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Correlation between faecal microbial community structure and cholesterol-to-coprostanol conversion in the human gut

Patrick Veiga, Catherine Juste, Pascale Lepercq, Katiana Saunier, Fabienne Béguet, Philippe Gérard *

Unité d'Écologie et Physiologie du Système Digestif, Institut National de la Recherche Agronomique, Bâtiment 405, Domaine de Vilvert, 78352 Jouy-en-Josas, France

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Abstract

Intensity of the cholesterol-to-coprostanol conversion in the intestine, as assessed by the coprostanol-to-cholesterol ratio in faeces, was found highly variable among 15 human volunteers, ranging from absent to almost complete cholesterol conversion. The number of coprostanoligenic bacteria in the same faecal samples, as estimated by the most probable number method, was found to be less than 10^6 cells g^{-1} of fresh stools in the low-to-inefficient converters and at least 10^8 cells g^{-1} of fresh stools in the highest converters, indicating that the population level of cultivable faecal coprostanoligenic bacteria correlated with the intensity of cholesterol-to-coprostanol conversion in the human gut. Microbial communities of the samples were profiled by temporal temperature gradient gel electrophoresis (TTGE) of bacterial 16S rRNA gene amplicons. Dendrogram analysis of the TTGE profiles using the Pearson product moment correlation coefficient and a unweighted pair group method with arithmetic averages (UPGMA) algorithm clearly separated banding patterns from low-to-inefficient and high converters in two different clusters suggesting a relationship between TTGE profiles and coprostanoligenic activity. Principal components analysis further demonstrated that a large subset of bands rather than some individual bands contributed to this clustering.

Keywords: Cholesterol; Coprostanol; Temporal temperature gradient gel electrophoresis; Principal component analysis; Faecal microbial community

1. Introduction

Cholesterol escaping absorption in the upper digestive tract can be extensively metabolised by the resident intestinal microbiota. Bacterial metabolism of cholesterol in the hindgut mainly involves an indirect pathway with 4-cholesten-3-one and coprostanone as the intermediates and coprostanol as the end-product [1]. The organisms responsible for this conversion are unknown in humans and only a few cholesterol-reducing strains have been isolated from animal faeces and assigned to the genus *Eubacterium* based on morphological and physiological properties [2,3]. Whereas unmetabolised cholesterol is subjected to extensive enterohepatic circulation, coprostanol is poorly absorbable and excreted in the faeces [4]. Therefore, it has been suggested that cholesterol-to-coprostanol conversion by the intestinal microbiota could facilitate the elimination of cholesterol from the body [5,6] and therefore decrease the risk of cardiovascular diseases [6]. However, epidemiological

* Corresponding author. Tel.: +33 1 34 65 24 28; fax: +33 1 34 65 24 92.

E-mail address: pgerard@jouy.inra.fr (P. Gérard).

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studies have revealed that high-risk populations for colon cancer have an increased number of total anaerobes and enhanced metabolism of neutral sterols [7], and coprostanol is thought to be associated with colorectal carcinogenesis [8,9].

Several epidemiological studies [10] and nutritional trials in humans [11] and animals [12] have demonstrated that dietary habits influence the intestinal conversion of cholesterol. Moreover, the distribution of this conversion among Americans was shown to be bimodal, with a vast majority of high converters and a minority of low-to-inefficient converters [13] but differences in the intestinal microbiota of both populations remain unexplored.

Most of the knowledge on bacterial diversity in the human gastrointestinal tract has been obtained by culture on selective media. However, several limitations are associated with culture-based approaches and up to 60–70% of intestinal bacterial species remain uncultivable by known classical methods [14,15]. Recently, our understanding of complex microbial communities has been greatly enhanced by the introduction of molecular techniques [16]. The major advances are based on the use of 16S rRNA as a phylogenetic marker for analysing community diversity [17]. In particular, denaturing gel electrophoresis (DGE) profiling of faecal 16S rRNA and rRNA gene amplicons was shown to be a powerful tool for analysing the dominant species diversity of the faecal bacterial community [18–20]. These methods separate DNA fragments of the same length on the basis of differences in base composition. The resulting banding patterns allow the overall and objective comparison of microbial communities from different habitats or under different conditions and avoid conclusions based on an a priori decision on the type of bacteria to be analysed [14]. Moreover, a computer-assisted characterisation of the banding patterns and the subsequent treatment of the data using a statistical approach can now be applied and lead to refined results.

The present study addresses the question whether a relationship exists between the overall structure of the intestinal microbial community and its ability to metabolise cholesterol in the human gut. For this purpose, bacterial communities from 15 human faecal samples were characterised using PCR–TTGE (temporal temperature gradient gel electrophoresis). Cholesterol-to-coprostanol conversion status was assessed by the coprostanol-to-cholesterol ratio in faeces, and cholesterol-to-coprostanol reducing bacteria were enumerated by the most probable number (MPN) estimation in the same faecal samples. Finally, hierarchical cluster analysis (HCA) and principal component analysis (PCA) of the TTGE profiles were performed and interpreted with the variations of the cholesterol-to-coprostanol conversion status.

2. Materials and methods

2.1. Faecal samples

Faeces from 15 healthy human subjects between 25 and 45 years of age were collected. Donors were on a Western European diet. None had any history of digestive pathology nor had received antibiotic, immunosuppressive or radiological treatments within the last three months. Donors with hypercholesterolemia (cut-off at 2.5 g l^{-1}) were excluded. Faecal samples were collected in sterile plastic boxes and kept under anaerobic conditions using an anaerocult® A (Merck, Nogent sur Marne, France) and stored at 4 °C for a maximum of 3 h before processing.

2.2. Most probable number enumeration of coprostanoligenic bacteria and determination of cholesterol and coprostanol contents in faeces

The MPN enumerations were performed using the strictly anaerobic technique of Hungate [21], with dilution solution and growth medium pre-reduced under O_2-free N_2. One g of stool was serially diluted 10-fold with dilution solution (casitone 2.0 g l^{-1}; yeast extract 2.0 g l^{-1}; NaCl 5.0 g l^{-1}; KH_2PO_4 1.0 g l^{-1}, pH 7.0) up to 10^{12}. One-millilitre aliquots of each dilution were then transferred in triplicate to the MPN culture tubes containing 9 ml of growth medium (brain heart infusion 10 g l^{-1}; yeast extract 10 g l^{-1}; L-cysteine 0.5 g l^{-1}; 0.1% aqueous hemin solution 10 ml l^{-1}, pH 7.4), enriched with cholesterol (0.2 g l^{-1}, Sigma–Aldrich Chimie) solubilised in L-α-phosphatidylcholine (1.0 g l^{-1}, Type IV-S, Sigma–Aldrich Chimie) as previously described [22]. After the MPN cultures had been incubated at 37 °C for 7 days, neutral sterols were extracted from 1 ml of each culture with 2 ml of *n*-hexane by magnetic stirring for 3 h [23]. The samples were centrifuged and the sterols in the hexane supernatant were analysed by gas chromatography (GC) as their silyl derivatives [24]. Numbers of coprostanoligenic bacteria were obtained from the highest dilutions showing bacterial growth associated with coprostanol production. The MPN results were calculated using a micro-computer program [25] and were expressed as cell number g^{-1} (fresh stools). Neutral sterols were extracted from faeces as previously described [26]. Briefly, total lipids from 2-g stool aliquots were extracted with ethanol for 48 h in a Soxhlet apparatus. Neutral sterols were analysed by GC as their silyl derivatives after they had been saponified and extracted 3 times with petroleum ether. Coprostanol and cholesterol contents in faeces were expressed in percentages of total neutral animal sterols.

2.3. DNA isolation, PCR and TTGE analysis

Total DNA was extracted for PCR–TTGE as previously described [27] from 0.2-g stool aliquots frozen at