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Review

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Neuroprotective Effects of Citrus Flavonoids

Sam-Long Hwang,[†] Ping-Hsiao Shih,[‡] and Gow-Chin Yen*[,‡]

[†]Food Industry Research and Development Institute, P.O. Box 246, Hsinchu 30012, Taiwan

[‡]Department of Food Science and Biotechnology, National Chung Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan

ABSTRACT: Recent attention has been given to the influence of dietary factors on health and mental well-being. Oxidative stress is associated with many diseases including neurodegenerative disorders. Dietary flavonoids exert cardioprotective, chemopreventive, and neuroprotective effects. The biological activities of flavonoids have been attributed to their antioxidant, anti-inflammatory, and signaling properties. A clear understanding of the mechanisms of action, as either antioxidants or signaling molecules, is crucial for the application of flavonoids as interventions in neurodegeneration and as brain foods. Citrus flavonoids exert little adverse effect and have low or no cytotoxicity to healthy, normal cells. The main citrus flavonoids can also traverse the blood−brain barrier; hence, they are promising candidates for intervention in neurodegeneration and as constituents in brain foods. In this review, we discuss the bioactivity, multiple neuroprotection mechanisms, and antioxidant and signaling properties of citrus flavonoids. Receptor-mediated neuroprotective actions and parallel signaling pathways are also explored. Finally, the induction of cellular defense proteins against oxidative stress and neurotoxicity by hesperetin, a main and widespread citrus flavonoid, are also discussed. It is suggested that citrus fruits, which are rich in abundant sources of hesperetin and other flavonoids, are promising for the development of general food-based neuroprotection and brain foods.

KEYWORDS: *neuroprotection, citrus, flavonoids, antioxidant, signaling*

1. INTRODUCTION

Many factors contribute to the degeneration of neural cells, leading to functional deterioration of neurons and neurodegenerative disorders. It is known that oxidative stress plays an important role in such events. Because of their high metabolic activities and low antioxidant defense capacities, neural cells in brains are more vulnerable to oxidative stress, particularly those neural cells in aging brains.[1−5] Generally, functional deterioration and neurotoxic substances cause the viscous cycle of oxidative stress and disruption of calcium-homeostasis during brain aging, which could result in neurotoxicity, memory decline, and neurodegenerative diseases.[6−9] In addition, hydrogen peroxide is produced in β-amyloid (Aβ) aggregation, dopamine oxidation, and brain ischemia/reperfusion. Aβ aggregation is known to cause oxidative damage in neurons, including protein and lipid oxidation and DNA damage.[10−12]

Increasing evidence has shown that dietary factors improve neuronal function and synaptic plasticity. Mechanisms underlying such actions have primarily been characterized through antioxidant and anti-inflammatory bioactivities and signaling regulation at the molecular level.[13] Citrus fruits and products have been consumed globally.[14] Citrus flavonoids exhibit antioxidant, anticarcinogenic, and anti-inflammatory bioactivities; they are mainly composed of flavanones, flavones, and polymethoxyflavones.[15,16] Major members of citrus flavonoids including hesperidin, neohesperidin, and hesperetin possess antioxidant activity and can traverse the brain−blood barrier. Thus, these citrus flavonoids have the potential to intervene in neurodegeneration and promote brain functions.[17−20]

Dietary flavonoids exhibit characteristics of both antioxidant and signaling molecules. As signaling molecules, flavonoids could interact with key cellular receptors or proteins (kinases and enzymes) that are involved in signaling cascades to catalyze or regulate signaling or regulatory pathways, resulting in physiological responses or gene expression.[10,21,22] It has been suggested that flavonoids are more likely to exert neuroprotective actions by modulation of intracellular signaling associated with neuronal survival, death, and differentiation as well as through interactions with mitochondria.[23] Therefore, understanding whether a flavonoid is involved more as an antioxidant or a signaling molecule against oxidative stress-related neurodegeneration is a key distinction to evaluate its potential and application for neuroprotection.[22]

2. DIETARY FLAVONOIDS AND NEUROPROTECTION

2.1. Dietary and Citrus Flavonoids. Natural antioxidants are primarily composed of vitamin C, vitamin E, carotenoids, and polyphenols. Among them, polyphenols are the most abundant natural antioxidants in people's diets.[24] The total dietary intake of antioxidants could be as high as 1 g/day, which is approximately 10 times higher than that of vitamin C and approximately 100 times higher than those of vitamin E and carotenoids.[25,26] The main class of polyphenols is flavonoids, of which the basic chemical structure contains a heterocyclic C6−C3−C6 skeleton. Based on the oxidization of the heterocyclic (C3) ring, flavonoids can be defined as flavanols, flavones, isoflavones, flavanols, and flavanones. Besides these, flavonoids also include anthocyanins, proanthocyanidins, stilbenes (resveratrols), and lignans.[24,27] Main dietary sources of flavonoids are fruits, vegetables, and plant-derived beverages such as fruit juices, tea, coffee, and red wine. Flavonoid content is high in

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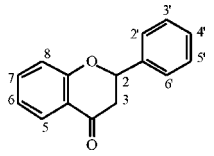
Table 1. Citrus Flavonoids[a]



compound	C3′	C4′	3	5	6	7	8	C2−C3
(a) hesperidin	OH	OCH_3	H	OH	H	rutinose[b]	H	single
(b) narirutin	H	OH	H	OH	H	rutinose	H	single
(c) eriocitrin	OH	OH	H	OH	H	rutinose	H	single
(d) isorhoifolin	H	OH	H	OH	H	rutinose	H	double
(e) diosmin	OH	OCH_3	H	OH	H	rutinose	H	double
(f) naringin	H	OH	H	OH	H	neohesperidose[b]	H	single
(g) neoeriocitrin	OH	OH	H	OH	H	neohesperidose	H	single
(h) neodiosmin	OH	OCH_3	H	OH	H	neohesperidose	H	double
(i) neohesperidin	OH	OCH_3	H	OH	H	neohesperidose	H	single
(j) diosmetin	OH	OCH_3	H	OH	H	OH	H	double
(k) luteolin	OH	OH	H	OH	H	OH	H	double
(l) isorhamnetin	OCH_3	OH	OH	OH	H	OH	H	double
(m) limocitrin	OCH_3	OH	OH	OH	H	OH	OCH_3	double
(n) kaempherol	H	OH	OH	OH	H	OH	H	double
(o) limocitrol	OCH_3	OH	OH	OH	OCH_3	OH	OCH_3	double
(p) tangeretin	H	OCH_3	H	OCH_3	OCH_3	OCH_3	OCH_3	double
(q) nobiletin	OCH_3	OCH_3	H	OCH_3	OCH_3	OCH_3	OCH_3	double
(r) natsudaidain	OCH_3	OCH_3	OH	OCH_3	OCH_3	OCH_3	OCH_3	double
(s) 3,5,6,7,8,3′,4′-heptamethoxyflavone	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3	double

[a]Modified from Manthey et al., 2001;[15] Gonzalez-Molina et al., 2010.[14]. [b]Neohesperidose, 2-*O*-α-L-rhamnopyranosyl-β-D-glucopyranose; rutinose, 6-*O*-α-L-rhamnopyranosyl-β-D-glucopyranose.

apples, cauliflower, carrots, tomatoes, soybeans, and citrus fruits.[25,28]

Citrus plants are the most important crop of fruit trees in the world. Their annual production is near 1.02 hundred million tons globally.[14] Citrus flavonoids are composed of three major subgroups including flavanones (mainly di- and tri-*O*-glycosides), flavone glycosides (mainly di- and tri-*O*-glycosides and *C*-glycosides), and polymethoxyflavones.[15] The citrus *O*-glycosides are mostly in the form of rutinosides or neohesperidosides (Table 1). The rutinosides such as hesperidin (a), narirutin (b), eriocitrin (c), isorhoifolin (d), and diosmin (e) are tasteless and mainly found in oranges (*Citrus sinensis* L.), tangerines (*C. reticulate* L.), and lemons (*C. limon* L.). Neohesperidosides such as naringin (f), neoeriocitrin (g), neodiosmin (h), and neohesperidin (i) are bitter and mainly found in hybrids of grapefruit (*C. paradise* Macf.) and pummelo (*C. grandis* L.). In the flavone aglycons, diosmetin (j) is present in general citrus plants, and luteolin (k) is present in lemons. Further, lemons (*C. limon* L.) contain flavonols (l−o), differentiating it from other citrus plants. The polymethoxyflavones such as tangeretin (p), nobiletin (q), natsudaidain (r), and heptamethoxyflavone (s) are rich in oranges, tangerines, and lemons and are less abundant in some grapefruits.[14,15] Recently, new polymethoxyflavones and hydroxylated polymethoxychalcones have also been separated from sweet oranges (*Citrus sinesis*) by Li et al.[29]

2.2. Antioxidant Activities of Dietary and Citrus Flavonoids. Antioxidative effects of flavonoids are thought to be mainly attributed to their scavenging of oxygen-derived free radicals.[30,31] Owing to the action of hydrogen donation, metal ion binding, and the resonance effect of phenoxyl radical stabilization, flavonoids can exhibit antioxidant activity.[28,32] Flavonoids function as reducing agents and metal chelators, reactive oxygen species (ROS) scavengers, chain-breaking antioxidants, quenchers of singlet oxygen formation, and protectors of ascorbic acid.[33]

Among dietary flavonoids, citrus flavonoids exert moderate to strong radical/reactive oxygen species (ROS) scavenging activities. However, citrus flavonoids had higher activity, inhibiting lipid peroxidation and scavenging benzoylperoxide radicals ($PhCOO^{\bullet}$), methyl methacrylate radicals ($R^{\bullet}$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), alkyperoxyl radicals ($ROO^{\bullet}$) and peroxynitrite ($ONOO^{-}$).[16,18,34−36] Kaempferol, luteolin, rutin, scutellarein and neoeriocitrin are known to exert strong antioxidant activities, while the most abundant and widespread citrus flavonoids such as hesperidin, hesperetin, neohesperidin, naringenin, and naringin exhibit moderate antioxidant activities. Usually, the antioxidant activities of citrus glycosides are weaker than those of the aglycons; however, the inhibition of hesperidin on the formation of $R^{\bullet}$ and polyunsaturated fatty acid-derived free radicals was better than that of hesperetin.[18,37] Hesperetin has also interacted with $ONOO^{-}$ and exhibited good intracellular $ONOO^{-}$-scavenging activity.[36,38] In a study of rats with diabetes, isorhamnetin acted as an antioxidant and effectively suppressed the peroxidation of lipids in the blood, liver, and kidneys of rats.[39]

2.3. Beneficial Bioactivities of Dietary and Citrus Flavonoids. *2.3.1. The Neuroprotection of Dietary Flavonoids.* Dietary flavonoids are beneficial for health because of their bioactivities including antioxidant action, inhibition of tumorigenesis, reduction of plasma cholesterol and blood sugar levels, reduction in blood pressure, anti-inflammation, and neuroprotection.[22,40] As novel neuroprotective agents against oxidative stress, flavonoids could directly scavenge free radicals, activate prosurvival regulatory pathways, or indirectly increase endogenous cellular antioxidant defenses via modulation

(activation/inhibition) of signaling cascades or upregulation of gene expression.[27,41,42] Briefly, flavonoids could mediate neuroprotective signaling by the modulation of PKA, Akt/PKB, PKC, ERK1/2, p38, and JNK pathways via receptors and upstream/downstream kinases. Cell and animal studies have shown that neuroprotective effects of flavonoids against oxidative damage and amyloid-derived neurotoxicity could be attributed to their antioxidant properties, anti-inflammation, and signaling regulation.[43−47] Furthermore, dietary flavonoids and flavonoid-rich extracts of strawberry, spinach, and blueberry have been shown to promote cognitive performances in animal and human studies.[48−53]

2.3.2. Bioactivities of Citrus Flavonoids. Citrus flavonoids possess beneficial bioactive qualities for health, such as antiallergic, anticancer, and anti-inflammatory activities, lowering blood pressure and plasma lipids and conferring cardioprotective and neuroprotective activities.[14,26,54,55] It is known that citrus flavonoids show chemopreventive effects on bladder, colon, liver, lung, prostate, mammary, and oral carcinogeneses.[56−59] For chemoprevention, citrus flavonoids can be used as suppressing, blocking, or transforming agents via diverse mechanisms including antiproliferation, anti-invasion, antiangiogenesis, and proapoptotic actions and cell-selective toxicity in cancer cells.[15,60] The relationship between chemical structure and function of citrus flavonoids is more obvious in the antiproliferation of cancer cells. The position and number of hydroxyl groups at the flavonoid A and B rings, number of methoxy groups, and low polar planar structure determine the antiproliferative activity of a citrus flavonoid. Promoting antiproliferative effects of the polymethoxyflavones, the hydroxyl at C-3 and the methoxy residue at C-8 are essential. Mechanisms underlying antiproliferative actions of citrus flavonoids act primarily via the inactivation of kinases and kinase inhibitors involved in cell cycle arrest and apoptosis.[60,61] However, citrus flavonoids typically exhibit low or no toxicity in animals, but show anti-inflammatory and chemopreventive characteristics, and thus have the potential for health-promoting activity in humans.[15,55,56,61,62]

2.3.3. Anti-Inflammatory Action of Citrus Flavonoids Conferring Neuroprotection. Inflammation (edema, fever, or ache) is a normal response to tissue trauma, bleeding, or pathogenic infection. In the inflammatory region, histamines, cytokines, chemokines, growth factors, interferons, and cytotoxic substances could be released to exert defensive actions such as blood vessel expansion, allergen resistance, virus resistance, or tissue repair.[58,63] However, the inflammatory response is often uncontrolled in chronic autoimmune diseases (e.g., rheumatoid arthritis and Crohn's disease). Additionally, excessive and sustained inflammation has been associated with many diseases such as asthma, inflammatory bowel syndrome, diabetes, atherosclerosis, sepses, cancers, and neurodegenerative disorders.[56,64] Different biological mediators influence each step of the inflammation cascade. Typically, an anti-inflammatory agent exhibits therapeutic properties by suppressing the actions or syntheses of these mediators.[15] Flavonoids have been shown to inhibit key reactions catalyzed by phospholipase A2, cyclooxygenase, and lipoxygenase in inflammatory responses.[65,66] These enzymes are involved in the syntheses of proinflammatory arachidonic acid derivates (AAD), such as prostaglandins E2, F2 and thromboxane A2. These AAD are essential for activating neutrophils and thus stimulate the formation of ROS in inflammatory tissues.[67−69] ROS-mediated inflammation and its mediators play central roles in many neurodegenerative disorders. It induces chronic inflammation and proinflammatory mediators including NO and tumor necrosis factor (TNF) in microglia. A high level of NO is neurotoxic because of the formation of peroxynitrite.[56] It is known that hesperidin and diosmin exhibit anti-inflammatory properties by blocking the synthesis and actions of ADD.[15] Apigenin and diosmin effectively inhibit NO formation and TNF-α release in lipopolysaccharide- or advanced glycation end-product-induced microglia.[70] Furthermore, some citrus flavonoids can inhibit the activation of phosphodiesterase and kinases involved in the initiation stage of inflammation. Activation of these enzymes can affect the expression of proinflammatory TNF-α and protein kinases. Some citrus flavonoids can inhibit the induction of endothelial cell adhesion molecules (ICAM-1, VCAM-1, and E-selectin) triggered by cytokines. They inhibit inflammatory responses by blocking the adhesion of neutrophils, monocytes, and other leukocytes from injured regions.[15,56]

2.3.4. The Neuroprotection of Citrus Flavonoids. Many studies have shown that citrus fruits exhibit neuroprotective effects. Heo and Lee[71] reported that the methanol extract of orange pulps was neuroprotective against oxidative damage in PC12 cells. Hesperetin (0.1 μM) protected cortical neurons from oxidative injury by activating prosurvival Akt and ERK1/2 signaling pathways, which inhibit the activation of proapoptotic proteins, such as apoptosis signal-regulating kinase-1 (ASK1), Bad, caspase-9, and caspase-3. It is suggested that such hesperetin-induced actions might occur via receptors.[72] A high dose of hesperetin protected cortical neurons against oxidative stress, Aβ-associated neurotoxicity, and glutamate-induced excitotoxicity.[73] In the brains of mice with long-term ingestion (50 mg/kg for 5 weeks) of hesperetin, the oxidation of lipids and proteins was obviously decreased. The expression of antioxidant enzymes including catalase, Mn-SOD, CuZn-SOD, glutathione (GSH) peroxide, and glutathione reductase, as well as the GSH/GSSG ratio, was obviously increased.[62] Additionally, naringenin, the main flavonoid of *Citrus junos*, has been proven to protect PC12 cells against Aβ-associated oxidative damage and enhance the memory performance of mice with scopolamine-induced amnesia.[74] Moreover, a recent study has shown that oral administration of hesperidin limits the extent of rat brain damage following stroke via the reduction of free radicals and its associated inflammation.[75]

2.4. Dietary Flavonoids: Antioxidants or Signaling Molecules? In recent years, studies on neuroprotection, cardioprotection, and chemoprevention by flavonoids have focused on the transfer from their antioxidant activities to signaling molecule properties.[22,23] The conventional antioxidant activities of flavonoids have been insufficient to explain their cellular bioactivity. Dietary flavonoids are generally metabolized to aglycons, glucuronides, sulfates, and *O*-methylated forms of metabolites in the liver and during absorption in small intestines, leading to obvious changes in their redox potentials.[76] Furthermore, concentrations of flavonoids (high nM) and their metabolites (low μM) are lower than those of small molecules of nutrients (high μM) such as ascorbic acid and α-tocopherol in plasma and brain in vivo. Their antioxidant activities are not comparable with those of such nutrients.[22] Many lines of evidence have suggested that flavonoids protect neural cells against oxidative stress via actions other than antioxidant activity. Schroeter et al.[77] reported that the neuroprotective effect of flavonoids against oxidative stress-associated apoptosis is better than that of

ascorbate with more than 10 times the dose of flavonoids in neurons. Like the protection of genistein against Aβ-induced apoptosis in primary hippocampal neurons,[46] the low dose (0.8 μM) of citrus flavonoids was more effective than the high dose (50 μM) in inhibiting caspase-3 activity and DNA damage in PC12 cells under oxidative stress.[78] In hydrogen peroxide-treated cortical neurons, the protection afforded by citrus flavanones (hesperetin and 5-nitrohesperetin) was not reflected by their antioxidant potential but via the activation of prosurvival Akt and ERK1/2 signaling pathways.[72] Based on higher ROS or intracellular calcium levels but lower caspase-3 and JNK activities found in hydrogen peroxide-treated PC12 cells after treatment with a high concentration (50 μM) of isorhamnetin or isosakuranetin than those with a low concentration (0.8 μM) of the flavonoid, it was also suggested that the flavonoids can act more as signaling molecules than antioxidants to protect cells from oxidative damage, even at the high dose level.[79] Because high ROS and intracellular calcium levels elevate caspase-3 and JNK activities, which could be suppressed by Akt signaling pathways,[72,80,81] hesperetin, isorhamnetin, and isosakuranetin have been shown to activate Akt signaling.[72,79]

2.5. The Bioavailability of Citrus Flavonoids. The application of dietary flavonoids in functional foods, besides bioactivities and unintended reactions (e.g., drug−flavonoid interactions), should also take into account the bioavailability of the flavonoids. The content of a flavonoid after its absorption and metabolism, as well as its metabolites, determines the bioactivity. To intervene in neurodegenerative disorders or improve brain function, the flavonoids must also penetrate the blood−brain barrier (BBB). It is known that the main members of citrus flavonoids such as hesperidin, neohesperidin, naringenin, hesperetin (also, a metabolite of hesperidin), isorhamnetin (also, a metabolite of quercetin), and other members including quercetin and isosakuranetin (also, a metabolite of naringin) can all traverse the BBB.[17,19,54,82,83]

After consumption of orange juice (8 mL/kg of body weight (bw)) the maximum plasma concentrations of naringenin and hesperetin in humans can reach 0.6 and 2.2 μM, respectively; after consumption of grapefruit juice (8 mL/kg bw) the maximum plasma concentration of naringenin in humans can reach 6 μM.[84] In a long-term study of human diets, the plasma concentration of hesperetin in subjects with diets high in fruits and vegetables (a cup of orange juice, half an orange, half a tangerine) was 0.3 μM, which was 27 times the plasma concentration of hesperetin in subjects with diets low in fruits and vegetables. The plasma concentration of naringenin in subjects with diets high in fruits and vegetables was 0.1 μM, whereas it was not detectable in the plasma of subjects with diets low in fruits and vegetables.[85] Additionally, after consumption of 0.5 or 1 L of orange juice (offering 444 mg/L hesperidin and 96.4 mg/L naringenin), flavanone metabolites appeared in the plasma of volunteers at 3 h, reaching maximum concentrations at 5−7 h, and declining to baseline levels at 24 h; the maximum plasma concentrations of hesperetin of subjects consuming 0.5 and 1 L of orange juice were 0.46 and 1.48 μM, respectively.[26] It was also observed that, after a single oral administration of the aglycons hesperetin and naringenin, the molecules were rapidly absorbed in 20 min in humans; the maximum plasma concentrations of hesperetin and naringenin were 2.7 and 7.4 μM, respectively.[86] Furthermore, eriocitrin is abundant in the peels of lemons. After consumption of water extracts of lemon peels and their glycosides, reiodictyol, homoeriodictyol, and hesperetin were bioavailable in humans, and incorporation with alcohol improved their bioavailability.[31]

3. MOLECULAR MECHANISMS UNDERLYING THE NEUROPROTECTIVE EFFECTS OF CITRUS FLAVONOIDS

3.1. Flavonoids as Pharmacological Neuroprotection Signaling Molecules? Nowadays, pharmaceutical therapies confer the major strategies for neuroprotective functions. Mechanism of action underlying the neuroprotection of pharmaceutical compounds is by their interactions with signaling-related transporters, receptors, or key enzymes. For examples, glutamate is the necessary excitatory neurotransmitter regulating brain functions. Excitatory amino acid transporter (EAAT)-2 is one of the major glutamate transporters primarily expressed in astroglial cells and is responsible for glutamate uptake.[87] It has been suggested that Ceftriaxone, one of the β-lactam antibiotics, is a stimulator of EAAT2 expression with neuroprotective effects.[88] Furthermore, estrogens and estrogen receptors (ER) are critical actors in the control of differentiation and survival of brain tissue.[89] Thus, estrogens may display neuritogenic effects during development and neuroprotective effects in the pathophysiological context of brain ischemia and neurodegenerative pathologies such as Alzheimer's disease or Parkinson's disease. Besides, rapamycin expresses strong antiaging effects in several species, including mammals.[90] By inhibiting the activity of mammalian target of rapamycin (mTOR), rapamycin, and some acetylcholine esterase inhibitor, act as pharmacological compounds that are able to provide neuroprotection in several experimental models

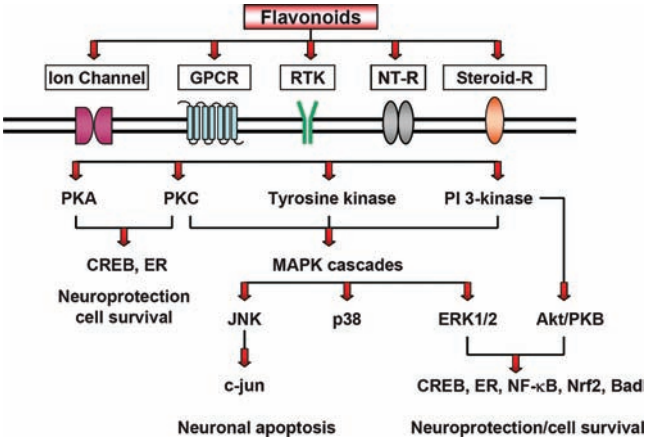


Figure 1. Potential signaling targets of flavonoids associated with neuroprotection. Flavonoid-binding sites on neurons include ligand-gated ion channels, G-protein-coupled receptors (GPCR), receptor tyrosine kinases (RTK), neurotransmitter receptors (NT-R), cell-surface steroid receptors (Steroid-R; membrane estrogen and testosterone receptors)[22,113]. It is suggested that flavonoids act as high-affinity receptor agonists to phosphorylate (activate/inhibit) downstream kinases at low concentrations (low to midnanomolar) and directly desensitize or inhibit the enzymes at higher concentration (high nanomolar to micromolar)[113]. Generally, the activation/inhibition within PKA, PKC, MAPK/ERK and Akt/PKB pathways could lead to neuroprotection/neuronal death. Inhibitory actions within JNK pathways are more likely to suppress neuronal apoptosis; inhibitory/activating p38 could lead to neuronal survival or apoptosis.[21,22,114]

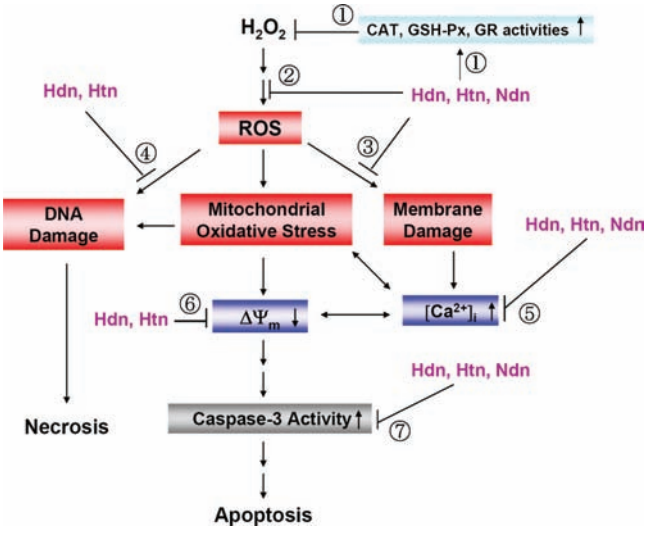


Figure 2. Possible mechanisms underlying the neuroprotection of citrus flavanones against H_2O_2-induced oxidative damage in PC12 cells: (1) increased catalase (CAT), glutathione peroxide (GSH-Px), and glutathione reductase (GR) activities to scavenge H_2O_2; (2) direct scavenging of ROS; (3) inhibition of membrane damage; (4) inhibition of DNA damage; (5) regulation of $[Ca^{2+}]_i$; (6) mitochondrial membrane potential (MMP) maintenance; and (7) inhibition of caspase-3 activity.

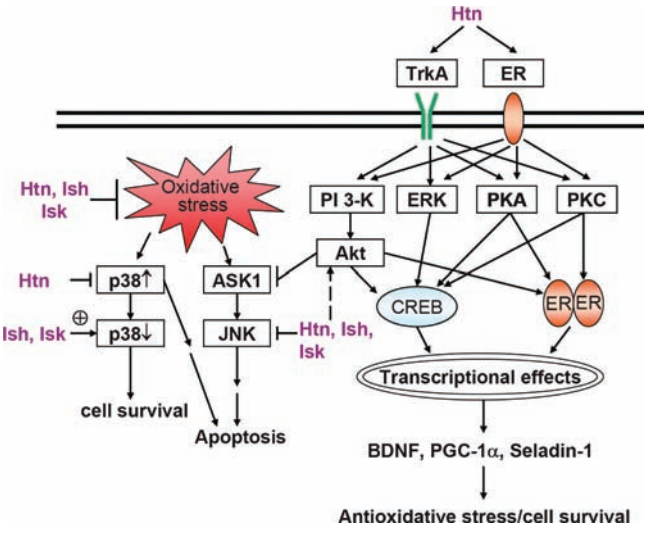


Figure 3. Molecular mechanisms of citrus flavonoids for neuroprotection against oxidative stress. Hesperetin (Htn, 0.1 to 1 μM) simultaneously triggers both estrogen receptor (ER)- and tyrosine receptor kinase A (TrkA)-mediated signaling pathways leading to antioxidative stress/cell survival and suppresses oxidative stress-induced apoptosis via PI 3-K/Akt/ASK1 signaling.[72,106,112] Htn, isorhamnetin (Ish), and isosakuranetin (Isk) scavenge ROS, activate Akt, inactivate JNK, and differentially modulate p38 activation (low concentration of Htn, 0.8 μM, inhibits p38 signaling for apoptosis; high concentration of Ish and Isk, 50 μM, activates p38 signaling for cell survival) to rescue cells from oxidative insult.[79,106]

of neurodegenerative diseases. The mTOR signaling inhibition leads to the suppression of neuronal apoptosis and the induction of autophagy.[91,92] Other FDA-proved drugs like nonsteroid anti-inflammation drugs (NSAIDs, inhibiting COX-1 and COX-2) also show neuroprotective benefits.[93] Although the pharmacological compounds possess sound action as neuroprotection signaling molecules, they generally exhibit side effects in humans. Hence, it increases the demand of flavonoids for intervening in neural disorders and neurodegeneration, because accumulative evidence has shown that flavonoids exert signaling properties in neuroprotection.

3.2. Neuroprotective Signaling Pathways of Flavonoids. Flavonoids may have the chemical structure affinity for bioactive proteins such as receptors, kinases, and proteins related to cellular physiology regulation.[22] It is known that flavonoids interact with proteins involved in protein kinase (PK) and lipid kinase signaling cascades that are associated with cellular survival and apoptosis, such as those of phosphatidylinositol 3-kinase (PI-3K), Akt/PKB, tyrosine kinase, PKC, and mitogen-activated protein kinases (MAPKs). Flavonoids can bind to the ATP-binding sites of proteins (e.g., mitochondria ATPase, calcium membrane ATPase, PKA, PKC, and topoisomerase) or benzodiazepine-binding sites of receptors (e.g., GABA-A, adenosine receptors) and consequently alter their activities. Calcium ions act as second messengers and trigger kinase signaling including Akt, extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) signaling, while mitochondria mediate many regulatory pathways in which ATP is required. Hence, flavonoids can also modulate cellular signaling by maintaining calcium homeostasis and mitochondrial function.[22] Studies have shown that hesperetin, naringenin, quercetin, and resveratrol inhibit kinase activity via their ATP-binding sites.[41,94−96] However, flavonoids may interact with an upstream kinase (MAPKKK) of JNK and the mitochondria permeability transition pore (MPTP; controlling the release of cytochrome C and Ca^{2+}) via their benzodiazepine-binding sites as well as mitochondria-related proapoptotic factors (DIABLO/SMAC). Such actions could account for the neuroprotection by flavonoids against apoptosis.[22,97−99]

Additionally, different cellular bioactivity could exist for a flavonoid between its high and low doses.[22] In neurons, a high dose of quercetin (>30 μM) inhibited the PI-3K/Akt procellular survival pathway, leading to a decrease in the phosphorylation of Bcl-2-associated death promoter (Bad) protein, but did not induce JNK-associated apoptotic signaling.[41,100] A low dose of quercetin (<20 μM) activated MAPK (ERK2, JNK1, and p38) pathways, leading to the induction of gene expression for cellular survival and defensive responses.[21] Many studies also showed that the neuroprotection by low doses (nM to low μM) of flavanols (epicatechin; EGC, epicatechin gallate; EGCG, epigallocatechin gallate) was through inhibition of JNK and caspase-3 activities and activating antiapoptotic MAPKs and PKC.[101−103] However, high doses of EGC and EGCG could trigger sustained activation of MAPK/JNK leading to the induction of apoptosis in cancer cells. Therefore, in intervening in neurodegenerative disorders, the effective dose of flavonoids and its cytotoxicity are important issues. Nevertheless, to select a flavonoid exerting neuroprotective action without cytotoxicity is possible. For example, citrus flavonoids are promising candidates because of their low to no cytotoxicity to normal cells. Potential flavonoid-mediated signaling pathways for neuroprotection are summarized in Figure 1.

3.3. Mechanisms Underlying the Neuroprotection of Citrus Flavonoids. Our studies have shown that the main citrus flavonoids, hesperidin, neohesperidin, and hesperetin, at physiological (0.4−4.0 μM) and high (20−50 μM) doses, all exhibit multiple mechanisms of neuroprotection against oxidative damage in PC12 cells, including the inhibition of

ROS formation and caspase-3 activity, decreases in membrane and DNA damage, enhancement of antioxidant enzyme activity, and the maintenance of calcium homeostasis and mitochondrial potential.[78] Such protective mechanisms are illustrated in Figure 2. We further investigated effects of hesperetin and its chemical structure counterparts, namely, isorhamnetin and isosakuranetin, on signaling regulation of cellular survival-related kinases in oxidatively stressed PC12 cells.[79] We showed that these flavonoids (0.8 and 50 μM) differentially activated prosurvival signaling kinases such as Akt/PKB and MAPK/p38 based on their chemical structures and doses. These flavonoids also suppressed the activation of the proapoptotic kinase, JNK. Therefore, signaling regulation was involved in the cytoprotection by these flavonoids against oxidative damage.

Studies have shown that hesperetin can activate the estrogen receptor (ER); physiological levels of estrogen exhibit neuroprotective effects via both ER- and tyrosine kinase receptor (Trks)-mediated signaling.[63,104,105] Our recent study demonstrated that only low, physiologically relevant concentrations (0.1 and/or 1.0 μM) of hesperetin exhibited neuroprotective effects against oxidative damage via both ER- and TrkA-mediated actions in cells, such as inhibiting the decrease in cell viability, scavenging ROS, maintaining calcium homeostasis, and suppressing caspase-3 activity.[106] Particularly, the TrkA-mediated actions of calcium homeostasis and caspase-3 inactivation in 1.0 μM hesperetin-treated cells were more obvious. Molecular mechanisms underlying the neuroprotection of a high concentration (50 μM) of hesperetin were different from such ER- and TrkA-mediated actions.

Furthermore, hesperetin (1.0 μM) also activated Akt/PKB, MAPK/ERK, and cAMP (cyclic adenosine monophosphate) response element-binding protein (CREB) and induced brain-derived neurotrophic factor (BDNF), peroxisome proliferators activated receptor γ (PPARγ) coactivator 1 α (PGC-1 α), and selective Alzheimer's disease indicator-1 (seladin-1). These proteins are inducible and known to be neuroprotective against apoptosis, oxidative injury, and Aβ-related neurotoxicity.[107−109] Our study showed that greater neuroprotective actions of hesperetin at physiological levels than those at high concentrations are attributed to its receptor-signaling molecule property. Additionally, PGC-1 α is highly inducible in most tissues. It is an almost ideal protector against the mitochondrial dysfunction-associated damage seen in Parkinson's and Alzheimer's diseases (AD).[109] ER and TrkA are also known to be expressed in most AD-vulnerable brain regions. Thus, hesperetin may have potential for intervention in neurodegenerative disorders, particularly for AD.

TrkA activation can trigger MAPK/ERK, PI3-K/Akt, phospholipase C γ (PLCγ)/PKC and cAMP/PKA pathways.[8,110] Membrane ER activation can also trigger parallel MAPK/ERK, PKA, Akt/PKB, and PKC pathways.[111] We further investigated whether hesperetin induced PGC-1 α (regulated by CREB) and seladin-1 (regulated by ER) and their signaling pathways via treating PC12 cells with antagonist or inhibitor to ER, TrkA, and related kinases under normal culture conditions.[112] The results showed that hesperetin rapidly induced PGC-1 α and seladin-1; it simultaneously triggered the activation of PI3-K, PKA, PKC, MAPK/ERK, and CREB via both ER and TrkA. These two receptor-mediated parallel pathways have cross-talk effects and can converge on different transcriptional factors and collaborate to affect (speed and promote) the expression of PGC-1 α and seladin-1. It not only supports the emerging principles of mER-mediated parallel signaling pathways[111] but also explains why hesperetin, which is known to have moderate antioxidant and low estrogen activity, could be neuroprotective against oxidative damage. Thus, an approach for a more complete intervention in oxidative damage-related neurodegeneration using a dietary flavonoid, with low or no cytotoxicity, is promising. Signaling pathways of the citrus flavonoids, leading to neuroprotection/cell survival, are illustrated in Figure 3.

4. CONCLUDING REMARKS

Citrus flavonoids have antioxidant and anti-inflammatory bioactivities. In vitro and in vivo studies showed that they exert neuroprotection at high or low doses. Some citrus flavonoids can even promote cognition. Benefits of main citrus flavonoids on brain health include high safety and bioavailability, the ability to traverse the blood−brain barrier, and multiple neuroprotective mechanisms. Physiological concentrations of hesperetin can trigger multiple prosurvival intracellular signaling pathways and induce proteins that promote cognition, prevent antioxidative stress, or are resistant to Aβ-associated neurotoxicity. Consequently, citrus fruits, that are rich in abundant sources of hesperetin and other flavonoids, are suitable and promising for the development of general food-type neuroprotection and brain foods.

AUTHOR INFORMATION

Corresponding Author

*Tel: 886-4-2287-9755. Fax: 886-4-2285-4378. E-mail: gcyen@nchu.edu.tw.

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