The Metabolism of Lithocholic Acid and Lithocholic Acid-3-α-sulfate by Human Fecal Bacteria

S.P. BORRIELLO,1 and R.W. OWEN,2, aBacterial Metabolism Research Laboratory, Central Public Health Laboratory, Colindale Avenue, Colindale, London NW9 5HT; and bDepartment of Chemistry and Biochemistry, Liverpool Polytechnic, Liverpool L3 3AF, England

ABSTRACT

Both lithocholic acid and lithocholic acid-3α-sulfate are metabolized by mixed fecal bacteria and by pure strains of the genus Clostridium. Mixed fecal bacteria metabolized lithocholic acid to 3-keto-lithocholic acid; lithocholic acid-3α-sulfate was metabolized to isolithocholic acid, 5β-cholanic acid and Δ3-cholenic acid under both aerobic and anaerobic conditions. The results indicate that a specific genus, the Clostridium, has a primary role in the metabolism of lithocholic acid-3α-sulfate to Δ3-cholenic acid.


INTRODUCTION

Lithocholic acid is formed in the distal intestine by the activity of bacterial 7α-dehydroxylase on the primary bile acid chenodeoxycholic acid. This major fecal bile acid may be present in above-average concentrations in the stools of subjects from populations at high risk for the development of colonic cancer (1). It has also been shown to enhance chemically induced tumorigenesis in the intestine (2) and liver (3) of rats, to enhance the mutagenicity of suboptimal amounts of 2-amino-anthracene (4) and benzo(a)pyrene (5) in the Ames mutagenicity test, and to transform Syrian hamster embryo cells (6). Lithocholic acid is conjugated in the liver to taurine or glycine (about 40%) (7) and also exists as the water-soluble 3α-sulfate, which can account for up to 80% of the biliary lithocholate (8). Sulfation occurs in the liver and is a detoxifying process that also apparently facilitates excretion, as the sulfate is poorly absorbed by the intestinal mucosa. Both lithocholic acid and lithocholic acid-3α-sulfate can escape the small bowel enterohepatic cycle and seep into the large bowel where they are available for metabolism by the gastrointestinal flora. Little is known about the further metabolism of this bile acid in the large bowel. This study was designed to investigate this question and to try and delineate the role of various components of the fecal flora in these activities. Metabolism of lithocholic acid-3α-sulfate probably represents a retoxifying process. The roles that these microbially produced metabolites may play in gastrointestinal disorders, such as colorectal cancer, are discussed.

MATERIALS AND METHODS

Synthesis of Lithocholic Acid-3α-Sulfate

Lithocholic acid-3α-sulfate (LASO4) was synthesized from lithocholic acid (Koch-Light Laboratories, Colnbrook, Bucks) as described by Tserng and Klein (9).

The product, mp 232 C, lit. mp 233-235 C; IR (KBr disc) 3450 (broad), 1560 (C=O), 1220, 1060 and 960 cm⁻¹ was obtained as a white crystalline solid. Thin layer chromatography (TLC) on 20 x 20 cm DC-Fertig Platten Kieselgel F 254 0.25 mm plastic backed plates (E. Merck, Darmstadt) in the solvent systems n-butanol/acetic acid/water (10:1:1, v/v) and methanol/dichloromethane (1:19, v/v) revealed that the product was free of residual lithocholic acid.

Fermentation Medium

LASO4 and lithocholic acid (LA) were both maintained as 1% (w/v) sterile stock solutions in distilled water. The LA was added to water as the sodium salt to overcome solubility problems. Aliquots of these stock solutions were added to brain-heart infusion broth (BHI) with added reducing agents (10) to a final concentration for LASO4 and LA of 0.05% (w/v) to yield BHI-LASO4 and BHI-LA, respectively ("Fermentation media").

Metabolism of LASO4 and LA by Mixed Cultures of Human Fecal Bacteria (MHFB)

Freshly voided feces were passed into an anaerobic chamber and suspended in BHI-
LASO₄ and BHI-LA to yield a 1:10 dilution (w/v). Serial 10-fold dilutions of these stock stool solutions were made over 10 steps in the fermentation media. This was performed in triplicate. Controls consisted of uninoculated BHI, BHI-LASO₄ and BHI-LA broths, and a 1:10 dilution (w/v) of stool in BHI broth. Incubations were performed under both aerobic and anaerobic conditions. Aerobic incubations consisted of 5-ml aliquots in loose capped bijoux bottles incubated on an orbital shaker. Anaerobic incubation was performed under an atmosphere of 90% hydrogen and 10% CO₂ in the presence of a cold 'D' catalyst (Englehard Industries, Cinderford, Gloucestershire, England). All broths were screened for the presence of metabolites after 16, 48 and 72 hr incubation. Fecal flora analysis was performed as described elsewhere (10) at 0 and 72 hr in an attempt to correlate metabolism with the presence of the major genera of bacteria.

Large-Scale Fermentation

The procedure just described was repeated. However, in this case, 5 g of freshly voided stool was inoculated into 1 ℓ of BHI-LASO₄ and incubated under anaerobic conditions for 72 hr. This large-scale fermentation process was to enable sufficient quantities of the metabolites to be isolated for more precise spectroscopic identification. After incubation, the medium was extracted twice with an equal volume of methanol/dichloromethane (1:19, v/v). The resultant extract was dried in vacuo at 50 C overnight yielding 110 mg of solid residue. The residue was dissolved in 5.0 ml of methanol/dichloromethane (1:19, v/v) and preparative TLC was performed on 20 x 20 cm glass plates coated with Silica Gel G (Anachem) to a thickness of 0.5 mm. The plates were irrigated in the solvent system methanol/dichloromethane (1:19, v/v). Observation of the plates under ultraviolet (UV) light (254 nm) did not reveal any products; however, spraying with anisaldehyde or heat for the selection of clostridial spores (13) or with vancomycin at a final concentration of 7.5 μg/ml to inhibit Gram-positive organisms. The treated MHFB suspensions were then added to BHI-LASO₄ fermentation media in universal bottles were inoculated with 2-3 drops of an actively growing culture in Robertson's cooked meat medium. Anaerobic isolates, which were all fresh fecal isolates, were incubated under anaerobic conditions. Facultative bacteria were incubated under both aerobic and anaerobic conditions. Controls consisted of uninoculated fermentation media and inoculated BHI broth. All broths were screened for the presence of metabolites after 16, 48 and 72 hr incubation.

Metabolism of LASO₄ and LA by Pure Strains of Facultative and Strict Anaerobes

Ten-ml aliquots of BHI-LASO₄ and BHI-LA media in universal bottles were inoculated with 2-3 drops of an actively growing culture in Robertson's cooked meat medium. Anaerobic isolates, which were all fresh fecal isolates, were incubated under anaerobic conditions. Facultative bacteria were incubated under both aerobic and anaerobic conditions. Controls consisted of uninoculated fermentation media and inoculated BHI broth. All broths were screened for the presence of metabolites after 16, 48 and 72 hr incubation.

Metabolism of LASO₄ by MHFB after Treatment with Alcohol, Heat or Vancomycin

The suspension of MHFB was treated with alcohol or heat for the selection of clostridial spores (13) or with vancomycin at a final concentration of 7.5 μg/ml to inhibit Gram-positive organisms. The treated MHFB suspensions were then added to BHI-LASO₄ fermentation medium prior to incubation under anaerobic conditions. Aliquots of all dilutions were tested for the presence of products of LASO₄ metabolism at 16, 48 and 72 hr.

Thin Layer Chromatography of LASO₄ and LA Metabolites

Metabolites of LASO₄ and LA were detected by TLC on 25 x 25 cm plastic-backed 0.25 mm plates. Ten μl from each fermentation was