Archives of Biochemistry and Biophysics 559 (2014) 12–16

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Review

Prenylation modulates the bioavailability and bioaccumulation of dietary flavonoids

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ARTICLE INFO

Article history:
Received 21 February 2014
and in revised form 3 April 2014
Available online 13 April 2014

Keywords:
Prenylflavonoids
Prenylation
Epithelial cells
Efflux
Uptake
Bioaccumulation

ABSTRACT

Prenylflavonoids are distributed widely in the plant kingdom and have attracted appreciable attention because of their potential benefits for human health. Prenylation may be a promising tool for applying the biological functions of flavonoids to clinical uses. The bioavailability and bioaccumulation of prenylflavonoids have not been clarified, but extensive studies have been accomplished on their biological functions. This review provides current knowledge on the bioavailability of prenylflavonoids, including their absorption and metabolism in the intestine, as well as their bioaccumulation in specific tissues. Despite higher uptake into epithelial cells of the digestive tract, the bioavailability of single-dose prenylflavonoids seems to be lower than that of the parent flavonoids. Efflux from epithelial cells to the blood circulation is likely to be restricted by prenyl groups, resulting in insufficient increase in the plasma concentration. Rodent studies have revealed that prenylation enhances accumulation of naringenin in muscle tissue after long-term feeding; and that prenylation accelerates accumulation of quercetin in liver tissue. Efflux from hepatocytes to blood and enterohepatic circulations may be restricted by prenyl groups, thereby promoting slow excretion of prenylflavonoids from the blood circulation and efficient uptake to tissues. The hepatotoxicity and other deleterious effects, taken together with beneficial effects, should be considered because unexpectedly high accumulation may occur in some tissues after long-term supplementation.

Introduction

Prenylflavonoids are naturally occurring flavonoids possessing ⩾1 C5 isoprene (dimethylallyl) unit(s) in their diphenylpropane structure. The geranyl group, farnesyl group and geranylgeranyl group are prenyl chains containing two, three and four C5 isoprene units, respectively.

Flavonoids are synthesized as plant secondary metabolites starting from malonyl CoA and *p*-coumaroyl CoA. Prenylation occurs in different positions of the aromatic ring owing to the catalytic action of prenyltransferase [1]. The nature of this enzyme has not been clarified, but Sasaki et al. [2] isolated prenyltransferase from the legume *Sophora flavescens* and succeeded in its molecular cloning. Therefore, various prenylflavonoids are expected to be prepared using prenyltransferase in the near future. After prenylation of the aromatic ring in flavonoids, the prenyl group can be modified through cyclization and hydroxylation [3]. At present, >1000 prenylflavonoids have been found in the plant kingdom [4].

The roots, leaves and seeds of *Moraceace*, *Leguminosae* and *Asteraceae* are major sources of prenylflavonoids. In recent years, considerable attention has been paid to the biological functions of this class of flavonoids, such as antibacterial activity, enzyme inhibition, estrogenic activity, and antioxidant activity [5]. Prenylation has been found to enhance the estrogenicity of naringenin and genistein [6], as well as the inhibitory effect of luteolin on melanin biosynthesis in cell cultures [7]. The hydrophobic prenyl chain can affect the cellular uptake and biological functions of flavonoids by accelerating interactions with the phospholipid bilayers of biomembranes or hydrophobic target proteins [8]. An *in vivo* rodent study demonstrated that prenylation enhanced the accumulation of naringenin in muscle tissue, resulting in the prevention of muscle atrophy [9]. A review article collected the bioavailability of prenylated isoflavonoids from the viewpoint of phytoestrogens [10]. However, how prenylation affects the bioavailability and bioaccumulation of flavonoids is not known. Although the intake level of prenylflavonoids from the diet has not been estimated yet, beer is the major dietary source of xanthohumol and related prenylflavonoids [11]. The daily intake of total prenylflavonoids would be about 0.14 mg, based on the assumption that average person in the United States consumed 225 mL of lager/pilsner beer per

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http://dx.doi.org/10.1016/j.abb.2014.04.002
0003-9861/

day [11]. In addition, considerable level of prenylflavonoids may be consumed from a variety of supplements, because herbs and natural ingredients frequently contain appreciable amounts of pharmacologically active prenylflavonoids such as 6-prenylquercetin (gancaonin P) in *Glycyrrhiza glabra* (licorice) and mulberrin in *Morus ihou* (mulberry) [12]. Thus, the impact of prenylation on the bioaccumulation of flavonoids should be taken into account to estimate the biological functions of prenylflavonoids originating from diets and supplements.

In this review article, we describe current knowledge on the intestinal absorption, transformation by intestinal microbiota, phase I/II metabolism, and tissue distribution of prenylflavonoids. We propose that prenylation enhances the bioaccumulation of flavonoids in specific tissues by modulating the balance between uptake and efflux in cells.

Discussion

Intestinal absorption and bioavailability of prenylflavonoids

The bioavailability of flavonoids varies considerably depending on the structure of each compound. The bioavailability of different classes of flavonoids has been assumed to decrease in the order isoflavones > flavan-3-ols > flavanones > flavonols [13]. Intestinal absorption may play a crucial part in determination of the bioavailability of flavonoids because absorptive cells in the intestine can act as barriers for xenobiotics. Flavanones, flavones and flavonols present in fruits and vegetables and other plant foods are present predominantly in glycosidic forms. Before intestinal absorption, monoglucosides can be converted into their aglycone in the small intestine by lactase phlorizin hydrolase (LPH)[1] on the surface of intestinal epithelial cells [14]. The resulting aglycone can pass enterocytes *via* passive transport because it is hydrophobic with high affinity for cellular membranes [15]. Glycosides other than monoglucosides seem to be absorbed only slightly at the small intestine because of the low substrate specificity of LPH [16]. They reach the large intestine intact and undergo hydrolysis and/or ring scission by enterobacteria [17]. Hydrolysis products (aglycone) and scission products (phenolic acids) may be transferred into intestinal epithelial cells by diffusion.

There are few data on the bioavailability of prenylflavonoids. However, it can be supposed that prenylation enhances cellular uptake of flavonoids in the intestine, resulting in higher bioavailability. Rodent studies imply that the prenylflavonoid xanthohumol, if administered orally, can be absorbed and transported in the blood stream in a comparable fashion as non-prenylated flavonoids [18]. However, *in vitro* studies of the acids in hops that cause their bitter taste (β-acid with three prenyl substituents and α-acid with two prenyl substituents) [19], and artepillin C (prenylated hydroxycinnamic acid and parent *p*-coumaric acid) [20] imply that prenylation decreases their bioavailability unexpectedly by attenuating the efficacy of intestinal absorption. An *in vivo* rodent study demonstrated that the area under the curve of 8-prenylquercetin and 8-prenylnaringenin was significantly lower than that of non-prenylated quercetin and naringenin, respectively, when they were administered intragastrically as a single dose for calculating their pharmacokinetic parameters in the plasma (Fig. 1) [9,21]. These results demonstrated clearly that the bioavailability of flavonoids is diminished by introduction of a prenyl group to their aromatic rings.

[1] *Abbreviations used:* LPH, lactase phlorizin hydrolase; ABC-transporters, adenosine triphosphate-binding cassette transporters; MRP-2, multidrug-resistant protein 2; BCRP, breast cancer-resistant protein; MRP-3, multidrug-resistant protein 3; COMT, catechol *O*-methyltransferase.

The discrepancy between high cellular uptake of prenylflavonoids (which is expected from their greater hydrophobicity) and experimental results of low bioavailability enables recognition of the role of efflux in intestinal cells in the determination of bioavailability (Fig. 2). The efficacy of intestinal absorption is dependent on the balance between uptake and efflux in intestinal epithelial cells. The flavonoid aglycone is subject to the action of phase-II enzymes in epithelial cells, and the resultant glucuronide and/or sulfate conjugates are returned to the digestive tract through adenosine triphosphate-binding cassette transporters (ABC-transporters) including multidrug-resistant protein 2 (MRP-2) [22] and breast cancer-resistant protein (BCRP) for efflux to the apical site [23]. Alternatively, glucuronide and/or sulfate conjugates can be transported into the portal vein through the ABC-transporter multidrug-resistant protein 3 (MRP-3) for efflux to the basolateral site [24]. A cell culture study found that 8-prenylquercetin accumulated within absorptive cells at higher amounts than non-prenylated quercetin, whereas a lower amount of 8-prenylquercetin was on the basolateral side, suggesting that prenylation enhances uptake (but reduces efflux) in cells [21]. The reason may be that the prenyl substituent suppresses the affinity of flavonoid conjugates to ABC-transporters, resulting in lower efflux from the cell. An alternative possibility is that the phase-II glucuronidation/sulfation reaction (which seems to be required for recognition by ABC-transporters) is diminished by the prenyl group in the structure. However, studies focusing on the effect of prenylation on the affinity of flavonoids to ABC-transporters and their substrate specificity to phase-II conjugation enzymes are lacking. Nevertheless, modulation of the balance between the uptake and efflux of cells should lead to the lower bioavailability of prenylflavonoids.

Transformation of prenylated flavonoids by intestinal microbiota

Microbiota has a major role in the modification of dietary flavonoids in the lower digestive tract. Phenolic acids such as 3′,4′-dihydroxyphenyl acetic acid, homovaleric acid and 3′-hydroxyphenyl acetic acid are produced as ring-scission products of flavonoids, together with flavonoid aglycones as the hydrolysis products [25]. Prenylflavonoids are likely to produce these ring-scission products through similar metabolic pathways if they reach the lower digestive tract and are subject to attack by microbiota. Isoxanthohumol is converted to its demethylation product 8-prenylnaringenin through the action of microbiota [26]. Beer consumption can induce unexpected intake of 8-prenylnarigenin because xanthohumol (a major prenylflavonoid originating from the hop *Humulus lupulus*) can be converted to its ring-closed product, isoxanthohumol, during beer manufacture [27]. It is reported that 40% of healthy postmenopausal Caucasian women carry 8-prenylnaringenin producer [28]. 8-Prenylnaringenin has been suggested to exert strong estrogenic activity *in vivo* [29,30]. In fact, 8-prenylnaringenin (0.78–4.83 pmol/g), together with xanthohumol (0.26–5.14 pmol/g) and isoxanthohumol (1.16–83.67 pmol/g) accumulated in human breast tissues, respectively, when a hop supplement (2.04 mg xanthohumol, 1.20 mg isoxanthohumol, and 0.1 mg 8-prenylnaringenin) was administered orally for 5 days [31].

Phase I/II metabolism of prenylflavonoids

During intestinal absorption, dietary flavonoids are converted to their conjugated metabolites and transferred into the liver, where further phase-II metabolism can take place before passing into the blood circulation. Prenylflavonoids are also subject to this metabolic process for conversion to conjugated metabolites in rodents [32,33]. In addition, *O*-methylation happened in the catechol group of the B-ring of 8-prenylquercetin as similarly to respective quercetin [21]. Nevertheless, studies focusing on the