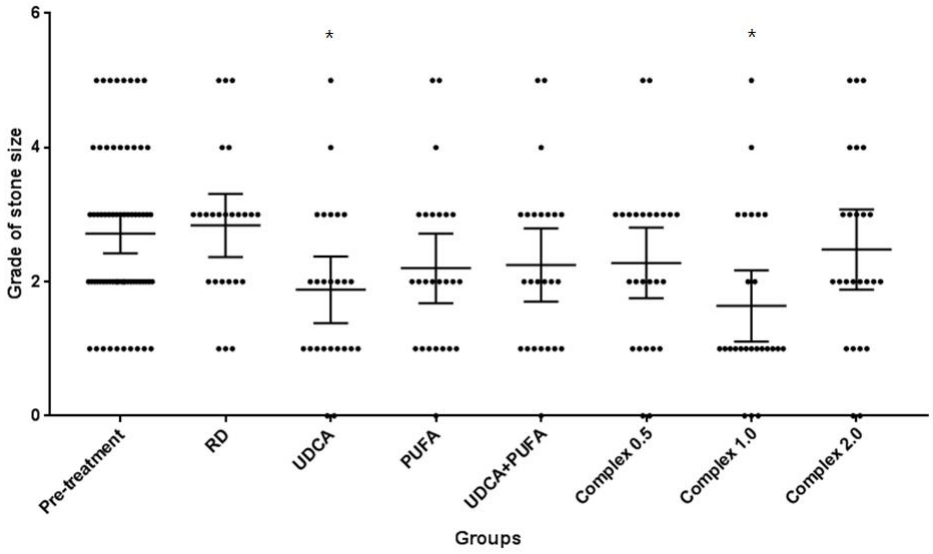

Figure 3. Grading of gallstone size

Gallstone sizes were compared for each group using a scoring system. In the ursodeoxycholic acid (UDCA) alone group and Complex 1.0 group, grading decreased significantly, signifying reductions in the gallstone sizes. (0 = clear bile; 1 = little sludge; 2 = wide-spread sludge; 3 = large levels of sludge; 4 = a few small formed stones; 5 = several formed stones; 6 = full of formed stones). (* p-value <0.05 compared with regular diet group)

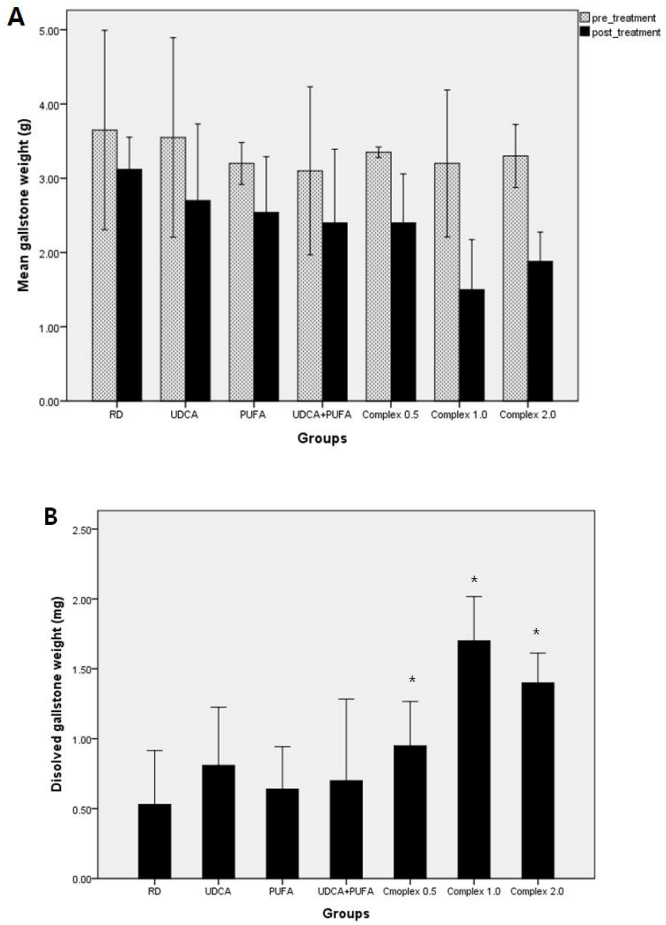

Figure 4. Difference of gallstone weight after administration of therapeutic agents Gallstone weights were decreased in all of the groups after the administration of therapeutic agents (A), but statistically meaningful reductions were observed only in the 3 groups that were treated with Complex agents. Gallstone weights decreased in a dose-dependent manner in Complex 0.5 and Complex 1.0, but not in Complex 2.0. (B). (* p-value <0.05 compared with regular diet group)

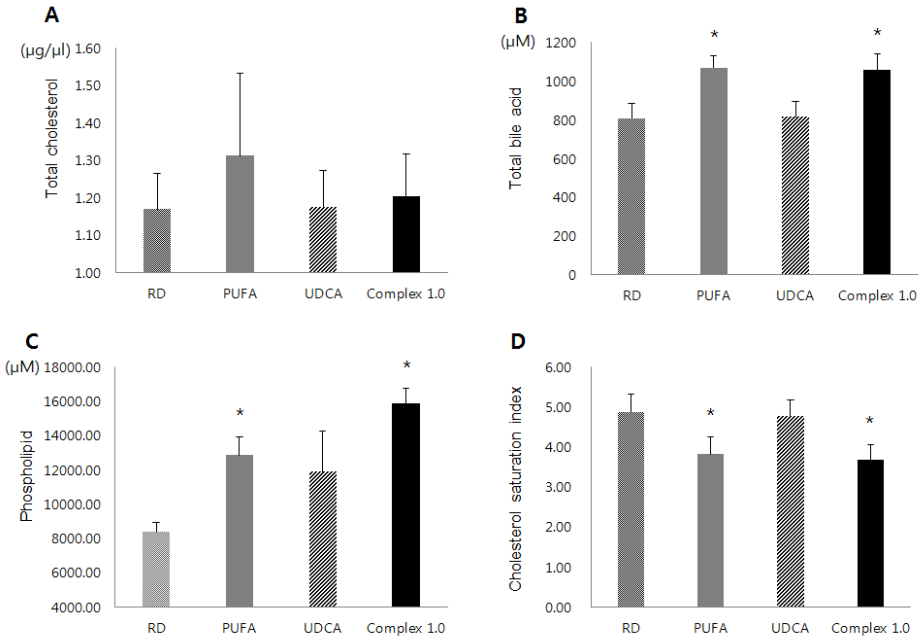

Figure 5. Total cholesterol, phospholipid, bile acid and cholesterol saturation index (CSI) in bile.

Total cholesterol in bile (A) did not change in each group, but phospholipid (B) and bile acid(C) decreased in the groups that were treated with PUFA. As a result, in the PUFA-treated groups, CSI decreased significantly (D). (* p-value <0.05 compared with regular diet group)

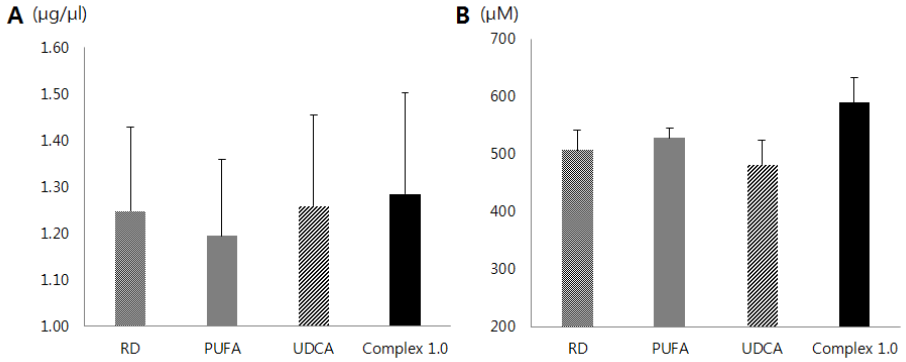

Figure 6. Total cholesterol and phospholipid in serum.

Total cholesterol and phospholipid in serum did not show any statistical differences between each group.

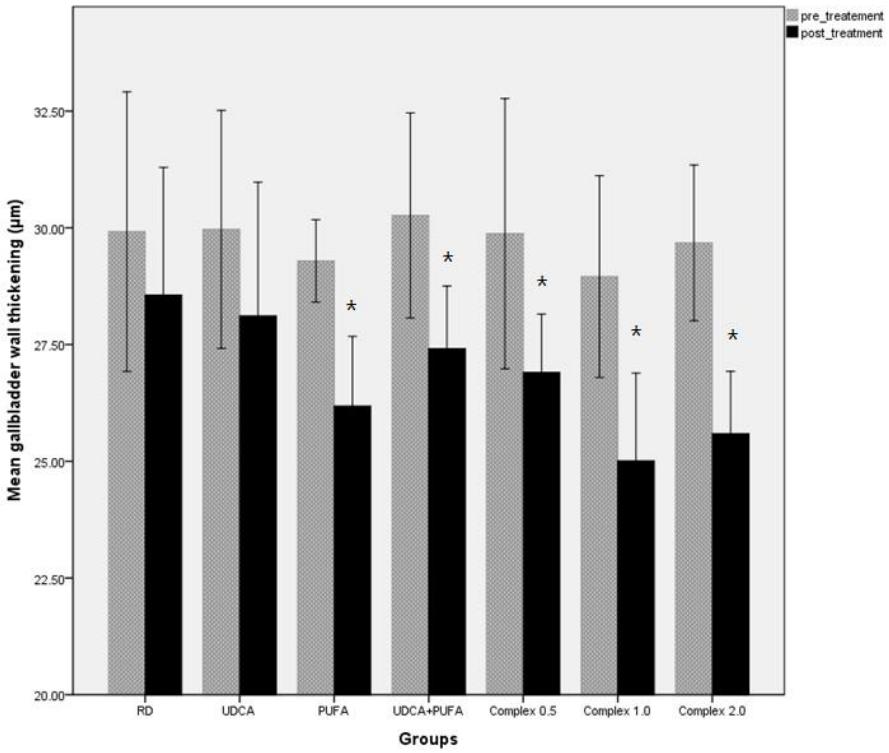

Figure 7. Total wall thickness of the gallbladder.

Measurements of total wall thickening of the bladder before and after the administration of therapeutic agents showed that regardless of UDCA administration, wall thickening was reduced in a statistically meaningful manner in the PUFA-treated groups. (* p-value <0.05 compared with regular diet group)

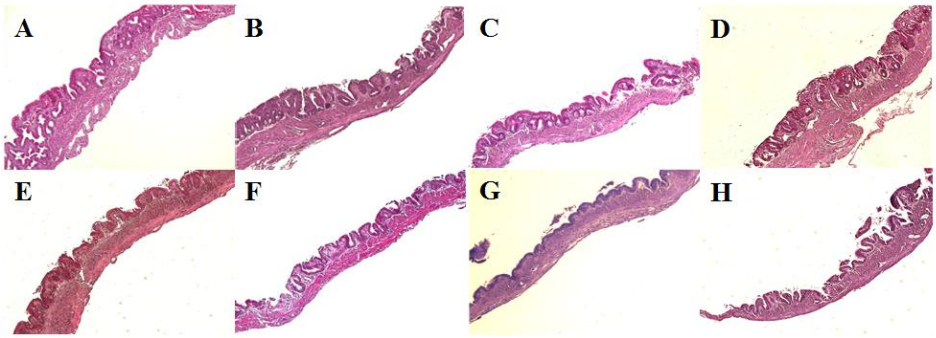

Figure 8 Histological mucosal changes and total wall thickness of the gallbladder.

(A) Microscopic feature of gallbladder after lithogenic diet ; (B) regular diet (RD); (C) RD+ ursodeoxycholic acid (UDCA); (D) RD + polyunsaturated fatty acids (PUFA); (E) RD + UDCA + PUFA; (F) RD + Complex 0.5; (G) RD + Complex 1.0; (H) RD + Complex 2.0. A lithogenic diet induced mucosal hypertrophy while PUFAs with or without UDCA administration displayed lower levels of hypertrophied gallbladder wall.

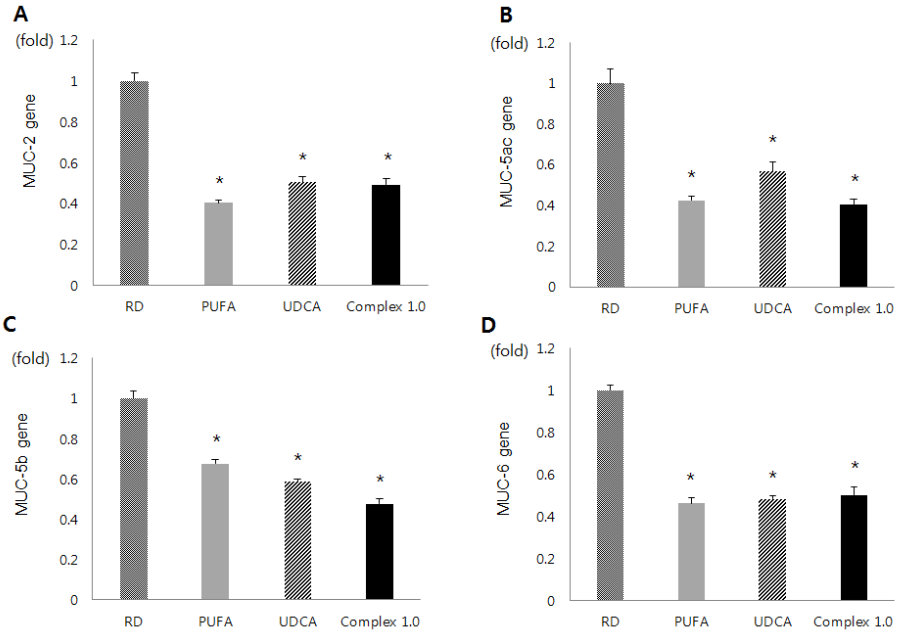

Figure 9 MUC gene expression in the gallbladder.

(A) MUC-2; (B) MUC-5AC; (C) MUC-5B; (D) MUC-6. PUFA with or without UDCA administration significantly inhibited multiple MUC gene expression in the gallbladder.

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ABSTRACT(IN KOREAN)

불포화지방산과 우르소데옥시콜릭산 복합제는 마우스에서 콜레스테롤 포화도를 낮추고 뮤신의 형성을 억제하여 콜레스테롤 담낭담석을 용해시킨다.

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배경 및 목적: 콜레스테롤 담낭담석 질환의 발생빈도가 증가함에 따라 보건건강에서 경제적 부담이 증가하고 있다. 우르소데옥시콜릭산(Ursodeoxycholic acid)은 현재까지 담석을 용해하는 유일하게 증명된 내과치료약제이다. 콜레스테롤 담낭담석에 대한 새로운 치료제를 연구하면서 마우스모델에서 고콜레스테롤이 포함된 고지방식이로 인해 유도된 콜레스테롤 담낭담석에서 불포화지방산의 치료효과를 우르소데옥시콜릭산과 비교하고자 하였다.

방법: 마우스는 7개 집단으로 나누었다. (가) 일반식이, (나) 우르소데옥시콜릭산, (다) 불포화지방산, (라) 우르소데옥시콜릭산 + 불포화지방산, (마) 복합제 0.5배, (바) 복합제 1배, (사) 복합제 2배. 8주간 고지방식이 후, 각 집단별로

10마리씩 적출하여 콜레스테롤 담석형성여부와 그 정성적 양을 측정하여 기준으로 삼았다. 이후 12주간 일반식이와 더불어 치료약제를 경구로 투여하였다. 포스포리피드, 콜레스테롤, 담즙산의 양을 측정하고 담석의 용해정도와 담낭벽 두께를 측정하였다. 그리고 담낭내의 MUC 유전자의 표현정도로 측정하였다.

결과: 실험 중 마우스의 체중과 간무게는 각 집단간 차이가 없어 치료약제들이 마우스에 전신적인 부작용을 일으키지 않는다는 것을 알 수 있었다. 복합제를 투여받은 집단들은 대조군인 일반식이를 시행한 집단에 비해 담석의 용해 정도가 통계적으로 유의하게 컸다. 복합제를 투여받은 집단들에서 담즙내 포스포리피드와 담즙산이 증가하여 결과적으로 콜레스테롤용해지표가 감소하였다. 비록 고지방식이한 마우스에서 담낭벽의 비후가 관찰되었으나, 우르소데옥시콜릭산 투여와 관계없이 불포화지방산을 투여받은 집단들에서는 이러한 비후가 감소하였다. MUC 2, 5ac, 6 의 유전자 발현도도 불포화지방산을 투여받은 집단들에서 의미있게 감소하였고, 이러한 효과는 우르소데옥시콜릭산의 투여와 관련없이

억제되었다.

결론: 불포화지방산과 우르소데옥시콜릭산의 복합제는 마우스에서 담즙내 포스포리피드와 담즙산의 양을 증가시켜 콜레스테롤용해지표를 낮추고, 담즙내 뮤신형성을 억제하여 콜레스테롤 담석을 용해시킨다. 이러한 복합제의 효과를 확인하기 위해서는 콜레스테롤 담낭담석을 가진 환자들을 대상으로 하는 임상연구가 필요하다.

핵심되는 말: 콜레스테롤 담낭담석, 불포화지방산, 우르소데옥시콜릭산, 뮤신, 콜레스테롤용해지표