Food Research International 88 (2016) 342–347

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Flavonoid–gastrointestinal mucus interaction and its potential role in regulating flavonoid bioavailability and mucosal biophysical properties

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ARTICLE INFO

Article history:
Received 10 July 2015
Received in revised form 23 December 2015
Accepted 27 December 2015
Available online 29 December 2015

Keywords:
Gastrointestinal mucus
Flavonoids
Bioavailability
Mucin
Microrheology
Macrorheology

ABSTRACT

Flavonoid bioavailability has attracted a lot of attention over the last decade due to the increasing evidence of their health-promoting properties. However, conflicting results appear in the literature, especially on the bioavailability of flavonoid glycosides in vitro versus in vivo. In in vitro studies, where Caco-2 cells are usually used, hydrophobic aglycones have been reported to be more bioavailable. On the contrary, in vivo studies suggest that increasing the aqueous solubility of flavonoids favors their bioavailability. In this paper, we aim to fill this gap by analyzing the role of the gastrointestinal mucus on flavonoid bioavailability. Mucus is a complex viscoelastic barrier that serves as the first line of defense against pathogens, particles and several toxins, while allowing nutrients to penetrate through and reach the epithelia. A mechanism by which mucus participates in flavonoid absorption is proposed. Also, the effect of flavonoids on the biophysical properties of the mucus layer is discussed. This article therefore reviews the complex interaction between flavonoids and mucus for the first time.

1. Introduction

Flavonoids are the most widely spread and diverse group of polyphenols. There are more than 8000 different flavonoids identified and some of the most abundant are quercetin and kaempferol, which often occur as complex conjugates with glycosides (commonly glucose, galactose and rhamnose) and acyl groups in nature (Fiol et al., 2012; Francisco et al., 2009; Olsen, Aaby, & Borge, 2010). Flavonoids consist of two aromatic rings and one heterocyclic ring containing an oxygen atom. Different classes of flavonoids then arise depending on the relative position of different functional groups in the 15-carbon skeleton (Schmidt et al., 2010). Recently, high regard has been placed on these metabolites due to their biological activity, which includes angiotensin-converting enzyme inhibitory activity (Actis-Goretta, Ottaviani, & Fraga, 2005; Balasuriya & Rupasinghe, 2012), several mechanisms against obesity (Hsu & Yen, 2008), and most popularly, antioxidant activity (Fiol et al., 2012; Pietta, 2000), among others.

Much research has been done to demonstrate the bioavailability of flavonoids following ingestion and the several barriers that hinder their intestinal absorption (Gonzales et al., in press; Manach, Williamson, Morand, Scalbert, & Rémésy, 2005). There are currently conflicting results derived from both in vitro and in vivo reports on the fate and efficiency of intestinal absorption of different flavonoid forms. For instance, while in vitro results point to the higher bioavailability of flavonoid aglycones, some in vivo experiments reported higher levels of flavonoid metabolites upon ingestion of flavonoid glucosides.

Certain gaps in our knowledge on the bioavailability of flavonoids and other phytochemicals have been reviewed (Bohn et al., 2015). For instance, the involvement of the mucus layer in most flavonoid digestion and bioavailability studies remains largely unexplained in current literature. In this review, we aim to examine the interaction of flavonoids and gastrointestinal mucus, and its potential to alter flavonoid bioavailability and mucosal biophysical properties. A mechanistic view on the role of intestinal mucus on flavonoid absorption is thereby proposed along with some future perspectives.

2. Mucus

Mucus is a highly complex viscoelastic secretion that covers epithelial surfaces, such as respiratory, ocular, reproductive and gastrointestinal (GI). In the GI tract, the mucus layer is the first line of protection against infection and intoxication, but also serves as a selective barrier to allow nutrients to diffuse to the epithelial layer. This selectivity is most important in the small intestine, where most of the nutrient absorption takes place and the mucus layer is thinnest. However, the rules governing this selective barrier function remain unclear (Lai, Wang, Wirtz, & Hanes, 2009; Macierzanka et al., 2014).

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http://dx.doi.org/10.1016/j.foodres.2015.12.023
0963-9969/

Mucus is composed of ~95% water with salts (up to 1%), lipids (1–2%), proteins (growth factors, lysozyme, immunoglobulins, etc), cellular debris, DNA (around 0.02%) and mucin, which is primarily responsible for its viscoelastic properties (Bansil & Turner, 2006; Lai et al., 2009). Although, extracellular DNA content in mucus has also been shown to increase its microrheology and barrier properties (Macierzanka et al., 2014). In the human GI tract, the mucus layer is thickest in the stomach (50–450 μm) and colon (110–160 μm), while the thickness in the small intestines varies depending on the diet and other digestive activities (Cone, 2009). Mucus of the GI tract is composed of both membrane-bound and secreted mucins, of which more than 20 are known, including MUC5AC and MUC2 that are secreted in the stomach and intestines, respectively (Ensign, Cone, & Hanes, 2012; Mackie, Round, Rigby, & Macierzanka, 2012). Mucins are large glycoproteins of 0.5–20 MDa in weight comprising of ~80% oligosaccharides that are attached to the protein core via *O*-glycosidic bonding to serine and threonine hydroxyl side chains, resembling a bottle brush-like structure (Bansil & Turner, 2006). Upon mucin secretion, a dense unstirred layer of mucus is rapidly built above the epithelial cells. Far from the epithelia, the mucus expands by proteolytic expansion causing it to loosen. In this loose layer, commensal microbes could thrive while the inner more dense mucus layer is thought to be largely impenetrable to bacteria in healthy conditions (Johansson et al., 2013; Malin E. V. Johansson et al., 2008).

The strength of interaction of mucin monomers, their degree of entanglement and the size of the pores in the network dictate the relative strength of the formed viscoelastic gel, which is characterized by its dynamic and shear-thinning properties (Ensign et al., 2012; Mackie et al., 2012). These properties give mucus the ability to maintain the dense unstirred layer on the epithelium despite the vigorous shearing in the intestinal lumen caused by peristalsis (Cone, 2009). To protect the epithelium, a rapid turnover of this layer occurs by continuous secretion of mucus, replacing both the tightly and loosely adherent layers. Particles and nutrients must therefore migrate upstream to reach the epithelium. Hence, a rapid and efficient diffusion of molecules, such as polyphenols, is a prerequisite to intestinal uptake. For a deeper discussion on mucus structure and properties, we recommend the reviews of Bansil, Stanley, and Lamont (1995); Cone (2009); Lai et al. (2009) and Mackie et al. (2012).

3. Emulsification increases flavonoid bioavailability: potential role of intestinal mucus in flavonoid absorption regulation

The low bioavailability of flavonoids remains a hurdle to their applicability as potent bioactive molecules. Thus, numerous studies have been made to understand the mechanisms of absorption as well as techniques to increase the intestinal absorption of flavonoids. Cellular models such as Caco-2 cells have been extensively used to simulate the intestinal permeability of flavonoids (Barrington et al., 2009; Tammela et al., 2004; Tian, Yang, Yang, & Wang, 2009). Results from these experiments revealed that membrane permeability is of great importance for flavonoid transport since passive diffusion is the dominant absorption route and thus, the hydrophobicity of the molecule plays a crucial role in intestinal absorption (Barrington et al., 2009; Gonzales et al., 2015; Liu & Hu, 2002; Tammela et al., 2004; Tian et al., 2009). Therefore, flavonoid glycosides are generally poorly absorbed through the intestinal cells/walls due to their hydrophilicity, thus reducing membrane permeability (Dai, Yang, & Li, 2008), unless highly actively transported. The involvement of active transporters in the transport of flavonoid glycosides still remains under debate and more research is needed to understand this mechanism.

Interestingly, animal and human studies suggest that certain flavonoid glucosides are absorbed more efficiently than their aglycone forms. In a feeding study in humans, it was found that quercetin glucoside is absorbed more than quercetin aglycone (Hollman, de Vries, van Leeuwen, Mengelers, & Katan, 1995; Hollman et al., 1997; Morand, Manach, Crespy, & Remesy, 2000b). The same observation was obtained from feeding studies using pigs (Cermak, Landgraf, & Wolffram, 2003), rats (Morand, Manach, Crespy, & Remesy, 2000a), and dogs (Reinboth, Wolffram, Abraham, Ungemach, & Cermak, 2010). While several hypotheses have been formulated, such as effective deglucosylation by bacteria or brush border enzymes of the epithelial cells, current literature has failed to provide a concrete explanation of this inconsistency. Cermak et al. (2003) hypothesized that the hydrophilic quercetin glucoside may have concentrated at the brush border, where deglucosylation occurs. This implies that the glucoside may have been able to penetrate through the mucus layer intact.

Flavonoid aglycones and glycosides belong to class II (low solubility and high permeability) and class IV (low solubility and low permeability) according to the biopharmaceutical classification system (BCS). Therefore, strategies to enhance the absorption of flavonoids include increasing their aqueous solubility (Kaur & Kaur, 2014). Permeability is not an issue for glycosides since active deglycosylation occurs in the brush border by β-glucosidases (lactase-phlorizin hydrolase), releasing a highly permeable aglycone (Day et al., 1998; Németh et al., 2003). Hence, attempts to increase the bioavailability of flavonoids generally involved methods to increase their aqueous solubility, such as micro- and nano-emulsions, and macromolecule complexation. Briefly, several techniques to increase bioavailability include incorporation of flavonoids to borneol/methanol eutectic mixtures, micro-emulsions, polyvinylpyrrolidone dispersion, lecithin complexation, and cyclodextrin complexation (Thilakarathna & Rupasinghe, 2013). The formulation of flavonoid aglycones into nanocrystals has also been previously described as an effective way of improving bioavailability (Li et al., 2013). All of these methods increase aqueous solubility of the flavonoid aglycone. However, the mechanisms of absorption enhancement remain unclear (Shen, Li, Li, & Zhao, 2011).

Dietary factors have also been shown to greatly improve the bioavailability of flavonoid aglycones. For instance, it has been shown that dietary fat increases the bioavailability of quercetin aglycones (Azuma, Ippoushi, Ito, Higashio, & Terao, 2002; Guo et al., 2013; Lesser, Cermak, & Wolffram, 2004). These reports attributed the enhanced bioavailability of the aglycone to its incorporation into mixed micelles, which permitted its diffusion through the unstirred mucus layer.

Given these findings, it is apparent that while hydrophobic flavonoid aglycones are favorably absorbed by intestinal cells, glycosylation and increasing aqueous solubility increase overall intestinal absorption. A logical mechanism therefore is the possible involvement of the mucus layer, which only allows the penetration of hydrophilic compounds (flavonoid glycosides or hydrophilic flavonoid complexes) and that deglycosylation occurs after the compound has penetrated through the mucus releasing the aglycone, which could then passively diffuse through the cell membrane. This hypothesis could be summarized in Fig. 1.

Hydrophobic flavonoid aglycones that reach the small intestines are unable to penetrate through the mucus layer and are thus pushed to the large intestines via peristalsis and are then metabolized (i.e. into smaller phenolic acid derivatives) by intestinal bacteria. However, in the presence of dietary fat and bile, micelles form and serve as carriers of aglycones through the mucus layer. They are then released upon contact with the brush border where passive diffusion is likely to occur through the cells. Soluble flavonoid glycosides on the other hand, are able to penetrate through the mucus layer to reach the epithelium. Upon contact with the brush border, β-glucosidases such as the lactase-phlorizin hydrolase (LPH) cleave-off the glucose moiety to release the aglycone, which could then passively diffuse through the cells. Since intestinal cells are unable to produce rhamnosidases, flavonoid containing rhamnose moieties, such as rutin (quercetin-3-rutinoside) and hesperidin (hesperetin-7-rutinoside), remain intact in the small intestines and are thus pushed to the large intestines, where fermentation by intestinal bacteria occurs or the action of secreted bacterial rhamnosidases