

Faecal sterol output is increased by arachidyl amido cholanoic acid (Aramchol) in rats

A. Leikin-Frenkel^{*1}, A.A Weinbroum[†], D. Leikin-Gobbi^{‡§}, L. Krupitzky[†], I. Goldiner[‡], L. Shafat^{*}, T. Gilat^{‡§} and F.M. Konikoff^{*‡}

^{*}Minerva Center for Cholesterol, Gallstones and Lipid Metabolism in the Liver, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel, [†]Animal Research Laboratory, Tel Aviv Sourasky Medical Center, Weizmann 6, Tel Aviv 64239, Israel, [‡]Department of Gastroenterology, Tel Aviv Sourasky Medical Center, Weizmann 6, Tel Aviv 64239, Israel, and [§]Galmed Medical Research Ltd, 8 Shaul HaMelech Boulevard, Tel Aviv 64733, Israel

Abstract

Fatty acid–bile acid conjugates (FABACs) were shown recently to have important and multiple effects on cholesterol metabolism. In human fibroblasts, they were found to markedly enhance cholesterol efflux by an ATP-binding cassette transporter A1-dependent pathway. In C57L/J mice, they increased CYP7A1 activity and RNA expression, while decreasing moderately 3-hydroxy-3-methylglutaryl-CoA reductase activity. In C57L/J mice and in hamsters, they also decreased serum cholesterol levels, whereas in other animals, this effect was not seen in short-term experiments. In the present study, we investigated potential mechanisms of action of arachidyl amido cholanoic acid (Aramchol), with particular reference to biliary and faecal sterol outputs in rats. Supplementation with Aramchol at a dose of 150 mg · kg⁻¹ · day⁻¹ increased neutral sterol output by approx. 50%, while the faecal outputs of bile salts and total sterols increased by almost 2-fold. Biliary lipid outputs were not significantly different between the control and FABAC-supplemented animals. These findings indicate an overall catabolic effect of FABACs on body cholesterol.

Introduction

Fatty acid–bile acid conjugates (FABACs) were shown recently to have important and multiple effects on cholesterol metabolism. In human fibroblasts, they were found to markedly enhance cholesterol efflux. This effect was shown to be ATP-binding cassette transporter A1 (ABCA1)-dependent [1]. In C57L/J mice, FABACs increased CYP7A1 activity and its mRNA levels 2–3-fold, while decreasing moderately 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity [2]. In C57L/J mice, they decreased serum cholesterol levels by 50% in three different experiments. In hamsters, they decreased serum cholesterol levels by 20–50%. In C57B16/J mice and in Wistar rats <3 weeks, however, this effect was not seen in short-term experiments (A. Leikin-Frenkel, I. Goldiner, F.M. Konikoff and T. Gilat, unpublished work). In FVB/N mice serum cholesterol levels decreased by approx. 30% within 5 days of FABAC administration (I. Goldiner and A. Groen, unpublished work). Because FABACs remove cholesterol from tissues and may also reduce its levels in blood, the question of mechanism arises. The present study was initiated to investigate potential mechanisms of action in rats and, in particular, effects on biliary and faecal sterol outputs. Arachidyl amido cholanoic acid (Aramchol), a conjugate of arachidic acid and cholic acid, using an amide bond at position 3 of the bile acid, was the FABAC used in most of the previous studies, as well as in the present study.

Materials and methods

Animals

Male Fisher 344 rats (400–500 g) were used in this study. All animals were fed a regular rodent chow diet containing maximum 4 g% of fat. The study was approved by the animal experimentation committee of our institution.

Experimental design

Eight animals were held in standard individual cages at an animal facility at room temperature (22°C) and under a 12-h light/dark cycle. Water was given *ad libitum* and animals were weighed weekly. Saline (0.5–1 ml · day⁻¹) was administered by gavage for 12 days. Stools were collected from day 8 until day 12. Aramchol (150 mg · kg⁻¹ · day⁻¹) was administered by gavage as a suspension in saline (0.5–1 ml) for the subsequent 12 days. Stools were collected as described above. The animals were fasted for 24 h, anaesthetized and the bile duct was cannulated. Bile was collected over a 3-h period. Similarly, bile was collected from another group of eight rats kept on a chow diet for 12 days and gavaged with saline. The Aramchol was prepared as previously reported [1] and was a generous gift from Galmed Medical Research Ltd (Tel Aviv, Israel).

Processing of biological samples

Stools were freeze-dried, pulverized and weighed. Lipids were extracted from stool aliquots and from bile aliquots by the method of Folch et al. [3]. Phospholipids were quantified in bile according to Bartlett's procedure [4]. Cholesterol and

Key words: bile, bile salts, cholesterol, faecal output, fatty acid–bile acid conjugate (FABAC).

Abbreviations used: ABCA1, ATP-binding cassette transporter A1; Aramchol, arachidyl amido cholanoic acid; FABAC, fatty acid–bile acid conjugate; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA.

[†]To whom correspondence should be addressed (e-mail alicial@post.tau.ac.il).

Figure 1 | Faecal outputs (mg/4 days) of neutral sterols, bile salts and total sterols in eight rats receiving saline and Aramchol during consecutive 12-day gavage periods

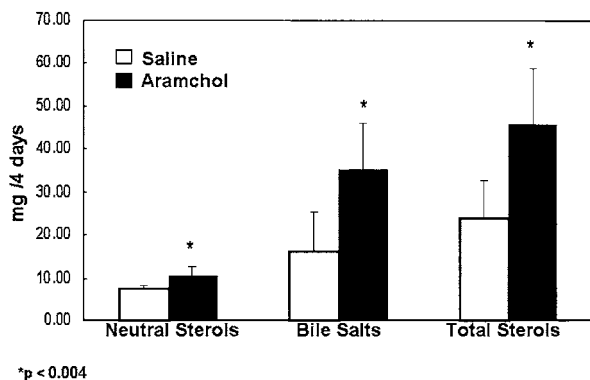
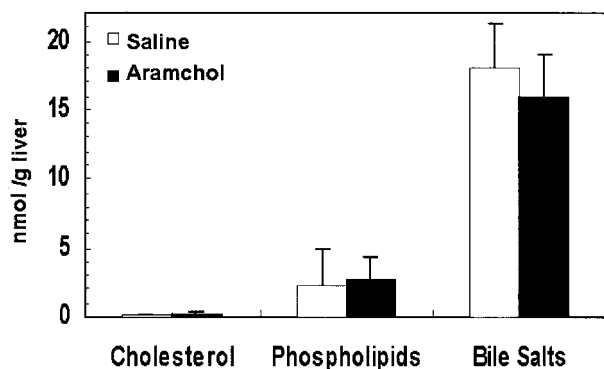


Figure 2 | Biliary lipid output during a 3-h period in two groups of saline-supplemented ($n = 8$) and Aramchol-supplemented ($n = 8$) rats



bile salts were determined in stools and bile by standard methods [5,6].

Results

Figure 1 shows faecal sterol outputs (mg/4 days) in rats on a regular diet gavaged initially with saline (control) and subsequently with Aramchol ($150 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$). Neutral sterol output increased from $7.58 \pm 0.76 \text{ mg/4 days}$ to $10.50 \pm 2.33 \text{ mg/4 days}$ ($P < 0.004$), bile salt output increased from $16.38 \pm 9.11 \text{ mg/4 days}$ to $35.23 \pm 10.85 \text{ mg/4 days}$ ($P < 0.002$) and total faecal sterol output increased from $23.96 \pm 8.68 \text{ mg/4 days}$ to $45.74 \pm 12.96 \text{ mg/4 days}$ ($P < 0.001$) during FABAC supplementation. Each animal served as its own control.

Neutral sterol output increased in each of the eight rats and faecal bile salt output increased in seven of the eight rats during FABAC supplementation.

Figure 2 shows the biliary output of bile salts, cholesterol and phospholipids in two groups of rats supplemented with either saline or Aramchol ($150 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) for 12 days. Bile flow was similar in the two groups (results not shown). Biliary cholesterol, bile salt and phospholipid outputs were

not significantly different between the control and FABAC-supplemented animals.

Discussion

FABACs were initially synthesized to solubilize biliary cholesterol. They were proven to delay or prevent cholesterol crystallization in model and human bile [7], and to prevent [8] and dissolve [9] cholesterol gall stones. Subsequently, they were shown to prevent and reduce diet-induced fatty liver in several animal species [10], and to reduce atherosclerosis in a mouse model [11].

These synthetic molecules, composed of a fatty acid and a bile acid, were shown to have marked effects on various human and animal transporters, enzymes and receptors.

In relation to cholesterol metabolism, the enhancement of cholesterol (and phospholipid) efflux from human fibroblasts has been well documented. The effect is dependent on a functioning ABCA1 transporter and is therefore non-existent in fibroblasts of patients with Tangier disease [1]. Stimulation of CYP7A and, to a lesser degree, down-regulation of HMG-CoA reductase, were demonstrated only in C57L/J mice on a lithogenic diet and require studies in other species [2]. Biliary lipid levels and their proportions in gall bladder bile from fasting animals were unchanged in several animal species [12]. The decrease in cholesterol blood levels in short-term experiments in wild-type animals was found in some species, but not in others. Enhancement of reverse cholesterol transport from tissues to blood would tend to increase serum cholesterol. So, where does that cholesterol go? Previous studies [12], as well as the present results, do not indicate an increased biliary lipid secretion.

The present findings that show increased output in the stools might point to the answer. The findings were very consistent and highly significant. They were found in almost every animal tested. Using each animal as its own control is arguably the most precise method for measuring an effect. It is noteworthy that the effect on bile-salt output was more marked than that on cholesterol output. Both effects are additive in relation to overall cholesterol metabolism.

A useful working hypothesis, which fits most of the presently known facts, is that sterol absorption or secretion in the gastrointestinal tract is impaired by Aramchol. In the case of bile salts, since secretion is normal, it is probably absorption/reabsorption that is impaired. Diminished absorption of bile salts could also affect sterol absorption. Cholesterol secretion from the bowel wall could also be affected. Absorption tests of bile acids and cholesterol are now under way. The effects of FABACs (Aramchol) on the IBAT and ABCG5/G8 in the intestine are being investigated in terms of expression and function.

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