Effects of Fatty Acid Bile Acid Conjugates (FABACs) on Biliary Lithogenesis: Potential Consequences for Non-Surgical Treatment of Gallstones

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Abstract: Fatty acid bile acid conjugates (FABACs) are novel synthetic lipid molecules, which were designed for the treatment of cholesterol gallstones. The rationale was to combine a cholesterol solubilizing moiety (a saturated fatty acid) with a bile acid (cholic acid) as a vehicle to enable secretion into bile and entry into the enterohepatic circulation. An amide bond was used to provide stability against intestinal degradation. Initial *in vitro* studies showed that FABACs are indeed cholesterol solubilizers, able to prevent biliary cholesterol crystallization. Arachidyl-amido-cholanoic acid (Aramchol) was found to be the most potent FABAC in these studies.

Animal studies revealed that Aramchol was absorbed after oral administration and could prevent cholesterol crystallization as well as dissolve preformed crystals in rodents fed a lithogenic diet. In gallstone susceptible mice, Aramchol prevented gallstone formation and dissolved gallstones. FABACs were found to be metabolically active substances, also able to decrease blood cholesterol, atherosclerotic plaques and fat accumulation in the liver in several animal species.

The underlying mechanisms of action are under active investigation, and several effects, e.g. on cholesterol and bile salt metabolizing enzymes as well as cholesterol efflux from cells have been discovered. These findings are, however, only the beginning of our understanding of the metabolic actions as well as the potential of use of FABACs as therapeutic agents.

INTRODUCTION

Gallstones are a major public health problem affecting some 15% of the population in most industrialized countries, with considerable ethnic and geographic variations [1]. Most of them are cholesterol gallstones. For a long time, and also currently, the only practical therapeutic option was surgery to remove the gallbladder with the stones.

The introduction of bile acid therapy in 1972 was a medical breakthrough proving that cholesterol gallstones in man could be dissolved by peroral medical means [2]. The specific bile acids used, first chenodeoxycholic acid (CDCA) and subsequently urseodeoxychodic acid (UDCA) were, however, found to be of low efficacy [3]. Moreover, most gallstones were at time of diagnosis noted to be unsuitable for this therapy. Stones were often calcified, too large, too numerous, and the gallbladder was sometimes obstructed or non-functioning. Complete dissolution was often protracted (1-2 years) or unachievable. Recurrence of dissolved stones, particularly multiple stones, was the rule. The advent of lithotripsy did not much modify these basic facts.

More recently, it became evident that bile acids are not the major cholesterol solubilizers in bile, particularly in the usually supersaturated human bile [4,5]. Phospholipids were shown to be the major physiologic cholesterol solubilizers in bile, able to normalize the rapid cholesterol crystallization in biles of patients with cholesterol gallstones [6]. Not only the concentration (absolute and relative) of the biliary phospholipids was a determining factor but also changes in phospholipid molecular species (without any change in concentration) could prolong or shorten the crystallization time (nucleation time) of model and human biles [7]. Phospholipids with saturated and/or long chain sn-2 fatty acids were particularly effective cholesterol solubilizers [8]. However, therapeutic attempts to increase the concentration of phospholipids in bile or modulate their molecular species failed. Feeding various phospholipids or their precursors left the concentration and composition of biliary phospholipids largely unchanged [9]. It was then investigated whether parts of the phospholipid molecule such as fatty acids and also mono- or diglycerides could act as cholesterol solubilizers. These studies revealed that saturated long chain fatty acids and some other parts of the phospholipid molecule had cholesterol solubilizing activity [10]. But again, there was no practical way to enrich bile with these compounds.

The FABACs (Fatty Acid Bile Acid Conjugates) were conceived and designed with the purpose of delivering a cholesterol solubilizing molecule (a long chain fatty acid) into bile using the very efficient enterohepatic circulation of bile salts. A scheme of the FABAC molecule is shown in Fig. (**1**). The concept of using bile salts as vehicles for the delivery of various drugs and molecules to the liver and bile was developed particularly by the group of Dr Werner Kramer [11,12]. Dr Kramer had in the past synthetized a conjugate of palmitic and cholic acids (Dr. Kramer personal communication) and cooperated with one of the authors

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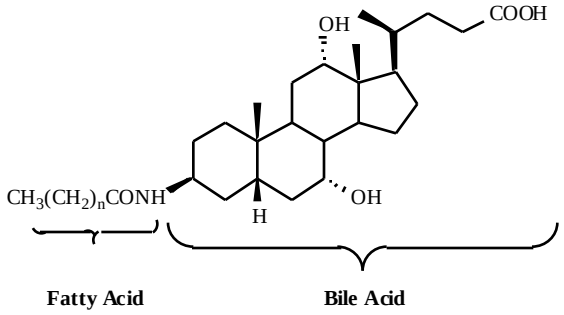

Fig. (1). The general scheme of Fatty Acid Bile Acid Conjugates (FABACs). A fatty acid is bound to a bile acid at position 3, by an amide bond in the beta-configuration.

(T.G.) on the conceptual aspects of the FABACs and their synthesis. Dr. David Kritchevsky had synthetized and published a whole series of fatty acid bile acid conjugates with the purpose of slowing down the bacterial degradation of CDCA and UDCA then used in gallstone dissolution [13]. He used an ester bond which was broken down during absorption, and the desired active moiety was the bile acid molecule itself, used to lower cholesterol saturation of bile. We postulated that an ester bond would be broken down during digestion/absorption resulting in the separate absorption of a fatty acid and a bile acid with almost complete loss of the biological activity of the FABAC. Therefore, an amide bond was chosen to provide better stability, more likely to resist brake down by intestinal bacteria, juices and enzymes.

In this review we will summarize the available information on FABACs with particular reference to their potential use in gallstone prevention and therapy.

IN VITRO EFFECTS

The first experiments with FABACs were undertaken to investigate the influences of FABACs on cholesterol solubility and cholesterol crystal formation [14]. In these studies cholic acid conjugates with saturated fatty acids of varying chain length (C6-C22) were investigated. In model bile, replacement of 20% of the bile acids by C16-, C18- and C20-FABAC significantly increased the crystal observation time and decreased the cholesterol crystal mass after 14 days of incubation. The effects were most marked with C18- and C20-FABACs. FABACs with fatty acids of a chain length of 14 carbons or less had little effect on cholesterol crystallization. When C16-, C18- and C20-FABACs were added to an enriched human bile at a concentration of 5mM, cholesterol crystal mass decreased by 60-90%, while a similar concentration of cholic acid had no effect on the crystal mass. In fresh human bile, the effects of C20- and C22-FABACs were even more prominent, leading to a fall in crystal mass of >90% after 3 weeks of incubation Fig. (**2**). In model as well as in native bile studies the inhibitory effects on cholesterol crystallization were mostly seen with FABACs having fatty acid chains lengths of 16 carbons or

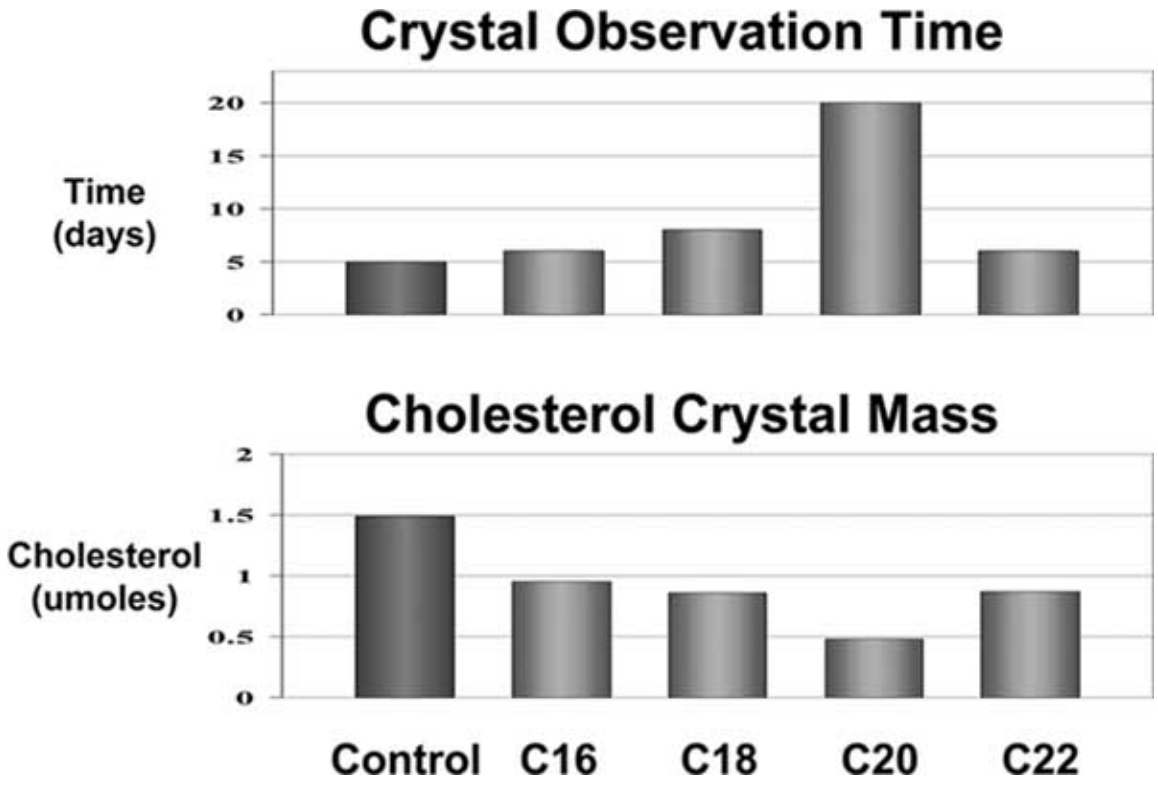

Fig. (2). The effect of FABAC fatty acyl chain length on biliary cholesterol crystallization. Five mM of FABACs were dissolved in fresh human gallbladder bile, obtained at cholecystectomy and the crystal observation time was determined by polarizing light microscopy (upper panel). Chlesterol crystal mass at 21 days of incubation is shown in the lower panel. FABACs with 16 to 22 carbons long saturated fatty acids are compared to the native control bile.

more. These studies led to the use of a C20-FABAC (Arachidyl-amido-cholanoic acid, or "Aramchol") in most subsequent studies.

Based on their molecular structure, FABACs were expected to have ampiphilic properties. The solubility of C20-FABAC was measured in various aqueous media, and found to be negligible in water or saline. The solubility in model bile was higher than in a pure bile salt solution, and significantly higher in native bile than in model bile solutions with comparable lipid compositions.

To investigate, whether FABACs were cholesterol solubilizers, a series of experiments in model and human biles were carried out [15]. In two different model biles the addition of 5mM of C20-FABAC increased the cholesterol solubilization capacity by at least two-fold as compared to that of a similar amount of taurocholate, and to a comparable extent to the addition of 5mM PC. In the supersaturated solution with a cholesterol saturation index >1 the FABAC was the most efficient solubilizer. Furthermore, when preformed cholesterol crystals were coincubated with C20-FABAC (7-30mM) in model biles and in human bile *ex vivo*, the crystal mass decreased in a dose-dependent manner. These data show that FABACs are indeed cholesterol solubilizers.

IN VIVO EFFECTS

The effects of FABACs on cholesterol crystallization and gallstone formation *in vivo* were studied in two different animal models – hamsters and mice [14-16]. In hamsters fed a lithogenic diet for ten weeks, all animals developed cholesterol crystals in their gallbladders, while animals supplemented with an oral FABAC (150mg/kg/day) had no crystals. A similar inhibitory effect was found in gallstone susceptible mice after 14 days on a lithogenic diet. In mice the effect was also noted with lower doses of FABAC (25 and 50mg/kg/day). Furthermore, when mice with cholesterol crystals in their gallbladders (after a lithogenic diet) were fed C20-FABAC, the crystals disappeared gradually in 25% and 75% of the animals within 2 and 4 weeks, respectively.

To test the potential effect of FABACs on gallstone prevention, inbred mice were fed a lithogenic diet with and without FABAC supplementation [15]. After 3 weeks all control animals had gallstones in their gallbladders, whereas the FABAC-fed animals had no stones. The preventive effect was noted with a dosage of FABAC ranging from 25 to 150mg/kg/day.

Finally, to test the therapeutic potential of FABACs, inbred mice were fed a lithogenic diet for two months to induce gallstones [16]. Thereafter, they were reverted to a regular chow diet, with and without FABAC supplementation (150mg/kg/day). The results of these experiments are summarized in Fig. (**3**). After two months, most (>90%) control animals still had gallstones, while less than 10% of FABAC supplemented animals had stones. In some of the FABAC treated animals, sludge was present in the gallbladders. The therapeutic effect of FABACs was dose dependent, with

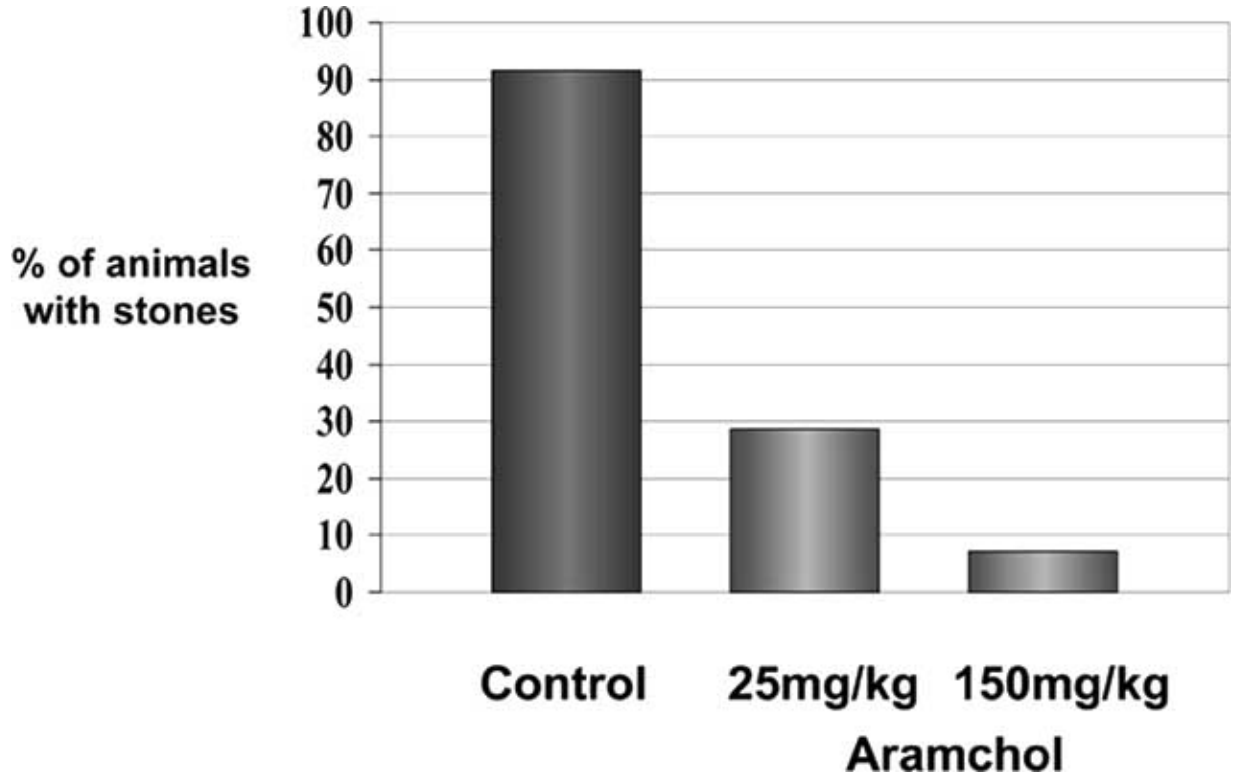

Fig. (3). Dissolution of gallstones in inbred gallstone susceptible (C57L/J) mice. Gallstones were induced in all animals by a lithogenic diet. Two months after stopping the lithogenic diet, 11 out of 12 (91.8%) control mice had gallstones in their gallbladders, compared to 2/7 (28.6%) and 1/14 (7.1%) of mice fed Aramchol at a daily dose of 25mg/kg and 150mg/kg, respectively. (Modified from ref 16).

dissolution rates of 71% and 93% with doses of 25mg/kg/day and 150mg/kg/day, respectively Fig. (**3**).

EFFECTS ON BILE

It was shown that FABACs are absorbed and secreted into bile, as measured by HPLC [14]. More recent data using tandem mass spectrometry have revealed that FABAC concentrations *in vivo* are lower than initially thought [17].

The effects of FABACs on biliary lipid composition were studied in hamsters, mice as well as in rats [18]. In hamsters, a decrease in cholesterol and bile salt concentration was noted after prolonged (10 weeks) FABAC feeding. This was accompanied by an increase in phospholipid concentration, resulting in an overall decrease in the CSI of the bile. After shorter periods, such as 4 weeks, these changes were not observed. In C57Bl/6 mice FABAC feeding resulted in a decreased concentration of all three biliary lipids, without influencing the CSI. Interestingly, in gallstone susceptible (C57L/J) mice, no significant changes in biliary lipid concentrations nor the CSI were noted despite the favorable effects on cholesterol crystal and gallstone formation. Bile flow has been so far measured only in rats and hamsters. FABAC feeding was not found to influence bile flow significantly. The effects of FABACs on other biliary constituents, such as biliary proteins or lipid subclasses remain to be studied.

NON-BILIARY EFFECTS OF FABACS

The FABACs are completely new synthetic molecules to which body cells were not previously exposed. (Esters of fatty acids with bile acids, particularly lithocholic acid, were found in the stools [19] but intact esters were not demonstrated in blood). Yet, the FABACs were found to have a range of effects on transporters, receptors and enzymes involved in lipid metabolism. Thus, FABACs may also lead to potential effects in several major non-biliary disease states.

Cholesterol Metabolism

FABACs were found to induce cholesterol efflux from human fibroblasts *ex vivo* [20]. This effect was dose dependent and was manifested at concentrations of less than 1μM. As this effect was absent in fibroblasts of patients with Tangiers disease it localized the effector molecule to the ABCA1 transporter [20]. It was a direct effect on ABCA1 independent of ApoA1 and HDL. This effect was also demonstrated in murine fibroblasts and macrophages (Dr I Goldiner and Dr A Groen, unpublished observations). It would result in increased reverse cholesterol transport, which is considered a desirable target in cholesterol homeostasis and atherosclerosis.

In C57L/J gallstone susceptible mice on a lithogenic diet Aramchol was shown to enhance 2-3 fold CYP7A1 activity while moderately (approx. 50%) decreasing HMGCoAR activity [21]. This would result in an increased cholesterol catabolism (to bile salts) while concomitantly decreasing its synthesis. This again is a desirable goal in the treatment of excess body cholesterol. It has to be confirmed in other animals.

Quite recently it has been demonstrated that FABACs may increase the fecal output of sterols and particularly bile salts [22]. The total fecal sterol output increased by close to 100% in rats. Biliary lipid output and relative concentrations were not changed. This suggests that the mechanism involves intestinal absorption. An increased conversion of cholesterol to bile salts coupled with an increased fecal bile salt loss would explain the normal biliary bile salts concentrations. The increased synthesis in the liver with resultant increased biliary secretion would be balanced by the increased fecal loss and hence result in decreased hepatic and biliary return. The overall effect of the above sequence is an increased loss of body sterols. Reduced serum cholesterol levels, during short term FABAC administration were noticed in some but not all animal species [22].

Atherosclerosis

FABACs were also documented to have a beneficial effect on atherosclerosis [23]. Additional studies in ApoE -/- mice and LDLR-/- mice also showed fewer lesions in the FABAC supplemented animals. Thus, FABACs may have potential in the treatment/prevention of atherosclerosis.

Fatty Liver

Animals receiving lithogenic and atherogenic diets developed fatty infiltration of their livers. It was then noted that the FABAC supplemented animals did not develop fatty livers. This was tested in controlled experiments in mice, rats, and hamsters [24]. The amount of fat in the liver was estimated by light microscopy and quantitatively measured by chemical means. The mg lipid/gm liver and mg lipid/mg protein in liver tissue were the parameters used. By all these measurements, FABAC supplementation markedly reduced liver fat in animals receiving various high fat diets. The decrease in liver fat was most notable in the triglyceride faction. Animals gained weight similar to controls, ate normally, and triglyceride secretion in VLDL was not increased. It is thus likely that triglyceride metabolism in the liver was altered with catabolism exceeding synthesis. The molecular mechanisms are being studied.

MECHANISM OF ACTION

In contrast to the quite impressive and promising biological effects of FABACs, the underlying mechanisms of action have been only partly elucidated. Even the effects on biliary cholesterol crystallization and gallstone formation appear to be much more complicated than originally thought. The FABACs are clearly cholesterol solubilizers. It was noted that FABAC concentrations needed to delay cholesterol crystallization or reduce the crystal mass were lower in human bile as compared to model biles composed of the 3 biliary lipids in water. This suggested that other components in bile enhanced the cholesterol solubilizing effect of the FABACs. *In vivo*, however, the FABAC concentrations found in gallbladder bile (less than 1 mM) were much lower than the minimal concentrations in bile ex vivo required to produce an effect on cholesterol solubility (~3 mM or more). Yet, these low concentrations in bile *in vivo* were effective in preventing or dissolving cholesterol gallstones [14,16].

With the discovery that FABACs are metabolically active compounds, it is plausible to assume that FABACs may have a multifactorial effect in gallstone disease. Specific effects that can play a role are: A physical-chemical effect as cholesterol solubilizers; An effect on bile flow; An effect on bile composition; An effect on the gallbladder. Of these, so far only the cholesterol solubilizing effect has been proven. However, as discussed above, it cannot account for the whole biological effect. Bile flow has been tested in rats and hamsters, but does not seem to be significantly influenced. Bile composition has been studied in terms of the three major biliary lipids – bile salts, cholesterol and phopholipids. Only in hamsters was an influence on lipid concentration, with a concomitant decrease in CSI, noted. In C57L/J mice, the biliary lipid composition remained unchanged, despite the significant biological effects on cholesterol crystals as well as gallstones. A possible explanation could be a change in the composition of bile salt or phospholipid species. A shift towards more hydrophobic bile salts or phospholipid species with more saturated acyl chains might increase the cholesterol solubilizing capacity of bile without changing the lipid concentration. FABACs could also influence biliary proteins, in particular by increasing proteins which have an antinucleating or cholesterol solubilizing effect. All these possibilities need to be investigated.

An additional effect, on as yet unidentified factors possibly in the gallbladder mucosa also remains to be explored.

Hence, despite a multitude of effects of FABACs, none can at present fully explain the preventive or therapeutic effects in gallstone disease.

CONCLUSION

FABACs were proven to be cholesterol solubilizers, able to prevent and dissolve cholesterol gallstones. For over 6 years no significant side effects have been noted. They are active following oral administration and possibly have more than a single mechanism of action. They seem to be suitable candidates for the treatment and prevention of cholesterol gallstones, following the appropriate animal and human studies. They were also found to have numerous non-biliary effects, some of which may enhance patient willingness to accept chronic therapy.

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