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Tuning constitutive and pathological inflammation in the gut *via* the interaction of dietary nitrate and polyphenols with host microbiome

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ABSTRACT

Chronic inflammation is currently recognized as a critical process in modern-era epidemics such as diabetes, obesity and neurodegeneration. However, little attention is paid to the constitutive inflammatory pathways that operate in the gut and that are mandatory for local welfare and the prevention of such multi-organic diseases. Hence, the digestive system, while posing as a barrier between the external environment and the host, is crucial for the balance between constitutive and pathological inflammatory events. Gut microbiome, a recently discovered organ, is now known to govern the interaction between exogenous agents and the host with ensued impact on local and systemic homeostasis. Whereas gut microbiota may be modulated by a myriad of factors, diet constitutes one of its major determinants. Thus, dietary compounds that influence microbial flora may thereby impact on inflammatory pathways. One such example is the redox environment in the gut lumen which is highly dependent on the local generation of nitric oxide along the nitrate-nitrite-nitric oxide pathway and that is further enhanced by simultaneous consumption of polyphenols. In this paper, different pathways encompassing the interaction of dietary nitrate and polyphenols with gut microbiota will be presented and discussed in connection with local and systemic inflammatory events. Furthermore, it will be discussed how these interactive cycles (nitrate-polyphenols-microbiome) may pose as novel strategies to tackle inflammatory diseases.

1. Introduction

Inflammation, a feared cascade of events mediated by critical signaling pathways, has been extensively studied in recent decades in an attempt to prevent, delay or cure a wide range of human diseases. In fact, chronic systemic inflammation has been associated with the greatest pandemics of the modern era: obesity, type 2 diabetes (T2D) and neurodegenerative disorders (Amor et al., 2014; Cani et al., 2007; Minihane et al., 2015). However, the human gut provides an exquisite environment in which constitutive inflammatory pathways are pivotal to maintain local and systemic homeostasis (Wallace, 2008). In fact, the gut mucosa is under a permanent yet physiological inflammatory status in order to rapidly counteract both exogenous and endogenous aggressors (Wallace, 2008; Wallace and Miller, 2000). Recent evidences suggest that this delicate crosstalk between external agents and the host is finely tuned by gut microbiota either by intrinsic bacterial interactions (*quorum sensing*) or by inter-kingdom signaling (Sekirov et al., 2010). In this regard, bowel disorders such as ulcerative colitis (UC), Crohn's disease (CD), irritable bowel syndrome (IBS) and colorectal cancer (CCR) have been associated with changes on gut microbial diversity (for comprehensive reviews see (Ahn et al., 2013; Bonaz and Bernstein, 2013)). Interestingly, dysbiosis (altered gut flora) has also been implicated in the ethiopathogeny of multiorganic disorders hallmarked by low-grade inflammation: T2D, metabolic syndrome and neurological conditions such as Parkinson's disease, multiple sclerosis and autism (Everard and Cani, 2013; Hsiao et al., 2013; Mazidi et al., 2016; Scheperjans et al., 2015; Turnbaugh et al., 2006). In any of these cases, strong evidence point to the notion that the initial trigger might be located in the gut, where endogenous events (*e.g.*,

Abbreviations: ARE, antioxidant response elements; CBP, CREB binding protein; CK2, casein kinase 2; Cul3, Cullin 3; IBD, inflammatory bowel disease; IFN-γ, interferon-γ; IKK, IkB kinase; IL-1 β, interleukine-1 β; iNOS, inducible nitric oxide synthase; JAKs, Janus Kinase; JNKs, c-Jun N-terminal kinases; LC8, 8-kDa dynein light chain protein; LPS, lipopolysaccharide; MLC, myosin light chain; MLCK, myosin light-chain kinase; NOX, NADPH-oxidase; NF-κB, nuclear factor-κB; Nrf2, nuclear factor-erythroid-2-related factor 2; PKC, protein kinase C; PTPs, protein tyrosine phosphatases; ROS, reactive oxygen species; SOCs, suppressors of cytokine signaling; STATs, Signal Transducer and Activator of Transcription; TJs, tight junctions; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-α; ZO, zonnula occludens.

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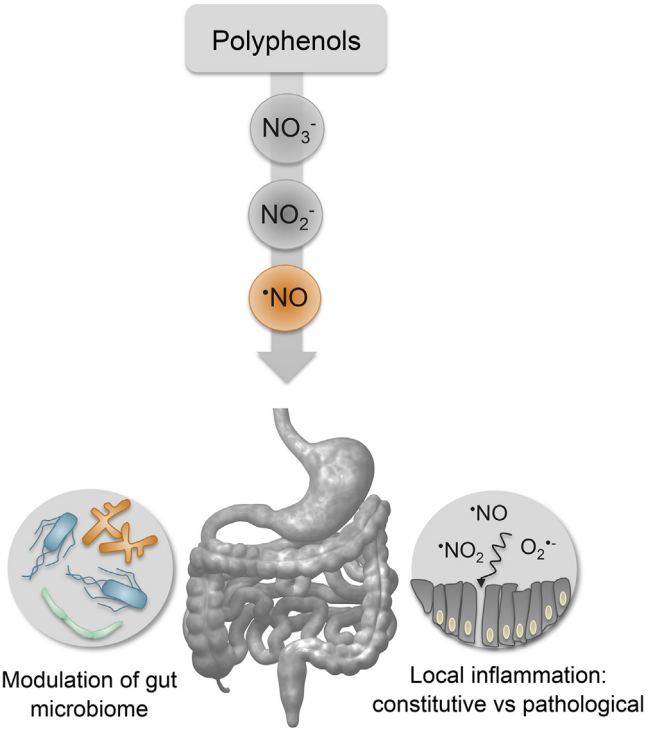

Fig. 1. Dietary nitrate and polyphenols interact in the human gastrointestinal lumen yielding different nitrogen oxides, depending on local conditions. The resulting species embark a series of reactions with the microbial community likely altering not only the profile of gut microbiota but also yielding microbial associated molecular patterns (MAMPs) that, in turn, trigger local inflammatory pathways within the gastrointestinal mucosa. The mechanisms underlying the activation of inflammatory events may rely on the production of nitrated (or nitrosated) proteins and alkenes, the disruption of epithelial tight junctions and the modulation of neurohumoral pathways.

accelerated intestinal transit, neurotransmitter secretion, mucus depletion) or exogenous stressors (*e.g.*, spices, bacteria) increase epithelial permeability and activate inflammatory signaling pathways within the gut mucosa while also allowing the translocation of bacterial and inflammatory products into the blood stream, thereby reaching all body systems (Ashida et al., 2012; Everard and Cani, 2013; Kaliannan et al., 2015).

Under physiological conditions, the composition of gut microbiome (the microbiota collection of genes) and thus the inflammatory status of the gastrointestinal (GI) mucosa are modulated by a myriad of factors including genetic characteristic, hygiene and lifestyle habits as well as nutrient intake (Sekirov et al., 2010; Turnbaugh et al., 2007). In this context, dietary polyphenols support a bidirectional communication with the human microbiota: in one hand bacteria digest the phenolics into physiologically-relevant metabolites and, on the other, polyphenols and their metabolites may also modulate microbial composition (Parkar et al., 2013; Valdes et al., 2015). Both edges of this axis are tightly controlled by electroactive molecules derived from nitrite as the highest concentration of nitric oxide (•NO) achieved *in vivo* is detected in the stomach through the non-enzymatic reduction of dietary nitrate and nitrite from green leaf vegetables (Jones et al., 2012; Lundberg et al., 2004).

This paper critically discusses the bidirectional interaction between redox-sensitive inflammatory pathways and the gut microbiome with impact on inflammatory bowel diseases and systemic welfare (Fig. 1).

1.1. Bidirectional interactions between gut microbiota and dietary polyphenols with impact on gut inflammatory status

Dietary polyphenols have raised a great deal of interest in the 1990′s when their health benefits have been associated with antioxidant properties. However, this mechanism is not straightforward *in vivo* and therefore other hypothesis, discussed bellow, have been proposed to explain the health-promoting effects of polyphenols (Fraga, 2007; Rocha et al., 2014). Some rely in the ability of the original molecules as well as specific metabolites to modulate inflammatory signaling pathways not only locally in the gut (right after a meal) but also systemically *via* inhibition of enzymatic activities, notably NADPH oxidases (Steffen et al., 2008). Others, as it will be detailed bellow, emphasize the ability of dietary phenols to induce the univalent reduction of nitrite anion to •NO in the human stomach and ensued •NO-related processes (Gago et al., 2007; Peri et al., 2005; Rocha et al., 2009). However, recent evidences show not only that bacteria metabolize dietary polyphenols into bioactive molecules but also that although not acting as direct antioxidants *in vivo*, polyphenols may rather exhibit similar properties by modulating critical redox signaling pathways. Interestingly to note, given the dietary sources of polyphenols and the insignificant metabolism in the proximal gut, in this local microenvironment the concentration of phenolics may be eventually high enough to allow direct antioxidant effect (Fraga, 2007). These findings triggered an intensive research on the metabolism of dietary polyphenols by gut bacteria into absorbable and effective metabolites, often endowed with greater biological activity than the initial molecules (Cheynier, 2005; Selma et al., 2009). For instance, different intestinal bacteria such as *Bifidobacterium* spp. metabolize daidzein (from soy) into bioactive metabolites (equol and O-desmethylangolensin) able to bind to estrogen receptors thereby exhibiting an estrogen-like activity; still, they may also inhibit iNOS activity and thus act as anti-inflammatory agents (Kang et al., 2007; Yokoyama and Suzuki, 2008).

Although very promising, two major questions need to be answered when addressing the formation of phenolic metabolites by gut bacteria: 1) given that most studies are conducted with an isolated polyphenol, how would the production of bacteria-dependent metabolites be influenced while consuming foods with several dozens of different polyphenols or embedded within particular food matrixes (*e.g.*, with fibers)? 2) if gut bacteria may decompose large phenolic molecules, how would polyphenols and/or their metabolites influence bacteria survival and ultimately, the microbiome profile? If any impact on microbiome is expected, what would be the outcome of dietary polyphenols for human health? Although still rhetorical, these questions may anticipate that the modulation of gut microbiome profile may be forwarded as an alternative mechanism underlying the *in vivo* effects of dietary polyphenols. Hence a *bidirectional microbiome-polyphenol axis* is now being proposed (Cardona et al., 2013; Duda-Chodak et al., 2015; Valdes et al., 2015). Indeed, the prolific ecology of the human gut impresses not only by outnumbering the number of human cells by a factor of 10 and the number of host genes by almost two hundredfold, but also by the profound impact that it has on GI and on multiorganic disorders (Hsiao et al., 2013; Sekirov et al., 2010; Turnbaugh et al., 2006). The human gut harbors up to 1000 different bacterial species that harvest otherwise indigestible nutrients, control epithelial cell renewal, modulate innate and adaptive immune responses and determine the disease risk in later life (Turnbaugh et al., 2007). Although humans share a common core microbiome, the overall profile of the gut flora is largely dependent on individual factors, such as antibiotic consumption, external stressors, recurrent infections, immunosenescence (reduced immune function) and the diet (Claesson et al., 2012; Turnbaugh et al., 2007). So, dietary polyphenols appear also to modulate this direction