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Review article

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FLAVONOIDS AND THE AGING BRAIN

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Like in all other organs, the functional capacity of the human brain deteriorates over time. Pathological events such as oxidative stress, due to the elevated release of free radicals and reactive oxygen or nitrogen species, the subsequently enhanced oxidative modification of lipids, protein, and nucleic acids, and the modulation of apoptotic signaling pathways contribute to loss of brain function. The identification of neuroprotective food components is one strategy to facilitate healthy brain aging. Flavonoids were shown to activate key enzymes in mitochondrial respiration and to protect neuronal cells by acting as antioxidants, thus breaking the vicious cycle of oxidative stress and tissue damage. Furthermore, recent data indicate a favorable effect of flavonoids on neuro-inflammatory events. Whereas most of these effects have been shown in vitro, limited data in vivo are available, suggesting a rather low penetration of flavonoids into the brain. Nevertheless, several reports support the concept that flavonoid intake inhibits certain biochemical processes of brain aging, and might thus prevent to some extent the decline of cognitive functions with aging as well as the development or the course of neurodegenerative diseases. However, more data are needed to assess the true impact of flavonoids on brain aging.

Key words: *brain aging, ROS, oxidative stress, flavonoids*

INTRODUCTION

As the elderly population increases, the prevalence of aging-related brain diseases, for example Alzheimer's disease (AD) or Parkinson's disease (PD), is likely to increase. Even if the cause of both neurodegenerative diseases is not yet finally known, enhanced oxidative stress seems to have an important contribution to brain aging in general and neurodegenerative diseases in specific. Therefore it becomes more and more important to develop drugs or neutraceuticals that

possibly are neuroprotective. The brain is the most susceptible organ to oxidative damage due to its high oxygen demand (1,2). Elevated oxygen consumption may lead to oxidative stress. The pathology of aging-associated neurodegeneration is increasingly linked to oxidative and nitrosative stress mediated by free radicals (3,4). Within the cell, free radicals, such as superoxide (O_2^-) and hydroxyl ($OH^\cdot$), are normal products of cellular oxygen metabolism. Moreover, other molecules like H_2O_2 and peroxynitrite ($ONOO^-$), although not themselves free radicals, can lead to generation of free radicals. Together, these highly reactive metabolites are referred to as reactive oxygen (ROS) and nitrogen species (RNS). Oxidative stress arises from an imbalance between cellular ROS/RNS production and the ability of cells to defend themselves against this stress. The attack of free radicals, ROS and RNS causes cellular damage by oxidizing proteins, membrane lipids, and DNA.

Normal cells have various mechanisms to protect themselves against oxidative and nitrosative attacks. Beside glutathione (GSH) and the vitamins C and E, all powerful antioxidant scavengers, the major cellular defense consists of antioxidative enzymes and bioactives such as carotenoids and flavonoids. Antioxidant enzymes in the brain include Cu/Zn superoxide dismutase (SOD-1) and Mn superoxide dismutase (SOD-2), both catalyzing the reaction of O_2^- to H_2O_2. Catalase and glutathione peroxidase (GPx) subsequently catalyze the conversion of H_2O_2 to H_2O. The brain contains the highest concentrations of ascorbic acid of all body tissues (5). Vitamin C is able to scavenge a broad spectrum of ROS and is therefore one of the most important exogenous antioxidants in the body. Vitamin E is a lipid-soluble molecule with chain-breaking properties, which by restoring ascorbate reduced form in a regenerative cycle reaction, enhances its antioxidant activity. Hence, the ratio of both vitamins determines their antioxidant efficacy. However, both vitamin E and C recently failed to exert protective effects on cognitive function in humans (6). Recent data from epidemiological studies and animal experiments indicate that flavonoids-rich diets may positively influence brain aging and lower the incidence of neurodegenerative disorders such as AD and PD (1). The present article reviews not only recent data from *in vitro* studies pointing out possible mechanisms responsible for the observed neuroprotective effect of flavonoids, but also the outcome of *in vivo* studies using flavonoid supplements.

BRAIN AGING

Changes in brain function, for example the slowing down of information processing, can already be detected in middle-aged humans (35-65 year old) and rodents (12-24 month old) without fulfilling the criteria of dementia. Whereas neurodegenerative diseases, such as AD, are accompanied by a devastating loss of neurons, certain features of normal brain aging often proceed without

significant brain atrophy (7). However, impairment of neuronal activity in different brain regions represents a common feature of normal brain aging (8). One of the most popular theories for explaining the aging process is the free radical theory, initially proposed by Denham Harman (9). As outlined before, the brain is especially vulnerable towards the accumulation of oxidative stress-induced damage (2). Although the brain accounts for less than 2% of the bodyweight it consumes 20% of the basal oxygen uptake. High oxygen consumption is linked to leakage of electrons along the respiratory chain with subsequent radical formation. A second reason is the high amount of polyunsaturated fatty acids (PUFAs) present in neuronal membranes (10). These PUFAs are especially prone to undergo lipid peroxidation reactions resulting in the formation of cytotoxic aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) (11). Furthermore, the insufficient supply of nutrients, caused by an impaired cerebromicrovascular function, contributes to the diminished neuronal capacity in the aging brain (12). Oxidative stress, deprivation of essential nutrient as well as age related and oxidative-stress induced damage hampers the activity of mitochondria, the key organelles for ATP production. Reduced energy supply will lead to reduced neuronal and finally brain function including impaired cognition.

FLAVONOID: BASIC PHARMACOKINETICS

Flavonoids belong to the broad group of polyphenols. Plants synthesize these secondary metabolites from the aromatic amino acids phenylalanine or tyrosin, or from malonate as defense against radiation and animals (13). Flavonoids contribute to the blue, orange or purple colors of fruits, leaves and flowers (14) and are mainly found in fruits, vegetables, nuts, grains, spices, seeds, wine, tea, and beer. By now, over 6000 different flavonoids have been identified (13) that can be divided in 6 classes due to their different structures: flavonoles, flavones, isoflavones, flavanoles (catechine), flavanones and anthocyanidins. Flavonoles, like quercetin and kaempferol, are most ubiquitous in food plants. The general flavonoid structure is a flavan nucleus, 15 carbon atoms arranged in three rings (A, B, C). The various classes differ in their level of oxidation and in their pattern of substitution of the c-ring (*Fig.1*).

Flavonoids are almost exclusively present in human diet as glycosylated derivates (β-glucosides) and not as aglycones, with the exception of catechins (flavanols). Flavonoid glycosides (FG), fairly large and highly polar molecules, are too hydrophilic to diffuse passively across biological membranes and the absorption of FGs was long believed negligible (15). Recent data, however, indicate that the bioavailability of FGs is determined by a complex network of absorption, metabolism and efflux processes (16-19). Despite rather high absorption rates, the bioavailability of flavonoids is comparatively low.

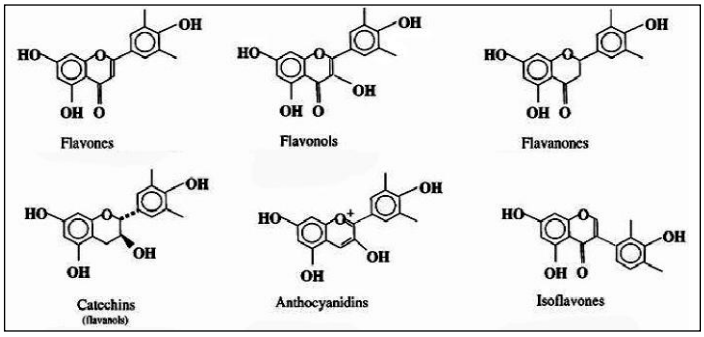

Figure 1: Main groups of flavonoids

Quercetin, for example, was found to reach 0.1 - 10 µmol/L in the circulation. The concentration of quercetin was mainly due to the presence of quercetin metabolites rather that its aglycone, as recently reviewed by Murota and Terao (20). Consequently, the action of flavonoid metabolites is attracting increasing scientific interest (17,21).

Effects on the blood brain barrier

In spite of the evidence for flavonoid-depending effects on brain function including neuroprotection, there is little information on their ability to cross the blood brain barrier (BBB) in order to reach the central nervous system (CNS). The BBB is formed by brain capillary endothelial cells tightly controlling the access of most small polar molecules and macromolecules into the brain. Youdim et al. (22) reported the uptake of several flavonoids and their relevant conjugated metabolites in a model of brain endothelial cells. Furthermore, the authors found that most of the tested flavonoids and respective metabolites are able to cross the BBB. The potential for BBB permeation is dependent on the compound's lipophilicity and the activity of efflux transporters, such as P-glycoprotein (Pgp), in the BBB (23). Inhibitory and stimulatory effects of quercetin on Pgp activity have been found *in vitro* and *in vivo*, recently (24,25). The exact mechanism, however, by which flavonoids modulate P-gp activity, is still unclear. Nonetheless, the ability of flavonoids to enter the brain has been confirmed by their identification in brain materials after p.o. and i.v. administration. Abd El Mohsen et al. (26) reported the presence of picomolar concentrations of epicatechin glucuronide and 3'-O-methyl epicatechin glucuronide in the brain of rats after oral supplementation with epicatechin (100 mg/kg bodyweight) for 1, 5 or 10 days. Similarly, naringenin and hesperetin have been detected in brain tissue after intravenous administration (27,28). These findings are supported by recent results from our laboratory, showing a biphasic modulatory effect of quercetin on the transport activity of Pgp in porcine brain capillary endothelial cells (PBCEC) and a human lymphocytic leukemia cell line expressing no Pgp (CEM) and their multidrug resistant variant (VLB) which express Pgp. At low concentrations (10 nM) quercetin activated the

transport of calcein-AM by Pgp and at high concentrations (1-3 µM) it significantly inhibited the transport in PBCEC cells (*Fig 2*). A similar, but not significant effect was observed in VLB cells. 30 nM quercetin activate the calcein transport by Pgp and 1-3 µM inhibit this transport (25).

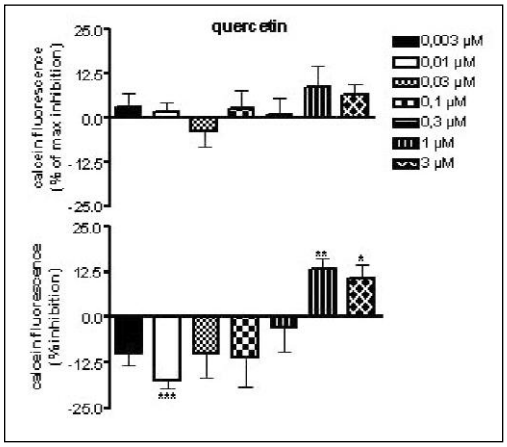

Figure 2: Influence of quercetin on the Calcein-AM uptake in VLB cells expressing Pgp (upper panel) and PBCEC cells expressing no Pgp (lower panel). Data are given as percent of calcein fluorescence under control condition and represent means ± SEM for n = 6 (PBCEC) / n = 5-6 (VLB) experiments performed in triplicates. * $p < 0,05$; ** $p < 0.01$; *** $p < 0.001$. Positive percentages indicate an inhibition of Pgp and negative values may be explained by activation of Pgp.

NEUROPROTECTION

Direct antioxidant activities

Flavonoids possess a highly reactive hydroxyl group that gets oxidized by electron-donation, thus stabilizing the radical to a less reactive molecule. One way of reaction is the direct scavenging of free radicals, for example superoxide anions, singulet oxygen and lipid peroxyl radicals. Structurally important features defining the reducing potential of flavonoids are the hydroxylation pattern, a 3',4'-dihydroxy catechol structure in the B-ring, the planarity of the molecule and the presence of 2,3-unsaturation in conjugation with a 4-oxo function in the C-ring. There is considerable evidence that flavonoids efficiently attenuate the deleterious effect of free radicals and ROS/RNS. Quercetin and some structurally related flavonoids, for example, showed a marked cytoprotective effect in PC12 cells exposed to H_2O_2 (29). Also in PC12 cells, the addition of flavonoids or flavonoid-rich extracts suppressed the hydroperoxide-induced increase in ROS, thus improving cell survival (30,31). Matsuoka et al. (32) showed that tea catechins protect cultured newborn-mouse cerebral nerve cells from ROS-induced cell death after addition of glucose oxidase. The authors further examined learning ability of mice by a step-down-type passive avoidance test and memory impairment of mice by intracisternal injection of glucose oxidase or induced cerebral ischemia. Glucose oxidase-induced memory impairment was improved by intracisternal injection of epicatechin and i.v.

injection of catechin or epicatechin attenuated memory impairment induced by cerebral ischemia. These results suggest that tea catechins ameliorate ROS-induced cytotoxicity through intracellular scavenging. Another beneficial effect resulting of this scavenging activity is to decrease oxidation of membrane lipids. For example, gossypin dramatically inhibited lipid peroxidation initiated by Fe2+ and ascorbic acid in rat brain homogenates. In addition, gossypin significantly attenuated the neurotoxicity induced by Aβ (25-35) (33). Anthocyanidins inhibited H_2O_2-induced lipid peroxidation in rat brain homogenates, too. The ID50 values of delphinidin, cyanidin, and pelargonidin for them were 0.7, 3.5, and 85 μM, respectively (34). Antioxidant effects of flavonoids have furthermore been detected in vivo. The volume of the lipid peroxidation marker lipofuscin was significantly reduced in the brain of adult rats chronically exposed to ethanol (35). These findings are supported by data from Zhang et al. (16,36) who reported a decrease of ROS production, thiobarbituric acid reactive substances content and nitrite/nitrate concentration in brain homogenates in a Mongolian gerbil stroke model after Crataegus flavonoid supplementation. Furthermore, pretreatment of the animals with green catechins increased the antioxidative level and amount of biologically available NO by scavenging of superoxide anion produced during reperfusion (36,37). Total infarction volume in the ipsilateral hemisphere of ischemia/reperfusion rats was significantly lowered by treatment with the flavonoid balicalein (50 mg/kg) given 2 h apart, promptly prior to and 2 h after reperfusion (38). Also significant ROS reduction in striatum of aged rats was found by Joseph et al. after 8 weeks of supplementing diet with either blueberry, strawberry or spinach extracts (39).

Indirect antioxidant activities

NADH-oxidase is a key enzyme in mitochondrial respiration. In a recent examination Hodnick et al. (40), compared hydroxylation and methoxylation patterns of flavonoids referring to their NADH-oxidase inhibition ability. The order of potency was robinetin, rhamnetin, eupatorin, baicalein, 7,8-dihydroxyflavone and norwogonin. Cos et al. (41) reported that flavones have higher xanthine oxidase inhibitory activity than flavonoles. Xanthine oxidase catalyses the oxidation of xanthine and hypoxanthine to uric acid. During the re-oxidation of the enzyme superoxide radicals as well as H_2O_2 are produced. The authors further showed that hydroxyl groups at C-3 and C-3' are essential for high superoxide scavenging. Recently, Suzuki et al. (37) administered catechin extract in drinking water to rats for five days before und during middle cerebral artery occlusion (MCAO) and reperfusion to examine their protective effects on various deteriorative processes following stroke. Catechins significantly ameliorated neurological deficits observed after reperfusion by the inhibition of iNOS expression and infiltration of neutrophiles. In addition, the formation of peroxynitrite was found to be decreased due to the potent radical scavenging

Table 1: Neuroprotective effects of flavonoids and flavonoid-rich extracts

Source	Treatment	Beneficial properties	Ref.
Strawberry extract, Spinach extracts, Vitamin E	9.5 g/kg 6.4 g/kg 500 IU/kg	Reduction of striatal ROS level in 6 month old rats after two weeks of supplementation	(62)
Crataegus flavonoid extract	0.5 mg/mL 2.5mg/mL	Protection against ischemia/reperfusion injury-caused delayed cell death in gerbils after 15 days of extract application through drinking water	(36)
Grape polyphenol extract	5 mg/dl	Prevention of ethanol-induced dopamine uptake activity decrease in young rats after grape polyphenol supplementation for 2 month	(63)
Catechin	3.4 µM 34 µM 340 µM	Reduction of lipid peroxidation in rat embryonic mesencephalic cell cultures after 6-OHDA-induced neuronal cell death	(64)
Resveratrol	30 mg/kg BW	Reduction of kainic acid-induced cell death in adult rat brains after i.p. administration for 5 days	(65)
(-)-epigallocatechine gallate	10 µM 25 or 50 m/kg i.p.	Attenuation of MDA formation in rat embryonic hippocampal neurons Reduction of postischemic brain edema in gerbils	(66)

properties of catechins. Komatsu et al. recently found increased SOD activity in the mitochondrial fraction of striatums and midbrains of aged rats after one-month administration of beta-catechin solution (42).

Anti-inflammatory activities

Many flavonoids are known to possess anti-inflammatory properties and suppression of the inflammatory response in chronic diseases may beneficially affect disease outcome. Therefore flavonoids are getting more and more in the focus as a possibility to help preventing these diseases. Several mechanisms for the anti-inflammatory effect of flavonoids have been proposed. Wogonin, for example, a flavonoid from medicinal herb, was recently found to be a potent neuroprotectant from natural source. In co-cultured PC12 cells as well as *in vivo*, wogonin showed inhibition of inflammatory activation and reduction in microglial cytotoxicity (43). Wogonin diminished lipopolysaccharide-induced tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta, and nitric oxide (NO) production *in vitro*. Latter effect of wogonin has been suggested to be facilitated by suppression of inducible NO synthase (iNOS) induction and NF-kappaB activation. The neuroprotective effect of wogonin was further demonstrated *in vivo* using two experimental brain injury models. In both animal models, wogonin conferred neuroprotection by attenuating the death of hippocampal neurons and the neuroprotective effect was associated with inhibition of the inflammatory activation of microglia. Also baicalein, a major flavonoid present in a traditional Chinese herb, showed potent anti-inflammatory properties on dopaminergic

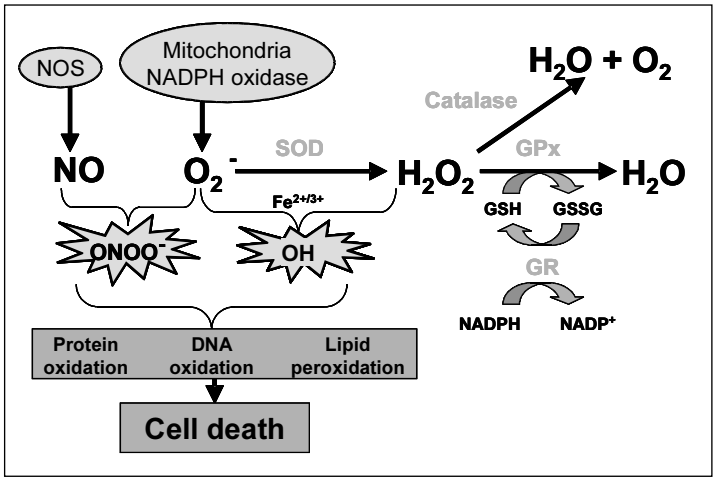

Figure 3: ROS formation and detoxification

neurons and primary midbrain neuron-glia cultures from rat. The maximum protective effect on lipopolysaccharide-induced damage to dopaminergic neurons evaluated by uptake capacity for [(3)H]dopamine and tyrosine hydroxylase (TH)-immunocytochemistry was observed at a concentration of 5 μM (44). Furthermore, baicalein prevented cell death in primary midbrain cultures, possibly due to its activity as a LOX-inhibitor (45). Quercetin and other flavonoids (e.g. catechin, chrysin, puerarin, naringenin, and genestein) also protected mesencephalic cultures from injury by MPP(+), which was shown by DNA fragmentation studies and tyrosine hydroxylase (TH) immunocytochemistry of mesencephalic dopamine (DA) neurons to occur by apoptosis. Similarly, catechin reduced cellular injury induced by hydrogen peroxide, 4-hydroxynonenal, rotenone, and 6-hydroxydopamine as shown by increases in cellular viability and [(3)H]DA uptake (46). Another polyphenol with anti-inflammatory effects is silymarin. This flavonoid recently demonstrated to exert neuroprotective effects against lipopolysaccharide (LPS)-induced neurotoxicity in mesencephalic mixed neuron-glia cultures (47).

Protection against Aβ neurotoxicity

Aging is the most important risk factor for Alzheimer's disease (AD). Its neuronal pathology is characterized by the abundance of intracellular neurofilament tangles and the extracellular deposition of β-amyloid peptide (Aβ) as senile plaques (48). The pathological effects of Aβ are significantly determined by its oxidative stress-inducing properties (49). As mentioned before, certain flavonoids are able to protect cells by acting as scavengers of ROS and RNS. Wang et al. (50) investigated in cortical neurons the effect of several flavonoids on Aβ-(25-35)-induced cytotoxicity. Pre-incubation with 50 μM of kaempferol, apigenin, luteolin, and quercetin significantly lowered the Aβ-mediated increase

in ROS production. Furthermore, apigenin and kaempferol - but not quercetin - decreased Aβ-induced neuronal death by alleviating the release of cytochrome c and the activation of the caspase cascade. Moreover, quercetin potentized the protective effect of apigenin. Neuroprotective effects of green tea and soy flavonoids due to their ability to scavenge Aβ-induced ROS have also been shown by Choi et al. (51) and Zeng et al. (52), respectively. An additional explanation for the neuroprotective activity of flavonoids has been presented, recently. Myricetin, morin, and quercetin dose-dependently not only inhibited the formation and extension of Aβ fibrils, but also destabilized preformed Aβ fibrils with effective concentrations (EC_{50}) in the order of 0.1-1 μM. The underlying mechanisms responsible for the observed effects, however, are still unclear (53). Bastianetto et al. showed that flavonoids-rich *Ginkgo biloba* and red wine extracts are able to significantly protect hippocampal cells against Aβ peptides- and/or oxidative stress-induced toxicities (37,54).

Improvement of cognition in aging and dementia

In a substantial number of studies about the effect of flavonoids or flavonoids-rich extracts on cognitive function in aged animals have been published (*Table 2*). Patil et al. (55) reported for aged but not young mice significant effects of quercetin supplementation on the step-through and transfer latency in the passive avoidance and elevated plus-maze task, respectively. Similarly, the learning time of aged mice supplied with catechin-enriched tab water was improved (56). Latter results are supported by Shirai and Suzuki (57) feeding adult and old mice with diets containing catechin. Time needed and distance travelled to reach the maze exit and the number of times strayed into blind alleys were significantly improved in the verum group. An extract of

Table 2: Cognition improvement properties of flavonoids and flavonoid-rich extracts

Source	Treatment	Beneficial properties	Ref.
Apigenin-7-glucoside Quercetin	5-20 mg/kg i.p. 25-100 mg/kg i.p.	Reduction of aging-induced impairment of Passive Avoidance Performance in aged mice after chronic administration for 7 days i.p.	(67)
DHA and Catechins	1.5 % 0,5 %	Enhancement of Maze behaviour in adult and aged mice after 3.5 month intake of DHA and catechin supplemented 5 % lard diet	(68)
Green tea catechins	0,02 % in tab water	Suppressive effect on cognitive dysfunction markers (passive avoidance and Y maze test) in accelerated senescence mice	(69)
Strawberry extract Spinach extract Blueberry extract	14.8 g/kg 9.1 g/kg 18.6 g/kg	Reversal of age-related declines in several cognitive and motor behavioural deficits in aged male rats after 8 weeks of extract intake	(39)
Quercetin	10-50 mg/kg	Amelioration of cognitive performance in aged mice after chronic quercetin intake for 24 days measured as retention performance in passive avoidance and elevated plus-maze test	(70)

purple sweet potato anthocyanin markedly enhanced cognitive performance, assessed by passive avoidance test in ethanol-treated mice (58). Water-maze performance of aged rats was significantly improved after strawberry, spinach and, blueberry supplementation for 8 month (39).

Up to now there are few studies showing significant effects of flavonoids on cognitive deficits in aged humans or demented patients. One of the few reports (*Table 3*) indicates that the development of dementia might be slowed down by flavonoid rich diet. The Paquid Study was done in 1991-1996 in south-west France. The authors concluded that the relative risk of incident dementia was inversely related to the mean antioxidant flavonoid intake during the last 5 years as determined by a questionair. After adjustment to age, gender, education, weight and vitamin C intake, the relative risk of dementia for the highest flavonoid intake percentile was 0.49, respectively (1).

Table 3: Effects of flavonoid intake on Dementia (Paquid Study, France) (1)

Study design	**Flavonoid consumption (mg/day)**	**Age-adjusted model**		
1367 subjects		**RR**	**95 % CI**	***p*-value**
	< 11.5 mg	1.00	-	-
	11.5, 16.2	0.50	0.27 – 0.94	0.03
	> 16.2 mg	0.59	0.33 – 1.06	0.08

Authors investigated whether flavonoid intake could be associated with a lower incidence of dementia in a cohort study of over 1300 subjects above 65 years of age. A questionnaire was used to evaluate their intake of flavonoids and subjects were followed-up for 5 years. Relative risk (RR) of dementia according to tertiles of flavonoid intake was estimated using a Cox model.

CONCLUSIONS

The constantly increasing number of people affected by neurodegenerative diseases is one of the major medical and social challenges of the 21st century. Although it is currently unknown whether oxidative stress is the primary cause of aging-related cognitive decline and dementia, the elevated release of ROS and RNS significantly contributes to the pathological changes observed in neurodegeneration (2). Flavonoids are ubiquitous in plant foods and beverages and can therefore be consumed with the daily diet (59). In numerous cell-free and cell-based experiments flavonoids demonstrated not only to possess excellent antioxidant activity but also to attenuate the accumulation of dementia-promoting metabolites, such as Aβ, and to suppress inflammation by interacting with pro-inflammatory enzymes. Whereas detailed data on flavonoid consumption are available for humans, unfortunately only a few studies investigated a possible activity of flavonoid intake as neuroprotectants (1). However, as summarized in *Table 1* and *2*, results obtained in animal studies support health-beneficial effects

of flavonoids in the aging brain on both, the reduction of ROS-induced damage to biomolecules and the improvement of cognitive performance. Discrepancies in the magnitude of effect due to flavonoids supplementation are due to the diversity of bioactives used, to the different modality of administration, to the diversity of utilized methods, and to the timing of treatment (60). Furthermore, the effect of metabolic changes in the structure of flavonoids and their interaction with the blood brain barrier have not been adequately studied, yet, to draw a clear picture of the possible neuroprotective mechanism linked to flavonoids intake.

Despite of all these findings there is not enough data on the special effects of flavonoids or flavonoid-rich extracts *in vivo* to come to a final conclusion. The majority of data which are considered as evidence for this neuroprotection, however, come from studies of complex mixtures of compound with high flavonoid contents, such as green tea and herbal medicines, which have been recently reviewed by Youdim et al (61). Considering the health benefiting effects of flavonoid supplements or nutraceuticals, the absorption and brain permeability of the containing flavonoids have to be taken into account. Some studies only worked with the flavonoid aglycones and not their naturally occurring glycosides or metabolites. Nevertheless available data suggest a positive role of supplementation with flavonoids to slow down neurodegeneration in pathological processes and aging.

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