1. Introduction

Bioflavonoids are polyphenolic compounds that are ubiquitously present in foods of plant origin (1). These constituents of the diet are believed to be important in the maintenance of health, especially to maintain the integrity of the cardiovascular system. The “French paradox” refers to the correlation of a high-fat and high-cholesterol diet with a lower incidence of coronary heart disease found in Mediterranean cultures contrasted with a higher incidence of coronary heart disease among most Western cultures (2). It has been shown that the French paradox may be attributable to regular consumption of red wine and that the unique antiatherogenic effects of red wine reside in the action of polyphenols (3). Moreover, it was demonstrated that the DASH (Dietary Approaches to Stop Hypertension) clinical trial diet that was rich in fruit, vegetables, and low-fat dairy products had a significant hypotensive effect (4). Recently the Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009) provides lifestyle modifications including increased intake of fruits and vegetables.
to prevent cardiovascular disease and risk factors (5). Quercetin (3,3′,4,5,7-pentahydroxyflavone), a member of the bioflavonoids family, is one of the most widely distributed dietary polyphenolic compounds in foods including vegetables, fruits, tea, and wine (6). Like other members of the bioflavonoids, quercetin has been shown to have biological properties consistent with its sparing effect on the cardiovascular system. It has been shown that quercetin possesses, anti-atherogenic, anti-inflammatory, anti-coagulative, and anti-hypertensive properties (6–8). In addition, within the bioflavonoid family, quercetin is the most potent scavenger of reactive oxygen species (ROS) (9). The anti-oxidant activity of quercetin has frequently been mentioned in connection with its pharmacological function in the cardiovascular system (10) because oxidative modification of plasma low-density lipoprotein (LDL) was suggested to be involved in the initial event of atherosclerosis, leading to coronary heart disease (11). In this review, we have summarized mainly our data regarding the pharmacological effects of quercetin, especially its metabolites, on cardiovascular diseases.

2. Metabolism of quercetin in vivo

Quercetin is a prime example of such a flavonoid and is bound to sugars in foods, mainly as β-glycosides. Quercetin is mostly present in the form of glycosides in vegetables and fruits, and dietary glycosides were believed to be converted to the respective aglycones in the large intestine by the glycosidase activity of intestinal bacteria (12). However, it was recently demonstrated that quercetin 3-O-β-D-glucuronide (Q3GA) and quercetin-3′-sulfate are the major quercetin conjugates in human plasma, in which aglycone could not be detected (13–15). Upon ingestion with the diet, quercetin glycosides are rapidly hydrolyzed during passage across the small intestine or by bacterial activity in the colon to generate quercetin aglycone, which is further metabolized in the so-called phase II reactions into the glucuronidated and/or sulfated derivatives (Fig. 1). Studies using rodents have also shown that orally administered quercetin is converted to its conjugates before accumulation in plasma (16, 17). In addition, it was reported that volunteer study clarified that conjugated metabolites of quercetin accumulate in human plasma in the concentration range of 10^{-7}–10^{-6} M after the periodic ingestion of onions with meals for 1 week (14). Therefore the pharmacological function of dietary quercetin, including antioxidant activity, should be exerted exclusively by its conjugated metabolites. Although most of the in vitro pharmacological studies have been carried out using only the quercetin aglycone form, experiments using Q3GA would be important to discover the mechanisms through which quercetin exerts its preventative effects on cardiovascular diseases in vivo. Therefore we examined the effects of the chemically synthesized Q3GA, as an in vivo form, on vascular smooth muscle cell (VSMC) disorders related to the progression of cardiovascular diseases.

3. The effects of Q3GA on migration, proliferation and hypertrophy in VSMCs

The primary cause of many fatal cardiovascular diseases is believed to be atherosclerosis (18). During atherogenesis and the progression of the disease, chronic inflammatory responses induce vascular wall remodeling or the generation of neointima and thickening of the tunica media, which leads to the development of plaque and artery stenosis (19). The neointima and thickened media are primarily composed of abnormally proliferating and migrating VSMCs (20–23). Therefore, we examined the effect of chemically synthesized Q3GA, as an in vivo form, on platelet-derived growth factor (PDGF)-induced cell migration in VSMCs. PDGF has been recognized as a major mitogen and one of the most important growth factors, and it also stimulates VSMC migration (24, 25). It has been reported that PDGF-induced VSMC
migration and proliferation are mediated by mitogen-activated protein (MAP) kinases, phosphatidylinositol 3-kinase (PI3-kinase)/Akt, and many other kinases (26–28). MAP kinases are protein kinases and play an important role in cell differentiation, growth, apoptosis, and the regulation of a variety of transcription factors and gene expressions (29, 30). The activation of these MAP kinase pathways has been shown to be involved in the promotion of VSMC proliferation, migration, and hypertrophy that relates to vascular remodeling and altered tone in hypertension. Q3GA pretreatment significantly suppressed PDGF (10 ng/ml, 6 h)-induced VSMC migration in a concentration-dependent manner (10 and 100 µM) (31). Also Q3GA significantly attenuated PDGF (10 ng/ml, 48 h)-induced rat aortic smooth muscle cell (RASMC) proliferation (10 and 100 µM) (31). It has been reported that PDGF-induced VSMC migration and proliferation mediated by MAP kinases and the PI3-kinase/Akt pathway. PDGF-induced c-Jun N-terminal kinase (JNK) and Akt activations were inhibited by Q3GA in a concentration-dependent manner (10 and 100 µM). In contrast, ERK1/2 and p38 MAP kinase activations were not influenced by Q3GA. These results suggested that JNK and Akt, but not extracellular signal–regulated kinase (ERK) 1/2 and p38 MAP kinase, were specifically sensitive to Q3GA in VSMCs. In this study, we used Q3GA at 1, 10, and 100 µM. Previously, in vitro studies using cultured cells required relatively higher concentrations (>>µM) of the flavonoid metabolites to exert their anti-atherosclerotic effects (32, 33). It has been shown that Q3GA is specifically localized in the arteries with atherosclerotic lesions. Thus, our results suggest that the prevention of cardiovascular diseases relevant to VSMC hypertrophy.

4. Localization of Q3GA in the atherosclerotic aorta

The endothelial injury, activation, or dysfunction is an early event during the development of atherosclerosis (42). A previous study showed that the cholesterol accumulation in the aorta of hypercholesterolemic rabbits was decreased by orally administered quercetin glucoside (43). It was also demonstrated that quercetin metabolites were detected in the atherosclerotic aorta using high performance liquid chromatography analysis (43). Moreover, it was reported that Q3GA is capable of inhibiting LDL oxidation in vitro (44, 45). To examine the localization of Q3GA associated with the anti-atherosclerotic effects in the aorta, human aortic tissues were immunohistochemically examined using monoclonal antibody mA14A2, a novel monoclonal antibody targeting the Q3GA (33). This investigation was carried out on aortic wall samples obtained during autopsy from patients with generalized arteriosclerosis. Immunohistochemical studies with mA14A2 demonstrated that the positive staining specifically accumulates in human atherosclerotic lesions, but not in the normal aorta (33). Increased permeability of endothelial cells with reduced barrier function has been observed during endothelial injury (46). These observations suggest that Q3GA in circulating blood can permeate through the injured endothelial cells in human atherosclerotic lesions. Thus, our results suggest that the Q3GA is specifically localized in the arteries with ath-
erosclerotic lesions.

5. Conclusions

In conclusion, we demonstrated that Q3GA, an active metabolite of quercetin, specifically inhibited PDGF-induced JNK and Akt activations and resultant cell migration and proliferation in VSMCs. Also we clarified that the preventing effect of Q3GA on Ang II–induced VSMC hypertrophy is attributable in part to its inhibitory effect on JNK and the AP-1 signaling pathway. It is suggested that the inhibition of these pathways by Q3GA may imply its usefulness for cardiovascular diseases in which VSMC growth may be involved. Although most of the in vitro pharmacological studies have been carried out using only the quercetin aglycone form, experiments with the in vivo form of quercetin would be important to elucidate the efficacy of orally administered antioxidants including quercetin. Considering the above results, Q3GA may possess preventing effects for cardiovascular diseases relevant to VSMC disorders. These findings of our study may shed light on the pharmacological basis for oral administration of bioflavonoids in cardiovascular diseases.

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