Toxicology and Applied Pharmacology 277 (2014) 138–145

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Effect of various antibiotics on modulation of intestinal microbiota and bile acid profile in mice

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ARTICLE INFO

Article history:
Received 10 January 2014
Revised 10 March 2014
Accepted 12 March 2014
Available online 20 March 2014

Keywords:
Bile acids
Antibiotics
Intestinal bacteria
Liver
Intestine
Enterohepatic circulation

ABSTRACT

Antibiotic treatments have been used to modulate intestinal bacteria and investigate the role of intestinal bacteria on bile acid (BA) homeostasis. However, knowledge on which intestinal bacteria and bile acids are modified by antibiotics is limited. In the present study, mice were administered various antibiotics, 47 of the most abundant bacterial species in intestine, as well as individual BAs in plasma, liver, and intestine were quantified. Compared to the two antibiotic combinations (vancomycin + imipenem and cephalothin + neomycin), the three single antibiotics (metronidazole, ciprofloxacin and aztreonam) have less effect on intestinal bacterial profiles, and thus on host BA profiles and mRNA expression of genes that are important for BA homeostasis. The two antibiotic combinations decreased the ratio of *Firmicutes* to *Bacteroidetes* in intestine, as well as most secondary BAs in serum, liver and intestine. Additionally, the two antibiotic combinations significantly increased mRNA of the hepatic BA uptake transporters (Ntcp and Oatp1b2) and canalicular BA efflux transporters (Bsep and Mrp2), but decreased mRNA of the hepatic BA synthetic enzyme Cyp8b1, suggesting an elevated enterohepatic circulation of BAs. Interestingly, the two antibiotic combinations tended to have opposite effect on the mRNAs of most intestinal genes, which tended to be inhibited by vancomycin + imipenem but stimulated by cephalothin + neomycin. To conclude, the present study clearly shows that various antibiotics have distinct effects on modulating intestinal bacteria and host BA metabolism.

Introduction

Primary bile acids (BAs) are synthesized from cholesterol in hepatocytes. The two primary BAs synthesized in the human liver are cholic acid (CA) and chenodeoxycholic acid (CDCA). Rodent livers can convert CDCA to form α-muricholic acid (αMCA) and βMCA (Botham and Boyd, 1983). Therefore, in addition to CA and CDCA, α- and β-MCA are also primary BAs in rodents. Primary BAs are then conjugated with glycine or taurine before secretion into bile and small intestine. In intestine, bacterial enzymes modify primary BAs to produce secondary BAs, which are reabsorbed and returned to the liver for further processing (Hofmann and Hagey, 2008). Deoxycholic acid (DCA) and lithocholic acid (LCA) are the two major secondary BAs in humans. The structure and nomenclature of various BAs are shown in Supplemental Fig. 1.

The BA profile is important not only to BA signaling pathways but also to BA-induced toxicity. BAs are endogenous ligands for farnesoid X receptor (FXR) (Makishima et al., 1999; Parks et al., 1999; Wang et al., 1999) and the plasma membrane-bound G-protein-coupled BA receptor 1 (TGR5 or GPBAR1) (Kawamata et al., 2003). Individual BAs differ markedly in their potency to activate BA receptors; the potency of BAs to activate FXR is CDCA > DCA > LCA > CA (Parks et al., 1999), and to activate TGR5 is LCA > DCA > CDCA > CA (Sato et al., 2008). However, these data are based on *in vitro* non-hepatocyte-derived cell cultures and do not necessarily reflect the *in vivo* BA potencies in the liver. Increased BA concentrations may cause toxicities to the liver and intestine due to their detergent properties. Secondary BAs are more hydrophobic than their corresponding primary BAs, and thus are thought to be more toxic. Our laboratory has shown that individual BAs produce hepatotoxicity with different potencies when fed to mice: LCA > DCA > CDCA > CA (Song et al., 2011).

The BA profile is largely dependent on activities of intestinal bacterial enzymes that mediate deconjugation, dehydrogenation, dehydroxylation and epimerization of primary BAs in the distal small intestine as well as large intestine (Ridlon et al., 2006). Bacterial bile salt hydrolases (BSH) mediate the deconjugation of BAs, whereas bacterial hydroxysteroid dehydrogenases (HSDH) mediate the oxidation and epimerization

Abbreviations: Abca1, ATP-binding cassette transporter a1; BA, bile acid; CA, cholic acid; Bsep, bile salt-export pump; CDCA, chenodeoxycholic acid; Cyp, cytochrome P450; DCA, deoxycholic acid; Fxr, farnesoid X receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HDCA, hyodeoxycholic acid; IS, internal standard; LCA, lithocholic acid; MCA, muricholic acid; MDCA, murideoxycholic acid; Mrp, multidrug resistance-associated protein; Ntcp, sodium taurocholate cotransporting polypeptide; Oatp/OATP, organic anion transporting polypeptide; Ost, organic solute transporter; 7-oxoDCA, 7-oxodeoxycholic acid; Shp, small heterodimer partner; TCA, tauro-cholic acid; T-12-epiDCA, tauro-12-epi deoxycholic acid; UDCA, ursodeoxycholic acid; UPLC, ultra performance liquid chromatography; WT, wild type.

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http://dx.doi.org/10.1016/j.taap.2014.03.009
0041-008X/

of the 3-, 7-, and 12-hydroxy groups of BAs. Despite trillions of bacteria in the intestine, only a few species have been examined for their ability to metabolize primary BAs, due to the limitations of cultivating anaerobic bacteria that make up the predominant intestinal microbiota (Ridlon and Hylemon, 2011; Ridlon et al., 2006).

Antibiotics can alter the intestinal bacterial composition, and thus they represent one strategy to investigate the role of intestinal bacteria on BA metabolism. Swann et al. (2010) treated rats with streptomycin and penicillin to assess the role of intestinal bacteria on the BA profile. Antunes et al. (2011) demonstrated that streptomycin markedly affects intestinal BA metabolism in mice. Kuribayashi et al. (2012) showed that ampicillin treatment increases total BA concentrations in intestine of mice. Recently, Sayin et al. (2013) showed that a cocktail of antibiotics (bacitracin, neomycin and streptomycin) increases primary BAs in gallbladder and decreases secondary BAs in serum of mice. However, little is known which antibiotics alter intestinal microbiota and thus bile acids.

In the present study, several popular antibiotics were selected to test our hypothesis that antibiotics have different effects on bile acid metabolism due to their different abilities to modify intestinal bacteria. Mice were treated with either single antibiotics (metronidazole, ciprofloxacin and aztreonam) or antibiotic combinations (vancomycin + imipenem and cephalothin + neomycin) that are expected to selectively eliminate intestinal bacterial genera. These antibiotics belong to various classes with different spectrums of activity and intestinal absorption (Table 1). The purpose of the present study was to systematically explore the impact of intestinal bacteria on BA profiles by modulating intestinal bacteria with various antibiotics.

Materials and methods

Chemicals and reagents

Aztreonam, cephalothin, ciprofloxacin, imipenem, metronidazole, neomycin, and vancomycin were obtained from Sigma-Aldrich (St. Louis, MO). CA, CDCA, αMCA, βMCA, DCA, LCA, and murideoxycholic acid (MDCA) were purchased from Steraloids, Inc. (Newport, RI) and Sigma-Aldrich (St Louis, MO). Other BA standards were generous gifts from Dr Alan F Hofmann (University of California, San Diego, CA). The structure and abbreviations of various BAs can be found in our previous manuscript (Zhang et al., 2012a).

Animals and chemical treatments

Eight week-old male C57BL/6 mice were purchased from Charles River Laboratories, Inc. (Wilmington, MA). All the animal maintenance and treatment protocols were in compliance with the Guide for Care and Use of Laboratory animals as adopted and promulgated by the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee. Mice were singly housed in the facility with a 14-h light/10-h dark-cycle, temperature-, and humidity-controlled environment and given *ad libitum* access to water and rodent chow (Harlan-Teklad 8064, Madison, WI). Mice were acclimated for one week before starting treatment. Mice (N = 4–5/group, singly housed) received either normal drinking water or drinking water supplemented with: metronidazole (0.1 mg/ml for 14 days), ciprofloxacin (30 mg/ml for 14 days), aztreonam (0.1 mg/ml for 7 days), vancomycin + imipenem (0.1 mg/ml of each for 14 days), or cephalothin + neomycin (2 mg/ml of each for 7 days). The dosages of antibiotics were according to previous studies (Goris et al., 1986; Hoentjen et al., 2003), and were found to have little effect on water and food consumption or body weight of mice in the present study (data not shown). Drinking water with and without antibiotics was prepared freshly and replaced daily. At the end of the treatment period, mice were anesthetized between 8:00 AM and 11:45 AM. Blood was collected by orbital sinus bleeding, and serum was obtained by centrifuging blood at 6000 *g* for 15 min. Livers were harvested after the gallbladders were removed from the same animals, washed with saline, frozen in liquid nitrogen, and stored at −80 °C. Intestinal contents were flushed using PBS containing 10 mM dithiothreitol (DTT) as the mucolytic agent. Intestinal segments were frozen in liquid nitrogen. All samples were stored at −80 °C until further analysis.

Bacterial DNA extraction

Luminal contents of intestine were collected in PBS containing 10 mM DTT and were centrifuged at 20,000 ×*g* for 30 min at 4 °C. Total genomic bacterial DNA was extracted from the pellet using QIAmp DNA® stool kit (Qiagen, Valencia, CA) following the manufacturer's instructions with slight modification where the entire volume of the lysate was processed instead of using only 200 μl as suggested by the manufacturer. The volumes needed for further processing were adjusted accordingly. The integrity, concentration, and quality of the total DNA were assessed by agarose gel electrophoresis, and determined by absorption at A_{260}, and A_{260} to A_{280} ratio, respectively. DNA solutions were stored at −20 °C until further analysis.

Quantification of bacteria

The bacteria quantified in the present study were chosen based on a previous publication on the major intestinal microbiota in mice (Salzman et al., 2002). A branched DNA (bDNA) assay based on the 16S rDNA gene was developed for 47 individual bacteria (Supplemental Table 1) belonging to *Firmicutes* (total 22 species), *Bacteroidetes* (total 14 species), and other bacteria (total 11 species). Compared with real-time qPCR and gene sequencing methodologies, the method developed in the present study is much simpler and more rapid. The 16S rDNA gene assay was performed using QuantiGene 2.0 Reagent System (Panomics/Affymetrix, Fremont, CA) according to the manufacturer's protocol. Because the majority of the bacteria residing in the intestine are not easily cultivable, the bacterial sequences are defined as the closest known relative in the phylogenic tree (Salzman et al., 2002). Accession numbers for the 16S-rRNA genes of the corresponding bacteria are given in Supplementary Table 1. Total bacterial DNA (1 ng/μl, 20 μl) was added to each well containing 80 μl of lysis buffer containing blocking reagent and each probe set. Sample DNA was allowed to hybridize to each probe set overnight at 55 °C. Subsequently, the plate was washed with washing buffer 3 times. Samples were hybridized

Table 1
Properties of antibiotics used in present study.

Antibiotics	Category	Mechanism	Spectrum	Intestinal absorption
Metronidazole	Nitroimidazole	Deactivation of critical enzymes	Narrow spectrum; anaerobic bacteria	Complete (>90%)
Ciprofloxacin	Fluoroquinolone	Inhibition of topoisomerase II and IV	Broad spectrum; gram-positive and negative	Well (>70%)
Aztreonam	β-Lactam	Inhibition of cell wall	Narrow spectrum; gram-negative	Poorly
Vancomycin	Glycopeptide	Inhibition of cell wall synthesis	Narrow spectrum; gram-positive	Poorly
Imipenem	β-Lactam	Inhibition of bacterial cell wall formation	Broad spectrum; gram-positive and negative	Poorly
Cephalothin	β-Lactam	Inhibition of bacterial cell wall formation	Broad spectrum; gram-positive and negative	Poorly
Neomycin	Aminoglycosides	Inhibition of protein synthesis in bacteria	Broad spectrum; excellent for gram-negative	Poorly