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Review

Dihydroquercetin: More than just an impurity?

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ABSTRACT

Dihydroquercetin (taxifolin) is a potent flavonoid found in onions, French maritime bark, milk thistle, tamarind seeds and commercially available semi-synthetic monoHER marketed as Venoruton®. This review focuses on the therapeutic promise of dihydroquercetin in major disease states such as cancer, cardiovascular disease and liver disease by reviewing the proposed mechanism(s) of action, including the activation of the antioxidant response element (ARE) and detoxifying phase II enzymes, inhibition of cytochrome P_{450} and fatty acid synthase in carcinogenesis. TNF-alpha and NF- B dependent transcription in hepatitis C infections, the scavenging effect of myeloperoxidase (MPO) derived reactive nitrogen species and subsequent effects on cholesterol biosynthesis as well as the effects on apob/apoA-I, HMG-CoA reductase and apoptosis are reviewed. The stereochemistry and pro-oxidant effect of dihydroquercetin are also considered. Although the majority of research on dihydroquercetin to date has focused on the identification of molecular targets *in vitro*, this review will bring together evidence of the potency and mode of action of dihydroquercetin and will propose a role for the therapeutic potential of flavonoid antioxidants.

Contents

1. Introduction

Dihydroquercetin, also known as taxifolin (Table 1), is a flavonoid commonly found in onions (Slimestad et al., 2007), milk thistle (Wallace et al., 2005), French maritime bark (Rohdewald, 2002) and Douglas fir bark (Kiehlmann and Edmond, 1995). To date dihydroquercetin (taxifolin) is rarely used as a single compound but it is found in complex preparations such as silymarin (Legalon™), Pycnogenol® and Venoruton®. Silymarin, for example, is an extract from the seeds of the Milk Thistle plant (*Silybum marianum*) licenced for the treatment of chronic liver disease in Germany (Blumenthal and Busse, 1998) and more recently for the prevention of recurrent hepatitis C in liver transplant recipients by the European Medicines Agency (EMEA, 2010). Initially silymarin was considered to be a pure compound (7-chromanol-3-methyl-taxifolin) (Hahn et al., 1968) but later introduced HPLC methods quantitatively determined 7 flavonolignans from the silymarin mixture (silybin A, silybin B, isosilybin A, isosilybin B, silychristin, isosilychristin and silydianin) (Ding et al., 2001) plus one flavonoid (dihydroquercetin) in addition to fatty acids and other polyphenolic compounds (Kim et al., 2003). Of these, silibinins A and B make up 50% of silymarin and are thought to be the most therapeutically active components responsible for the majority of therapeutic effects (Comelli et al., 2007). Although dihydroquercetin can clearly be purified and is present in the original extract of the milk thistle plant it is dismissed as unimportant as it is "barely discernable in the HPLC chromatogram of silymarin" (Kroll et al., 2007). Thus investigation of silymarin has devoted attention to the major constituents' silibinins A and B, rather than dihydroquercetin in the quest to identify possible modes of action. A paper published in 2003, was the

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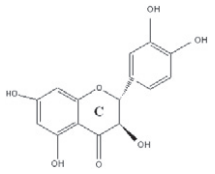
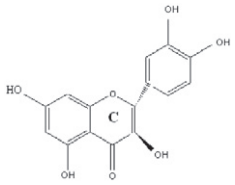
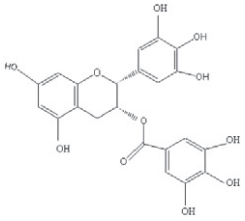
first to suggest that silymarin may contain "impurities" which have a more potent antioxidant capacity than any of the flavonoid isomers themselves (Kvasniča et al., 2003). As the attention for silymarin in the treatment of liver diseases has shifted to its therapeutic promise in cancer treatment (Flaig et al., 2007, 2010), the investigation of dihydroquercetin as a therapeutic agent is attracting more and more interest. Although the majority of research on dihydroquercetin to date has focused on the identification of molecular targets *in vitro* it is conceivable that dihydroquercetin, although a minor component in extracts such as silymarin, Pycnogenol® and Venoruton® may contribute to their therapeutic efficacy.

This review aims to look at the evidence that dihydroquercetin is a potent flavonoid antioxidant and its therapeutic promise in major disease states such as cancer, cardiovascular disease and liver disease. The chemical composition and the molecular targets of action will be discussed with reference to these specific disease states. The effects of stereochemistry and pro-oxidant effects are considered bringing together evidence for the potency and molecular sites of action of dihydroquercetin.

2. Dihydroquercetin—Chemistry and pharmacokinetics

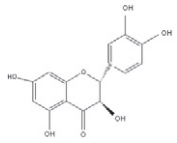
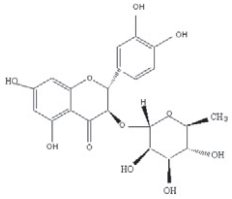
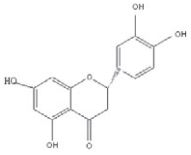
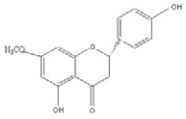
Dihydroquercetin (2*R*,3*R*)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-2, 3-dihydrochromen-4-one) (Table 1) is a flavonoid first isolated from Douglas fir bark (*Pseudotsuga taxifolia*) and later Dahurian and Siberian larch (*Larix sibirica* Leder. and *Larix gmelini Rupr*. (Rupr), syn *Larix dahurica Turoz* (Pinaceae) (Pew, 1948). HPLC analysis has revealed that it can exist in both *trans* and *cis* forms and that it crystallises as two independent molecules in a cell (Nifant'ev et al., 2006). The stearic structure of the dihydroquercetin crystal is, $C_{15}H_{12}O_7 \cdot 2.5H_2O$, monoclinic; at 25 °C, *a* 23.612, *b* 5.206, *c* 25.495 Å; β 103.18O, *V* 3046.3 $Å^3$, d_{calc} 1.520 g cm^{-3}, *Z* 8, space group *C*2 (Nifant'ev et al., 2006). Reported melting points for dihydroquercetin range from 218 to 253 °C (Kiehlmann and Edmond, 1995) and both dihydroquercetin isomers are soluble in water–alcohol solutions and polar solvents. (+) *trans*-Dihydroquercetin is known to oxidize more actively, donating hydrogen atoms, resulting in the oxidation product quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one) (Rogozhin and Peretolchin, 2009) (Table 1). Dihydroquercetin and quercetin differ in one single structural element, which is the presence/absence of a C2,C3-double bond in the C-ring. This has a considerable impact on the structure–activity relationship (SAR) of both compounds especially with respect to their antioxidant potency (Silva et al., 2002). The effect of molecular structure on biological systems such as DNA degradation, growth and proliferation of malignant cells and inhibition of gastric acid secretions through the inhibition of the H^+, K^+ ATPase in gastric parietal cells is well documented (Chen et al., 2002; Choi et al., 2002) and the direct impact of the SAR on the therapeutic properties of dihydroquercetin is reported (common dihydroquercetin derivatives are shown in Table 2). Dihydroquercetin is classed as an antioxidant (Kurth and Chan, 1951) and meets two of the three criteria for effective radical scavenging ability, the presence of the *o*-dihydroxy structure in the B ring conferring stability and the 5- and 7-OH groups with 4-oxo function in the A and C rings which are responsible for a maximum radical scavenging potential (Salah et al., 1995). It does however lack the 2,3 double bond in conjunction with the 4-oxo function in the C ring rendering it less potent than other flavonoid antioxidants containing this 2,3 double bond such as its oxidation product and planar hydrophobic equivalent, quercetin (Seyoum et al., 2006). Dihydroquercetin (taxifolin) (100 mg/kg) was found to have a similar anti-oxidant activity profile to α-tocopherol (Teskin et al., 1996), inhibiting superoxide anion production, protecting mitochondria from peroxyl radical damage and inhibiting the activation of NADPH-dependent cytochrome P_{450} reductase induced by microsomal lipid peroxidation (Haraguchi et al., 1996). The knowledge about its cellular uptake, transport profile and interaction with drug-transporters is very limited. Dihydroquercetin (10–100 μM) is thought to increase the expression of P-glycoprotein (P-gp) and Multidrug resistance Protein 2 (MRP2) in an *in vitro* study by Wang et al. (2009) but both further *in vivo* and *in vitro* studies identifying the exact mechanism(s) involved in its cellular uptake and bioavailability are not available.

Table 1
Flavonoid antioxidant structures.

Compound Structure
Dihydroquercetin (Taxifolin) (2*R*,3*R*)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-2,3-dihydrochromen-4-one 
Quercetin 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one 
Epigallocatechin gallate (EGCG) (2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-chromen-3-yl 3,4,5 -trihydroxybenzoate 

Dihydroquercetin is barely water soluble and a slow dissolution rate results in only trace amounts being detectable in plasma of experimental rats after oral (*po*) administration of 50 and 500 mg/kg as a single dose. Intravenous (i.v.) administration results in non-linear pharmacokinetic behaviour (Voskoboinikova et al., 2006). The dissolution kinetics of dihydroquercetin can be enhanced by preparing a solid nanodispersion of dihydroquercetin dihydrate in polyvinylpirrolidone. This achieves 90% dihydroquercetin release after 30 min (Shikov et al., 2009). Furthermore the development of a more reliable reverse phase (RP)-HPLC method including UV detection, together with a lipid formulation of dihydroquercetin (0.1 g dihydroquercetin in 10 ml Labrasol) has shown to increase the bioavailability (*F*) of dihydroquercetin to 36% in rat plasma (*po*—8 and 80 mg/kg) (Pozharitskaya et al., 2009). As a result a new liposomal formulation of dihydroquercetin has been developed (Flamena D). It has an elimination half life ($T_{(1/2)}$) of 1.3 h with a relative *F* of 159% (rat plasma), compared to the parent substance dihydroquercetin, following *po* administration of 50 mg/kg (Zherdev et al., 2010). Absorption of polyphenols is accompanied by extensive conjugation and metabolism. Identified metabolites of dihydroquercetin include 3,4-dihydroxyphenyl-acetic, *m*-hydroxyphenylacetic, and 3-methoxy-4-hydroxyphenylacetic acids. These are excreted following *po* ingestions

Table 2
Common dihydroquercetin derivatives.

Compound Structure
Dihydroquercetin (2*R*, 3*R*)-2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-2, 3-dihydrochromen-4-one 
Astilbin (2*R*, 3*R*)-2-(3, 4-dihydroxyphenyl)-5, 7-dihydroxy-3-[(2*S*, 3*R*, 4*R*, 5*R*, 6*S*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxy-2, 3-dihydrochromen-4-one 
Eriodictyol (2*S*)-2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4-chromanone 
Sakuranetin (2*S*)-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-2, 3-dihydrochromen-4-one 

of 2 g dihydroquercetin by human volunteers. Dihydroquercetin has shown to be non-toxic in long term feeding trials to albino rats (Booth and Deeds, 2006) but no further toxicity studies have been carried out.

3. Dihydroquercetin—molecular targets for anti-cancer therapy

By far the largest number of citations for dihydroquercetin during the more recent years has been surrounding its effect on cancer cell models and the prevention of drug toxicity related effects (for a full list of all experimental models and dihydroquercetin concentrations cited see Table 3). Proliferative changes of cells, differentiation, apoptosis as well as altered expression of transcription factors and proteins essential in cell cycle regulation result in the development of abnormal cell growth and the development of carcinogenic tissues. The protection against certain types of cancers afforded by many common foods has been well established (Sutandyo, 2010), with flavonoids showing distinct anti-inflammatory responses with beneficial implications for cardiovascular disease and cancer (Garcia-Lafuente et al., 2009). Aside from the more obvious antioxidant and free radical scavenging effects of many flavonoids which afford cytoprotection to cells, evidence suggests that dietary flavonoids can affect the process of carcinogenesis through multiple mechanisms. These include the induction of phase II detoxifying enzymes and resultant detoxification of carcinogenic intermediates, the suppression of cytochrome P_{450}-dependent monooxygenases, apoptosis and disruption of cancer cell cycle progression (Antosiewicz et al., 2008). The potential anti-cancer mechanisms of dihydroquercetin are summarised in Fig. 1.

The generation of oxidative stress leads to the production of reactive oxygen species (ROS) and electrophiles which are implicated in the

Table 3
Experimental models and dihydroquercetin concentrations cited.

Experimental model	Dihydroquercetin concentration	Reference
Cancer		
In vitro		
OVCAR-3 human ovarian cancer	0–160 μM	Luo et al. (2008)
HCT116 colon cancer	3.9–250 μM	Lee et al. (2007)
TA102 *Salmonella typhimurium*	0.01% g/100 ml	Makena and Chung (2007)
Hepa-1c1c7 mouse hepatoma	0–100 μM	Boerboom et al. (2006)
LNCaP human prostate cancer	0–100 μM	Brusselmans et al. (2005)
MDA-MB human breast cancer	0–100 μM	Brusselmans et al. (2005)
T47D human breast carcinoma	0–100 μM	Plísková et al. (2005)
COLO205, COLO320HSR, COLO320 DM, and HT29 cells	100–200 μM	Shen et al. (2004)
Male BALB/c nu/nu mice	100–200 μM	Shen et al. (2004)
Rat and bovine liver	0–60 μM	Haraguchi et al. (1996)
Mitochondria		
Squamous cell carcinoma (HTB43)	2–8 μg/ml	Kandaswami et al. (1991)
Gliosarcoma (9L) cell	2–8 μg/ml	Kandaswami et al. (1991)
In vivo	N/A	
CV disease		
In vitro		
OVCAR-3 human ovarian cancer	0–160 μM	Luo et al. (2008)
HeLa	100 μM	Trantafyllou et al. (2008)
HepG2 human liver carcinoma	0–200 μM	Casaaschi et al. (2004)
HepG2 human liver carcinoma	0–100 μM	Gebhardt (2003)
Rat heart and liver microsomes	0–60 μM	Miura et al. (2003)
Human blood serum (healthy volunteers)	1% g/100 ml	Kostyuk et al. (2003)
HepG2 human liver carcinoma	0–200 μM	Theriault et al. (2000)
RHCR—RHCR from rabbit heart	Not known	Imamura et al. (2000)
In vivo		
Long-Evans rats	0.1 and 1.0 μg/kg, i.v. 60 min	
Male adult		Wang et al. (2006)
Male Wistar rats	20 mg/kg in diet for 6 days	Plotnikov et al. (2003)
Male Wistar rats 5-week old	0.05% to basal diet for 10 days	Igarashi et al. (1996)
Liver disease		
In vitro		
Huh-7.5.1 cells	0–150 μM	Polyak et al. (2010)
Liver homogenate (genus unknown)	0–100 mM	Vladimirov et al. (2009)
Liver mitochondria, male Wistar rats	0–50 μM	Dorta et al. (2005)
In vivo		
Human peripheral blood mononuclear cells	0–150 μM	Polyak et al. (2010)
Male Wistar rats	100 mg/kg s.c. for 4 days	Teselkin et al. (2000)

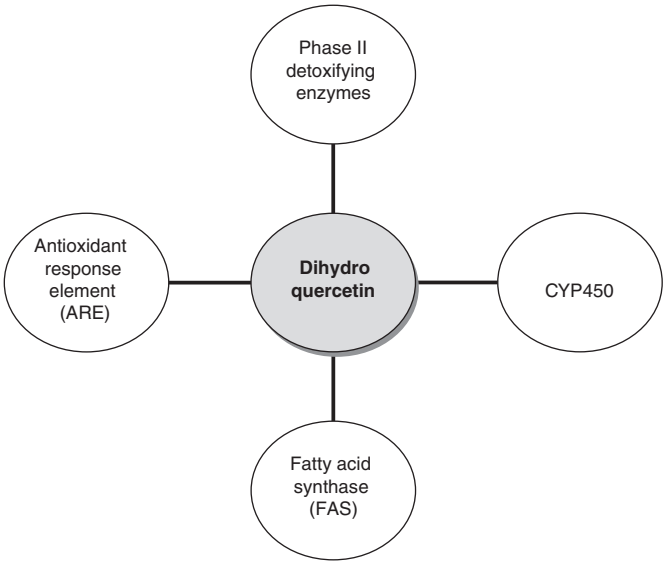


Fig. 1. Potential anti-cancer mechanisms of dihydroquercetin.

development of cancer and other diseases (Breimer, 1990). The generation of oxidative stress leads to the expression of defensive genes targeted at detoxifying ROS and the promotion of cell survival. This transcriptional response is mediated by a *cis*-acting transcriptional enhancer termed antioxidant-response element (ARE) (also referred to as electrophile response element—EpRE) (Wasserman and Fahl, 1997). The ARE is found in the promoter regions of genes encoding two major phase II detoxifying enzymes, glutathione S-transferase A2 (GSTA2) and NADPH: quinone oxidoreductase 1 (NQO1) (Favreau and Pickett, 1991; Frilling et al., 1990). These detoxifying enzymes promote cell survival, signal transduction, proliferation and immunological defence reactions (Kaspar et al., 2009). Phase II detoxifying enzymes are also responsible for the elimination of activated carcinogens unregulated by phase I enzymes such as CYP1A1 and CYP2E1 (Lee et al., 2007). A study by Lee et al. (2007) looking at the differential gene regulation by dihydroquercetin in colon cancer HT116 cells, identified that dihydroquercetin activated ARE at a concentration range of 31.1–65 μM. They demonstrated that chemopreventative phase II enzymes, NQO1 and GSTM1 are upregulated in the presence of dihydroquercetin while the phase I enzyme CYP2E1 was downregulated. The implication is that dihydroquercetin exerts a chemopreventative effect through an ARE dependent mechanism however the effect on Nrf2 (erythroid 2-related factor), an essential transcription activator of ARE was not assessed. These observations stand in contrast to reports by Boerboom et al. (2006) who found that dihydroquercetin (0–100 μM) completely lacked the ability to stimulate gene expression *via* the EpRE in Hepa-1c1c7 (mouse hepatoma) cells. This was argued to be due to the absence of the C2,C3 double bond in the C-ring and seemingly in line with earlier studies detailing that the C2,C3 double bond is essential for a flavonoid to induce NQO1 expression (Uda et al., 1997). NQO1 induction by non planar flavonoids requires an aryl hydrocarbon receptor (Ah) implicating the xenobiotic response element (XRE) in its regulation (Valerio et al., 2001). Lee et al. (2007) however found that dihydroquercetin activates ARE but not XRE, leaving the differential gene regulation of Phase I and II enzymes by dihydroquercetin open for debate.

Cytochrome P_{450}s are a family of diverse haem-containing enzymes, catalysing various Phase I xenobiotic reactions including oxidation and dealkylation (Coon, 2005). Cytochrome P_{450}s are responsible for metabolising chemical carcinogens encountered in the environment to highly reactive carcinogenic conversion products (Guengerich and Shimada, 1991). Occupational exposure to benzidine, for example, has been shown to result in an increased risk of developing bladder cancer (Agency for Toxic Substances and Disease Registry, 1995) caused by mutations introduced into the genome by the bioactivation of cytochrome P_{450} (Josephy, 1986) and subsequent oxidation to its N-hydroxyl derivative by CYP1A2 (Murata et al., 2001). A study looking at the mutagenic potential of benzidine on a Salmonella tester strain TA102 and the protective effect of various plant polyphenols found, that dihydroquercetin was one of seven flavonoids inhibiting benzidine induced mutations (Makena and Chung, 2007). It seems unclear however, whether the protection afforded by dihydroquercetin is a result of the direct inhibition of P_{450} isoenzymes or the inhibition of DNA adducts. An earlier study carried out by Haraguchi et al. (1996) looked at oxidative damage in *Engelhardtia chrysolepis* and found a dihydroquercetin dependent cytochrome P_{450} inhibition. As dihydroquercetin shows a significant potential to reduce benzidine induced lipid peroxidation in this salmonella tester strain, Makena and Chung (2007) suggested that dihydroquercetin acts as a potent iron chelator of the haem-iron resulting in the inhibition of cytochrome P_{450} and subsequent lipid peroxidation.

Fatty acid synthase (FAS) is a metabolic enzyme essential in the synthesis of long chain fatty acids providing lipids for membrane repair as well as energy generation through β-oxidation and lipid modification of proteins (Wakil, 1989). As altered cellular metabolism is one of the hallmarks of cancer it is not surprising that numerous studies have shown a marked increase in FAS level in many different types of cancers (Alo et al., 1996; Piyathilake et al., 2000; Swinnen et al., 2002). Initial reports found that tumour cells display enhanced glycolytic activity (Warburg, 1956) leading to an excess production of pyruvate which along with acetyl Co-A carboxylase (ACC) and FAS are essential precursors for fatty acid synthesis. Since up-regulation of FAS occurs early on in the tumour development, it stands to reason that disruption of glycolysis and/or FAS could be promising targets for specific anti-cancer agents. Among the compounds earmarked as potential FAS inhibitors are cerulenin (antifungal antibiotic), C75 (fatty acid synthase inhibitor) and orlistat (lipase inhibitor), all of which have shown to effectively suppress cell growth and induce apoptosis (Flavin et al., 2010; Pizer et al., 2000). The best characterized natural product however is epigallocatechin gallate (EGCG), the principal polyphenol component of green tea (Wang and Tian, 2001) (Table 1). As EGCG has been shown to work through a plethora of different mechanisms, such as inhibiting the KR domain of the FAS protein (Li et al., 2006) and mimicking NADPH-dependent FAS activity (Hiipakka et al., 2002), it is perceived as being a viable lead compound for the development of other naturally occurring chemopreventative agents (Kridel et al., 2007). In 2003, a study, looking at human prostate LNCaP cells, showed that EGCG successfully inhibited the FAS enzyme resulting in growth arrest and cell death (Brusselmans et al., 2003). The same study group subsequently published a second paper identifying dihydroquercetin as one of only six polyphenols, including EGCG, to show potent inhibitory properties on lipogenesis in LNCaP prostate and MDA MB-231 breast cancer cells (Brusselmans et al., 2005). Dihydroquercetin was less potent than quercetin, luteolin and kaempferol but since all six flavonoids had a marked effect on cell growth and apoptosis, the authors hypothesised that FAS inhibition may be one of the mechanisms involved through which flavonoids such as dihydroquercetin mediate their effect.

Inhibition in ovarian cancer cell growth (OVCAR-3) in response to dihydroquercetin has also been reported together with a dose-dependent cessation in vascular endothelial growth factor (VEGF) expression (Luo et al., 2008). VEGF plays a key role in pathological angiogenesis mediating cell survival *via* the (PI3K)-Akt pathway commonly found in response to hypoxic conditions. Hypoxia, low pH and nutrient starvation are characteristic for tumour cells and have both been linked to FAS expression in cancer (Furuta et al., 2008) signposting a second possible mechanism of action for dihydroquercetin on tumour cell arrest. A third tangible mechanism is the involvement of oestrogen receptors (ER) in the complex regulation of FAS upstream of (PI3K)-Akt and mitogen-activated protein kinase (MAPK) *via* the sterol regulatory

element-binding protein 1c (SREBP-1c) (Lupu and Mendez, 2006; Mashima et al., 2009). Plísková et al. (2005) showed that dihydroquercetin is a strong ER agonist. No isolated experiments however looking at the consequences of this effect in cancer cells have to date been carried out leaving the mechanistic action of dihydroquercetin on cancer cell arrest open to further scrutiny.

Almost four decades after Otto Warburg first reported his observation, there is now a growing body of evidence which challenges his findings (Moreno-Sánchez et al., 2007) illustrating that more complex metabolic control mechanisms are key to tumour progression and therapeutic intervention. Each particular cancer cell carries its own mutations and there is no unique mechanism which explains one single effect. This may go some way towards explaining the contrasting findings where dihydroquercetin has not shown to have any beneficial anti-tumour effects in a variety of cancerous cell lines including colorectal carcinoma cells (HT29, COLO25, COLO320HR) (Shen et al., 2004), HTB43 squamous carcinoma cells (Kandaswami et al., 1992) or 9L gliosarcoma cells (Kandaswami et al., 1991). As no *in vivo* experiments to further verify any of the above reported effects of dihydroquercetin have to date been published, the evidence for a therapeutic promise of dihydroquercetin in anti-cancer therapy is "suggestive" at best. The idea that flavonoids contribute to cancer prevention through other mechanisms such as radical scavenging (Lambert and Elias, 2010), detoxification (Uda et al., 1997) and kinase inhibition (Davies et al., 2000) should be born in mind.

4. Dihydroquercetin in cardiovascular disease

As with cancer pathology, the synthesis of long chain fatty acids is essential in maintaining a functioning cardiovascular (CV) system through membrane repair and energy generation (Wakil, 1989). One of the main risk factors for the development of coronary heart disease (CHD) is the elevated low density lipoprotein (LDL) cholesterol level (Nabel, 2003). This plays a pivotal role in the development of artherosclerosis (Chisolm and Steinberg, 2000) as it leads to a rise in the adherence of circulating monocytes to arterial endothelial cells, induction of necrotic and apoptotic pathways, smooth muscle cell migration and proliferation, prevention of endothelial cell migration and endothelial-mediated relaxation, and induction of pro-coagulation properties (Steinberg, 1997). In contrast to LDL cholesterol, high density-lipoprotein (HDL) cholesterol prevents atherosclerosis development *via* the ABCA1 and ABCG1 (members of the ATP binding cassette transporters) mediated cholesterol efflux from macrophages (Shao et al., 2010). Recent findings suggest that HDL cholesterol may loose this cardioprotective effect if subjected to oxidative and compositional changes. Both LDL and HDL cholesterols are subject to myeloperoxidase (MPO) induced reactive nitrogen species ($ONOO^-$, NO_2^-; $NO_2\bullet$) (Gaut et al., 2002) and resulting oxidative damage. Kostyuk et al. (2003) demonstrated that dihydroquercetin inhibited the oxidation of LDL cholesterol in blood serum of healthy human volunteers *via* the scavenging of MPO-derived NO_2 radicals (Kostyuk et al., 2003) in human blood serum, thereby lowering total LDL cholesterol levels. This cholesterol lowering effect of dihydroquercetin is also reported by Gebhardt (2003) who reported an inhibitory effect of dihydroquercetin on hepato-cellular cholesterol biosynthesis in HepG2 cells. Theriault et al. (2000) suggested that dihydroquercetin inhibits HMG-CoA reductase activity which is the essential rate limiting enzyme in cholesterol synthesis and similar to the mechanism of the well established licenced lipid-regulating drug Simvastatin. They also reported a decrease in the synthesis and secretion of the lipid apolipoprotein B-100 (apoB) and subsequent increase in apolipoprotein A-I (apoA-I) following dihydroquercetin treatment (HepG2 cells).

ApoB is the apolipoprotein of LDL cholesterol responsible for carrying cholesterol to the tissues acting as a ligand for LDL receptors throughout the body. ApoA-I in contrast is the apolipoprotein for HDL cholesterol responsible for promoting cholesterol efflux from tissues to the liver for excretion. Two recently published large scale studies, INTERHEART (Yusuf et al., 2004) and AMORIS (Walldiu et al., 2001), advocate the superiority of measuring the apoB/apoA-I ratio to predict the cardiovascular risk (Walldiu et al., 2001; Yusuf et al., 2004). By preferentially altering this risk prediction ratio, dihydroquercetin is ideally placed to warrant further studies into its potential to reduce the CV disease risk in dyslipidemic patients. A marked reduction in apoB secretion following the addition of dihydroquercetin to HepG2 cultured cells was also reported by Casaaschi et al. (2004). He hypothesised a mechanism by which dihydroquercetin reduces apoB secretion by limiting triglyceride availability *via* diacylglycerol acyltransferase and microsomal triglyceride transfer protein (Casaaschi et al., 2004). Further follow up studies were however not carried out. Despite these reports signposting the cholesterol lowering effects of dihydroquercetin, it remains unclear if dihydroquercetin acts by inhibiting HMG-CoA reductase or by beneficially influencing the apolipoprotein ratio by limiting triglyceride availability. A study investigating the effect of dihydroquercetin on serum lipid concentration and liver antioxidant enzyme activity *in vivo* found that in addition to lowering total liver cholesterol, dihydroquercetin also significantly reduced serum and liver thiobarbituric acid reactive substance (TBARS) concentration (Igarashi et al., 1996). TBARS are an index of lipid peroxidation and oxidative stress useful in the determination of the antioxidant potency of compounds. The role of oxidative events in the proartherogenic and prothrombic pathogenesis of the arterial wall in CV disease has been well established (Stocker and Keaney, 2004) as has the inhibitory effects of antioxidants, vitamins and mineral supplementation in CV pathology (Kris-Etherton et al., 2010). Imumara et al. (2000) reported that dihydroquercetin (conc. not given) inhibited rabbit heart carbonyl reductase (RHCR) thereby preventing the formation of superoxide anion radicals. These findings were backed up by reports that dihydroquercetin inhibited carbonyl reductase (NADPH) dependent microsomal lipid peroxidation in male Wistar rat heart (Miura et al., 2003). The findings, however, also highlight the lacking antioxidant potency of dihydroquercetin compared to its closely related oxidation product quercetin. This is thought to be due once again to the absent C2,C3-double bond in the C ring and this absence was confirmed by NMR analysis (Sawai et al., 2005). Despite the presence of a catechol-group (3′,4′-OH) in the B-ring, a determinant of high antioxidant capacity, dihydroquercetin remains less potent than the flavonoids quercetin, catechin and kaempferol which possess identical hydroxylation patterns (Silva et al., 2002) rendering dihydroquercetin less favourable for the prevention of arterial oxidative events in CV pathology.

Along with the proartherogenic and prothrombic events in CV pathology, hypoxia plays a key role in the pathology of congestive heart diseases (CHD) such as stroke, transient ischemic attacks (TIA) and myocardial infarction (MI). Very few publications looking at the effect of dihydroquercetin on hypoxia related CHD exist but, as discussed previously, dihydroquercetin has been reported to have a dose-dependent cessation effect on VEGF expression, a key mediator for cell survival *via* the (PI3K)-Akt pathway in response to hypoxia (Luo et al., 2008). VEGF is the only growth factor to be specific and critical for blood vessel formation stimulating endothelial cell proliferation, microvascular permeability, vasodilation and angiogenesis (Ferrara, 1999; Ferrara and Davis-Smyth, 1997). In response to cell/tissue hypoxia, such as myocardial infarct or transient ischemic attacks, the transcription factor HIF-1 (hypoxia-inducible factor 1) is activated and then translocates into the nucleus where it induces the transcription of VEGF (Semenza, 2000). Coronary artery occlusion has been shown to induce VEGF mRNA in rat hearts (Maruyama et al., 1999). In a study by Triantafyllou et al. (2008), dihydroquercetin failed to induce HIF-1α expression in HeLa cells which may explain its observed inhibitory effect on VEGF, detrimental to cell survival. The study, however, looked at dihydroquercetin induced HIF-1α expression under normal oxygen pressure to demonstrate the iron-chelating properties of flavonoids in

the regulation of HIF-1 expression and is therefore not representative of dihydroquercetin activity under hypoxic conditions. In addition dihydroquercetin has been reported to reduce blood viscosity after myocardial infarction in rat heart when combined with ascorbic acid (Plotnikov et al., 2003) as well as improving cerebral ischemic-reperfusion injury in rats (Wang et al., 2006). Proposed mechanisms include a decrease in the content of plasma fibrinogen and erythrocyte aggregation, inhibition of leukocyte infiltration, COX-2 and iNOS expression, prevention of Mac-1 and ICAM-1 expression, inhibition of NF-kappaB activity and antagonized production of ROS and NO. Supportive evidence for these proposed mechanisms is however distinctly lacking rendering the beneficial involvement of dihydroquercetin in CV disease and CHD hypothetical at best. The potential effects of dihydroquercetin on cardiovascular mechanisms are summarised in Fig. 2.

5. Dihydroquercetin in liver disease

The investigation of the therapeutic properties of dihydroquercetin in hepatological disorders is probably the most intuitive on the one hand and the least well researched on the other. Dihydroquercetin is the only flavonoid found in the licenced hepatoprotective drug silymarin (Legalon®) for the treatment of toxic liver damage and the supplementary treatment of chronic inflammatory liver disease and liver cirrhosis (Blumenthal and Busse, 1998). As such it makes intuitive sense to investigate its properties in isolation. However research on dihydroquercetin in this context is minimal. This may be due to the lack of knowledge surrounding the mechanism involved in the hepatoprotective properties of silymarin itself. To date, four main hepatoprotective mechanisms of silymarin have been identified (i) antioxidant radical scavenger; (ii) cell membrane stabiliser and permeability regulator; (iii) promotor of ribosomal RNA synthesis (i.v.); transformation inhibitor of stela fibroblasts into myofibroblast (Franschini et al., 2002). Recently a study aimed to identify the hepatoprotective flavonolignans from the silymarin compound, including dihydroquercetin (Polyak et al., 2010). The potential hepatoprotective mechanisms of dihydroquercetin are summarised in Fig. 3.

After establishing that silymarin blocked chronic hepatitis C virus (HCV), inhibited TNF-alpha and NF- B dependent transcription as well as suppressing proliferation and production of inflammatory cytokines from T-cells (Wagoner et al., 2010), Polyak et al. (2010) screened the seven major flavonolignans and one flavonoid (dihydroquercetin) of silymarin *in vivo* to establish their antiviral actions in human peripheral blood mononuclear cells. He established that dihydroquercetin has a superior antiviral activity which may influence the course of HCV disease in some patients. An earlier study looking at experimentally induced tetrachloromethane hepatitis in Wistar rats also identified that dihydroquercetin offered protection against hepatitis hypothesising that the antioxidative defences of dihydroquercetin protect against lipid peroxidation (Teselkin et al., 2000).

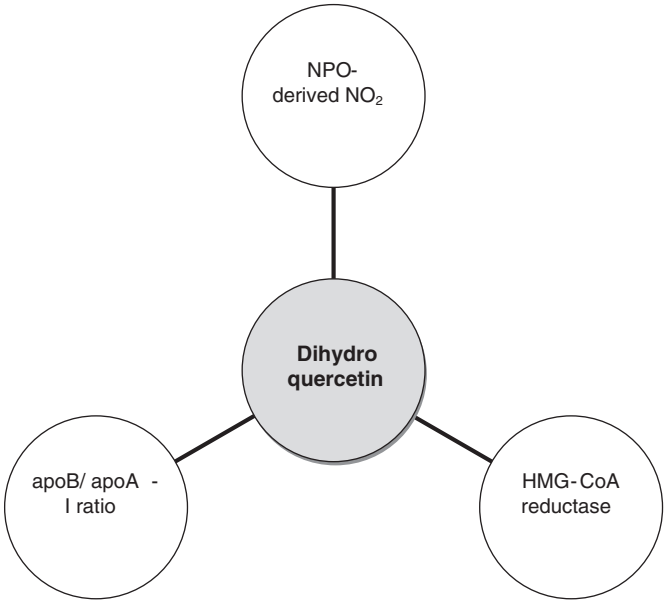


Fig. 2. Effect of dihydroquercetin on cholesterol biosynthesis.

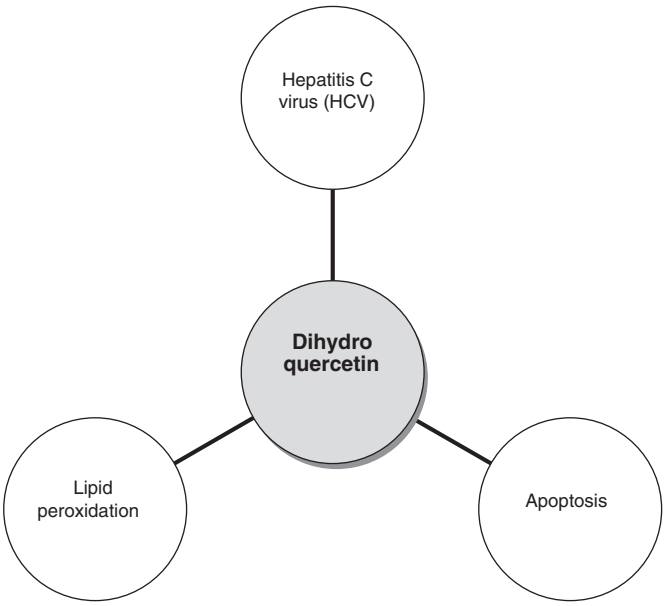


Fig. 3. Potential hepato-protective mechanisms of dihydroquercetin.

Lastly two conflicting results have been published looking at the apoptotic inhibitory potential of dihydroquercetin in rat liver mitochondria. Vladimirov et al. (2009) published results identifying that dihydroquercetin inhibits two radical-producing reactions responsible for apoptosis onset. Namely the formation of radicals by the cytochrome c–cardiolipin complex in the presence of hydrogen peroxide or lipids, and chain lipid peroxidation resulting in cytochrome c release from mitochondria and initiation of the apoptotic cascade. These results stand in contrast to an earlier publication identifying that dihydroquercetin has a high potential to induce apoptosis in isolated rat liver due to its lacking C2,C3 double bond (Dorta et al., 2005).

6. Perspectives

This review attempts to summarize the recent advances and the potential therapeutic promise of dihydroquercetin in major disease states such as cancer, cardiovascular disease and liver disease on a molecular level. The most striking observation is the plethora of documented mechanisms identified in *in vitro* models coupled with the distinct lack of experimental *in vivo* data. This raises the question how one compound can be involved in so many different yet independent mechanisms. Closer analyses reveal that it seems to be the inhibitory effect of dihydroquercetin on lipid peroxidation that results in the most promising chemopreventative and chemotherapeutic action across all three disease states. Since inhibition of lipid peroxidation is also a measure of the antioxidant potency of a compound, it is not only seemingly dihydroquercetin's greatest strength but also pinpoints its greatest weakness. The absent C2,C3-double bond and resulting stereochemical behaviour have been suggested to be responsible for the lack of antioxidant potency of dihydroquercetin compared to its oxidation product quercetin and many other flavonoids with identical hydroxylation patterns. Quercetin has therefore been recognised as a multipotent flavonoid with great potential for the prevention and

treatment of diseases (Bischoff, 2008) while dihydroquercetin has been overlooked as an inconsequential impurity of some flavonoid compounds. The evidence for a therapeutic promise of dihydroquercetin to date is "suggestive" at best and while *in vitro* evidence for an effect of dihydroquercetin on apoptosis and pathological angiogenesis mediated cell survival may be beneficial in certain cancer models, the same effects are detrimental in other disease states such as hypoxia mediated cardiovascular disease. However this review highlights that dihydroquercetin works on many other molecular targets, resulting in some interesting preliminary data of its effects on certain anti-cancer mechanisms, cholesterol biosynthesis and antiviral activity (HCV). Further research into the exact mechanisms of dihydroquercetin in physiological systems and disease pathology both *in vitro* and *in vivo* are warranted.

Statement of conflict of interest

The authors are not aware of any conflict of interest.

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