Review Article

Vegetable flavonoids and cardiovascular disease

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Studies have suggested that dietary flavonoids are helpful in the prevention of atherosclerosis and cardiovascular disease. Antioxidant activity should be noted as underlying mechanism of their health impact in the vascular system, as atherosclerosis is closely related to oxidative events such as oxidized LDL accumulation in the macrophages. Vegetables contain a variety of flavonoids, such as flavonols, flavones and anthocyanidins. We focused on quercetin (3,3',4',5,7- pentahydroxyflavone), a major flavonoid in onion, and its anti-atherosclerotic effect was examined from the aspect of the bioavailability and translocation to the target site. Although quercetin exists as its glucoside form in onion, it is metabolized into several glucuronides and/or sulfate conjugates with or without methylation during its intestinal absorption. We found that these metabolites circulating in the human blood stream were mostly localized in plasma albumin fraction, *but not* LDL fraction. Onion consumption failed to enhance the antioxidant activity of plasma fraction against LDL oxidation, indicating that the level of quercetin metabolites bound to albumin is insufficient to exert the antioxidative effect *in vivo*. In contrast, we discovered that quercetin metabolites accumulate in the aorta tissue and exerted their antioxidant activity, when rabbits were fed with quercetin glucoside and high cholesterol diet. Furthermore, quercetin metabolites were detected in human atherosclerotic aorta exclusively. These imply that quercetin metabolites are incorporated into the atherosclerotic region and act as complementary antioxidants, when oxidative stress is loaded in the vascular system. It is likely that plasma albumin is a carrier for translocation of quercetin metabolites to vascular target.

Key Words: atherosclerosis, quercetin, onion, antioxidative effect, LDL oxidation, aorta tissue

INTRODUCTION

Physiological function of plant polyphenols has attracted much attention in relation to the prevention of vascular diseases. Flavonoids containing diphenylpropane structure are major polyphenols present in vegetables and fruits. In 1936, Szent-Gyoigi[1] first claimed that citrus flavonoids (hesperidin ad rutin) reduced capillary fragility and permeability in human blood vessel, as similarly to vitamin C. Thereafter, a lot of works have been carried out on pharmacological function of flavonoids isolated from medicinal and edible plants. In 1993, Hertog *et al*[2] found that flavonoid intake was inversely correlated with cardiovascular heart disease (CHD) mortality in elderly men. Nowadays, epidemiological studies strongly suggest that the intake of flavonoids from diet is helpful in the prevention of atherosclerosis and its related events including CHD. However, the molecular mechanism for their anti-atherosclerotic action and absorption mechanism related to their bioavailability should be clarified for practical use of dietary flavonoids in lowering the risk of atherosclerosis. Our research group are focusing on quercetin, a typical and major flavonoid present in vegetables, and investigating the mechanism of intestinal absorption as well as metabolic conversion, distribution of their metabolites in blood plasma and their antioxidant activity. In addition, we have indicated that their metabolites apparently accumulate in the aorta tissue, the site of atherosclerosis. Some of these studies were already accomplished and reported elsewhere.[3-6] Here we summarise these results and review a role of vegetable flavonoids on the prevention of atherosclerosis from the viewpoint of bioavailability and molecular mechanism for exerting anti-atherosclerotic action in the target site.

CHARACTERIZATION OF VEGETABLE FLAVONOIDS

A wide variety of flavonoids are distributed in vegetables. In general, flavonoids are categorized as flavone, flavonol, flavanone, flavanol, anthcyanin, chalcone and isoflavone, depending on the C_3 structure in C_6-C_3-C_6 carbon skeleton. One of the major subgroups ubiquitously occurring in vegetables is flavonol-type flavonoids including kaempferol, quercetin, and myricetin. Quercetin and other flavonoids are present in vegetable as their glycosides. Onion is a typical vegetable rich in quercetin. Interestingly, quercetin 4'-glusoside (Q4'G) and quercetin 3,4'-diglucoside (Q3',4'diG) are exclusively present in onion, whereas quercetin 3-glucoside (isoquercitrin) and quercetin-3-rutinoside (rutin) are predominant glycosides in common vegetables.

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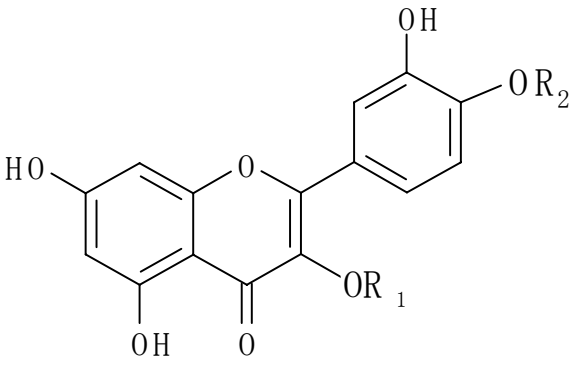

Figure 1. Structures of quercetin glycosides present in vegetables. R_1=H, R_2=H quercetin; R_1=β-*D*-glucose, R_2=H isoquercitrin; R_1=β-*D*-rutinose, R_2= H Rutin; R_1=H, R_2=β-*D*-glucose G4'G; R_1=R_2=β-*D*-glucose Q3,4'-diG.

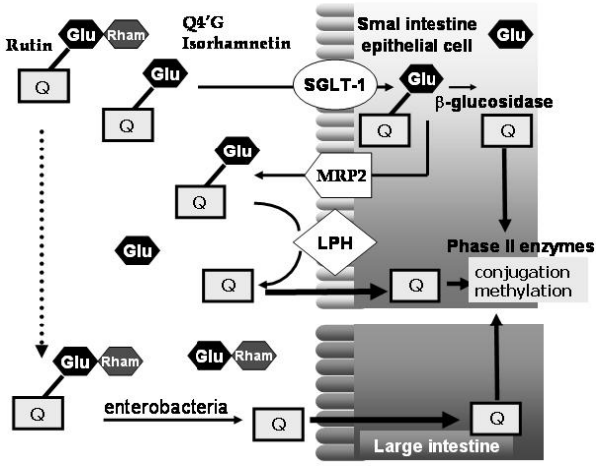

Figure 2. Intestinal absorption of quercetin glycosides. Q: quercetin aglycon, Glu: glucose, Glu-Rham: rutinose, SGLT-1: Na-dependent glucose transporter, MRP2 multidrug resistance-associated protein-2, LPH lactose phlorizin hydrolase

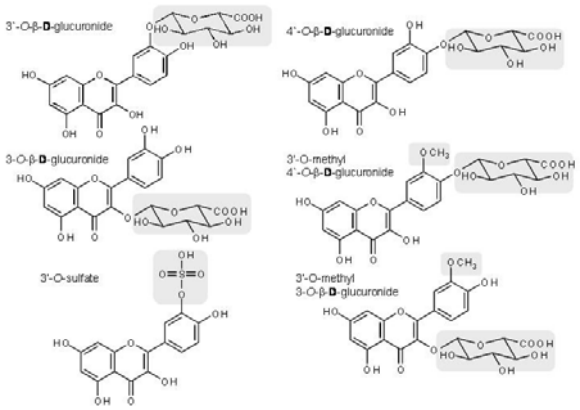

Figure 3. Structures of quercetin metabolites found in human plasma[8]

INTESTINAL ABSORPTION AND METABOLISM[7]

It has long been suggested that dietary flavonoids are scarcely absorbed into the body. However, recent studies demonstrated that a variety of flavonoids are capable of being absorbed from the digestive tract with or without metabolic conversion, although the site of their absorption, absorption rate and absorption mechanism are different from each other depending on their inherent structures. In the case of quercetin glycosides, rutin and other glycosides containing disaccharide or oligosaccharide are absorbed at the lower part of the digestive tract (large intestine) as aglycons through deglycosidation by enterobacteria. On the other hand, quercetin monoglucosides such as isoquercitrin and Q4'G are absorbed at the upper part (small intestine) after enzymatic hydrolysis with β-glucosidase and/or lactose phlorizin hydrolase (LPH) occurring at intestinal mucosa. A part of quercetin monoglucosides may be absorbed via sodium-dependent glucose transporter-1 (SGLT-1) pathway. In either case, quercetin is converted into its conjugated metabolites or their methylated derivatives by the action of phase II enzymes. Although these metabolites are at least excreted into the digestive tract via multidrug-resistance-associated protein 2 (MRP-2), these are also transported into the liver through portal vein and subject to secondary metabolism. Recently we have demonstrated that orally administered quercetin is partly absorbed into the body via lymph pathway.[4] Thus, fat-soluble components coexisting in the diet seem to affect the efficacy of quercetin absorption.

DISTRIBUTION OF QUERCETIN METABOLITES IN HUMAN PLASMA AND THEIR ANTIOXIDANT ACTIVITY

Oxidative modification of low-density lipoprotein (LDL) is believed to be an initial step for foam cell formation leading to atherosclerosis. Therefore antioxidants involving those from dietary origin are strongly expected to act as effective anti-atherosclerotic agents. A variety of quercetin metabolites are known to be present in the circulation when quercetin-rich diet is supplied into the body. Fig.3 shows the structures of quercetin metabolites found in human plasma.[8] We have previously shown that one of these metabolites, quercetin-3-*O*-β-glucuronide (Q3GA) possesses a considerable antioxidant activity and is capable of inhibiting cupper ion-induced LDL oxidation.[9] We recently confirmed that more than 80% of quercetin metabolites, which are localized in the fraction that contains concentrated serum albumin and albumin-bound Q3GA, exerts the antioxidant effect against LDL oxidation.[6] However, our human-intervention study failed to demonstrate that the intake of quercetin-rich onion enhances the antioxidant activity of albumin fraction because of their insufficient supply into the circulation. On the other hand, our preliminary results indicate that onion consumption elevates the resistance of HDL against reactive oxygen species (ROS). Thus, HDL may instead, be a target for the antioxidant activity of quercetin metabolites in plasma compartment.

ACCUMULATION OF QUERCETIN METABOLITES IN THE AORTA

An ultimate target for dietary quercetin as anti-atherosclerotic agent is undoubtedly the blood aorta where plaque formation happens in relation to atherosclerotic injury.However, work on the accumulation of dietary flavonoids in this target site is scarce. We designed

Table 1. Accumulation of lipid peroxidation products and quercetin metabolites in the aorta of high cholesterol – loaded rabbit

Diet	TBARS (nmol/g protein)	ChE-OOH (nmol/mol ChE)	Quercetin metabolites (nmol/mg protein)
Control diet	0.29 ± 0.08[a]	0.13± 0.09[a]	ND
High-cholesterol diet	2.02 ± 0.64[b]	1.85± 0.54[b]	ND
High-cholesterol + quercetin diet	0.72 ± 0.38[a]	0.47± 0.13[a]	0.70±0.20

Data are cited from ref 3. RC-4 rabbit chaw with 2.0% (w/w) cholesterol with or without 0.1% (w/w) quercetin-3-glucoside were fed to NZW rabbits for one month. All values are mean ± SD (n=4). Values with different superscripts ($^{a–c}$) are significantly different (p<0.05).

an experiment using high cholesterol-fed rabbits to examine whether or not dietary quercetin actually accumulates in the aorta tissue and exerts antioxidant activity.[3] By HPLC and HPLC-MS analyses, we detected quercetin metabolites in the aorta tissue of quercetin glucoside-fed rabbits as shown in Table 1. TBARS contents and the cholesteryl ester hydroperoxides (ChE-OOH) level in the aorta tissue were also suppressed by the administration of quercetin glucosides, indicating that quercetin metabolites exert an antioxidant activity in cholesterol-rich aorta. Hypercholesterolemia is known to increase endothelial superoxide production via xanthin oxidase (XOD).[10] Some quercetin metabolites other than Q3GA possess XOD inhibiting activity.[11] It is therefore likely that high cholesterol diet induces oxidative stress in the blood vessel and quercetin metabolites act as a member of antioxidant network for preventing ROS-induced injury directly or indirectly. A question is raised on the translocation of quercetin metabolites from the plasma albumin fraction to the aorta tissue. Shimoi *et al*[12] suggested that flavonoid aglycons are released from their metabolites by enhanced β-glucuronidase activity during inflammation. There is a possibility that quercetin performs as aglycon form in the target site. We recently prepared novel monoclonal antibody which recognizes quercetin glucuronide specifically and succeeded in detecting quercetin glucuronides in human atherosclerotic aorta by immunohistochemical approach. This implies that dietary quercetin at least partly accumulates in the atherosclerotic site as glucuronide metabolites. The significance of the accumulation of quercetin metabolites in the target site should be further clarified to assess the efficacy of dietary quercetin and its containing vegetables as dietary anti-atherosclerotic factors.

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AUTHOR DISCLOSURES

Junji Terao, Yoshichika Kawai and Kaeko Murota, no conflicts of interest.

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