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A Review of Flavonoid Quercetin: Metabolism, Bioactivity and Antioxidant Properties

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Abstract: Flavonoids belong to a category of polyphenolic compounds that are produced exclusively in plants. These compounds are able to bring about various biological and pharmacological activities in animal cells. It is generally believed that most mammals need to consume polyphenols from plants due to their antioxidant properties in order to stay healthy. The antioxidants are essential for protecting the cells from undergoing chemical damage due to oxidation. Many compounds are known to have both antioxidant and pro-oxidant properties. The pro-oxidant properties can either be favourable or detrimental to biological systems and can affect the progress of chronic diseases in human beings. Quercetin, the most plentiful flavonoid, acts as both an antioxidant and a pro-oxidant. Currently, there is no reliable method for maintaining a balance between these two activities in the whole organism. Therefore, this review focuses onMetabolism, bioactivity and both the antioxidant and pro-oxidant effects of flavonoids.
Keywords: Quercetin, Nrf2, Phase II enzyme, Antioxidant.

Introduction

Generally, studies on flavonoids are inclined to focus on quercetin (QUR)[1]. QUR is widely available and easy to extract, isolate and detect [2].Commonly found in vegetables and fruits in the form of a glycoside (with an exceptionally high concentration in onions, apples, tea, broccoli and red wine), a quercetin is in every sense a polyphenol (3, 3', 4', 5, 7-pentahydroxyflavone)(chemical structure and natural sources shown in Fig. 1).

Recent evidence indicates that QUR has multiple beneficial effects[3,4].Among the many attributes of QUR are its antioxidative[5-10]; anti-inflammation [11] and anti-apoptosis[12, 13]; qualities. Furthermore, there is reason to believe that QUR can shield the liver from damage brought about by hepatoxins[6, 14-16].

Metabolism and bioactivity of quercetin

Once QUR is absorbed in the gastrointestinal tract, it is processed by Phase II enzymes in the epithelial cells of the stomach and intestines. The combined metabolites are then processed further in the liver and kidney [17, 18]. The B-ring catechol structure undergoes methylation at the 3′or 4′ hydroxyl site by catechol-O-methyl transferase (COMT) to form isorhamnetin and tamarixetin, respectively[19]. QURmetabolites even seem to build up in tissues shortly after quercetin-rich vegetables are eaten[20]. In vitro studies have shown that QUR metabolites, that are produced in enterocytes and the liver, function as antioxidants by raising the resistance of low-density lipoprotein (LDL) cholesterol to oxidation[20].

It is assumed that the bioactivity of QUR is due to its metabolization from various naturally occurring conjugated isoforms in the intestines and/or liver that are absorbed and extensively distributed in animal tissues[18, 21, 22]. However, the use of QUR as a healing agent has been impeded by its poor water solubility, short biological half-life, and low oral bioavailability[23,24]. Although it has been reported that increased solubility will considerably improve both the bioavailability and the clinical impact of QUR[23], its relatively high molecular weight together with its high melting point and poor water-solubility still pose a major challenge[25]. Since QUR glycosides are metabolised into aglycone and absorbed in the intestinal tract[26, 27], perhaps an innovative delivery method could be developed to indirectly improve the solubility of QUR[28].

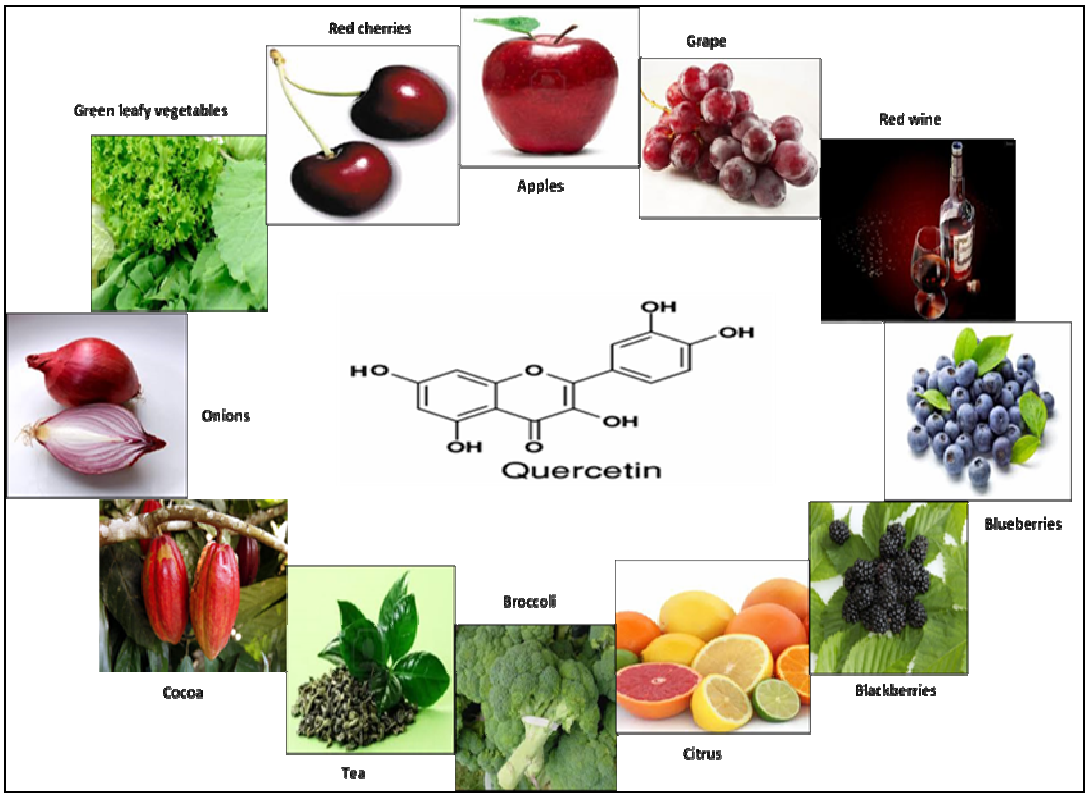

Figure1: Chemical structure and natural sources of quercetin

Structural Criteria for the Antioxidant Action of quercetin

Flavonoid antioxidants limit the negative effects of free radicals through the quick transfer of hydrogen atoms to the radicals. In a recent appraisal by [29], it was observed that many studies on the antioxidant activity of flavonoids have endeavoured to ascertain the relationship between the structure of flavonoids and their tendency for radical-scavenging[30-33].

QUR, owing to its specific chemical structure, counteracts oxidative stress generated as a result of reactive oxygen species (ROS), which contributes to the genesis of atherosclerosis, diabetes, ischemic heart disease, heart failure, and hypertension [34-36]. Flavonoids which have a 3-OH and 3`,4`-catechol are known to be ten times more potent towards peroxynitrite than ebselen, a renowned RNS scavenger [37].

The predominance of QUR in preventing both metal and non-metal-induced oxidative damage is partly attributed to its free 3-OH substituent [38, 39], which is believed to increase the stability of the flavonoid radical. The catechol group is also directly connected to the chelating action of QUR, as has been proven by various studies in which quercetin suppresses lipid peroxidation by the scavenging action of its free radical.

QUR is considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions, while at the same time undergoing an oxidation process to generate a semiquinone radical. The labile semiquinone radical goes through another oxidation reaction to cause the formation of a quercetinquinone. Quinone reacts intensely with protein thiols and it is eradicated by glutathione, which, in turn, reduces its level [40]. Compared to the related aglycons, the glycosylation of flavonoids diminishes their*in vitro* antioxidant activity[41-45]. The glygosylation of quercetin also considerably decreases its superoxide

scavenging ability[46], hypochlorite scavenging activity[47] and power to reduce Fe (III) to Fe[48] [determined by FRAP assay [49]].

Antioxidant and pro-oxidant properties of quercetin

QUR can reduce inflammation by scavenging free radicals. Free radicals can activate transcription factors that generate pro-inflammatory cytokines, which are often found elevated in patients that suffer from chronic inflammatory diseases[50].

QURcan also protect against the more apparent activities that contribute to the presence of free radicals in the environment, such as smoking. It has been discovered that free radicals, which come from cigarette tar, destroy the membranes of red blood cells. According to Begum and Terao[51]the quercetin aglycone and its conjugate metabolites (Quercetin -3-O-β-glucuronide and Quercetin -3-O- β -glucoside) are able to prevent damage to the membranes of red blood cells due to smoking.A superoxide is both a weak oxidant and a weak reducing agent that reacts with very few biological molecules by comparison[52]. A superoxide is often produced in large quantities when organic radicals, such as semiquinones, react with oxygen in a process known as a redox cycling [53].In simple terms, superoxides react with other radicals to generate non-radical products [54].

However, certain findings refute the antioxidant properties of QUR, and suggest that QUR also scavenges nitric oxide (NO) while producing superoxide anions according to physiological conditions such as pH, $O2^-$concentration and superoxide anion concentration[55, 56].QUR has been proven to be a better scavenger of $O2^-$ than of NO under conditions of increased $O2^-$in the smooth muscles of blood vessels [57].QURbehaves as a protective agent in the corpus cavernosumof mice by increasing the bioavailability of exogenous NO by shielding it from superoxide anions[58].

It has been proven that at a concentration of 0.2 –1 mM, QUR scavenges super oxide anions, singlet oxygen and lipid peroxyl radicals [59] and at the same time suppresses Cu-catalysed oxidation and cytotoxicity of low density lipoproteins (LDL) in vitro[60].

It has been discovered that flavonoids suppress the expression of inducible nitric oxide synthase (NOS) without suppressing its activity. Quite a few flavonoids, including QUR, bring about a reduction of ischemia-reperfusion injury by restricting the activity of inducible NOS [61].

Cell membranes and low-density lipoproteins (LDL) in humans contain a primary antioxidant known as α-tocopherol, which shields lipoprotein particles from the harmful effects of oxidation. Liu, Liao, Yin, Li, Liu, Li, Huang, Yang, He [62]stated that flavonoids can contribute hydrogen atoms to the α-tocopheryl radical, which is a potential pro-oxidant, and by their interaction the oxidation of LDL can be slowed down tremendously. Flavonoid QUR shows varying degrees of protection against the reduction of α-tocopherol in LDL [63].

Cellular studies have shown that QUR can generate both antioxidant and pro-oxidant effects according to its concentration[64]. It has been proposed that cellular oxidative balance and GSH (Glutathione) content play a crucial role in these effects. GSH is a tripeptide consisting of glutamate, cysteine, and glycine, and the antioxidant and conjugation properties of GSH are derived from the sulfhydryl moiety of the cysteine deposit. GSH is able to directly hunt down the cellular ROS in a non-enzymatic manner and is also able to function as a co-factor for GSH peroxidase (GPx) in the reduction of H_2O_2 and other peroxide types. GSH can also be employed in reactions involving disulphide exchanges to form mixed protein-glutathione disulphides, and the direct post-translational conversion of proteins via the glutathionylation of the protein sulfhydryl groups is being recognized as a significant signal transduction mechanism for controlling various cellular processes[65-67].

Glutathione is also known to promote damage to DNA through QUR[68]. Spencer, Kuhnle, Williams, Rice-Evans [69]demonstrated that QUR concentrations of up to 10 μM provided a defence against fibroblast damage stimulated by oxidative stress, and that following pre-treatment with QUR concentrations of 30μM, cytotoxicity occurred instead. It has also been reported that low concentrations of quercetin (1–10 μM) have been successful in curbing oxidative DNA damage in a concentration-dependent way for the in vitro pre-treatment of human lymphocytes [70].

When rat hepatoma cells were treated with QUR at concentrations of 5–100 μM, the activities of enzymatic antioxidants like manganese superoxide dismutase, glutathione peroxidase and the mRNA expression levels of copper zinc superoxide dismutase were reduced by 30–40% [71]. In a recent study, Cao, Liu,

Tuo, Shen, Chan[72] found that QUR increased cell viability and mitochondrial function of H_2O_2-exposed ARPE-19 cells by over 100%.

Many drugs and naturally occurring chemopreventive phytochemicals are known to increase the transcription of these phase II genes in cell culture and animal models[73]. Flavonoids have the ability to stimulate phase II detoxifying enzymes such as NAD (P) H-quinoneoxidoreductase, glutathione S-transferase and UDP-glucuronosyltransferase. These enzymes are positioned at the forefront in the defence against electrophilic toxicants and oxidative stress.

On the biological level, these agents share the ability to activate expression of a battery of genes that are regulated, in part, by the presence of cis-acting DNA sequences referred to as the antioxidant response elements (ARE)[74, 75].

Nrf2-mediated ARE activation has become a target for chemoprevention since Nrf2 binds to ARE located in the promoter region of many phase II detoxifying or antioxidant enzymes such as NQO1, GSR,HO-1,SRXN1,GCLC/M, AKR1C2 and TXNRD1[76].Thus, the stimulation of these cell-protecting enzymes by way of Nrf2-ARE monitoring is an effective way of ensuring that cells are protected from a range of electrophilic cancer causing agents and other reactive poisons that are produced by humans,as well as ROS[77]. Studies have shown recently that it is important to activate this pathway in order to prevent human diseases, such as cancer, neurodegenerative disease, diabetes, pulmonary fibrosis, cardiovascular diseases, ischemia, and inflammatory diseases [78].

NAD(P)H: quinoneoxidoreductase 1 (NQO1) is acknowledged as a flavoprotein that is able to accelerate the metabolic detoxification of quinones and safeguard cells from fruitless redox cycling, oxidative stress and neoplasia. The NQO1 agent is stimulated by QUR through the ARE/EpRE-dependent mechanisms[79].

In an experiment to examine the impact of QUR on the expression and enzymatic activity of NQO1 in the MCF-7 human breast carcinoma cells, it was discovered that when these cells were treated for 24 hours with 15 μM of quercetin, the NQO1 protein levels and enzyme activity doubled, and the NQO1 mRNA expression increased by three to four times [79]. This research group also observed a similar increase in NQO1 protein levels after treatment with 25 μM of quercetin for 24 hours [80]. The increase in the transcription of NQO1 as a result of the reaction to quercetin indicates that nutritive plant polyphenols can promote the transcription of phase II detoxifying systems, possibly through an ARE-dependent mechanism [79].

However, Boots, Bast, Haenen[81] reported that NQO1 is unable to provide any protection against QUR. On the whole, there have been contradictory reports thus far regarding the connection between NQO1 and quercetin. And in hepatocellular carcinoma HepG2 cells, quercetin activated Nrf2–ARE-mediated NQO1 transactivation by way of enhancing the level of Nrf2 at both transcriptional and posttranslational levels, and meanwhile reducing the level of Keap1 through 26S proteasome independent degradation [48]. Therefore, both Nrf2 up-regulation and Keap1 down-regulation are essential for ARE-mediated NQO1 activation induced by quercetin.

QUR raises the production of γ-glutamylcysteinesynthetase (GCS), which is a rate limiting enzyme, in the synthesis of the vital endogenous cellular antioxidant, GSH, which is needed for various cell functions and delivers bio-reducing equivalents for numerous cellular reactions. In the case of COS-1 cells, 5–25μM QUR enhanced the mRNA of GCL and induced a reporter gene controlled by GCL promoter fragments with at least one EpRE [82]. Nagata, Takekoshi, Takagi, Honma, Watanabe [83]examined the ability of QUR to protect cells against hydrogen peroxide cytotoxicity in cultured rat BL-9 hepatocytes, which are cells that produce a high level of cytosolic glutathione peroxidase (GPx).

The microsomal enzyme, Hemeoxygenase (HO-1), accelerates the rate limiting procedure in the breakdown of the heme into biliverdin, carbon monoxide (CO), and free iron[50]. It can be induced by a variety of oxidativeinducing agents, including H_2O_2. Recently, numerous *invitro* and *in vivo* studies have shown that induction of HO-1 is an important cellular protective mechanism against oxidative injury [84-86].

QUR may increase the response to the production of HO-1 through the introduction of Nfr2 nuclear translocation, which attaches to the antioxidant response element [13] in the promoter region of the HO-1 gene and various other phase II detoxifying enzymes (Tanigawa et al., 2007). HO-1 induction by QUR is facilitated particularly by way of the MAPK transduction pathway (Yao et al., 2007).

QUR (50µM) explicitly displayed a protective attitude towards RAW264.7 macrophages against H_20_2-induced apoptosis by upregulating Nrf2-directed enzymes including HO-1[87]. QUR also raised the level of phosphorylation of ERK in RAW264.7 cells through the utilization of a mechanism that is yet to be defined.

Kimura, Warabi, Yanagawa, Ma, Itoh, Ishii, Kawachi, Ishii [88]indicate that QUR protected cells from UVA oxidative damages by elevating intracellular antioxidant activity by enhancing the activation of transcription factor Nrf2. Further, Johnson, Maher, Hanneken[89] found in RPE cells that citrus flavonoid (eriodictyol) provided long-term protection against oxidative stress by the activation of Nrf2, and the induction of phase II enzymes.

Conclusion

There has been a growing interest in the possible health benefits of quercetin due to *in vitro* observations of its powerful antioxidant and free radical scavenging properties. Nevertheless, there is less evidence available on the antioxidant efficiency of quercetin *in vivo* and in fact, its pro-oxidant properties have been defined *in vivo*. Because of its pro-oxidant properties, quercetin can result in oxidative damage when it reacts with different biomolecules, such as lipids, proteins and DNA.

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