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Microbial metabolism of catechin stereoisomers by human faecal microbiota: Comparison of targeted analysis and a non-targeted metabolomics method

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Abstract

Microbial metabolism of the stereoisomers (+)-catechin and (−)-epicatechin was compared by two analytical techniques, GC/MS for quantitative targeted analysis and GC×GC-TOF for global characterization of the metabolome, using human faecal microbiota as an inoculum of converting microbiota. The ring-fission site changed when the inocula originated from two different groups of donors, but dehydroxylation progressed similarly regardless of the inoculum. Whereas GC/MS proved to be an appropriate tool for the study of specific expected metabolites of catechin stereoisomers, GC×GC-TOF-based metabolomics analysis also revealed new metabolites not included in the targeted analyses. Quantitation and verification of identification can also be performed in a metabolomics platform, if authentic standards are available.

Keywords: Metabolomics; (+)-Catechin; (−)-Epicatechin; GC/MS; GC×GC-TOF; Colonic microbiota

1. Introduction

Dietary phenolic compounds, including catechins, are ubiquitous plant-derived secondary metabolites which are metabolised extensively during digestion. Monomeric catechins are abundant in tea as (+)-catechin and (−)-epicatechin (Fig. 1) and their galloylated derivatives and condensed catechins (proanthocyanins) are abundant in red wine, tea and cocoa and in various fruits and berries (Hollman & Arts, 2000; Santos-Buelga & Scalbert, 2000). Catechins undergo ring-fission in the colon by the microbiota and are known to be transformed to phenolic acids and lactone derivatives. Eleven (+)-catechin metabolites have hitherto been identified in human urine. The three major metabolites are 3-hydroxyphenylpropionic acid, δ-(3,4-dihydroxyphenyl)-γ-valerolactone and δ-(3-hydroxyphenyl)-γ-valerolactone. The metabolites are excreted in urine in both free and glucuronidated forms and to a lesser degree as ethereal sulphates (Das, 1971). (−)-Epigallocatechin gallate, the major green tea catechin, is converted to di- and trihydroxyphenyl derivatives of γ-valerolactone and excreted in human urine after consumption of green tea solids. Galloylation affects the hydroxylation pattern of the metabolites. The ring-fission metabolites account for 1.5–16% of the ingested catechins. Consumption of both black and green tea results in an increase in the excretion of hippuric acid, which is a glycinated benzoic acid, into human urine. The results suggest both ring-fission in the colon and β-oxidation of the resulting phenolic acids (Meng et al., 2002).

3-Hydroxyphenylpropionic acid and 3-hydroxyhippuric acid were also identified in rat urine and faeces after ingestion of (+)-catechin (Griffiths, 1964). Das and Sothy (1971) showed that 3- and 4-hydroxyphenylpropionic acids as well as δ-(3,4-dihydroxyphenyl)- and δ-(3-hydroxyphenyl)-γ-valerolactones originate from the action of intestinal microorganisms and that they undergo enterohepatic circulation, reflected by conjugation of the metabolites. Identification of the metabolites in the early studies was performed by paper chromatography and semi-quantitatively using spectrophotometry (Griffiths, 1964; Das & Griffiths, 1969; Das, 1971; Das & Sothy, 1971), whereas the later studies involved NMR techniques (Meselhy, Nakamura, & Hattori, 1997) and LC/MS (Meng et al., 2002; Mulder, Rietveld, & van Amelsvoort, 2005).

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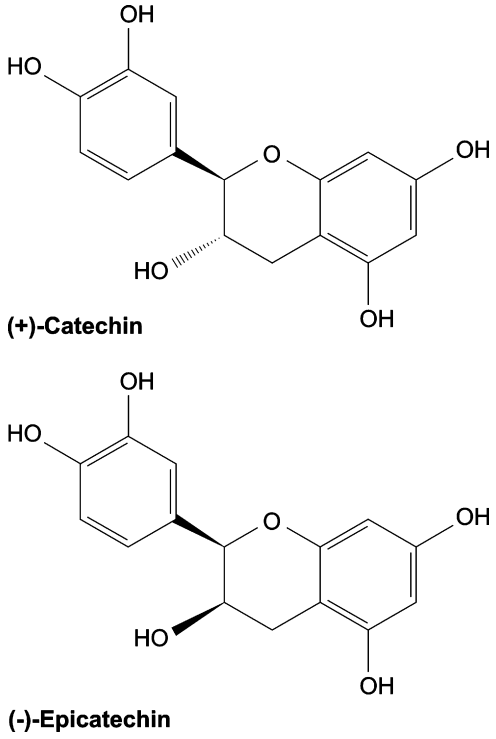

Fig. 1. Structures of catechin stereoisomers (+)-catechin and (−)-epicatechin.

The majority of the colonic microbial species are strict anaerobes which actively participate in the degradation and decomposition of the non-absorbable intake (Kleessen, Bezirtzoglou, & Mättö, 2000). The individual variation of microbiota in faeces is affected by age, diet, intestinal diseases and medication, particularly antibiotics (Rowland, Wiseman, Sanders, Adlercreutz, & Bowe, 1999; Kilkkinen et al., 2002). The differences in microbiota also lead to varying concentrations of microbial metabolites (Rowland et al., 1999; Cerda, Tomas-Barneran, & Espin, 2005). Involvement of the microbiota in the metabolism of catechins has been confirmed by *in vitro* incubations with human faecal or animal caecal suspensions (Scheline, 1970; Meselhy et al., 1997). Furthermore, there are differences in the degradation products of catechins between microbiota from humans and different animals (Das & Griffiths, 1969; Meselhy et al., 1997). Thus differences in metabolism occur due to the origin of the microbiota and possibly due to the structure of the precursor.

In this paper, an *in vitro* colon model was used to compare the microbial metabolism of two stereoisomers, (+)-catechin and (−)-epicatechin. The stereoisomer metabolites were analysed using targeted quantitative gas chromatography coupled to mass spectrometry (GC/MS) and a metabolomics strategy was applied utilizing the two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC×GC-TOF) technology. The advantages and limitations of these two different analytical approaches are discussed.

2. Results and discussion

Selected microbial metabolites were first analysed using GC/MS analysis. Transient formation of 3,4-dihydroxyphenylpropionic acid (3,4-diOHPPr) was observed for (+)-catechin, but was absent from samples containing (−)-epicatechin (Fig. 2a). The main microbial metabolites of both (+)-catechin and (−)-epicatechin were 3-hydroxyphenylpropionic acid (3-OH-PPr) and 3-phenylpropionic acid (3-PPr), showing differences in metabolite concentrations during the first 8 h of incubation (Fig. 2b and c). The differences in metabolite concentrations at the latest time point 24 h were most likely due to dying microbiota. 3,4-dihydroxyphenylacetic acid (3,4-diOHPAc) and 3-hydroxyphenylacetic acid (3-OHPAc) were not formed in the presence of either of the monomeric catechins (data not shown). Extent of fermentation for (+)-catechin and (−)-epicatechin were 91.4% and 69.8%, respectively.

The experiment was repeated using faeces from a new group of donors and analysis was performed using GC×GC-TOF coupled with bioinformatics tools. GC×GC-TOF has major advantages over GC/MS. The instrument is equipped with a non-polar and a polar column to produce a three-dimensional chromatogram. This means improved resolution and more accurate identifications (Welthagen et al., 2005). The two-column set-up also enhances sensitivity, which enables the use of scan measurement.

The alignment programme was able to separate 980 peaks, of which 161 were unknown peaks. Derivatives such as lipids and nitrogen heterocycles, expected to be formed from the faecal microbiota, were excluded. A total of 448 metabolites, of which 287 were identified, were selected for multivariate statistical analysis using partial least squares discriminant analysis (PLS/DA). PLS/DA is a pattern recognition technique that correlates variation in the data set with class membership. The projection model showed five latent variables (LV; $Q^2 = 63\%$) focusing on maximum separation (“discrimination”), which could be clearly distinguished based on substrate (i.e. (+)-catechin, (−)-epicatechin and faecal suspension “No addition”) and time points of 0–24 h. As shown in Fig. 3, the two first LV scores followed a time course within the same substrate group.

Furthermore 61 metabolites were selected for closer investigation. Data from the selected compounds were drawn as a profile on a time course and compared with the faecal suspension without added catechin isomers. The time course of 3,4-dihydroxyphenylvaleric acid formation (Fig. 4a) and that of a corresponding dehydroxylation product, 3-hydroxyphenylvaleric acid (Fig. 4b), were profiled. Notably, only (−)-epicatechin was a precursor of 3-hydroxyphenylvaleric acid. In a previous study Scheline (1970) was able to detect hydroxyphenylvaleric acid as a fermentation product of (+)-catechin by rabbit intestinal microbiota *in vitro*, but its formation by human microbiota has not been shown before. In the presented study hydroxyphenylpropionic acids formed using the inoculum A were not detected using the inoculum B, indicating inoculum-dependent change in the ring-fission site.

In another previous study by Das and Griffiths (1969) ^{14}C-labelled (+)-catechin showed that labelled phenyl-γ-valerolactones and carbon dioxide were derived from the A-ring and phenolic acids from the B-ring. Administration of [^{14}C]δ-(3-hydroxyphenyl)-γ-valerolactone gave rise to a labelled