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Interactions of black tea polyphenols with human gut microbiota: implications for gut and cardiovascular health[1–4]

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ABSTRACT

Epidemiologic studies have convincingly associated consumption of black tea with reduced cardiovascular risk. Research on the bioactive molecules has traditionally been focused on polyphenols, such as catechins. Black tea polyphenols (BTPs), however, mainly consist of high-molecular-weight species that predominantly persist in the colon. There, they can undergo a wide range of bioconversions by the resident colonic microbiota but can in turn also modulate gut microbial diversity. The impact of BTPs on colon microbial composition can now be assessed by microbiomics technologies. Novel metabolomics platforms coupled to de novo identification are currently available to cover the large diversity of BTP bioconversions by the gut microbiota. Nutrikinetic modeling has been proven to be critical for defining nutritional phenotypes related to gut microbial bioconversion capacity. The bioactivity of circulating metabolites has only been studied to a certain extent. Bioassays dedicated to specific aspects of gut and cardiovascular health have been used, although often at physiologically irrelevant concentrations and with limited coverage of relevant metabolite classes and their conjugated forms. Evidence for cardiovascular benefits of BTPs points toward antiinflammatory and blood pressure–lowering properties and improvement in platelet and endothelial function for specific microbial bioconversion products. Clearly, more work is needed to fill in existing knowledge gaps and to assess the in vitro and in vivo bioactivity of known and newly identified BTP metabolites. It is also of interest to assess how phenotypic variation in gut microbial BTP bioconversion capacity relates to gut and cardiovascular health predisposition. *Am J Clin Nutr* doi: 10.3945/ajcn.113.058263.

INTRODUCTION

Black tea is one of the most consumed beverages and accounts for a significant part of polyphenol intake in the world population (1–3). Black tea differs from green tea by a fermentation process during which the catechins in tea leaves (*Camellia sinensis*) undergo extensive oxidation and oligomerization. In the past years a body of epidemiologic evidence has been built for the reduction in risk of stroke (4, 5) and cardiovascular diseases (6–8) with sustained green and black tea intake. For black tea, there is now convincing evidence from intervention studies for effects on surrogate cardiovascular endpoints. Black tea consumption may lower systolic and diastolic blood pressure (BP)[5] in subjects with mildly elevated BP (9, 10). Perhaps even more convincing are the acute and chronic effects of black tea on endothelium-dependent vasodilation, which may contribute to a healthy blood flow (11). There are data (although inconclusive) suggesting that consumption of black and green tea may positively affect platelet function, inflammatory tone, and weight management (12, 13); the evidence for the latter, however, is stronger for green tea (14). There is no evidence for systemic antiinflammatory or antioxidant effects of black tea (15, 16); hence, more local mechanisms at a vascular level are being pursued. The compounds in tea most likely responsible for the vascular benefits are the polyphenols, which may exert vascular relaxation via multiple pathways (17). The responsible polyphenols in black tea for mediating these effects have still not been identified. Whereas in unfermented green tea the catechins represent 80–90% of total flavonoids, in black tea they only represent 20–30%. Nevertheless, the plasma concentration of different types of catechins increases after black tea consumption (15), but metabolites of larger tea polyphenols

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[5] Abbreviations used: BP, blood pressure; BTP, black tea polyphenol; FMD, flow-mediated dilation; NMR, nuclear magnetic resonance; SHIME, Simulator of the Human Intestinal Microbial Ecosystem.

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may potentially also contribute to the vascular benefits. Black tea polyphenol (BTP) composition is dominated by theaflavins and thearubigens (60–70%) (18). Theaflavins consist of concatenated catechin rings with molecular weights up to 700 Da, which explains their low direct bioavailability (19). Thearubigens are larger polymeric structures with molecular weights of not more than 2 kDa (20), which is too high for direct bioavailability. Hence, a major portion of BTPs predominantly persist in the colon where they undergo extensive bioconversion by colonic microbiota (21) to metabolites that can be further absorbed by the human body. The BTPs in their turn can also modulate gut microbial diversity. We currently discern 2 hypothetical mechanisms by which BTPs may exert their health benefits:

1) Microbial bioconversion of BTPs in the colon (22): This process brings high amounts of bioconverted BTP metabolites into the circulation, although these are still too few to support direct antioxidant mechanisms (23). Instead, multiple specific biological effects and mechanisms have been proposed (21). In this review we focus on those effects related to gut and cardiovascular health.

2) Modulation of the colonic microbiota: BTPs are well known for their antimicrobial activity. Because some bacterial groups are more resistant than others toward BTPs, these resistant bacteria could take advantage of available niches left open by susceptible microbes (eg, *Bifidobacterium*). This can beneficially affect the indigenous microbial composition and activity (24–26).

The symbiotic interactions between the gut microbiota, its metabolites, and the host have led to the recognition of humans as superorganisms (22). The unraveling of the interactions of BTPs with the human superorganism has been hampered by the sheer complexity of gut microbial interactions. Currently, there are knowledge gaps in the following areas: *1*) the bioavailability of gut microbial BTP metabolites, *2*) their bioactivity in the human host, and *3*) the role of the gut microbiota. We first discuss recent progress in enabling omics technologies to assess events at the level of both the metabolome and the microbiome in the human superorganism. Next, we describe how in vitro colon microbial fermentation models can complement human intervention studies. We also discuss in vitro assays for the assessment of the bioactivity of circulating BTP metabolites. The in vitro and in vivo work performed so far on the interaction of BTPs with gut microbiota is reviewed, and implications for the impact on gut and cardiovascular health are discussed in a critical manner.

EMERGING ENABLING TECHNOLOGIES

Metabolic profiling

Until recently, polyphenol bioavailability studies typically focused on a few predefined metabolites. Such approaches have inherent limitations when the massive gut microbial bioconversions of polyphenols and their subsequent metabolic fate in the human host need to be covered (27, 28). Analytic profiling approaches have become the method of choice for simultaneous assessment of the large range of polyphenol metabolites in urine, plasma, or in vitro models (29). In a so-called targeted profiling approach, a large range of preidentified, conjugated, polyphenol-derived metabolites could be detected simultaneously in a semiquantitative manner (30–32). These methods relied on extraction and fractionation by means of solid phase extraction of the complex biofluid matrix. Further sensitivity was gained by using targeted detection in multiple reaction monitoring mode on liquid chromatography tandem mass spectroscopy systems. With the use of these mass spectrometry methods, absolute quantification is hampered because authentic standards, in particular for conjugated polyphenol metabolites, are mostly not available. The use of compounds that are structurally related to the preidentified analytes as a standard can at best only result in semiquantification. As an alternative to liquid chromatography tandem mass spectroscopy–based approaches, untargeted gas chromatography profiling, focused on phenolic compounds, can be used. Such a platform has successfully been used for capturing microbial bioconversion products in in vitro models, feces, urine, and plasma (33). A disadvantage of this approach is the rather laborious sample pretreatment, which also involves a deconjugation step, discarding all information on host conjugative mechanisms. An advantage is that preidentified (deconjugated) phenolic compounds can readily be quantified by making use of commonly available standards (26). For global untargeted metabolite profiling of urine, so far only nuclear magnetic resonance (NMR) spectroscopy has been able to meet the requirements of nonselective detection and quantification in an unbiased manner (34). Although NMR is often presented as a relatively insensitive technique, it has successfully been used to identify BTP metabolites in in vitro (35, 36) and in vivo (37–39) studies. Now that most BTP gut microbial bioconversion products appear to be known (35, 36, 40), the next step is to identify their fate in the human host where they can undergo extensive conjugation. With the advent of sensitive high-resolution mass spectrometers such as quadrupole time-of-flight mass spectrometry and Orbitrap fourier transform mass spectrometry instruments, we can now witness a significant improvement in the untargeted coverage of conjugated phenolic metabolites in plasma and urine (41). However, for absolute structural elucidation, NMR also needs to be involved. By on-line coupling (hyphenation) of liquid chromatography to solid phase extraction, mass spectrometry, and NMR, a large range of urinary conjugated valerolactones and phenolic acids have recently been successfully identified and quantified at micromolar concentrations (41).

Nutrikinetic modeling

A number of complicating factors hamper assessment of BTP bioavailability as follows: *1*) the diversity and concentration ranges of metabolites that are produced by phase I and II metabolism; *2*) large interindividual variation in produced metabolites due to the interaction between BTPs, the food matrix, the gut microbiota, and the host; and *3*) the background diet, which continuously provides baseline amounts of polyphenol metabolites. Hence, we introduced nutrikinetics, which is an extension of the classical pharmacokinetic concept with explicit model adaptations (42). The concept relies on integrated deployment of metabolic profiling, multilevel data analysis, and population-based single-compartment modeling. It has already been successfully used to recognize nutritional phenotypes with different gut microbial bioconversion capacity for BTPs (38). Nutrikinetic

modeling also allowed for making in vivo associations between valerolactone production and the involved microbial species (22, 43).

Microbiomics

In the past decade, advances in sequencing technology and the development of metagenomic and bioinformatic methods have revolutionized studies of composition and activity of the gut microbiota (44). Microbial compositions can be assessed in a high-throughput manner on the basis of amplification and next-generation sequencing of 16S ribosomal RNA genes for bacteria and can determine bacterial groups both quantitatively (in relative abundance) and qualitatively (targeting all detectable microbial groups) (45, 46). Some techniques are purely quantitative (ie, quantitative polymerase chain reaction) and only provide information on targeted groups. The use of shotgun sequencing of whole genomes has also advanced in particular to study the functionality of complex communities (47), and a gene catalog was established for the gut microbiota (48). The development of such techniques affords the opportunity to better understand how food compounds affect the gut microbiota (49, 50).

IN VITRO MODELS FOR INTERACTIONS OF BTPs WITH GUT MICROBIOTA

In vivo intervention trials of dietary polyphenols hold inevitable practical and ethical limitations for elucidating mechanistic interactions between BTPs and the gut microbiota. Hence, in vitro models have been developed that mimic conditions in the gastrointestinal tract. These models allow for elucidation of microbial polyphenol bioconversion processes and vice versa, modulation of microbial composition by polyphenols. Most model work has focused on the colon; there has only been one model study of ileum microbial bioconversion of catechins (51). Because simulating physiologic complexity has budgetary and operational repercussions, acceptable trade-offs need to be made. Simple, static gut models are relatively easy to operate, are cost-effective, have a fair throughput, and allow for parallel screening. Hence, they have been widely applied to assess interindividual variation in polyphenol bioconversion (36, 40, 52–54) or to compare the effects of different food sources (40, 55). However, these static gut models are only adequate for simulating short-term conditions in the gut; for assessment of long-term adaptations of the gut microbial community, more complex dynamic models are needed. By using the Reading model (56), the Simulator of Human Intestinal Microbial Ecosystem (SHIME) (57), or the TNO in vitro model (57), gut microbiota can be cultured for long periods (days to weeks) in multiple connected vessels that represent different compartments of the human colon. The SHIME model has been used to monitor bioconversion of BTPs on a single bolus dosage (35) as well as effects of multiple BTP dosing on microbial composition (25).

IMPACT OF BTPs ON THE GUT MICROBIOTA

So far, there is a paucity of in vivo studies on the modulation of gut microbiota by black tea. One intervention in healthy humans, a randomized, double-blind crossover trial, indicated that shifts in the fecal microbiota had occurred; however, the community profiling and quantification methods were insufficient in sensitivity and depth to effectively identify the changes (58). The dosage of tea was also not described, and low dosage could account for the subtle changes. Most studies collecting data on the effect of black tea extracts on the intestinal microbiota have been performed with the use of in vitro experiments. A review summarizing in vitro data on the effects of BTPs clearly showed their strong antimicrobial, antitoxin, and antiviral effects (59). Black tea extract can also affect the virulence traits of the food-borne pathogen *Shigella* and enteropathogenic *Escherichia coli* strains (60). A synergistic action between theaflavin and epicatechin was even discovered when tested on nosocomial pathogens such as *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* (61). Moreover, microbial metabolites of black tea, such as benzoic, phenylacetic, and phenylpropionic acids (62) and urolithins (63), showed mainly antimicrobial properties against several bacterial species. Overall, numerous in vitro tests show the potency of BTPs and their end-products on a diversity of human commensals and pathogens. A few mechanisms of actions have been proposed and have been previously reviewed (25). The most common one is believed to be related to membrane disruption. Polyphenols can bind to membrane proteins and form a complex that might act in a bacteriocidal or bacteriostatic manner. Other hypotheses, such as inhibiting glucose inward transport or complexing free iron, have also been considered (64).

With the use of a physiologically relevant dosage, a bifidogenic effect of black tea and specifically black tea extracts enriched in thearubigins and flavonol glycosides has been observed (24). Pure catechins (devoid of polysaccharide content) have previously been linked to bifidogenic effects in vitro (65). The mode of action for this effect of thearubigin-rich fractions requires further research. Producing thearubigin-rich fractions is, however, a major challenge, and their characterization is part of ongoing studies (20). Alternatively, the possibility that both plant fibers and polyphenols act in synergy to provide a prebiotic bifidogenic effect has been proposed for a cocoa extract (66). Further studies using advanced technologies, as well as mechanistic studies, are needed to determine the in vivo impact of black tea on the human microbiota and potential links to human health.

IMPACT OF THE GUT MICROBIOTA ON BTPs

In the past few years a number of studies have appeared that proposed colon microbial degradation pathways for different flavonoids (21, 41, 67). These pathways have been summarized in **Figure 1** and pertain to monomeric catechins. No clarity exists on the first degradation step of thearubigins into smaller fragments. For oligomeric procyanidins, a direct colon microbial conversion to valerolactones has been proposed (68). In vitro experiments assessing the colon microbial bioconversion of BTPs have also shown the direct appearance of valerolactones, yet no intermediate forms were observed (35, 36). In vitro model fermentations in the 3-stage SHIME model showed that BTP bioconversions were colon-region dependent (35) for both a single bolus dosage as well as for sustained dosing. Several in vitro studies (25, 67) associated polyphenol bioconversion capacity with members of the Clostridia class, especially *Eubacterium ramulus* and *Clostridium orbiscindens* (reclassified as *Flavonifractor plautii*), and Actinobacteria (28, 69). Only recently have adequate in vivo nutrikinetic modeling approaches

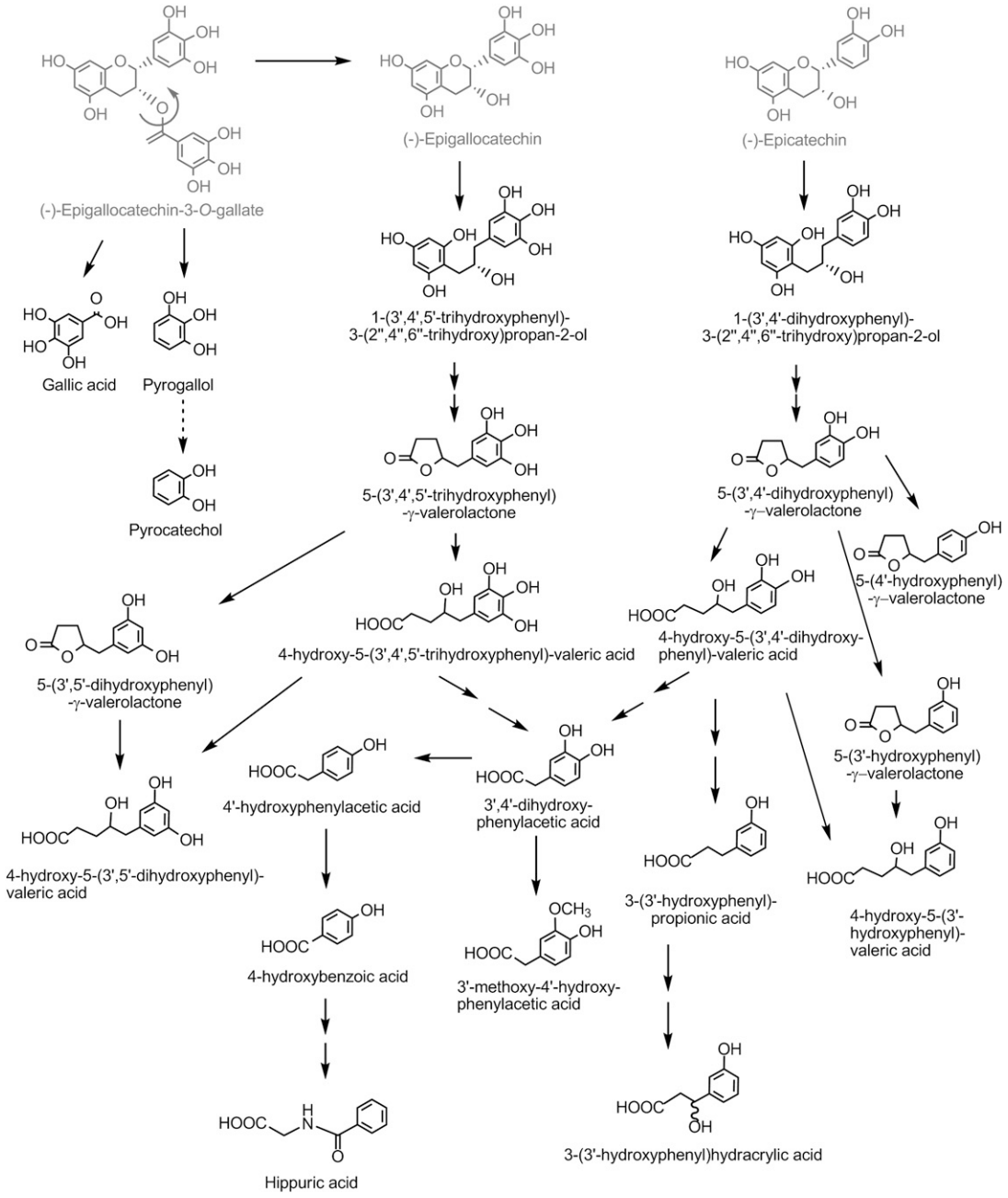

FIGURE 1. Schematic presentation of colon microbial degradation pathways of (epi-)catechins. Note that these metabolites are depicted as they are formed in the colon; within systemic circulation they will primarily appear in conjugated forms. Reprinted with permission from reference 41.

been introduced to associate circulating BTP metabolites with specific gut microbial phylotypes (22, 43).

HEALTH IMPLICATIONS OF BTP-MICROBIOTA INTERACTIONS

Gut microbial bioconversion products: from systemic exposure to cardiovascular effect

Thanks to powerful targeted and untargeted profiling platforms we are now able to get a fair overview of BTP metabolites and the concentrations at which they appear in systemic circulation (**Table 1**). The evidence of in vivo activity of these circulating species mostly originates from animal intervention studies. These studies have shown in vivo beneficial effects of 3,4-dihydroxy-benzoic acid and 4-hydroxy-cinnamic acid on monocyte infiltration (97) and platelet aggregation (87), respectively. For 3,4-dihydroxy-cinnamic acid (caffeic acid), antiinflammatory, anticoagulant, platelet activation inhibition and BP-lowering effects have been described in mice and rats (98–100). Most bioactivity studies, however, have been carried out in in vitro assays. These assays have focused on different aspects of cardiovascular health as follows: *1*) oxidative stress and LDL oxidation, which contributes to accumulation of lipid material in the vessel wall; *2*) impaired endothelial function, an early marker for atherosclerosis; *3*) macrophage activity and other inflammatory processes that may accelerate plaque formation; *4*)

TABLE 1
Overview of identified microbial bioconversion products of BTPs in vitro (colon bioconversion models) and in vivo (human interventions)[1]

	Bioavailability		Bioactivity						
	In vivo	In vitro	In vitro gut health bioactivity			In vitro cardiovascular health bioactivity			
Compound (alternative names)	Human interventions	Colon models	Assay	Concentration	Ref	Effect	Assay	Concentration	Ref
				μmol/L				*μmol/L*	
Gallic acids									
3,4,5-TriOH-benzoic acid (gallic acid)	Urine (70)	(36, 40)				PF	Inhibition of platelet aggregation	100	(71)
						PF	Prevents inhibition of platelet aggregation	10	(72)
						IP	Decreases MCP-1, ICAM-1, VCAM-1 secretion	≥10	(73)
						BP	Inhibition of ACE activity	>100	(73)
						EF	Inhibition of vasorelaxation	10	(74)
						OS	Inhibition of oxidation of LDL and erythrocytes	10–100	(75)
4-OH-methylgallic acid (4-OMGA)[2]	Plasma, urine (43, 70)	(36)				IP	Inhibition of NF-κB and ICAM-1, VCAM-1 expression	25	(76, 77)
						BP	Decreases angiotensin-1 receptor expression	1	(78)
Phenylalcohols									
1,3-DiOH-benzene (resorcinol)		(36)				PF	Inhibition of platelet aggregation	100	(71)
1,3,5-TriOH-benzene (phloroglucinol)		(36, 40)				OS	Inhibition of oxidative stress–induced cytotoxicity	1–10	(79)
1,2,3-TriOH-benzene (pyrogallol)		(36, 40)				PF	Inhibition of platelet aggregation	100	(71)
						EF	Enhanced vasodilation	0.01–1	(80)
						EF	Enhanced vasoconstriction	10–100	(80)
Pyrogallol-2-*O*-sulfate[2]	Urine (41, 81)								
Pyrogallol-2-*O*-glucoronide[2]	Urine (41)								
Benzoic acids									
Hippuric acid[2]	Urine (41, 81)					PF	Inhibition of platelet aggregation	100	(71)
3-OH-benzoic acid		(36)	Antiinflammatory protection of colon fibroblasts	100	(82)				
4-OH-benzoic acid (4 HBA)		(35, 36)				EF	Increased eNOS expression	10–33	(83)
4-OH-hippuric acid[2]	Urine (38)								
3,4-DiOH-benzoic acid (protocatechuic acid)		(36, 40)				IP	iNOS inhibition	25	(73)
						IP	Inhibition of NF-κB and ICAM-1, VCAM-1 expression	150	(84)
						OS	Inhibition of oxidation LDL and erythrocytes	10–100	(75)
2,3-DiOH-benzoic acid (3,4 dHBA)		(36, 35)							
2,6-DiOH-benzoic acid		(36)							
3,5-DiOH-benzoic acid		(36)							

(Continued)

TABLE 1 (*Continued*)

	Bioavailability		Bioactivity						
	In vivo	In vitro	In vitro gut health bioactivity			In vitro cardiovascular health bioactivity			
Compound (alternative names)	Human interventions	Colon models	Assay	Concentration	Ref	Effect	Assay	Concentration	Ref
Phenylproprionates									
3-Phenylproprionate (PPA)		(35, 36)	Antiinflammatory protection of colon fibroblasts	100	(82)				
3-(4′-OH-phenyl)-proprionic acid (4-HPPA), hydrocinnamic acid		(35, 36)	Antiinflammatory protection of colon fibroblasts	100 0.1	(82) (85)				
3-(3′-OH-phenyl)-proprionic acid (3-HPPA), phloretic acid		(35, 36)	Antiinflammatory protection of colon fibroblasts	100 0.1	(82) (85)				
3-(3′,4′-diOH-phenyl)-proprionic acid (3,4-dHPPA), dihydrocaffeic acid (HCAF)		(35, 40)	Antiinflammatory protection of colon fibroblasts	100 0.1	(82) (85)	IP PF	Decrease cytokine excretion by PBMCs Inhibition of platelet aggregation	1	(86) (71)
Phenylacetic acids									
Phenylacetic acid (PAA)		(36)	Antiinflammatory protection of colon fibroblasts	100	(82)				
3′,4′-diOH-phenylactetic acid (3,4 dHPAA) homoprotocatechuic acid, diOH-phenyl acetic acid (dOHPA)		(35)				IP	Decrease cytokine excretion by PBMCs	1	(86)
2′-OH-phenylacetic acid (mandelic acid)		(36)							
4′-OH-phenylacetic acid (4-HPAA, 4-OHPA)		(35, 36)	Antiinflammatory protection of colon fibroblasts	100	(82)				
3′-OH-phenylacetic acid (3-HPAA, 3OHPA)		(35, 36)	Antiinflammatory protection of colon fibroblasts	100	(82)				
3′-Methoxy-4′-OH-phenyl acetic acid, homovanillic acid		(40)				OS	Inhibition of NADPH oxidase	8	
Cinnamic acids									
4′-OH-cinnamic acid, p-coumaric acid		(36, 40)	Antiinflammatory protection of colon fibroblasts	0.1	(85)	PF BP PF OS IP EF	Inhibition of platelet activation ACE inhibition Inhibition of platelet aggregation Inhibition of LDL oxidation Inhibition iNOS in LPS-stimulated macrophages Increase eNOS expression	<100 >100 500 10–50 50 10–33	(71) (73) (87) (88) (89) (83)

(Continued)

TABLE 1 (*Continued*)

	Bioavailability		Bioactivity						
	In vivo	In vitro	In vitro gut health bioactivity			In vitro cardiovascular health bioactivity			
Compound (alternative names)	Human interventions	Colon models	Assay	Concentration	Ref	Effect	Assay	Concentration	Ref
3′,4′-DiOH-cinnamic acid, caffeic acid		(36)	Antiinflammatory protection of colon fibroblasts	0.1	(85)	PF	Inhibition of platelet activation	100	(71)
						BP	ACE inhibition	>100	(73)
						EF	Inhibition of vasorelaxation	10	(74)
						IP	Inhibition of NF-κB and ICAM-1, VCAM-1 Inhibition of oxidation	100	(90)
						OS	LDL and erythrocytes	10–100	(75)
							Inhibition of LDL oxidation	100	(88)
							Pro- and antioxidant effects for LDL	≥0.1	(91)
							Cholesterol efflux macrophages	0.5	(92)
							NADPH inhibition in HUVECs	15	(93)
Valerolactones and valeric acids									
[δ-(3′,4′-diOH-phenyl]-γ-valerolactone (M6, 3,4 DHPVL)	Plasma (43), urine[3](41)	(35, 40)				IP	Inhibition of iNOS expression and nitrite formation in macrophages	1–10	(94)
[δ-(3′,4′-diOH-phenyl]-γ-valerolactone) and its 3′-*O*-methyl derivative (M2)	Plasma (43), urine[3](41)								
5-(3′-OH-phenyl)-γ-valerolactone, 5-(3′,4′,5′-triOH-phenyl)-γ-valerolactone	Urine[3](41)	(40)							
5-(4′-OH-phenyl)-γ-valerolactone, 5-(3′,5′-diOH-phenyl)-γ-valerolactone	Urine[3](41)								
4-OH-γ–phenylvaleric acids	Urine[3](41)								
Ellagic acids (derived)									
Urolithin A			Antipathogenic; antiinflammatory protection of colon fibroblasts	4–8; 40	(63, 95)				
Urolithin A 3- and 8-*O* glucuronides[2]	Urine[4](41)					IP	Inhibition of monocyte adhesion and endothelial cell migration	5–10	(96)

[1] Bioactivity was surveyed for in vitro assays for gut health and cardiovascular health aspects. Cardiovascular aspects encompass EF, PF, BP regulation, IPs, and OS. ACE, angiotensin-converting enzyme; BP, blood pressure; BTP, black tea polyphenol; EF, endothelial function; eNOS, endogenous nitric oxide synthase; HUVEC, human umbilical vein endothelial cells; ICAM-1, intracellular adhesion molecule 1; iNOS, inducible nitric oxide synthase; IP, inflammatory process; NF-κB, nuclear transcription factor κB; OH, hydroxy; OS, oxidative stress; PBMC, peripheral blood mononuclear cell; PF, platelet function; Ref, reference; VCAM-1, vascular cell adhesion molecule 1.

[2] Conjugated microbial bioconversion products identified in vivo.

[3] One or more conjugates (sulfonates, glucuronidates) of the listed metabolites were identified in urine (41). No data were available on the in vitro bioactivity of these conjugates.

[4] The exact configuration of glucuronidate is unknown.

smooth muscle cell proliferation, which is relevant for vascular remodeling and BP-regulating processes; and *5*) platelet activity and aggregation (platelet function). Studies with in vitro bioassays and occasionally in vivo interventions indicate that BTP metabolites may *1*) reduce LDL oxidation, *2*) improve endothelial function by increasing nitric oxide bioavailability and vasorelaxation, *3*) reduce the production or expression of inflammatory mediators [eg, intracellular adhesion molecule 1 (ICAM-1), IL-1β] and inhibit monocyte adhesion and macrophage activation, *4*) inhibit the activity of enzymes and expression of receptors involved in hypertension (angiotensin-converting enzyme, angiotensin-1 receptor), and *5*) inhibit (collagen-induced) platelet aggregation and activation (P-selectin expression) (Table 1). Here we need to consider that the human host is capable of extensive phase 2 conjugation reactions of gut microbial products, such as glucuronidation, sulfonation, methylation, and glycination. Despite early recommendations to assess polyphenol in vitro bioactivity only with relevant circulating, ie, bioconverted and conjugated, species at relevant physiologic concentrations (101), these conditions have not become a common standard. As shown in Table 1, most of the known gut microbial bioconversion products have been tested in relevant in vitro models for cardiovascular effects, but unfortunately often at physiologically irrelevant concentrations. Exceptions are phenylpropionic and phenylacetic acids for which well-designed in vitro studies show antiinflammatory effects at relevant physiologic concentrations. The need for efficacy data on compounds at physiologically relevant concentrations is shown by experiments performed with pyrogallol, which can act as a vasodilator and vasoconstrictor, depending on concentration. For compounds such as valerolactones and valeric acids, data on in vitro bioactivity is scarce, although these compounds appear early in circulation at high plasma concentrations and their complex molecular structures suggest specific mechanisms of action. The lack of in vitro bioactivity data on valerolactones and valeric acids is most likely because of the practical and financial difficulties of obtaining these compounds in their pure form (102). The same consideration also pertains to the almost complete lack of in vitro activity data on conjugated forms of gut microbial bioconversion products. It has been argued that circulating conjugated phenolic species may undergo deconjugation at the site of action (103), but this mechanism has not been proven as a general mechanism.

Available data on the in vivo effect of black tea on flow-mediated dilation (FMD) (9) show both an acute (less than a few hours) as well as a chronic effect. The time scale of the acute effect of FMD does not match with the appearance of gut microbial bioconversion products of BTPs in systemic circulation. For procyanidins, a similar observation has been made (104), and it was argued that the gut microbial metabolites are not responsible for the acute effect, but they may explain the chronic FMD effects. The same reasoning may apply for the chronic effects of black tea on FMD (11, 105) for which gut microbial BTP bioconversion products could be the responsible bioactive species.

Gut microbial bioconversion products: from colonic exposure to local effect

Sustained dosing experiments in the SHIME model indicate that BPT bioconversion products reach high steady state concentrations in the colon (35). A range of these compounds (Table 1) can exert in vitro antiinflammatory protection to colon fibroblasts and have been implicated in gut health maintenance (82, 85, 106). Moreover, for 2 gut microbial BTP metabolites (hydrocaffeic and 3,4-dihydroxyphenylacetic acid), antiinflammatory protection was confirmed in vivo in a mouse model of colitis (82). The steady state concentrations of acetate and propionate observed on sustained dosage of BTPs in the SHIME model (35) may be linked to protective effects against Enterobacteriaceae infection in mouse models (107).

Modulation of the gut microbiota: health implications

It has been hypothesized that the antimicrobial activities of tea could contribute toward an antidiarrheal activity. For centuries, tea has been linked to digestive health, and there is growing evidence from animal studies that suggest that compounds of black tea can play a role in either the prevention of or recovery from diarrhea (108, 109). The potential bifidogenic effect of BTPs may play a role in this. Whereas the production of polyphenol metabolites can be attributed to microbial fermentation, changes in specific bacterial composition and levels linked to specific health benefits are still to be proven. Alterations in intestinal microbiota composition are being increasingly associated with health or chronic conditions (110). It is, however, too soon to conclude whether BTPs can affect the profile and level of the intestinal microbiota and whether the produced metabolites might affect gut health status. Deeper insights using the latest analytic tools described above will allow for further hypotheses to be generated and tested.

PERSPECTIVES

We envisage 2 system biology routes for establishing links between BTP bioavailability and bioactivity. In bottom-up approaches, the point of departure is the exometabolome of BTPs in systemic circulation. In top-down approaches, the departure point is a holistic assessment of molecular/cellular processes in the human superorganism by metabolomics and microbiomics tools (111).

Bottom up

In vitro bioconversion experiments and in vivo human intervention trials are now showing an increasing number of BTP metabolites that appear at high concentrations in the colon and in systemic circulation. One in vitro model study has shown that microbes from the ileum can bioconvert catechins (51), but whether they are also capable to do so with BTPs remains to be investigated. Most of the known conjugated BTP metabolites have been identified in urine (Table 1), and there is now an urgent need to assess their quantitative concentrations and nutrikinetic signatures in plasma. Bioactivity studies for cardiovascular effects of BTP metabolites differ widely in testing conditions (Table 1), which makes it difficult to compare bioactivities of the different circulating species. There is a clear need for well-designed studies that compare bioactivities of the different circulating species in standardized bioassays. Moreover, when taking the next steps of establishing the bioactivity of BTP metabolites, physiologic concentrations and the circulating conjugated forms need to be considered (101). This recommendation

appears to be addressed by recent studies on protective effects of BTP metabolites on the colon wall (Table 1). Given the wide range of chemical structures of BTP metabolites in systemic circulation, synergistic effects also need to be considered.

Top down

The joint deployment of nutrigenomics tools [metabolomics, microbiomics, transcriptomics, and proteomics (112)] provides a powerful strategy to unravel the role played by BTPs in maintaining gut and cardiovascular health. Comprehensive nutrigenomics assessment of critical homeostatic processes in the human host needs to be linked with the nutrikinetic signatures of microbiota-mediated BTP metabolites (42). In this respect, the use of metabolic challenge tests has been proposed to obtain sensitive read-outs of the long-term modulation of homeostatic resilience by dietary ingredients (42, 113). We further envisage that the identification of nutrikinetic phenotypes (114) will allow for stronger associations between nutritional phenotypes and the bioactivity of polyphenols. The comprehensive human gut microbiome projects that are currently underway around the world (44) will enable assessment of the contribution of colonic microbiota to the nutritional phenotype and ultimately gut and cardiovascular health and disease predisposition.

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