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Pharmacokinetics of black tea-derived phenolic acids in plasma

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ABSTRACT

Little is known about the pharmacokinetics of black tea metabolically-derived phenolic acids (PAs). This knowledge is required to better understand their putative role in human health. Plasma concentrations of 18 PAs were measured after intake of 2650 mg black tea extract, combined with a 50 mg dose of three selected mass-labelled PAs. Levels of 15 PAs remained constant at low-µmol/L range. In contrast, those of 4–O–methylgallic acid, gallic acid, and hippuric acid peaked at up to 6.5 µmol/L after 1.3 to 8.8 h. Absorption and elimination half-lives of the mass-labelled PAs ranged from 0.35 to 1.24 h. A wide range of PAs were accurately quantified in plasma after black tea extract intake. Their mostly constant plasma concentrations may be due to slow formation and fast elimination, as indicated by the fast pharmacokinetics of mass-labelled PAs. These findings support the physiological significance of identified black tea metabolites.

1. Introduction

1.1. Tea and vascular health

Globally, tea is a widely consumed beverage, only second to water (Graham, 1992). Polyphenols as in black tea (BT) and green tea (GT) are associated with a range of health effects, whereas at the same time their bioavailability is poor (Arab, Liu, & Elashoff, 2009; Chow & Hakim, 2011; de Mejia, Ramirez-Mares, & Puangpraphant, 2009; Duffy et al., 2001; Manach, Scalbert, Morand, Remesy, & Jimenez, 2004; Quinones, Miguel, & Aleixandre, 2013; Ras, Zock, & Draijer, 2011). The approach to improve tea polyphenol bioavailability via derivatization to yield bioavailable, lipophilic, fatty acid derivatives has shown to be effective for specific catechins, such as EGCG (Zhong & Shahidi, 2011).

To create a stronger mechanistic link between the putative health effects and these teas, a better understanding of the absorption, distribution, metabolism, and excretion properties of tea polyphenols is warranted. While the bioavailability of catechins from GT is fairly well understood, significant gaps

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Abbreviations: AIC, Akaike information criterion; $AUC^{0\rightarrow inf}$, area under the plasma concentration-time curve from 0 to infinite h; $AUC^{0\rightarrow 30}$, area under the plasma concentration-time curve from 0 to 30 h; BBRLE, Brook Bond red label extract; BT, black tea; *bw*, body weight; C_p, plasma concentration; C_{max}, maximum plasma concentration; Cl_{tot}, total clearance; F_{abs}, absolute bioavailability; GT, green tea; $t_{1/2,\lambda}$, the half-life time associated with the terminal elimination phase; PA, phenolic acid; *t*, time; $t_{1/2,a}$, half-life time of absorption; $t_{1/2,e}$, half-life time of elimination; t_{max}, time of maximum plasma concentration; V_{hyp}, hypothetical volume of distribution

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remain to be filled for the more complex BT polyphenol compositions (Butt et al., 2014; Chen, Lee, Li, & Yang, 1997; Singh, Arseneault, Sanderson, Murthy, & Ramassamy, 2008). Specifically for BT, the condensed polyphenol forms are likely not absorbed, given their unfavourable absorption profile but undergo in the gut microbial conversion. The generated low molecular weight compounds are likely well absorbed as confirmed by ample presence in urine after BT administration. It could thus equally well be that the absorbed breakdown products from BT, either formed by microbiota or human metabolism, are also involved in the molecular mechanisms behind the reported health effects. The identification and quantification of those metabolites in human plasma, derived from BT, have not been reported to a large extent.

1.2. *Black tea polyphenols: composition and disposition*

The majority of BT polyphenols are condensation products of catechins like theaflavins and thearubigens (approximately 30% dry weight), whereas a minor fraction of the BT polyphenols are monomeric catechins themselves (approximately 5% dry weight). The only reported phenolic acid (PA) in BT is gallic acid, which is present in the one to two percent range dry weight (Graham, 1992; Li, Lo, Pan, Lai, & Ho, 2013; Ramdani, Chaudhry, & Seal, 2013). Biotransformation of black tea polyphenols has been described in numerous reviews (Clifford, Van der Hooft, & Crozier, 2013; Khan & Mukhtar, 2013; Qiao, Kong, Kong, & Han, 2014). The current view on the fate of BT polyphenols is that catechins are only fractionally absorbed from the small intestine (Chow & Hakim, 2011; Crozier, Del Rio, & Clifford, 2010; Del Rio, Costa, Lean, & Crozier, 2010; Lambert, Sang, & Yang, 2007; Manach, Williamson, Morand, Scalbert, & Remesy, 2005; Zhang, Zuo, & Lin, 2007). Additionally, they are highly metabolized by phase II enzymes during first-pass in enterocytes and hepatocytes, forming glucuronides, sulphates, and methyl conjugates of catechins (Qiao et al., 2014). Subsequently, a minor fraction of metabolized polyphenols may be subject to enterohepatic cycling due to active efflux (Schantz, Erk, & Richling, 2010; Silberberg et al., 2006; Zhang et al., 2007). Recent studies have shown that catechins are also present in plasma in non-conjugated form, but only at picomolar to low nanomolar concentrations, with the exception of epigallocatechin. This catechin reaches maximum plasma concentrations (C_{max}) of approximately 600 nmol/L, 1 to 3 h after tea consumption. Approximately 4 to 8 h after consumption aforementioned compounds have been eliminated from plasma via bile and urine (Chow & Hakim, 2011; Chow et al., 2005; Daykin et al., 2005; Renouf et al., 2011).

1.3. *Formation, disposition and selection of phenolic acids*

It has been hypothesized that the majority of catechins and their condensed forms, which are apparently not absorbed, as derived from the very low plasma levels, remain in the gut and are converted by gut microbiota to ring scission products like valerolactones and PAs (Crozier et al., 2010; Del Rio, Calani, et al., 2010). *In vitro* studies using faecal slurry fermentation confirm this hypothesis (Schantz et al., 2010). It has been shown that these microbiota products are absorbed from the colon followed by conjugation by phase II metabolic enzymes, reaching maximum plasma concentrations approximately 5 to 10 h after tea consumption (Van Duynhoven et al., 2011; Van Velzen et al., 2008, 2009). In addition, urinary recovery of PAs after BT administration implies the formation and absorption of those compounds.

The compounds $^{13}C_1$-D_3-*p*-4-methoxybenzoic acid, $^{13}C_1$-D_3-4-hydroxy-3-methoxybenzoic acid, and $^{13}C_6$-benzoic acid were chosen as model molecules to study the pharmacokinetic behaviour of tea-derived PAs. Selection was based on: (1) prior identification of these PAs in human urine samples after BT consumption (Van Velzen et al., 2008), (2) being unique in structure, (3) having a high predicted metabolic stability and (4) technical feasibility to synthesize the mass-labelled forms.

PAs to be studied have primarily been selected for various reasons: because they are part of the catechin metabolism pathway (van der Hooft et al., 2012) or confirmed in *in-vitro* microbial conversion tests (Clifford et al., 2013; Van Duynhoven et al., 2013). The selection is: 2-benzamidoacetic acid (hippuric acid) (van der Hooft et al., 2012), 3,4,5-trihydroxybenzoic acid (gallic acid) (van der Hooft et al., 2012), 3,4-dihydroxybenzoic acid (Van Duynhoven et al., 2013), 3,4-dihydroxyphenylpropionic acid (Van Duynhoven et al., 2013), 3-methoxy-4-hydroxyphenylpropionic acid (van der Hooft et al., 2012), 3-O-methylgallic acid (Loke et al., 2009), 4-hydroxy-4-methoxybenzoic acid including the $^{13}C_1$, D_3-analogue thereof, 4-hydroxy-4-methoxybenzoic acid, 4-hydroxybenzoic acid (van der Hooft et al., 2012; Van Duynhoven et al., 2013), 4-hydroxyphenylacetic acid (van der Hooft et al., 2012), 4-hydroxyphenylpropionic acid, 4-methoxybenzoic acid including the D_4-analogue thereof, 4-O-methylgallic acid (Loke et al., 2009; Van Duynhoven et al., 2013), benzoic acid including the $^{13}C_6$-analogue thereof, 3-(3,4-dihydroxyphenyl)-2-propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid, phenylacetic acid (Van Duynhoven et al., 2013), phenylpropionic acid. In addition 1,2,3-benzenetriol and pyrogallol (van der Hooft et al., 2012) were measured. The latter two compounds are not PAs, but are part of a polyphenol catabolism pathway anyhow (Manach et al., 2005).

Finally, tea-derived PAs like 4-hydroxyphenylacetic acid and 3-hydroxyphenylacetic acid have molecular weights of approximately 200 g/mol and physico-chemical properties which make them suitable for uptake by enterocytes and subsequent entry into the systemic circulation (Henning et al., 2013; Lipinski, Lombardo, Dominy, & Feeney, 2001). Little is known, however, about the identity, the true, and the apparent pharmacokinetics of tea-derived PAs in human plasma (Henning et al., 2013). It should be noted that similar PAs were detected in urine after consumption of other sources of polyphenols like red wine and grape juice (Appeldoorn, Vincken, Aura, Hollman, & Gruppen, 2009; Del Rio, Calani, et al., 2010; Graham, 1992; Henning et al., 2013; Van Dorsten et al., 2010).

Thus, we aimed to address in a clinical study whether and to which extent PAs circulate in human plasma after a single oral dose of a BT extract. Concomitantly with administration of this extract, three mass-labelled versions of BT-derived PAs were administered as such to give their true PK and interpret better the apparent pharmacokinetics of identical PAs originating from the BT extract via microbiota conversion.