

REVIEW

Understanding metabolic conversions and molecular actions of flavonoids *in vivo* : toward new strategies for effective utilization of natural polyphenols in human health

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Abstract : Many papers have suggested the health-beneficial activity of natural dietary polyphenols to prevent chronic diseases and aging processes in humans. It is generally recognized that polyphenols are absorbed from the intestines and metabolized into the phase-II conjugates, *i.e.*, the glucuronides and sulfates. For example, a major dietary flavonoid, quercetin, abundant in onion and buckwheat, is metabolized after oral intake into its conjugates, such as quercetin-3-*O*-glucuronide and quercetin-3'-*O*-sulfate, whereas no aglycone was found in the human plasma. Therefore, to understand the mechanisms of the biological activity of quercetin *in vivo*, we should focus on the molecular actions of these conjugates. In the last decade, we have demonstrated the unique actions of quercetin-3-*O*-glucuronide at sites of inflammation, including specific accumulation in macrophages and the following deconjugation into active aglycone, catalyzed by the macrophage-derived β -glucuronidase. This review summarizes recent findings regarding the anti-inflammatory mechanisms of quercetin conjugates in macrophages and propose a possible strategy for the effective utilization of natural polyphenols in our daily diet for prevention of age-related chronic diseases. *J. Med. Invest.* 65 : 162-165, August, 2018

Keywords : polyphenol, flavonoid, glucuronide, inflammation, atherosclerosis

INTRODUCTION

Many people now recognize that naturally-occurring bioactive compounds, called phytochemicals, could play important roles in the maintenance of homeostasis and prevention of chronic human diseases. Among the various phytochemicals, polyphenols are the most popular family widely distributed in fruits, vegetables, beverages, and cereals. They are defined according to the nature of their backbone structures ; *i.e.*, phenolic acids, flavonoids, stilbenes and lignans. Flavonoids are the most abundant polyphenols in plant foods, and are further divided into several classes ; *i.e.*, flavones, flavonols, isoflavones, flavanols, anthocyanins, proanthocyanidins, flavanones, etc. Over eighty years ago, Rusznyák and Szent-Györgyi discovered that citrus flavonoids attenuated capillary fragility and permeability in blood vessels (1, 2). Flavonoids were first believed to be a member of a new class of vitamins, and were designated as vitamin P, but it has not been proven that flavonoids meet the requirements to be called a vitamin. Quercetin (3,3',4',5,7-pentahydroxyflavone) is a major flavonoid in our daily diet, and most of the quercetins in plants is in the glycoside form. The quercetin glycosides are particularly abundant in onion (0.3 mg/g fresh weight) (3), tea (10-25 mg/L) (4), and buckwheat (up to 3% dry weight as rutin) (5). Epidemiological studies suggested the tight links between the intake of flavonoid-rich diets and the decreased incidence of various chronic age-related diseases (6-11). However, the bioavailability of flavonoids is generally low due to limited absorption, extensive metabolism, and rapid excretion. Therefore, the molecular mechanisms underlying the health-beneficial actions of flavonoids *in vivo* have not been fully clarified.

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BIOAVAILABILITY OF DIETARY FLAVONOIDS

The bioavailability of quercetin and other polyphenols in humans, as well as in rodents, has been well investigated. The flavonoid glycosides, after oral intake, are rapidly hydrolyzed into the aglycones by lactase-phlorizin hydrolase or cytosolic β -glucosidase in the small intestine or by bacterial glycosidases in the colon. The aglycones are further metabolized through the phase II reactions into their glucuronides by UDP-glucuronosyltransferase and/or sulfates by sulfotransferase, and if they have the catechol moiety, some are also methylated by catechol-*O*-methyltransferase (COMT) (12, 13). In the case of quercetin, it should be noted that non-conjugated aglycones, including methylquercetins, are scarcely detected in the human plasma (14). For example, previous human volunteer studies with onion intake have clearly shown that the quercetin conjugates, such as quercetin-3-*O*-glucuronide (Q3GA) and quercetin-3'-*O*-sulfate (Q3'S), are detected in human plasma, while the aglycones could not be detected (14, 15). Quercetin diglucuronide, sulfoglucuronide (diconjugate with sulfate and glucuronide), and isorhamnetin (3'-methylquercetin)-3-*O*-glucuronide, as well as Q3GA and Q3'S, were also detected in human plasma after the oral intake of onion (16). The total quercetin concentrations in human plasma after onion intake are up to several μ M at maximum (15, 16). It is generally recognized that phase-II reactions produce more polar, hydrophilic, biologically inactive molecules that are more readily excreted into the urine. Indeed, the phase-II metabolism of flavonoids introduces hydrophilic functionalities, such as glucuronic acid and sulfuric acid, onto the relatively hydrophobic flavonoid structures and facilitates urinary excretion. Therefore, there have been controversies about how the inactive phase-II metabolites of flavonoids exhibit the health-beneficial activity *in vivo*.

DEVELOPMENT OF ANTIBODIES TO FLAVONOIDS

Thus, to resolve these controversies, we tried to identify the localization and target sites of the flavonoid conjugates *in vivo*. We speculated that there might be specific interactions of the flavonoid conjugates with the target sites during blood circulation. However, analytical methods for the localization of flavonoids in tissues and cells have been limited to chromatographies (such as liquid chromatography combined with a UV/visible spectrophotometer, electrochemical detector or mass spectrometer). To overcome the difficulties, we developed specific antibodies to the flavonoid conjugates to immunohistochemically visualize their localizations *in vivo*. We finally developed a monoclonal antibody, mAb14A2, by immunizing mice with the Q3GA-keyhole limpet hemocyanin complex. This antibody significantly recognized Q3GA, but neither the quercetin aglycone, the 3'-methylated metabolite isorhamnetin, nor a quercetin sulfate (17). This was the first report for the development of antibodies to the *in vivo* metabolites of flavonoids. Our laboratory has previously reported that orally administered quercetin accumulated as conjugates in the aorta of hypercholesterolemic rabbits with significant inhibition of the development of atherosclerotic lesions (18), suggesting that quercetin conjugates could play important roles regarding the anti-atherosclerotic effects in the aorta. We then immunohistochemically examined the localization of Q3GA in human aortic tissues using this novel antibody and found that the immune-positive staining was specifically detected in atherosclerotic lesions, mainly co-localized in the macrophage-derived foam cells, but not in the normal aorta (18). In contrast, the endothelial cells, smooth muscle cells, and extracellular matrix in the subendothelial layer, the so-called intima, were scarcely stained with the anti-Q3GA antibody. On the other hand, since we have previously revealed the presence of quercetin conjugates, associated with the anti-oxidative activity, in rat brain tissue by liquid chromatography-mass spectrometry (19), we then immunohistochemically examined the localization of Q3GA in the human brain and observed specific localization of Q3GA, especially in macrophages, at different stages of cerebral infarction (20). These results suggested that Q3GA could accumulate in macrophages, not only in the aorta, but also in the brain tissue. Thus, immunohistochemical application of a novel monoclonal antibody strongly supported the presence of Q3GA in specific sites, especially macrophages, *in vivo*.

In contrast to the observation that no quercetin aglycone was detected in human plasma, tea catechins, such as (-)-epicatechin-3-gallate (ECg) and (-)-epigallocatechin-3-gallate (EGCg), can be detected in human plasma as the aglycones after the intake of green tea (21). We have also developed a monoclonal antibody specific to the ECg aglycone and found the specific localization of the immunoreactivity in macrophages in human atherosclerotic lesions (22). These results suggest that macrophages could be the specific target not only of the conjugated metabolites, such as Q3GA, but also perhaps of the aglycones including ECg. The significant accumulation of Q3GA and ECg was also reproduced in cultured macrophages *in vitro*, resulting in suppression of the mRNA expression of scavenger receptors, scavenger receptor class-A (SR-A) and CD36 (17, 22). These results suggest that the interaction between flavonoids and macrophages might be crucial for the anti-atherosclerotic actions of flavonoids in the aorta.

INTERACTION BETWEEN MACROPHAGE AND QUERCETIN CONJUGATES

We further investigated in detail the interaction of Q3GA with macrophages *in vitro* (Figure 1) and demonstrated that Q3GA is bound to the cell surfaces on the macrophages (23). In contrast, the

quercetin aglycone could be incorporated into cells, rather than bound to the cell surface, presumably *via* simple diffusion due to its hydrophobicity. It was of interest that the quercetin and the methylated quercetins, not only Q3GA itself, were also significantly accumulated in macrophages during incubation with Q3GA (17, 20, 23). Intracellular accumulation of quercetin and methylquercetins in the Q3GA-treated macrophages reflects the presence of the enzymatic activities of β -glucuronidase and COMT in the cells. These non-conjugated aglycones were specifically detected in the macrophages after Q3GA-treatment, but not detected in the vascular endothelial cells (human umbilical vein endothelial cells and bovine aortic endothelial cells) and many other cell lines from different tissues (23). Based on the general information that the COMT activity is present inside the cells (24) and that Q3GA cannot be incorporated into the cells, we concluded that the β -glucuronidase-catalyzed deconjugation of Q3GA is the first step in the extracellular fluid or cell surface and the formed quercetin aglycone can enter the cells, followed by the COMT-catalyzed partial methylation of the catechol moiety. To the best of our knowledge, the possible demethylation of methylquercetins into quercetin has not yet been revealed *in vivo*.

We demonstrated that the anti-atherosclerotic and/or anti-inflammatory effects of Q3GA in activated macrophages are tightly linked with the β -glucuronidase-mediated deconjugation (17, 20, 23). It is generally recognized that quercetin with a catechol moiety is more bioactive (typically as an antioxidant) than the methylquercetins. However, we observed that the COMT activity enhanced the inhibitory effects of Q3GA on the SR-A expression in macrophages (17), suggesting that partial methylation of quercetin by COMT could provide alternative bioactive compounds, as well as quercetin, inside the cells. Although the possibility as to whether the glucuronides of other polyphenols could also be deconjugated into their aglycones remains unproven, these results suggest that β -glucuronidase is a key enzyme for the "reactivation" of inactive flavonoid conjugates *in vivo*. The *in vivo* expression of β -glucuronidase is also immunohistochemically demonstrated in the foamy macrophages in the atherosclerotic aorta of the apolipoprotein E-deficient mice (25), showing the tight link between the pro-atherogenic conditions and the deconjugation-mediated anti-atherosclerotic of flavonoids *in vivo*. We also demonstrated that the deconjugation of Q3GA was enhanced in the lipopolysaccharide-stimulated macrophages (23). In addition, a significant expression of β -glucuronidase in ICR mice was observed in the thymus, liver and spleen, which may contain inflammatory cells, such as lymphocytes, kupffer cells (in liver), and/or macrophages (23). Therefore, tissues with β -glucuronidase activity could be the potential sites where the deconjugation of the flavonoid glucuronides and the following actions of the aglycones could occur *in vivo*.

It should be noted that, as far as we investigated, the sulfate metabolites of quercetin could not be deconjugated during interaction with the macrophages at least under our experimental conditions (23). Galindo *et al.* reported that the intravenous injection of Q3GA or isorhamnetin-3-*O*-glucuronide, but not Q3'S, progressively reduced the mean blood pressure in spontaneously hypertensive rats, and furthermore, that the hypotensive effect of Q3GA was abolished in rats treated with the β -glucuronidase inhibitor (26). These observations suggest that the β -glucuronidase-mediated deconjugation of glucuronide metabolites, rather than the sulfates, might represent a major pathway for exhibiting the biological activity *in vivo* after the quercetin intake. On the other hand, the biological activity linked with sulfate metabolites has also been suggested by the observations that the sulfate metabolites of resveratrol, a stilbene found in grape seed, induced autophagy and senescence in human cancer cells and these effects were abrogated by a sulfatase inhibitor (27). However, the presence and

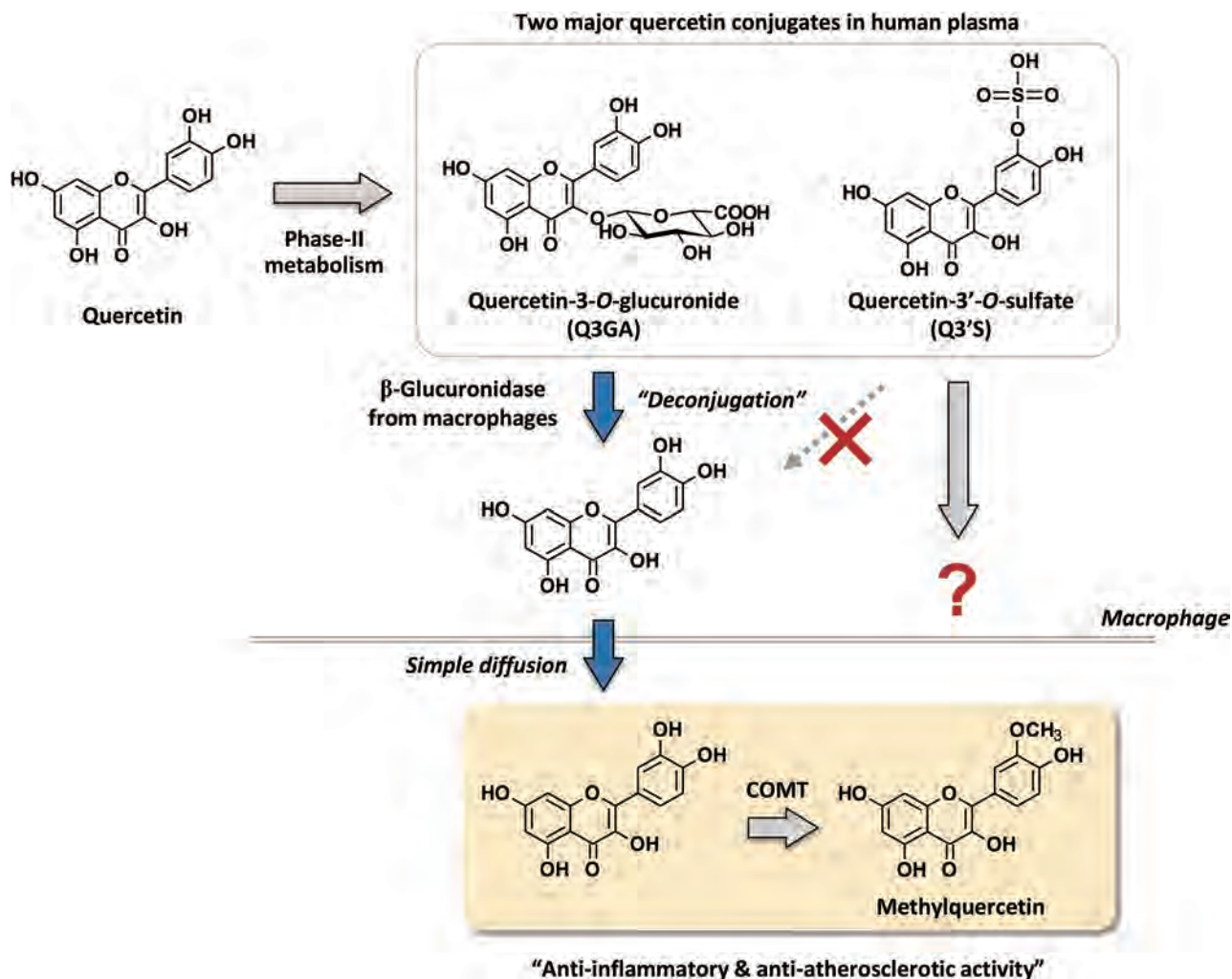


Figure 1 Metabolic conversions of quercetin during oral intake, absorption, and interaction with macrophages.

localization of sulfatase for deconjugating sulfate metabolites have not yet been fully characterized. In human plasma, Q3'S is more abundant than Q3GA after the oral intake of quercetin (16). Therefore, possible regulation of the sulfation/glucuronidation ratio of the flavonoids by coexisting food components might optimize the biological activity of the flavonoids *in vivo*. Application of natural sulfotransferase inhibitors (28) to our daily diet will have the possibility to enrich the glucuronidation of flavonoids resulting in the enhanced biological activity through the β -glucuronidase-mediated deconjugation.

COI

We have no conflict of interest to declare

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