QUERCETIN: AN OVERVIEW OF BIOLOGICAL EFFECTS AND RECENT DEVELOPMENT OF DRUG DELIVERY SYSTEMS

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Abstract. The numerous beneficial pharmacological properties of quercetin justify its future clinical application. Nevertheless, the use of quercetin as a safe and effective therapeutic agent in the treatment of various diseases is limited because of the poor water solubility and membrane permeability, low bioavailability and instability. Several approaches have been undertaken to improve the bioavailability of quercetin, including the loading in different drug-delivery systems such as different types of micro and nanoparticles, nanocrystals, polymeric micelle, liposomes, microemulsions, etc. This review summarizes the beneficial biological properties of quercetin, discusses the problems of its bioavailability and clinical use, and provides an overview of possible ways to overcome these problems by its loading in perspective drug-delivery systems.

Key words: quercetin, bioavailability, drug delivery systems, nanoparticles.

Introduction

Flavonoids are a large group of phenolic compounds that are widely spread in different plant foods. There are many classes of flavonoids, for example flavones, flavonones, isoflavones, flavonols, flavanonols, and anthocyanins. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a common dietary flavonol with a wide range of pharmacological activities including antioxidant, antiplatelet, antiinflammatory, neuroprotective, antimutagenic, anticarcinogenic, antiangiogenic, antibacterial, antitumor, antiviral, antianxiety and hepatoprotective. The beneficial biological properties of quercetin make it a promising therapeutic agent. Nevertheless, the pharmacokinetic studies show that poor absorption and rapid metabolism are the major problems and reasons for its low bioavailability. A possible approach to overcome this problem is loading of quercetin in appropriate drug delivery systems. The solubility of poor water-soluble drugs could be improved by decreasing crystallinity or incorporation in compexes using compounds such as cyclodextrines. Encapsulating quercetin in nano-sized drug delivery systems, such as polymeric micro- and nanoparticles, liposomes, micelles, phospholipid complexes are novel approaches to enhance the solubility and permeability and to resolve the pharmacokinetics and some safety problems (1). Therefore, we focus our review on discussing the promises of quercetin use, the problems in bioavailability and the new approaches to resolve them.

Biological effects of quercetin

Antioxidant effects. The beneficial effects of quercetin are closely related to its antioxidant activity by the following mechanisms: scavenging reactive oxygen and singlet oxygen species (2), inhibiting enzymes (xanthine oxidase, protein kinase C, cyclooxygenase, glutathione S-transferase) or chelating trace elements, upregulating or protecting antioxidant defenses (3). Quercetin has been reported to have antioxidant activity in several *in vivo* studies. It has been found to inhibit the oxidative stress caused by streptozotocin-induced diabetes in rats (4), to show antioxidant effects against lipid peroxidation, induced by tert-butylhydroperoxide in human sperm cells (5), and to reduce effectively oxidative damage caused by an industrial compound CCl_4 using methanolic extract of *Heterotheca inuloides* containing quercetin (6). Despite of the well-known antioxidant activity of quercetin, in certain circumstances it may act as a pro-oxidant (Fig. 1). This phenomenon, better-known as "quercetin paradox" is first explained by Boots et al. (7). When quercetin scavenges free radicals, it is chemically converted into oxidation products (such as ortho-quinone/quinone methide intermediates). These products display a high reactivity towards thiols and this can be a reason to the loss of some protein functions. Boots et al. found that quercetin pre-treatment can protect DNA from oxidative damage. However, it was found that quercetin, in presence of hydrogen peroxide, led to significant lactate dehydrogenase (LDH) leakage and intracellular glutathione depletion, respectively affecting cell viability. Thus, depending of the dose, the apparent antioxidative protection offered by quercetin could be trade-off with other type toxicity.

Protection in neurodegenerative disorders. Because of its antioxidant property, increased glutathione levels and antioxidant enzyme function, quercetin has been reported to increase the intracellular GSH levels in many diseases, including Alzheimer's disease (8, 9). Quercetin has been reported to reduce oxidative stress induced by 6-hydroxydopamine in the striatum and reduced the dopaminergic neuronal loss in the rat model of Parkinson's disease (9). However, prolonged use of quercetin should be better evaluated and studied.

Cardiovascular protection. Cardioprotective properties of quercetin have been reported in patients. The beneficial effects were supported by several studies completed in cellular and animal models (10). It has been shown to decrease blood pressure and heart rate in spontaneously hypertensive rats (11), to prevent calcium chloride in-

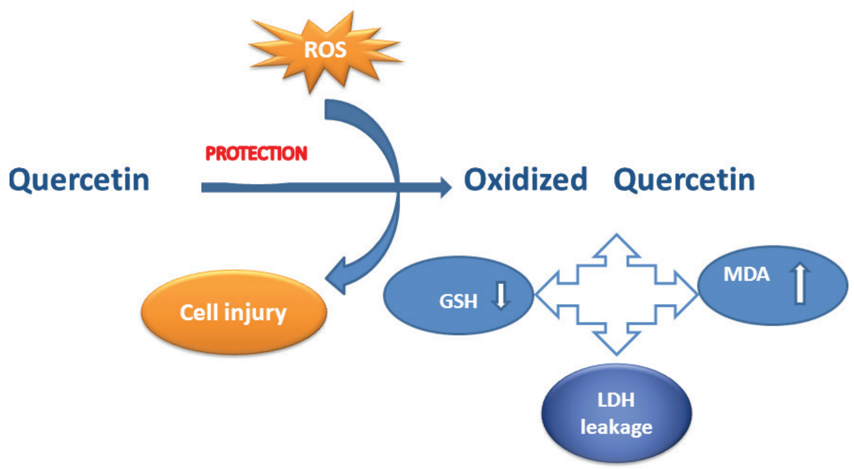

Fig. 1. Dual anti-oxidant and pro-oxidant effects of quercetin.

In absence of quercetin, cell viability is decreased by ROS – induced oxidative stress cell damages. Quercetin, used in low concentrations, scavenges free radicals and protects cells [6, 32]. However, during this process quercetin is converted into its oxidized thiol reactive form. The excessive dose of quercetin (<100 µM) leads to increased GSH consumption, increase in products of lipid peroxidation (MDA) and lactate dehydrogenase (LDH) leakage.

duced abdominal aortic aneurism (12), to reduce the levels of total cholesterol, triglycerides and free fatty acids, serum phospholipids, as well as reduced levels of serum LDL and significantly increased serum HDL in a model of isoproterenol cardiotoxicity in rats (13) .

Anticancer and antiviral activity. It is considered that quercetin inhibits the growth and proliferation of cancer cell lines of different origin, induces senescence, inhibits telomerase, induces autophagy in cancer cells, possesses anti-angiogenic activity and it can also activate immune destruction (10). Quercetin has been reported as a potent antiviral agent in *in vitro* studies (14). This effect is probably related to its ability to reduce viral growth, inhibit DNA fragment of infected cells regardless of its antioxidant activity (15), inhibit viral polymerase and bind viral nucleic acid or viral capsid proteins.

Anti-inflammatory activity. Quercetin can inhibit several enzymes that are activated in certain inflammatory conditions. Quercetin has been shown to inhibit nitric oxide production, iNOS expression, inflammatory causing agents like NO synthase, reactive C-protein, and cyclooxygenase (COX-2) in several *in vivo* and *in vitro* studies (16).

Hepatoprotective activity. It is considered that quercetin has beneficial effects on liver damage. Quercetin has been reported to protect hepatocytes against oxidative stress via phosphonoside 3-kinase activation and to maintain Ca^{2+} level in the endoplasmic reticulum in mice (17). Furthermore quercetin decreases oxidative damage induced by ethanol in rat hepatocytes (18).

Clinical applications and safety

There are some *in vivo* human studies showing that quercetin improve endothelial function in patients with hypertension and cardiovascular diseases (19), inhibit LDL oxidation after *in vivo* supplementation (20) and was effective in prevention and therapy of allergic and inflammatory diseases (21, 22). Quercetin showed beneficial effects in cancer prevention. Experimental data suggest its role in breast cancer and prostate cancer, known to have hormone sensitive component. Knekt et al. (23) demonstrated an inverse relation between total flavonoid intake (a majority of which quercetin ~95%), and overall cancer incidence, as well as the incidence of lung cancer. Quercetin possesses a good safety profile. Clinical and epidemiological studies of products containing a standardized quercetin indicate that the flavonol is well-tolerated in humans; no significant severe adverse events were reported during the studies (24-26).

Pharmacokinetic properties and problems in bioavailability

As it was discussed in a previous section, quercetin possesses a good efficacy, intrinsic activity and has a strong potential as a therapeutic agent. Despite quercetin's efficacy and safety, some pharmacokinetic limitations, like poor bioavailability, continues to be mentioned as a major concern of it's use in a clinical practice. In generally, the main reasons for reduced bioavailability of biologically active substances are poor absorption, low intrinsic activity, high rate of metabolism, inactivity of metabolic products and/or rapid elimination and clearance from the body (27). Several studies have revealed quercetin's poor absorption and rapid metabolism.

The water-solubility of quercetin is very low: about 1μg/mL in water, 5.5μg/mL in simulated gastric fluid and 28.9 μg/mL in simulated intestinal fluid. Chen et al. (2002) reported that, only 20% of the radiolabeled quercetin was absorbed following administration of an oral dose to male rats (28). In experimental pharmacokinetic study, male Sprague-Dawley rats were treated with quercetin in a dose 50 mg/kg body weight, p.o., once daily. The plasma concentration of the free quercetin was found to be 0.27 μg/mL; 93% of the active substance was metabolized after one hour (29). Low concentrations of free quercetin were identified in liver and kidney tissues (i.e., less than 8% of total quercetin) following p.o. treatment of rats.

A study in ileostomy patients showed that 24% of total quercetin was absorbed following ingestion of 100 mg of the quercetin aglycone (30), while in healthy subjects provided 100 mg of radiolabeled quercetin, up to 53% of the total administered radioactivity was absorbed (31).

Approaches for development of quercetin delivery systems

The successful realization of quercetin's health benefits depends on technological approaches for improving its unfavorable ADME characteristics while retaining its desired pharmacological effects. In the present review such strategies are summarized in Table 1.

Nanocrystals. Nanosizing of the active substance is achieved by wet media milling, high-pressure homogenization, precipitation or a combination of methods. This approach allows an achievement of greater and faster dissolution (34). For example, quercetin nanosuspension stabilized with Tween 80, produced by combined bead milling and high-pressure homogenization

Table 1. *Strategies for improving quercetin's pharmacokinetics.*

Biopharmaceutical strategy	Quercetin preparation (example)	Major outcome
Nanocrystals	SmartCrystals® stabilized with Tween® 80[35]	↑↑dispersion stability ↑water solubility
Microemulsions	Nanosized emulsion produced [37]	↑ Caco-2 cells permeability ↑ intestinal absorption in rats
Prodrug(40)	Pentamethyleter conjugate [38]	↓ water solubility ↑ permeability coeff. nude mouse skin (26 times)
	Glutamic acid conjugate[40]	↑ water solubility, ↑MDCK cells permeability
	Butyl ester conjugate [39]	↑ water solubility, ↑ skin penetration
Inclusion complexes	Modified β-cyclodextrin inclusion complexes[43, 44]	↑↑water solubility, ↑dissolution rate, ↑antioxidant
Liposomes	Liposomes[48, 51]	Preserve antioxidant; ↑anti-inflammatory; CNS delivery (nasal route)
Polymeric micelles	MPEG-PCL micelles[54] PEG-phosphatidylethanolamine micelles[55] PEG-b-oligo(ε-caprolactone)[56] Pluronic P123 micelles[57] Mixed micelles[60]	↑antiproliferative effects; preserve antioxidant activity[60]
Polymer NPs	PLGA[73] PLGA, mannose/folic acid conjugated [64,65]	preserve antioxidant activity; cancer ligand targeting
	PLA[63]	preserve antioxidant activity; sustained release
	Chitosan[68]	preserve antioxidant activity; mucoadhesive
	Chitosan-alginate[70,71]	↑hypoglycaemic; mucoadhesive
	Alginate[74]	Pb(II) waste water recovery; adhesive
Inorganic NPs	NH_2-MSN[75]	↑antiproliferative effects

had 7.56 fold higher saturation solubility than crude quercetin (35).

Microemulsions (ME) are optically isotropic and thermodynamically stable solutions of oil, water, surfactant/s and a cosurfactant (36) Hädrich et al. created a nanosized emulsion by the hot solvent diffusion method with phase inversion technique (PIT). Oral administration to rats was non-toxic and resulted in significant reduction of paw edema, comparable to diclofenac (10 mg/kg, i.p.) (37).

Prodrugs. The five hydroxyl groups of quercetin enable its conjugation with a variety of chemical moieties. However, the capping of these groups may improve solubility at the cost of decreased pharmacological activity. A study comparing quercetin glycosides and its 3,5,7,3',4'-pentamethyl ether concluded that its ether was less water-soluble but had superior skin permeability and anti-inflammatory activity (inhibition of superoxide anion activated neutrophils)(38). Among acyl esters at C3, propyl and butyl ones notably improved water solubility (9- and 64-fold respectively) and penetration through excised human skin (enhancement factor of 3.9 and 11.9 respectively) (39). The research on amino acids conjugates with different charge to the aromatic B-ring showed that negatively charged aspartic and more notably, glutamic acids had increased water solubility (45,2- and 53-fold respectively) and permeability across MDCK cell layers (2,9- and 3-fold respectively) (40).

Inclusion complexes. Cyclodextrins (CD) are capable of forming inclusion complexes with hydrophobic drugs. β-CD is inappropriate for parenteral administration due to nephrotoxicity but is non-toxic when administered orally (41). Nowadays, cyclodextrins were modified in order to obtain cyclodextrins which are safer and appropriate for i.v. route (42) The complexation of quercetin with methyl-β-cyclodextrin led to increase in aqueous solubility (254-fold), dissolution rate (3-fold) and antioxidant activity (10%)(43). Furthermore, a comparison of methylated β-cyclodextrin with sulfobutyl ether-β-cyclodextrin (SBE-β-CD) and hydroxypropyl-β-cyclodextrin (HP-β-CD) concluded that quercetin's antioxidant activity was strongest in the SBE-β-CD complex (44).

Liposomes are lipid bilayer vesicles with great potential and application in clinical drug delivery. A major disadvantage of liposomal preparations is the high cost of industrial scale and difficult aseptic manufacturing (45). There is plenty of research on liposome-loaded quercetin. This approach preserves its pharmacological effects (46-50). Noteworthy, Priprem et al. (51) suggested that such a formulation is effective in delivering quercetin to the CNS through the nasal route. Based on their synergism in adipocyte apoptosis induction and adipogenesis inhibition, Cadena et al. proposed that encapsulation of quercetin and resveratrol in sodium deoxycholate-elastic liposomes could be an approach for local fat reduction by subcutaneous injection (47).

Polymer micelles are composed of amphiphilic block copolymers with critical micelle concentrations (CMC) in the order of 10^{-6} to 10^{-7} M (1000 times less than classical, low molecular weight surfactants) (52). They allow very efficient loading of compounds of a wide lipophylicity range either incorporating them in the hydrophobic core or hydrophilic corona (53). Quercetin loading in Pluronic® P104 micelles revealed that the drug could be protected from oxidation or the instantaneous phase II metabolism in cells or plasma (52, 53). Many reports show that the incorporation of quercetin in micelles can amplify its antiproliferative effects on cell lines (54-59). Recent study also reported that polymer micelles can preserve quercetin from oxidation and release it in a sustained manner (60).

Polymer nanoparticles. Polymeric nanoparticles (NPs) are valuable drug delivery platforms due to their mechanical strength, uptake by cells, slow drug release and potential for multifunctional modification (1, 61). Some of the most studied polymers for drug delivery are poly(lactic-co-glycolic acid) (PLGA) and poly-d,l-lactide (PLA) which are biocompatible and biodegradable. PLGA NPs have attracted considerable attention due to their approval by FDA and EMA as parenteral drug delivery systems (62). Encapsulation of quercetin in PLA NPs preserved antioxidant activity and showed sustained release under physiological conditions (63). *In vitro* cytotoxicity assays and in vivo experiments with IGROV-1 tumor-bearing mice showed that folate surface-modified PLGA are capable of tumor-targeted quercetin delivery (64, 65). Chitosan and alginate are natural biopolymers that are biodegradable and biocompatible. Chitosan NPs are of interest because of its natural abundance (chitin), low toxicity and mucoadhesive properties. Its positive charge facilitates its cell internalization (66). Encapsulation of quercetin in chitosan NPs preserved and protected its antioxidant activity (67, 68). Dual drug loading, quercetin and 5-fluorouracil, in chitosan NPs exerted synergistic cytotoxic effects on pancreatic cancer cell line, MiaPaCa2 3D culture and primary mouse fibroblast cell line (L929) 2D culture (69). Alginate is a water soluble linear polysaccharide extracted from brown sea weed. Like chitosan it is biodegradable and mucoadhesive but with negative surface charge. Thus, a simple method for preparation of nanoparticles is by electrostatic interaction between both polyelectrolytes. Quercetin was effectively loaded in such polymer formulation and had an improved hypoglycaemic effect *in vivo* compared to its free form (70, 71).

Conclusion

Besides numerous beneficial biological activities, the use of quercetin is limited by its poor solubility and low bioavailability. The development of drug delivery strategies is crucial for its use in a clinical practice. Therefore, novel delivery strategies (micro- and nanoparticles, polymeric micelles, liposomes) are promising and offer a good opportunity to enhance the bioavailability and clinical applications of quercetin.

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