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RAPID COMMUNICATION

IN VITRO TRANSFORMATION OF CHENO- AND URSODEOXYCHOLIC ACIDS AND THEIR 7-OLEYL ESTERS BY HUMAN INTESTINAL MICROFLORA

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Abstract

Chenodeoxycholic and ursodeoxycholic acid are used widely for the treatment of gallstones. A possible drawback to their utility is their conversion to lithocholic acid, which has displayed histotoxicity and mutagenicity. The 7-oleyl esters of cheno- and ursodeoxycholic acid are not degraded by fecal bacteria and may represent safer means of treatment.

Introduction

Cheno and ursodeoxycholic acids, which are used in the treatment of gallstone disease, are to a large extent, transformed into lithocholic acid by human intestinal microflora (1). This transformation is potentially dangerous in view of histotoxic and mitogenic properties of lithocholic acid (2). We have recently been successful in synthesizing 7-acyl derivaties of both cheno- and ursodeoxycholic acids (3) and have shown them to inhibit lithogenesis in hamsters (4). We thought it would be of interest to determine whether the esters at the 7 position of the bile acids were as susceptible to bacterial transformation as were the parent substances. A litholytic derivative of cheno-or ursodeoxycholic acid, which was not readily converted to lithocholic acid, could have great therapeutic advantage. This communication describes results of studies in which cheno- and ursodeoxycholic acids and their 7-oleyl derivatives were incubated in vitro with human fecal preparations.

Material and Methods

Sterile plastic containers were used to collect stool samples which were obtained from two healthy male subjects who had not received antibiotic therapy for at least six months prior to the collection. Experimental procedures were carried out under rigorous oxygen-free, anaerobic conditions and were performed within 2h after stool collection. Specimens were introduced into

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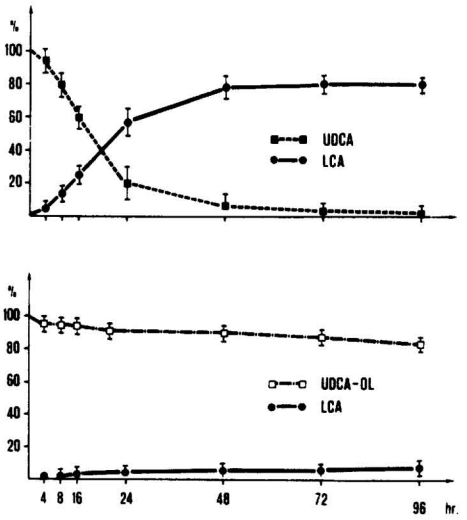
an anaerobic glove cabinet (Biolife, Milan, Italy) in which the air had been replaced by an atmosphere of 85% N_2, 10% H_2 and 5% CO_2 that had been sterilized by filtration (FHLP 02500, Millipore Corp., Bedford, MA). All media were kept in a GasPak 100 jar anaerobic system (BBL Microbiology Systems, Cockeysville, MD) until use.

About 10g of fecal material taken from three different sampling sites were homogenized in 100ml of Aranki solution (5). Two ml of suspension were inoculated into tubes containing 18ml of Marcus-Talalay broth (6). This system had been shown previously (7) to maintain adequate microbial balance in mixed cultures of intestinal bacteria and to support microbiological transformation of bile acids. Chenodeoxy and ursodeoxycholic acids were introduced at a concentration of 0.01% and their 7-oleyl derivatives were used at concentrations of 0.02, 0.01 and 0.005%. The tubes containing the stool homogenates and bile acids were incubated at 37°C and samples taken at 0, 4, 8, 12, 24, 48, 72 and 96h. The samples were acidified and extracted with 60ml of a mixture of methylene chloride and ethyl acetate (1:1, v/v). The organic extract was dried over anh. Na_2SO_4 and taken to dryness under N_2. The residue was dissolved in methanol and aliquots used for analysis by thin layer (TLC) or gas liquid (GLC) chromatography.

TLC was carried out on silicic acid plates using chloroform:acetone:acetic acid (7:2:1, v/v) as developing solvent and using a ferric chloride spray 2g $FeCl_3$ $6H_2O$ in butyl alcohol (83ml) -con H_2 SO_4 (15 ml). For gas chromatographic analysis, each sample was acidified to pH 1 with con HCl, a known quantity of an internal standard (3,12-dihydroxy-7-keto-5-beta-cholanoic acid) was added and the mixture extracted with ethyl acetate. The extracts were dried over anh Na_2SO_4 and evaporated to dryness. The bile acids were esterified using 5% HCl in anh methanol. After several washings with benzene-methanol (86:14, v/v) to remove final traces of HCl the bile acid methyl esters were converted to the respective trimethylsilyl (TMS) esters using Sil-Prep (Applied Science Laboratories, Inc., State College, PA). Chromatography was carried out using a Fractovap GLC (Carlo Erba, Milan, Italy) equipped with a flame ionization detector. The column (10 ft x 3 mm i.d.) was packed with OV-1 (0.75%) on Chromasorb G AW-DMCS, 100-120 mesh (Supelco, Inc., Belelfonte, PA). Operating conditions: 6°C/min from 230-295°C with a N_2 flow of 40 ml/min.

Results and Discusssion

Cheno- and ursodeoxycholic acids gave similar results. The top panels of figures 1 and 2 show the falling concentrations of urso- (UCDA) and chenodeoxycholic (CDCA) acids, respectively, with concommitant increase of lithocholic acid (LCA). After 48 hours of incubation, more than 80% of the ursodeoxycholic acid had been converted to other metabolites, mostly to lithocholic acid. This result parallels earlier findings with chenodeoxycholic acid (8).



Results of incubation of bile acids with intestinal bacteria.

Figure 1: Upper panel, UDCA, ursodeoxycholic acid; LCA, lithocholic acid; lower panel, UDCA-OL, 7-beta oleylursodeoxycholic acid.

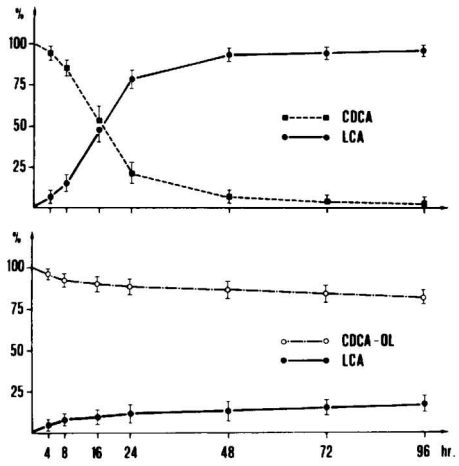


Figure 2: Upper panel, CDCA, chenodeoxycholic acid; lower panel, LCA; CDCA-OL.

The 7 oleyl derivatives, however, are transformed minimally in this in vitro system. As the lower panel of figure 1 shows, 85% of the 7-beta oleyl-ursodeoxycholic acid (UDCA-OL) is unmodified after 96 hours. The 7-alpha oleylchenodeoxycholic acid (CDCA-OL) was 90% intact at this time (figure 2). The percentage of lithocholic acid present in the incubation was less than 10% even after 4 days of incubation.

These studies have permitted the evaluation of the in vitro metabolic conversion of the bile acids used therapeutically in gallstone disease. Both cheno- and ursodeoxycholic acids were converted principally to lithocholic acid, a substance with serious hepatotoxic potential (9). The 7 oleyl derivatives of these bile acids are almost totally refractory to metabolic transformation by intestinal bacteria and, consequently, represent far lower hepatic threat. If these derivatives are as effective litholytic agents in man as they are in hamsters (4) they may offer a safer mode of gallstone treatment.

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