

## Quercetin Overview

Quercetin is a water-soluble plant pigment called a flavonoid typically found in red wine, green tea, onions, apples, and leafy vegetables. In the body, quercetin is a potent antioxidant and anti-inflammatory activity, protecting cellular structures and blood vessels from the damaging effects of free radicals. It improves blood vessel strength (capillary fragility). Quercetin inhibits the activity of an enzyme (catechol-O-methyltransferase) that breaks down the neurotransmitter norepinephrine. This effect may lead to elevated levels of norepinephrine and an increase in energy expenditure (thermogenesis) and fat oxidation. It also means quercetin acts as an antihistamine leading to relief of allergies and asthma. As an antioxidant, it reduces LDL cholesterol and offers protection from heart disease. Quercetin blocks an enzyme that leads to accumulation of sorbitol, which has been linked to nerve, eye, and kidney damage in diabetics. It may prevent cataract formation. Quercetin is considered a phytoestrogen.

**Dietary Sources:** Quercetin is found in apples, green tea, black tea, and onions, with smaller amounts in green vegetables and beans.

**Dosage:** Although there are no widely recognized standard dosage recommendations for quercetin, intakes of as high as 100-1000mg per day, consumed in 2-3 divided doses have been used with no apparent adverse side effects.

**Safety:** No adverse side effects have been identified relating to typical doses of quercetin in dietary supplements.

(Source: [www.supplementwatch.com](http://www.supplementwatch.com))

## Research Overview

The scientific literature on flavonoids, including quercetin, shows the following areas of research.

1. Chronic treatment with flavonoids, including quercetin, reverses cognitive deficits in aged and LPS-intoxicated mice.
2. Quercetin successfully prevented the premature senescent phenotype and up-regulation induced by hydrogen peroxide.
3. Dietary antioxidants, including quercetin, could play a significant role in the reduction of inflammatory responses.
4. Quercetin in particular, paired with ascorbic acid, may be of therapeutic benefit in protecting neurovasculature structures in skin from oxidative damage.
5. In human platelet-rich plasma, quercetin prevented the secondary aggregation and blocked ATP release from platelets induced by epinephrine or ADP.
6. Flavonoids inhibit platelet function by blunting hydrogen peroxide production and, in turn, phospholipase C activation.
7. Flavonoids in regularly consumed foods may reduce the risk of death from coronary heart disease in elderly men.
8. The habitual intake of flavonoids and their major source (tea) may protect against stroke.
9. Quercetin shows potential in the prophylaxis of cardiovascular diseases.
10. Quercetin reduces the elevated blood pressure, the cardiac and renal hypertrophy and the functional vascular changes in rats.
11. Oral low dose quercetin is cardioprotective, possibly via a mechanism involving protection of mitochondrial function.
12. Two flavonoids, quercetin and silybin, characterized as free radical scavengers, exert a protective effect preventing the decrease in the dehydrogenase/oxidase ratio observed during ischemia-reperfusion.
13. Quercetin shows therapeutic potential in CsA-induced nephrotoxicity.
14. Dietary quercetin and metabolites are active in inhibiting oxidative damage in the lens and thus could play a role in prevention of cataract formation.
15. Quercetin provided its strong antioxidant property of protecting cells against H<sub>2</sub>O<sub>2</sub>-induced oxidative stress and calcium dysregulation.
16. Daily administration of quercetin for 5 days markedly prevented the cardiac hypertrophy in AAC mice.
17. Citrus flavonoids are effective inhibitors of human breast cancer cell proliferation in vitro, especially when paired with quercetin, which is widely distributed in other foods.
18. Quercetin glycosides are capable of inhibiting lipoxygenase-induced LDL oxidation more efficiently than ascorbic acid and alpha-tocopherol.

## Quercetin Abstracts (30)

1. Pharmacology. 2003 Oct;69(2):59-67. Protective Effect of Flavonoids against Aging- and Lipopolysaccharide-Induced Cognitive Impairment in Mice. Patil CS, Singh VP, Satyanarayan PS, Jain NK, Singh A, Kulkarni SK. Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India.

Flavonoids, naturally occurring polyphenolic compounds, are known to inhibit both lipopolysaccharide (LPS) stimulated tumor necrosis factor alpha and interleukin 6 release which modulate the proinflammatory molecules that have been reported in many progressive neurodegenerative disorders, including Alzheimer's disease (AD), viral and bacterial meningitis, AIDS dementia complex, and stroke. The present experiments were performed to study the possible effects of exogenously administered flavonoids (apigenin-7-glucoside and quercetin) on the cognitive performance in aged and LPS-treated mice (an animal model for AD) using passive avoidance and elevated plus-maze tasks. Aged and LPS-treated mice showed poor retention of memory in step-through passive avoidance and in plus-maze tasks. Chronic administration of the flavonoids apigenin-7-glucoside (5-20 mg/kg i.p.) and quercetin (25-100 mg/kg i.p.) dose dependently reversed the age-induced and LPS-induced retention deficits in both test paradigms. However, flavonoids after chronic administration in young mice did not show any improvement of memory retention in both paradigms. Apigenin-7-glucoside showed more efficacy as compared with quercetin in both models that may be probably due to its greater efficacy to inhibit cyclooxygenase-2 and inducible nitric oxide synthase. Chronic treatment with flavonoids did not alter the locomotor activity in both young and aged mice; however, aged mice showed improvement of performance on Rota-Rod test. The results showed that chronic treatment with flavonoids reverses cognitive deficits in aged and LPS-intoxicated mice which suggests that modulation of cyclooxygenase-2 and inducible nitric synthase by flavonoids may be important in the prevention of memory deficits, one of the symptoms related to AD. Copyright 2003 S. Karger AG, Basel

2. Clin Pharmacokinet. 2003;42(5):437-59. Clinical pharmacokinetics of antioxidants and their impact on systemic oxidative stress. Schwedhelm E, Maas R, Troost R, Boger RH. Institute of Experimental and Clinical Pharmacology, Clinical Pharmacology Unit, University Hospital of Hamburg-Eppendorf, Hamburg, Germany. schwedhelm@uke-hamburg.de

Dietary antioxidants play a major role in maintaining the homeostasis of the oxidative balance. They are believed to protect humans from disease and aging. Vitamin C (ascorbic acid), vitamin E (tocopherol), beta-carotene and other micronutrients such as carotenoids, polyphenols and selenium have been evaluated as antioxidant constituents in the human diet. This article addresses data provided from clinical trials, highlighting the clinical pharmacokinetics of vitamin C, vitamin E, beta-carotene, lycopene, lutein, quercetin, rutin, catechins and selenium. The bioavailability of vitamin C is dose-dependent. Saturation of transport occurs with dosages of 200-400 mg/day. Vitamin C is not protein-bound and is eliminated with an elimination half-life ( $t_{1/2}$ ) of 10 hours. In Western populations plasma vitamin C concentrations range from 54-91 micro mol/L. Serum alpha- and gamma-tocopherol range from 21 micro mol/L (North America) to 27 micro mol/L (Europe) and from 3.1 micro mol/L to 1.5 micro mol/L, respectively. alpha-Tocopherol is the most abundant tocopherol in human tissue. The bioavailability of all-rac-alpha-tocopherol is estimated to be 50% of R,R,R-alpha-tocopherol. The hepatic alpha-tocopherol transfer protein (alpha-TTP) together with the tocopherol-associated proteins (TAP) are responsible for the endogenous accumulation of natural alpha-tocopherol. Elimination of alpha-tocopherol takes several days with a  $t_{1/2}$  of 81 and 73 hours for R,R,R-alpha-tocopherol and all-rac-alpha-tocopherol, respectively. The  $t_{1/2}$  of tocotrienols is short, ranging from 3.8-4.4 hours for gamma- and alpha-tocotrienol, respectively. gamma-Tocopherol is degraded to 2, 7, 8-trimethyl-2-(beta-carboxyl)-6-hydroxychroman by the liver prior to renal elimination. Blood serum carotenoids in Western populations range from 0.28-0.52 micro mol/L for beta-carotene, from 0.2-0.28 for lutein, and from 0.29-0.60 for lycopene. All-trans-carotenoids have a better bioavailability than the 9-cis-forms. Elimination of carotenoids takes several days with a  $t_{1/2}$  of 5-7 and 2-3 days for beta-carotene and lycopene, respectively. The bioconversion of beta-carotene to retinal is dose-dependent, and ranges between 27% and 2% for a 6 and 126mg dose, respectively. Several oxidised metabolites of carotenoids are known. Flavonols such as quercetin glycosides and rutin are predominantly absorbed as aglycones, bound to plasma proteins and subsequently conjugated to glucuronide, sulfate, and methyl moieties. The  $t_{1/2}$  ranges from 12-19 hours. The bioavailability of catechins is low and they are eliminated with a  $t_{1/2}$  of 2-4 hours. Catechins are degraded to several gamma-valerolactone derivatives and phase II conjugates have also been identified. Only limited clinical pharmacokinetic data for other polyphenols such as resveratrol have been reported to date.

3. Neurobiol Aging. 2002 Sep-Oct;23(5):891-97. Natural extracts as possible protective agents of brain aging. Bastianetto S, Quirion R. Department of Psychiatry and Pharmacology and Therapeutics, Douglas Hospital Research Centre, McGill University, 6875 LaSalle Boulevard, Verdun, Que, Canada H4H 1R3.

A growing number of studies suggest that natural extracts and phytochemicals have a positive impact on brain aging. We examined the potential of the Ginkgo biloba extract EGb 761 and red wine-derived constituents on cell death produced by beta-amyloid (A $\beta$ ) peptides and oxidative stress, with respect to their possible deleterious role in age-related neurological disorders. We found that EGb 761, possibly through the antioxidant properties of its flavonoids, was able to protect hippocampal cells against toxic effects induced by A $\beta$  peptides. Moreover, we showed that an exposure of rat hippocampal cells to the nitric oxide (NO) donor sodium nitroprusside (SNP) resulted in a decrease in cell survival and increase in reactive oxygen species (ROS) accumulation. However, EGb 761 and red wine-derived polyphenols protected against these events, due to their antioxidant activities, and their ability to block SNP-stimulated activity of protein kinase C (PKC). Taken together, these results support the hypothesis that dietary intake of natural substances may be beneficial in normal aging of the brain. Copyright 2002 Elsevier

4. *Mol Biol Cell*. 2002 Jul;13(7):2502-17. Expression of caveolin-1 induces premature cellular senescence in primary cultures of murine fibroblasts. Volonte D, Zhang K, Lisanti MP, Galbiati F. Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261, USA.

Caveolae are vesicular invaginations of the plasma membrane. Caveolin-1 is the principal structural component of caveolae in vivo. Several lines of evidence are consistent with the idea that caveolin-1 functions as a "transformation suppressor" protein. In fact, caveolin-1 mRNA and protein expression are lost or reduced during cell transformation by activated oncogenes. Interestingly, the human caveolin-1 gene is localized to a suspected tumor suppressor locus (7q31.1). We have previously demonstrated that overexpression of caveolin-1 arrests mouse embryonic fibroblasts in the G(0)/G(1) phase of the cell cycle through activation of a p53/p21-dependent pathway, indicating a role of caveolin-1 in mediating growth arrest. However, it remains unknown whether overexpression of caveolin-1 promotes cellular senescence in vivo. Here, we demonstrate that mouse embryonic fibroblasts transgenically overexpressing caveolin-1 show: 1) a reduced proliferative lifespan; 2) senescence-like cell morphology; and 3) a senescence-associated increase in beta-galactosidase activity. These results indicate for the first time that the expression of caveolin-1 in vivo is sufficient to promote and maintain the senescent phenotype. Subcytotoxic oxidative stress is known to induce premature senescence in diploid fibroblasts. Interestingly, we show that subcytotoxic level of hydrogen peroxide induces premature senescence in NIH 3T3 cells and increases endogenous caveolin-1 expression. Importantly, quercetin and vitamin E, two antioxidant agents, successfully prevent the premature senescent phenotype and the up-regulation of caveolin-1 induced by hydrogen peroxide. Also, we demonstrate that hydrogen peroxide alone, but not in combination with quercetin, stimulates the caveolin-1 promoter activity. Interestingly, premature senescence induced by hydrogen peroxide is greatly reduced in NIH 3T3 cells harboring antisense caveolin-1. Importantly, induction of premature senescence is recovered when caveolin-1 levels are restored. Taken together, these results clearly indicate a central role for caveolin-1 in promoting cellular senescence and they suggest the hypothesis that premature senescence may represent a tumor suppressor function mediated by caveolin-1 in vivo.

5. *Mech Ageing Dev*. 2000 Dec 20;121(1-3):217-30. Antioxidants may contribute in the fight against ageing: an in vitro model. Hu HL, Forsey RJ, Blades TJ, Barratt ME, Parmar P, Powell JR. Molecular Physiology, Unilever Research Laboratory Colworth, Sharnbrook, Bedford MK44 1LQ, UK.

Elderly humans have altered cellular redox levels and dysregulated immune responses, both of which are key events underlying the progression of chronic degenerative diseases of ageing, such as atherosclerosis and Alzheimer's disease. Poorly maintained cellular redox levels lead to elevated activation of nuclear transcription factors such as NFkB and AP-1. These factors are coordinately responsible for a huge range of extracellular signalling molecules responsible for inflammation, tissue remodelling, oncogenesis and apoptosis, processes that orchestrate many of the degenerative processes associated with ageing. It is now clear that levels of endogenous anti-oxidants such as GSH decrease with age. This study aimed to investigate the potential of exogenous anti-oxidants to influence inflammatory responses and the ageing process itself. We investigated the potential of the dietary antioxidant, quercetin, to reverse the age related influences of GSH depletion and oxidative stress using in vitro human umbilical vein endothelial cells (HUVEC) and human skin fibroblast (HSF) cell models. Oxidative stress-induced inflammatory responses were investigated in a GSH depletion and a Phorbol 12-myristate 13-acetate (PMA)-induced stress model. As measured with a sensitive HPLC fluorescence method, GSH in HUVEC was depleted by the addition of L-buthionine-[S,R]-sulfoximine (BSO), a gamma-glutamylcysteine synthetase inhibitor, to the culture medium at a concentration of 0.25 mM. Time course studies revealed that the GSH half-life was 4.6 h in HUVEC. GSH depletion by BSO for 24 h led to a slight increase in intracellular adhesion molecule - 1 (ICAM1) expression and prostaglandin E2 (PGE2) secretion in both types of cells. However, GSH depletion markedly enhanced PMA-induced ICAM and PGE2 production in HUVEC. Responses were progressively elevated following prolonged BSO treatment. Inhibition studies showed that 1-(5-Isoquinoliny)sulfonyl)-2-methylpiperazine (H7), a protein kinase C (PKC) inhibitor, not only abolished most of PMA-induced ICAM-1 expression and PGE2, production, but also eliminated GSH depletion-enhanced PMA stimulation. This enhancement was also inhibited by supplementation with quercetin. The results clearly demonstrate that GSH depletion increased the susceptibility of vascular endothelial cells and fibroblasts to oxidative stress associated inflammatory stimuli. This increased in vitro susceptibility may be extrapolated to the in vivo situation of ageing, providing a useful model to study the influence of micronutrients on the ageing process. In conclusion, these data suggest that dietary antioxidants could play a significant role in the reduction of inflammatory responses.

6. *Eur J Clin Nutr*. 2000 May;54(5):415-7. Quercetin intake and the incidence of cerebrovascular disease. Knekt P, Isotupa S, Rissanen H, Heliovaara M, Jarvinen R, Hakkinen S, Aromaa A, Reunanen A. National Public Health Institute, Helsinki, Finland. paul.knekt@ktl.fi

**OBJECTIVE:** To study the relation between intake of the antioxidant flavonoid quercetin and subsequent incidence of cerebrovascular disease (CVA). **DESIGN:** A cohort study carried out among 9208 Finnish men and women 15 y or more of age and initially free from cardiovascular disease. During a 28 y follow-up period in 1967-1994, a total of 824 cases with CVA were diagnosed. **METHODS:** Food consumption data were collected using a dietary history interview method covering the total habitual diet during the previous year. **RESULTS:** Quercetin intake was not associated with CVA incidence. The relative risk of CVA adjusted for age, serum cholesterol, body mass index, smoking, hypertension, diabetes, geographical area, occupation and intake of beta-carotene, vitamin E, vitamin C, fibre, various fatty acids, and energy between the highest and lowest quartiles of quercetin

intake was 0.99 (95% confidence interval (CI)=0.71-1.38) for men and 0.85 (CI=0.60-1.21) for women. In contrast, apples, the major source of quercetin in the study population, showed a significant inverse association both in men and women, mainly due to an association with thrombotic or embolic stroke. The relative risks of thrombotic stroke after further adjustment for quercetin intake were 0.59 (CI=0.35-0.99; P=0.45) and 0.61 (CI=0.33-1.12; P for trend=0.02) for men and women, respectively. CONCLUSIONS: The results suggest that the intake of apples is related to a decreased risk of thrombotic stroke. This association apparently is not due to the presence of the antioxidant flavonoid quercetin.

7. *Free Radic Biol Med.* 1997;22(4):669-78. Quercetin protects cutaneous tissue-associated cell types including sensory neurons from oxidative stress induced by glutathione depletion: cooperative effects of ascorbic acid. Skaper SD, Fabris M, Ferrari V, Dalle Carbonare M, Leon A. Researchlife S.c.p.A., Castelfranco Veneto, Italy.

Oxidation reactions are essential biological reactions necessary for the formation of high-energy compounds used to fuel metabolic processes, but can be injurious to cells when produced in excess. Cutaneous tissue is especially susceptible to damage mediated by reactive oxygen species and low-density lipoprotein oxidation, triggered by dysmetabolic diseases, inflammation, environmental factors, or aging. Here we have examined the ability of the flavonoid quercetin to protect cutaneous tissue-associated cell types from injury induced by oxidative stress, and possible cooperative effects of ascorbic acid. Human skin fibroblasts, keratinocytes, and endothelial cells were cultured in the presence of buthionine sulfoximine (BSO), an irreversible inhibitor of glutathione (GSH) synthesis. Depletion of intracellular levels of GSH leads to an accumulation of cellular peroxides and eventual cell death. Quercetin concentration-dependently (EC50: 30-40 microM) reduced oxidative injury of BSO to all cell types, and was also effective when first added after BSO washout. BSO caused marked decreases in the intracellular level of GSH, which remained depressed in quercetin-protected cells. Ascorbic acid, while by itself not cytoprotective synergized with quercetin, lowered the quercetin EC50 and prolonged the window for cytoprotection. The related flavonoids rutin and dihydroquercetin also decreased BSO-induced injury to dermal fibroblasts, albeit less efficaciously so than quercetin. The cytoprotective effect of rutin, but not that of dihydroquercetin, was enhanced in the presence of ascorbic acid. Further, quercetin rescued sensory ganglion neurons from death provoked by GSH depletion. Direct oxidative injury to this last cell type has not been previously demonstrated. The results show that flavonoids are broadly protective for cutaneous tissue-type cell populations subjected to a chronic intracellular form of oxidative stress. Quercetin in particular, paired with ascorbic acid, may be of therapeutic benefit in protecting neurovasculature structures in skin from oxidative damage.

8. *Exp Gerontol.* 1982;17(3):213-7. Quercetin, flavonoids and the life-span of mice. Jones E, Hughes RE.

A dietary supplement of 0.1% quercetin significantly reduced the life span of mice. The effect was predominantly on the 'shorter living' males. A blackcurrant juice extract, containing a mixture of flavonoids in addition to quercetin, prolonged significantly the life span of the 'older dying' females. The significance of these results vis-a-vis aging mechanisms and the dietary intake of quercetin is discussed.

9. *Biochem Pharmacol.* 1992 Mar 17;43(6):1167-79. Effects of flavonoids on immune and inflammatory cell functions. Middleton E Jr, Kandaswami C. Department of Medicine, State University of New York, Buffalo 14203.

No doubt can remain that the flavonoids have profound effects on the function of immune and inflammatory cells as determined by a large number and variety of in vitro and some in vivo observations. That these ubiquitous dietary chemicals may have significant in vivo effects on homeostasis within the immune system and on the behavior of secondary cell systems comprising the inflammatory response seems highly likely but more work is required to strengthen this hypothesis. Ample evidence indicates that selected flavonoids, depending on structure, can affect (usually inhibit) secretory processes, mitogenesis, and cell-cell interactions including possible effects on adhesion molecule expression and function. The possible action of flavonoids on the function of cytoskeletal elements is suggested by their effects on secretory processes. Moreover, evidence indicates that certain flavonoids may affect gene expression and the elaboration and effects of cytokines and cytokine receptors. How all of these effects are mediated is not yet clear but one important mechanism may be the capacity of flavonoids to stimulate or inhibit protein phosphorylation and thereby regulate cell function. Perhaps the counterbalancing effect of cellular protein tyrosine phosphatases will also be found to be affected by flavonoids. Some flavonoid effects can certainly be attributed to their recognized antioxidant and radical scavenging properties. A potential mechanism of action that requires scrutiny, particularly in relation to enzyme inhibition, is the redox activity of appropriately configured flavonoids. Finally, in a number of cell systems it seems that resting cells are not affected significantly by flavonoids but once a cell becomes activated by a physiological stimulus a flavonoid-sensitive substance is generated and interaction of flavonoids with that substance dramatically alters the outcome of the activation process.

10. *Thromb Res.* 1991 Oct 1;64(1):91-100. Inhibition of platelet aggregation by some flavonoids. Tzeng SH, Ko WC, Ko FN, Teng CM. Department of Pharmacology, Taipei Medical College, Taiwan.

The inhibitory effects of five flavonoids on the aggregation and secretion of platelets were studied. These flavonoids inhibited markedly platelet aggregation and ATP release of rabbit platelets induced by arachidonic acid or collagen, and slightly those by platelet-activating factor. ADP-induced platelet aggregation was also suppressed by myricetin, fisetin and quercetin. The IC50 on arachidonic acid-induced platelet aggregation was: fisetin, 22 microM; kaempferol, 20 microM; quercetin, 13 microM; morin, 150

microM less than IC50 less than 300 microM. The thromboxane B2 formations were also inhibited by flavonoids in platelets challenged with arachidonic acid. Fisetin, kaempferol, morin and quercetin antagonized the aggregation of washed platelets induced by U46619, a thromboxane A2/prostaglandin endoperoxides mimetic receptor agonist. In human platelet-rich plasma, quercetin prevented the secondary aggregation and blocked ATP release from platelets induced by epinephrine or ADP. These results demonstrate that the major antiplatelet effect of flavonoids tested may be due to both the inhibition of thromboxane formation and thromboxane receptor antagonism.

11. Am J Clin Nutr. 2000 Nov;72(5):1150-5. Erratum in: Am J Clin Nutr 2001 Feb;73(2):360. The flavonoids quercetin and catechin synergistically inhibit platelet function by antagonizing the intracellular production of hydrogen peroxide. Pignatelli P, Pulcinelli FM, Celestini A, Lenti L, Ghiselli A, Gazzaniga PP, Violi F. Department of Experimental Medicine and Pathology, Institute of 1st Clinical Medicine, University La Sapienza, National Institute for Nutrition, Rome, Italy. gazzaniga@uniroma1.it

**BACKGROUND:** Epidemiologic studies have shown an inverse relation between moderate consumption of red wine and cardiovascular disease. Studies have shown that red wine and its component flavonoids inhibit in vivo platelet activation, but the underlying mechanism has not yet been identified. **OBJECTIVE:** Because we showed previously that collagen-induced platelet aggregation is associated with a burst of hydrogen peroxide, which in turn contributes to stimulating the phospholipase C pathway, the aim of this study was to investigate whether flavonoids synergize in inhibiting platelet function and interfere with platelet function by virtue of their antioxidant effect. **DESIGN:** We tested the effect of 2 flavonoids, quercetin and catechin, on collagen-induced platelet aggregation and hydrogen peroxide and on platelet adhesion to collagen. **RESULTS:** Catechin (50-100 micromol/L) and quercetin (10-20 micromol/L) inhibited collagen-induced platelet aggregation and platelet adhesion to collagen. The combination of 25 micromol catechin/L and 5 micromol quercetin/L, neither of which had any effect on platelet function when used alone, significantly inhibited collagen-induced platelet aggregation and platelet adhesion to collagen. Such a combination strongly inhibited collagen-induced hydrogen peroxide production, calcium mobilization, and 1,3,4-inositol triphosphate formation. **CONCLUSIONS:** These data indicate that flavonoids inhibit platelet function by blunting hydrogen peroxide production and, in turn, phospholipase C activation and suggest that the synergism among flavonoids could contribute to an understanding of the relation between the moderate consumption of red wine and the decreased risk of cardiovascular disease.

12. Lancet. 1993 Oct 23;342(8878):1007-11. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study. Hertog MG, Feskens EJ, Hollman PC, Katan MB, Kromhout D. National Institute of Public Health and Environment Protection, Bilthoven, Netherlands.

Flavonoids are polyphenolic antioxidants naturally present in vegetables, fruits, and beverages such as tea and wine. In vitro, flavonoids inhibit oxidation of low-density lipoprotein and reduce thrombotic tendency, but their effects on atherosclerotic complications in human beings are unknown. We measured the content in various foods of the flavonoids quercetin, kaempferol, myricetin, apigenin, and luteolin. We then assessed the flavonoid intake of 805 men aged 65-84 years in 1985 by a cross-check dietary history; the men were then followed up for 5 years. Mean baseline flavonoid intake was 25.9 mg daily. The major sources of intake were tea (61%), onions (13%), and apples (10%). Between 1985 and 1990, 43 men died of coronary heart disease. Fatal or non-fatal myocardial infarction occurred in 38 of 693 men with no history of myocardial infarction at baseline. Flavonoid intake (analysed in tertiles) was significantly inversely associated with mortality from coronary heart disease ( $p$  for trend = 0.015) and showed an inverse relation with incidence of myocardial infarction, which was of borderline significance ( $p$  for trend = 0.08). The relative risk of coronary heart disease mortality in the highest versus the lowest tertile of flavonoid intake was 0.42 (95% CI 0.20-0.88). After adjustment for age, body-mass index, smoking, serum total and high-density-lipoprotein cholesterol, blood pressure, physical activity, coffee consumption, and intake of energy, vitamin C, vitamin E, beta-carotene, and dietary fibre, the risk was still significant (0.32 [0.15-0.71]). Intakes of tea, onions, and apples were also inversely related to coronary heart disease mortality, but these associations were weaker. Flavonoids in regularly consumed foods may reduce the risk of death from coronary heart disease in elderly men.

13. Arch Intern Med. 1996 Mar 25;156(6):637-42. Dietary flavonoids, antioxidant vitamins, and incidence of stroke: the Zutphen study. Keli SO, Hertog MG, Feskens EJ, Kromhout D. Department of Chronic Disease and Environmental Epidemiology, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

**BACKGROUND:** Epidemiological studies suggested that consumption of fruit and vegetables may protect against stroke. The hypothesis that dietary antioxidant vitamins and flavonoids account for this observation is investigated in a prospective study. **METHODS:** A cohort of 552 men aged 50 to 69 years was examined in 1970 and followed up for 15 years. Mean nutrient and food intake was calculated from cross-check dietary histories taken in 1960, 1965, and 1970. The association between antioxidants, selected foods, and stroke incidence was assessed by Cox proportional hazards regression analysis. Adjustment was made for confounding by age, systolic blood pressure, serum cholesterol, cigarette smoking, energy intake, and consumption of fish and alcohol. **RESULTS:** Forty-two cases of first fatal or nonfatal stroke were documented. Dietary flavonoids (mainly quercetin) were inversely associated with stroke incidence after adjustment for potential confounders, including antioxidant vitamins. The relative risk (RR) of the highest vs the lowest quartile of flavonoid intake ( $> \text{or} = 28.6 \text{ mg/d}$  vs  $<18.3 \text{ mg/d}$ ) was 0.27 (95% confidence interval [CI], 0.11 to 0.70). A lower stroke risk was also observed for the highest quartile of beta-carotene intake (RR, 0.54; 95% CI, 0.22 to 1.33). The intake of vitamin C and vitamin E was not associated with stroke risk. Black tea contributed about 70% to flavonoid intake. The RR for a daily consumption of 4.7 cups or more of tea vs less than 2.6 cups of tea was 0.31 (95% CI, 0.12 to

0.84). CONCLUSION: The habitual intake of flavonoids and their major source (tea) may protect against stroke.

14. Surgery. 2002 Feb;131(2):198-204. Quercetin inhibits human vascular smooth muscle cell proliferation and migration. Alcocer F, Whitley D, Salazar-Gonzalez JF, Jordan WD, Sellers MT, Eckhoff DE, Suzuki K, Macrae C, Bland KI. Department of Surgery, University of Alabama at Birmingham, 35294-0007, USA.

BACKGROUND: The French paradox has been associated with regular intake of red wine, which is enriched with flavonoids. Quercetin, a flavonoid present in the human diet, exerts cardiovascular protection through its antioxidant properties. We hypothesized that the beneficial effect of quercetin also could be related to the inhibition of vascular smooth muscle cell proliferation and migration. METHODS: Human aortic smooth muscle cells (AoSMC) were grown in culture in the presence of serum. Quercetin inhibited the serum-induced proliferation of AoSMC. This inhibition was dose-dependent and not attributed to toxicity. Cell cycle analysis revealed that quercetin arrested AoSMC in the G(0)/G(1) phase. The effect of quercetin on AoSMC migration was examined using explant migration and Transwell migration assays. Quercetin significantly decreased migration in both assays in a consistent manner. Finally, Western blot analysis of AoSMC exposed to quercetin demonstrated a significant reduction in the activation of mitogen-activated protein kinase, a signaling pathway associated with the migration of vascular smooth muscle cells. CONCLUSIONS: Quercetin inhibits the proliferation and migration of AoSMC, concomitant with inhibition of mitogen-activated protein kinase phosphorylation. These findings provide new insights and a rationale for the potential use of quercetin in the prophylaxis of cardiovascular diseases.

15. Mol Pharmacol. 2001 Oct;60(4):656-65. Quercetin inhibits Shc- and phosphatidylinositol 3-kinase-mediated c-Jun N-terminal kinase activation by angiotensin II in cultured rat aortic smooth muscle cells. Yoshizumi M, Tsuchiya K, Kirima K, Kyaw M, Suzaki Y, Tamaki T. Department of Pharmacology, The University of Tokushima School of Medicine, Tokushima, Japan. yoshizu@basic.med.tokushima-u.ac.jp

Angiotensin II (Ang II) induces vascular smooth muscle cell (VSMC) hypertrophy, which results in various cardiovascular diseases. Ang II-induced cellular events have been implicated, in part, in the activation of mitogen-activated protein (MAP) kinases. Although it has been proposed that daily intake of bioflavonoids belonging to polyphenols reduces the incidence of ischemic heart diseases (known as "French paradox"), the precise mechanisms of efficacy have not been elucidated. Thus, we hypothesized that bioflavonoids may affect Ang II-induced MAP kinase activation in cultured rat aortic smooth muscle cells (RASMC). Our findings showed that Ang II stimulated rapid and significant activation of extracellular signal-regulated kinase (ERK) 1/2, c-Jun N-terminal kinase (JNK), and p38 in RASMC. Ang II-induced JNK activation was inhibited by 3,3',4',5,7-pentahydroxyflavone (quercetin), a major bioflavonoid in foods of plant origin, whereas ERK1/2 and p38 activation by Ang II were not affected by quercetin. Ang II caused a rapid tyrosine phosphorylation of Src homology and collagen (Shc), which was inhibited by quercetin. Quercetin also inhibited Ang II-induced Shc.p85 association and subsequent activation of phosphatidylinositol 3-kinase (PI3-K)/Akt pathway in RASMC. Furthermore, LY294002, a PI3-K inhibitor and a quercetin derivative, inhibited Ang II-induced JNK activation as well as Akt phosphorylation. Finally, Ang II-induced [(3)H]leucine incorporation was abolished by both quercetin and LY294002. These findings suggest that the preventing effect of quercetin on Ang II-induced VSMC hypertrophy are attributable, in part, to its inhibitory effect on Shc- and PI3-K-dependent JNK activation in VSMC. Thus, inhibition of JNK by quercetin may imply its usefulness for the treatment of cardiovascular diseases relevant to VSMC growth.

16. Br J Pharmacol. 2001 May;133(1):117-24. Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. Duarte J, Perez-Palencia R, Vargas F, Ocete MA, Perez-Vizcaino F, Zarzuelo A, Tamargo J. Department of Pharmacology, School of Pharmacy, University of Granada, 18071 Granada, Spain.

1. The effects of an oral daily dose (10 mg kg<sup>-1</sup>) of the flavonoid quercetin for 5 weeks in spontaneously hypertensive (SHR) and normotensive Wistar Kyoto rats (WKY) were analysed. 2. Quercetin induced a significant reduction in systolic (-18%), diastolic (-23%) and mean (-21%) arterial blood pressure and heart rate (-12%) in SHR but not in WKY rats. 3. The left ventricular weight index and the kidney weight index in vehicle-treated SHR were significantly greater than in control WKY and these parameters were significantly reduced in quercetin-treated SHR in parallel with the reduction in systolic blood pressure. 4. Quercetin had no effect on the vasodilator responses to sodium nitroprusside or to the vasoconstrictor responses to noradrenaline or KCl but enhanced the endothelium-dependent relaxation to acetylcholine (E(max)=58±5% vs 78±5%, P<0.01) in isolated aortae. 5. The 24 h urinary isoprostane F(2 alpha) excretion and the plasma malonyldialdehyde (MDA) levels in SHR rats were increased as compared to WKY rats. However, in quercetin-treated SHR rats both parameters were similar to those of vehicle-treated WKY. 6. These data demonstrate that quercetin reduces the elevated blood pressure, the cardiac and renal hypertrophy and the functional vascular changes in SHR rats without effect on WKY. These effects were associated with a reduced oxidant status due to the antioxidant properties of the drug.

17. Free Radic Biol Med. 2002 Jun 1;32(11):1220-8. Mitochondrial function in response to cardiac ischemia-reperfusion after oral treatment with quercetin. Brookes PS, Digerness SB, Parks DA, Darley-Usmar V. Department of Pathology, University of Alabama at Birmingham, Birmingham, AL 35294-2180, USA. brookes@uab.edu

Polyphenolic compounds present in red wines, such as the flavonol quercetin, are thought capable of cardioprotection through

mechanisms not yet clearly defined. It has been established that mitochondria play a critical role in myocardial recovery from ischemia-reperfusion (I-R) damage, and in vitro experiments indicate that quercetin can exert a variety of direct effects on mitochondrial function. The effects of quercetin at concentrations typically found in 1-2 glasses of red wine on cardiac I-R and mitochondrial function in vivo are not known. Quercetin was administered to rats (0.033 mg/kg per day by gavage for 4 d). Isolated Langendorff perfused hearts were subjected to I-R, and cardiac functional parameters determined both before and after I-R. Mitochondria were isolated from post-I-R hearts and their function assessed. Compared to an untreated control group, quercetin treatment significantly decreased the impairment of cardiac function following I-R. This protective effect was associated with improved mitochondrial function after I-R. These results indicate that oral low dose quercetin is cardioprotective, possibly via a mechanism involving protection of mitochondrial function during I-R.

18. Proc Natl Acad Sci U S A. 2000 Aug 1;97(16):9052-7. The NF-kappa B signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation. Hajra L, Evans AI, Chen M, Hyduk SJ, Collins T, Cybulsky MI. Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto General Research Institute, Toronto, Ontario, M5G 2C4, Canada.

Atherosclerotic lesions form at distinct sites in the arterial tree, suggesting that hemodynamic forces influence the initiation of atherogenesis. If NF-kappaB plays a role in atherogenesis, then the activation of this signal transduction pathway in arterial endothelium should show topographic variation. The expression of NF-kappaB/IkappaB components and NF-kappaB activation was evaluated by specific antibody staining, en face confocal microscopy, and image analysis of endothelium in regions of mouse proximal aorta with high and low probability (HP and LP) for atherosclerotic lesion development. In control C57BL/6 mice, expression levels of p65, IkappaBalpha, and IkappaBbeta were 5- to 18-fold higher in the HP region, yet NF-kappaB was activated in a minority of endothelial cells. This suggested that NF-kappaB signal transduction was primed for activation in HP regions on encountering an activation stimulus. Lipopolysaccharide treatment or feeding low-density lipoprotein receptor knockout mice an atherogenic diet resulted in NF-kappaB activation and up-regulated expression of NF-kappaB-inducible genes predominantly in HP region endothelium. Preferential regional activation of endothelial NF-kappaB by systemic stimuli, including hypercholesterolemia, may contribute to the localization of atherosclerotic lesions at sites with high steady-state expression levels of NF-kappaB/IkappaB components.

19. Clin Exp Allergy. 2000 Apr;30(4):501-8. Effects of luteolin, quercetin and baicalein on immunoglobulin E-mediated mediator release from human cultured mast cells. Kimata M, Shichijo M, Miura T, Serizawa I, Inagaki N, Nagai H. Department of Pharmacology, Gifu Pharmaceutical University, Gifu, Japan.

**BACKGROUND:** Flavonoids have a variety of activities including anti-allergic activities, and are known to inhibit histamine release from human basophils and murine mast cells. **OBJECTIVE:** The effects of luteolin, a flavone, on the immunoglobulin (Ig) E-mediated allergic mediator release from human cultured mast cells (HCMCs) were investigated and compared with those of baicalein and quercetin. **METHODS:** HCMCs were sensitized with IgE, and then treated with flavonoids before challenge with antihuman IgE. The amount of released mediators was determined as was mobilization of intracellular Ca<sup>2+</sup> concentration, protein kinase C (PKC) translocation and phosphorylation of intracellular proteins were detected after anti-IgE stimulation. **RESULTS:** Luteolin, baicalein and quercetin inhibited the release of histamine, leukotrienes (LTs), prostaglandin D<sub>2</sub> (PGD<sub>2</sub>), and granulocyte macrophage-colony stimulating factor (GM-CSF) from HCMC in a concentration-dependent manner. Additionally, the three flavonoids inhibited A23187-induced histamine release. As concerns Ca<sup>2+</sup> signalling, luteolin and quercetin inhibited Ca<sup>2+</sup> influx strongly, although baicalein did slightly. With regard to PKC signalling, luteolin and quercetin inhibited PKC translocation and PKC activity strongly, although baicalein did slightly. The suppression of Ca<sup>2+</sup> and PKC signalings might contribute to the inhibition of mediator release. The activation of extracellular signal-regulated kinases (ERKs) and c-Jun NH<sub>2</sub>-terminal kinase (JNK), that were activated just before the release of LTs and PGD<sub>2</sub> and GM-CSF mRNA expression in IgE-mediated signal transduction events, were clearly suppressed by luteolin and quercetin. In contrast, the flavonoids did not affect the activation of p38 mitogen-activated protein kinase (p38 MAPK) pathway. **CONCLUSION:** These results indicate that luteolin is a potent inhibitor of human mast cell activation through the inhibition of Ca<sup>2+</sup> influx and PKC activation.

20. Res Commun Chem Pathol Pharmacol. 1992 Nov;78(2):211-8. Changes in the xanthine dehydrogenase/xanthine oxidase ratio in the rat kidney subjected to ischemia-reperfusion stress: preventive effect of some flavonoids. Sanhueza J, Valdes J, Campos R, Garrido A, Valenzuela A. Unidad de Bioquimica Farmacologica y Lipidos, INTA, Universidad de Chile, Santiago.

The enzyme xanthine oxidase has been implicated in the tissue oxidative injury after ischemia-reperfusion. This enzyme, which is a source of oxygen free radicals, is formed from a dehydrogenase form during ischemia. The ratio dehydrogenase/oxidase of rat kidney homogenates decreases during the ischemia and the reperfusion. Two flavonoids, quercetin and silybin, characterized as free radical scavengers, exert a protective effect preventing the decrease in the dehydrogenase/oxidase ratio observed during ischemia-reperfusion. The mechanism of this effect and the role of flavonoids in the ischemia-reperfusion tissue damage is discussed.

21. Methods Find Exp Clin Pharmacol. 2001 May;23(4):175-81. Quercetin, a bioflavonoid, protects against oxidative stress-related renal dysfunction by cyclosporine in rats. Satyanarayana PS, Singh D, Chopra K. Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India.

Nephrotoxicity is the most common and clinically important side effect of cyclosporine (CsA). Recent evidence suggests that reactive oxygen species (ROS) play an important role in CsA nephrotoxicity. This study was designed to demonstrate the role of oxidative stress and its relation to renal dysfunction and to investigate the effects of quercetin, a bioflavonoid with antioxidant properties, in CsA-induced nephrotoxicity. Quercetin (0.5 and 2.0 mg/kg i.p.) was administered 24 h before and concurrently with CsA (20 mg/kg s.c.) for 21 days. Tissue lipid peroxidation was measured as thiobarbituric acid reacting substances (TBARS). Renal function was assessed by estimating plasma creatinine, blood urea nitrogen (BUN), creatinine and urea clearance. Renal morphological alterations were assessed histopathologically. Pretreatment with CsA (20 mg/kg s.c.) for 21 days produced elevated levels of TBARS and deteriorated renal function as assessed by increased plasma creatinine, BUN and decreased creatinine and urea clearance as compared to vehicle-treated rats. The kidneys of CsA-treated rats showed severe striped interstitial fibrosis, arteriopathy, glomerular basement thickening, tubular vacuolization and hyaline casts. Quercetin (2 mg/kg) markedly reduced elevated levels of TBARS and significantly attenuated renal dysfunction and morphological changes in CsA-treated rats. It is likely that quercetin, due to its antioxidant properties, prevented CsA-induced ROS and consequently CsA nephrotoxicity. These results clearly demonstrate the pivotal role of oxidative stress and its relation to renal dysfunction, and also point to the therapeutic potential of the natural antioxidant quercetin in CsA-induced nephrotoxicity.

22. Free Radic Biol Med. 2002 Jul 1;33(1):63-70. Quercetin metabolism in the lens: role in inhibition of hydrogen peroxide induced cataract. Cornish KM, Williamson G, Sanderson J. School of Biological Sciences, University of East Anglia, Norwich, Norfolk, UK.

Oxidative stress is implicated in the initiation of maturity onset cataract. Quercetin, a major flavonol in the diet, inhibits lens opacification in a lens organ culture oxidative model of cataract. The aim of this research was to investigate the metabolism of quercetin in the lens and show how its metabolism affects the ability to prevent oxidation-induced opacity. The LOCH model (Free Radical Biology & Medicine 26:639; 1999) was employed, using rat lenses to investigate the effects of quercetin and metabolites on hydrogen peroxide-induced opacification. High-performance liquid chromatography analysis showed that the intact rat lens is capable of converting quercetin aglycone to 3'-O-methyl quercetin (isorhamnetin). Over a 6 h culture period no further metabolism of the 3'-O-methyl quercetin occurred. Loss of quercetin in the lens was accounted for by the increase in 3'-O-methyl quercetin. Incubation with 3,5-dinitrocatechol (10 microM), a catechol-O-methyltransferase (COMT) inhibitor, prevented the conversion of quercetin to 3'-O-methyl quercetin. The presence of both membrane-bound and soluble COMT was confirmed by immunoblotting. The results demonstrate that in the rat lens COMT methylates quercetin and that the product accumulates within the lens. Quercetin (10 microM) and 3'-O-methyl quercetin (10 microM) both inhibited hydrogen peroxide- (500 microM) induced sodium and calcium influx and lens opacification. Incubation of lenses with quercetin in the presence of COMT inhibitor revealed that the efficacy of quercetin is not dependent on its metabolism to 3'-O-methyl quercetin. The results indicate dietary quercetin and metabolites are active in inhibiting oxidative damage in the lens and thus could play a role in prevention of cataract formation.

23. Free Radic Biol Med. 1999 Sep;27(5-6):683-94. Structure-activity relationships of quercetin in antagonizing hydrogen peroxide-induced calcium dysregulation in PC12 cells. Wang H, Joseph JA. Jean Mayer United States Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, USA. wang\_us@hnrc.tufts.edu

Oxidative stress can induce neurotoxic insults by increasing intracellular calcium (Ca<sup>2+</sup>), which has been implicated in various neurodegenerative diseases in aging. Previously, we showed that hydrogen peroxide induced calcium dysregulation in PC12 cells, as evidenced by (i) an increase in calcium baselines, (ii) a decrease in depolarization-induced calcium influx, and (iii) a failure to recover the Ca<sup>2+</sup> levels. In the present experiments, we investigated whether a dietary flavonoid, quercetin, can antagonize the effects of hydrogen peroxide in the same cell model. We also investigated the possible structure-activity relationships of quercetin by comparing the results with four other flavonoids, each having a slightly different structure from quercetin. Our results indicated that two structural components, including (i) 3', 4'-hydroxyl (OH) groups in the B ring and (ii) a 2,3-double bond in conjugation with a 4-oxo group in the C ring, along with the polyphenolic structures were crucial for the protection. These structural components are found in quercetin, and this compound was also the most efficacious in reducing both the H<sub>2</sub>O<sub>2</sub>-induced Ca<sup>2+</sup> dysregulation in cells and oxidative stress assessed via the dichlorofluorescein assay. Collectively, these data indicated that the particular polyphenolic structural components of quercetin provided its strong antioxidant property of protecting cells against H<sub>2</sub>O<sub>2</sub>-induced oxidative stress and calcium dysregulation.

24. Zhongguo Yao Li Xue Bao. 1999 May;20(5):426-30. Quercetin decreased heart rate and cardiomyocyte Ca<sup>2+</sup> oscillation frequency in rats and prevented cardiac hypertrophy in mice. Wang Y, Wang HY, Yuan ZK, Zhao XN, Wang JX, Zhang ZX. School of Medicine, State Key Laboratory of Coordination Chemistry, Nanjing University, China.

AIM: To study the effects of quercetin (Que) on myocardial excitation-contraction coupling and cardiac remodeling. METHODS: Left ventricles and femoral arteries of rats were cannulated for hemodynamic recording. Mouse cardiac hypertrophy was induced by abdominal aortic coarctation (AAC). Cultured myocardial cells in neonatal rats were loaded with Fura 2-AM. The intracellular calcium ([Ca<sup>2+</sup>]<sub>i</sub>) and spontaneous [Ca<sup>2+</sup>]<sub>i</sub> oscillations ([Ca<sup>2+</sup>]<sub>i</sub>-SO) were tested by AR-CM-MIC cation measurement system. RESULTS: Que 3 or 25 mg.kg<sup>-1</sup> i.v. in rats decreased heart rate from (420 +/- 19) to (390 +/- 15) and (314 +/- 18) beat.min<sup>-1</sup>, respectively, accompanied with very modest changes in both left ventricular pressures (LVP) and its differential dpLV/dt<sub>max</sub>. Que 10, 50, 250 mumol.L<sup>-1</sup> concentration-dependently slowed the frequency of [Ca<sup>2+</sup>]<sub>i</sub>-SO in cultured myocardial cells from (26 +/- 4) to

(25 +/- 3), (18 +/- 4), and (12 +/- 3) time.min<sup>-1</sup>, respectively, but did not change their resting [Ca<sup>2+</sup>]<sub>i</sub> or amplitudes of [Ca<sup>2+</sup>]<sub>i</sub>-SO. Similarly, the increases in frequency of [Ca<sup>2+</sup>]<sub>i</sub>-SO caused by either isoproterenol (Iso) or ouabain (Oua) were prevented by Que 100 μmol.L<sup>-1</sup>, while the simultaneous increases in amplitude of [Ca<sup>2+</sup>]<sub>i</sub>-SO remained. Besides, [Ca<sup>2+</sup>]<sub>i</sub> rises excited by angiotensin II (Ang II) but not high [K<sup>+</sup>]<sub>o</sub> were prevented by Que 100 μmol.L<sup>-1</sup>. Daily administration of Que 120 mg.kg<sup>-1</sup> i.g. for 5 d markedly prevented the cardiac hypertrophy in AAC mice, without effects on the ventricular mass to body weight ratio (VM/BW) in sham-operated mice. CONCLUSION: Quercetin decreased myocardial [Ca<sup>2+</sup>]<sub>i</sub>-oscillation frequency and prevented cardiac remodeling, but had no direct effect on cardiac excitation-contraction coupling.

25. Zhongguo Yao Li Xue Bao. 1995 May;16(3):223-6. Effects of quercetin on aggregation and intracellular free calcium of platelets. Xiao D, Gu ZL, Bai JP, Wang Z. Department of Pharmacology, Suzhou Medical College, China.

AIM: To study the effects of Que on the intraplatelet free calcium concentration and the effects of calcium on the inhibition of platelet aggregation by Que. METHODS: Using Quin-2 fluorescence technique. RESULTS: Que inhibited the platelet aggregation and the rise of [Ca<sup>2+</sup>]<sub>i</sub> induced by thrombin in platelets. The values of IC<sub>50</sub> and 95% confidence interval were 146.2 (92.4 - 231.3) and 78.5 (49.5 - 124.4) μmol.L<sup>-1</sup>, respectively. The inhibitory effects of Que on platelet aggregation induced by thrombin were reduced by adding calcium to the medium, and Que had no effect on thrombin-induced internal Ca<sup>2+</sup> release from dense tubular system. CONCLUSION: The inhibitory effects of Que on aggregation and the rise of [Ca<sup>2+</sup>]<sub>i</sub> in platelets was mainly due to an inhibition of Ca<sup>2+</sup> influx.

26. Antiproliferative potency of structurally distinct dietary flavonoids on human colon cancer cells Kuo SM. Nutrition Program, State University of New York at Buffalo, 14214, USA. smkuo@acsu.buffalo.edu Cancer Letters (Ireland), 1996, 110/1 2 (41 48) Dietary flavonoids are known to be antiproliferative and may play an important role in cancer chemoprevention, especially cancers of the gastrointestinal tract, because of a direct contact with food. This study was designed to compare the antiproliferative potency of several structurally distinct dietary flavonoids in colon cancer cells, Caco 2 and HT 29, and in rat nontransformed intestinal crypt cells, IEC 6. Flavonoids varied significantly in their antiproliferative potency depending on the structural features but the observations were consistent among the three cell lines studied. Of the two most potent flavonoids, quercetin and genistein, the effect was found to be dose dependent and chromatin condensation, an indication of apoptosis, was noticed. Quercetin was found to distribute throughout the cell with higher amounts in the perinuclear and nucleoli areas. The lack of specific cell membrane enrichment by quercetin was consistent with its lack of effect on the transepithelial resistance. While several flavonoids including quercetin were found to be unstable, the chemical instability did not correlate with the antiproliferative potency, although it may contribute to the antiproliferative effect.

27. Preferential requirement for protein tyrosine phosphatase activity in the 12-O-tetradecanoylphorbol-13-acetate-induced differentiation of human colon cancer cells. Kuo ML, Huang TS, Lin JK. Institute of Toxicology, College of Medicine, National Taiwan University, Taipei. Biochem Pharmacol; 50(8):1217-22 1995

Some lines of colon cancer cells are forced to undergo differentiation by 12-O-tetradecanoylphorbol-13-acetate (TPA). The increases in activities of both protein tyrosine phosphatase (PTP) and protein tyrosine kinase (PTK) have been reported to be associated with the TPA-induced differentiation of HL-60 leukemia cells. In the present study, a 2-fold increase in PTP activity was observed in SW620 human colon cancer cells after 30 min of TPA treatment; a maximal level (4- to 5-fold) was reached at 60 min and continued for more than 6 hr. In addition, two TPA-induced differentiated characteristics, morphological alteration and release of cellular surface proteoglycan, were effectively blocked by PTP inhibitors, such as sodium orthovanadate (50 μM), zinc chloride (100 μM), and iodoacetate (250 μM), but not by the protein serine/threonine phosphatase inhibitor okadaic acid (20 nM). On the other hand, although TPA induced a transient slight increase in PTK activity (1.4-fold) at 60 min, four PTK inhibitors (genistein, herbimycin A, tyrphostin-23 and quercetin) had different effects on the TPA-induced release of cell surface proteoglycan. Genistein (60 μM) potentiated this process, but in contrast, quercetin (45 μM) could partially inhibit the TPA effect. Taken together, these observations suggest that both PTP and PTK activities were increased in SW620 cells in response to TPA; however, the activation of PTP seems to be preferentially required for the TPA-induced differentiation of SW620 human.

28. Effect of Quercitrin on acute and chronic experimental colitis in the rat De Medina F.S.; Galvez L.-H.; Romero J.A.; Zarzuelo A. F.S. De Medina, Department of Pharmacology, School of Pharmacy, University of Granada, 18071 Granada Spain Journal of Pharmacology and Experimental Therapeutics (USA) , 1996, 278/2 (771-779)

Quercitrin was tested for acute and chronic anti-inflammatory activity in trinitrobenzenesulfonic acid-induced rat colitis. The inflammatory status was evaluated by myeloperoxidase, alkaline phosphatase and total glutathione levels, leukotriene B<sub>4</sub> synthesis, in vivo colonic fluid absorption, macroscopical damage and occurrence of diarrhea and adhesions. Treatment with 1 or 5 mg/kg of quercitrin by the oral route reduced myeloperoxidase and alkaline phosphatase levels, preserved normal fluid absorption, counteracted glutathione depletion and ameliorated colonic damage at 2 days. Increasing or lowering the dose of the flavonoid resulted in marked loss of effect. The acute anti-inflammatory effect of quercitrin is unrelated to impairment of neutrophil function or lipoxygenase inhibition, and it may be caused by mucosal protection or enhancement of mucosal repair secondary to increased defense against oxidative insult and/or preservation of normal colonic absorptive function. When tested in chronic colitis (2 and 4 weeks), quercitrin treatment (1 or 5 mg/kg . day) decreased colonic damage score and the incidence of diarrhea, and normalized the colonic fluid transport. All other parameters were unaffected. The chronic effect of the flavonoid is apparently related to its

action on colonic absorption, although it can be partly secondary to its acute beneficial effect.

29. Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus juices So FV, Guthrie N, Chambers AF, Moussa M, Carroll KK. Department of Pharmacology and Toxicology, University of Western Ontario, London, Canada. *Nutrition and Cancer (USA)* , 1996, 26/2 (167-181)

Two citrus flavonoids, hesperetin and naringenin, found in oranges and grapefruit, respectively, and four noncitrus flavonoids, baicalein, galangin, genistein, and quercetin, were tested singly and in one to one and growth of a human breast carcinoma cell line, MDA MB 435. The concentration at which cell proliferation was inhibited by 50% (IC50), based on incorporation of (3H)thymidine, varied from 5.9 to 140 microg/ml for the single flavonoids, with the most potent being baicalein. IC50 values for the one to one combinations ranged from 4.7 microg/ml (quercetin + hesperetin, quercetin + naringenin) to 22.5 microg/ml (naringenin + hesperetin). All the flavonoids showed low cytotoxicity (>500 microg/ml for 50% cell death). Naringenin is present in grapefruit mainly as its glycosylated form, naringin. These compounds, as well as grapefruit and orange juice concentrates, were tested for their ability to inhibit development of mammary tumors induced by 7,12 dimethylbenz(a)anthracene (DMBA) in female Sprague Dawley rats. Two experiments were conducted in which groups of 21 rats were fed a semipurified diet containing 5% corn oil and were given a 5 mg dose of DMBA intragastrically at approximately 50 days of age while in diestrus. One week later, individual groups were given double strength grapefruit juice or orange juice or fed naringin or naringenin at levels comparable to that provided by the grapefruit juice; in the second experiment, the rats were fed a semipurified diet containing 20% corn oil at that time. As expected, rats fed the high fat diet developed more tumors than rats fed the low fat diet, but in both experiments tumor development was delayed in the groups given orange juice or fed the naringin supplemented diet compared with the other three groups. Although tumor incidence and tumor burden (grams of tumor/rat) were somewhat variable in the different groups, rats given orange juice had a smaller tumor burden than controls, although they grew better than any of the other groups. These experiments provide evidence of anticancer properties of orange juice and indicate that citrus flavonoids are effective inhibitors of human breast cancer cell proliferation in vitro, especially when paired with quercetin, which is widely distributed in other foods.

### 30. Quercetin glycosides inhibit lipoxygenase-induced LDL oxidation

Inhibition of mammalian 15-lipoxygenase-dependent lipid peroxidation in low-density lipoprotein by quercetin and quercetin monoglucosides. Luiz da Silva E, Tsushida T, Terao J. National Food Research Institute, Ministry of Agriculture, Forestry, and Fisheries, Ibaraki, Japan. *Arch Biochem Biophys.* 1998 Jan 15;349(2):313-20.

Lipoxygenase is suggested to be involved in the early event of atherosclerosis by inducing plasma low-density lipoprotein (LDL) oxidation in the subendothelial space of the arterial wall. Since flavonoids such as quercetin are recognized as lipoxygenase inhibitors and they occur mainly in the glycoside form, we assessed the effect of quercetin and its glycosides (quercetin 3-O-beta-glucopyranoside, Q3G; quercetin 4'-O-beta-glucopyranoside, Q4'G; quercetin 7-O-beta-glucopyranoside, Q7G) on rabbit reticulocyte 15-lipoxygenase (15-LOX)-induced human LDL lipid peroxidation and compared it with the inhibition obtained by ascorbic acid and alpha-tocopherol, the main water-soluble and lipid-soluble antioxidants in blood plasma, respectively. Quercetin inhibited the formation of cholesteryl ester hydroperoxides (CE-OOH) and endogenous alpha-tocopherol consumption effectively throughout the incubation period of 6 h. Ascorbic acid exhibited an effective inhibition only in the initial stage and LDL preloaded with fivefold alpha-tocopherol did not affect the formation of CE-OOH compared with the native LDL. CE-OOH formation was inhibited by both quercetin and quercetin monoglucosides in a concentration-dependent manner. Quercetin, Q3G, and Q7G exhibited a higher inhibitory effect than Q4'G (IC50: 0.3-0.5 microM for quercetin, Q3G, and Q7G and 1.2 microM for Q4'G). While endogenous alpha-tocopherol was completely depleted after 2 h of LDL oxidation, quercetin, Q7G, and Q3G prevented the consumption of alpha-tocopherol. Quercetin and its monoglucosides were also exhausted during the LDL oxidation. These results indicate that quercetin glycosides as well as its aglycone are capable of inhibiting lipoxygenase-induced LDL oxidation more efficiently than ascorbic acid and alpha-tocopherol.

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