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Inhibition of the intestinal absorption of bile acids using cationic derivatives: Mechanism and repercussions

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ABSTRACT

To pharmacologically interrupt bile acid enterohepatic circulation, two compounds named BAPA-3 and BAPA-6, with a steroid structure and 1 or 2 positive charges, were obtained by conjugation of *N*-(3-aminopropyl)-1,3-propanediamine with one or two moieties of glycocholic acid (GC). Both BAPA-3 and BAPA-6 inhibited Na^+-dependent taurocholate (TC) uptake by *Xenopus laevis* oocytes expressing rat Asbt, with K_i values of 28 and 16 μM, respectively. BAPA-3 reduced V_{max} without affecting K_m. In contrast, BAPA-6 increased K_m, with no effect on V_{max}. Uptake of [^{14}C]-GC by the last 10 cm of the rat ileum, perfused *in situ* over 60 min, was inhibited to a similar extent by unlabeled GC, BAPA-3 and BAPA-6. However, the intestinal absorption of these compounds was lower (BAPA-6) or much lower (BAPA-3) than that of GC. When administered orally to mice, both compounds (BAPA-3 > BAPA-6) reduced the bile acid pool size, which was accompanied by up-regulation of hepatic Cyp7a1 and Hmgcr and intestinal Ostα/Ostβ. A tendency towards a decreased expression of hepatic Ntcp and an enhanced expression of intestinal Asbt was also observed. Serum biochemical parameters were not affected by treatment with these compounds, except for a moderate increase in serum triglyceride concentrations. In sum, our results suggest that these compounds, in particular BAPA-3, are potentially useful tools for inhibiting the intestinal absorption of bile acids in a non-competitive manner.

1. Introduction

Hypercholesterolemia is one of the most important risk factors for the development of cardiovascular diseases owing to the increased probability of atheromatose lesions [1]. This justifies the enormous efforts carried out in the last decades to obtain new cholesterol-lowering drugs. One of the strategies that have produced the best results has been the one based on the inhibition of the key enzyme involved in cholesterol synthesis, i.e., hydroxymethyl glutaryl coenzyme A reductase (HMGCR), by statins. However, in spite of its efficacy, this family of compounds is not the definitive solution. The long-term side effects are only partially understood and its good tolerability cannot be extended to children with genetic disorders affecting cholesterol homeostasis [2].

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Abbreviations: ASBT, apical sodium-dependent bile acid transporter; BAPA, bile acid-polyamine derivative; BSEP, bile salt export pump; CDCA, chenodeoxycholic acid; GC, glycocholic acid; NTCP, Na^+-taurocholate-cotransporting polypeptide; OST, organic solute transporter; TC, taurocholic acid

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Alternative pharmacological approaches include the stimulation of cholesterol biotransformation into bile acids subsequent to an increased loss of these compounds due to their sequestering in the intestinal lumen using specific gels or resins. Cholesterol-7α-hydroxylase (CYP7A1), the key enzyme in bile acid synthesis [3], is down regulated by bile acids [4]. Thus, by depleting the bile acid pool an enhancement in the expression of cholesterol-7α-hydroxylase occurs, which is subsequently accompanied by enhanced removal of cholesterol, which is then biotransformed into bile acids [5]. Although the conceptual basis of this therapy is interesting, the pharmaceuticals available are difficult to manipulate and administer. Moreover they are not devoid of adverse consequences [6,7].

Since the molecular mechanisms accounting for intestinal bile acid re-absorption are being elucidated [8,9], the alternative of favouring the foecal elimination of these steroids by inhibiting the plasma membrane carriers responsible for bile acid uptake has emerged as a promising alternative. The apical sodium-dependent bile acid transporter (ASBT), located at the brush-border membrane of epithelial cells in the ileal mucosa, is a member of the family 10 of solute carriers encoded by the gene *SLC10A2* [10]. Since this is the main transport system involved in the active reabsorption of bile acids during the intestinal transit of their enterohepatic circulation, the inhibition of bile acid uptake by this carrier would result in increased fecal loss of bile acids.

Several investigators and pharmaceutical companies have developed ASBT-inhibitors with very different chemical structures. Among them, the following can be mentioned as examples: SC-635 [11], S-8921 [12], 2164U90 [13], 2,3-disubstituted-4-phenylquinolines [14]. Regarding the use of bile acid derivatives, several studies carried out in the 70 s using everted hamster gut sac as an experimental model revealed that cationic bile salt derivatives were able to interact with the ileal bile acid transport system in such a way as to inhibit the transport of natural bile salts [15]. This interaction seemed to involve two recognition components; one includes the steroid moiety, the other a coulombic interaction between the anionic bile salt and a cationic membrane site [16].

In later studies, using isolated perfused rat liver [17] and *in vivo* measurement of the absorption from the jejunum and ileum of anesthetized guinea pigs [18], it was shown that fully positively charged bile acid derivatives or zwitterionic bile acid derivatives were not appreciably taken up by the liver or the intestine.

More recently, once the transporters involved in these processes were cloned and expressed in cell lines, it has been possible to investigate substrate specificity of sodium/bile acid co-transporters that are the main responsible for bile acid uptake by the liver (Na^+-taurocholate co-transporting polypeptide, NTCP, gene symbol *SLC10A1*) and the ileum (ASBT). Studies carried out in rabbit orthologues have suggested that the side chain of bile acids is important for their interaction with the recognition site of ASBT and NTCP [19]. Regarding human ASBT, a recent study suggests that C-24 conjugation and steroidal hydroxylation pattern modulate native bile acid interaction with human ASBT, with the effect of conjugation dominating that of steroidal hydroxylation. Moreover the results of that study indicate that bile acid binding to human ASBT may be the rate-limiting step in the apical transport of bile acids [20].

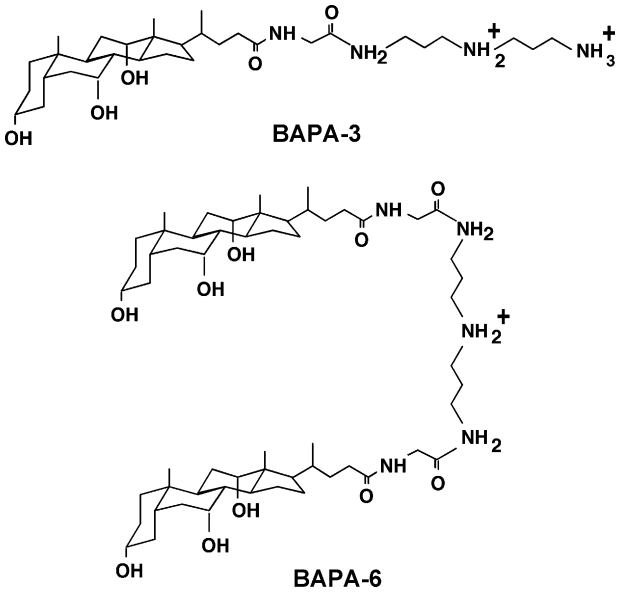

Fig. 1 – Schematic representation of the molecular structure of conjugates of N-(3-aminopropyl)-1,3-propanediamine with one or two glycocholic acid moieties to obtain BAPA-3 and BAPA-6, respectively.

Based on the results mentioned above, the conceptual base of the present work was that a negative charge in the lateral chain is needed for natural bile acids to interact with the main intestinal carrier accounting for bile acid uptake from the intestinal lumen, i.e., ASBT [18]. This prompted us to design and synthesize steroids with different sizes and net positive charges (Fig. 1) and to evaluate their ability to inhibit ASBT-mediated bile acid intestinal absorption and to affect several physiological aspects related to the enterohepatic circulation of these compounds.

2. Methods and materials

2.1. Reagents

FITC-Dextran-40 kDa, N-(3-aminopropyl)-1,3-propanediamine (PA) and sodium salts of glycocholic acid (GC), taurocholic acid (TC) and chenodeoxycholic acid (CDCA) more than 95% pure by thin-layer chromatography were purchased from Sigma–Aldrich (Madrid, Spain). [^{14}C]-GC (specific radioactivity 46.7 mCi/mmol) and [^{3}H]-TC (specific radioactivity 3.0 Ci/mmol) were obtained from Perkin-Elmer Life Science (Izasa, Barcelona, Spain). All other chemicals were from Merck Eurolab (Barcelona, Spain). They were of high purity and were used as purchased without any further purification. The GC and PA conjugates named BAPA-3 and BAPA-6 whose molecular structures are shown in Fig. 1, were obtained by an adaptation of a previously described procedure for the