Available online at www.sciencedirect.com

SCIENCE DIRECT®

Journal of Nutritional Biochemistry 16 (2005) 641–649

Journal of Nutritional Biochemistry

REVIEWS: CURRENT TOPICS

Soy isoflavones and cognitive function☆

Yoon-Bok Lee[a,b], Hyong Joo Lee[b,*], Heon Soo Sohn[a]

[a]*Central Research Institute, Dr Chung's Food Co., Ltd., Chungjoo-si 361-782, South Korea*
[b]*School of Agricultural Biotechnology and Center for Agricultural Biomaterials, College of Agriculture and Life Sciences, Seoul National University, Gwanak-gu 151-742, South Korea*

Received 25 April 2005; received in revised form 22 June 2005; accepted 27 June 2005

Abstract

There is growing interest in the physiological functions of soy isoflavones, especially in whether they affect cognitive function and have beneficial effects on neurodegenerative diseases. Here we review the recent evidence from clinical and experimental studies supporting a role for soy isoflavones in cognitive function. Soy isoflavones may mimic the actions and functions of estrogens on brain, and they have been shown to have positive effects on the cognitive function in females; however, studies on their effects on spatial memory have not provided consistent results in males. Although data from humans, cultures, and animal models are currently insufficient for elucidating the metabolism of soy isoflavone actions on cognitive function and the nervous system, we suggest two putative pathways; (1) an estrogen receptor-mediated pathway and (2) via the inhibition of tyrosine kinase, in particular by genistein, which is one of the soy isoflavones. Although soy isoflavones appear to have a positive effect on brain function, further research is needed to determine not only the efficacy but also the safety of soy isoflavones on the nervous system and cognitive function.

Keywords: Soy isoflavones; Cognitive function; Estrogen receptor; Tyrosine kinase

1. Introduction

Soy has been consumed as a traditional food in Asia for a long time. In contrast, in Western countries, soy still plays a minor dietary role despite it representing a good source of protein, dietary fiber and a variety of phytochemicals. Soy contains numerous phytochemicals including isoflavones, phytic acid, trypsin inhibitors and saponins [1]. Among soy phytochemicals, soy isoflavones have attracted considerable attention because of their healthful effects.

Soy isoflavones are referred to as phytoestrogens because they bind to the estrogen receptor (ER) and affect estrogen-mediated processes [2]. Two types of ER have been found in the nucleus: ERα and ERβ [3]. ERα is expressed in various tissues and organs, and, similarly, ERβ has also been found to be expressed in bone [4], the cardiovascular system [5], and the brain [6]. Soy isoflavones contain genistein, daidzein, glycitein and their respective glucosidic conjugates, and they have different binding affinities to ERs based on their structures and ER types. Genistein was reported to have higher affinities than other derivatives and significantly higher affinities for ERβ than for ERα [7].

Soy isoflavones can exert both agonistic and antagonistic estrogenic effects [8], and have inhibitory effects on tyrosine kinase, topoisomerase and angiogenesis, which might reduce the risk of cancer [9]. Soy protein containing isoflavones has been observed to have several beneficial effects on cardiovascular health; a metaanalysis study showed that total cholesterol was decreased by 9.3%, triglyceride by 10.5% and LDL cholesterol by 12.9% when an average of 47 g of soy protein was consumed daily [10]. Soy isoflavones were shown to have antiosteoporosis effects. In a case-control study, 80 mg of isoflavones in the daily diet prevented lumbar spine bone loss in perimenopausal women [11], and it has also been observed that the consumption of extracted soy isoflavones by ovariectomized (OVX) rats prevented decreases in bone density [12].

While there have been numerous studies on the potential beneficial effects of soy isoflavones in various age-related

☆ This study was supported by the technology development program for agriculture and forestry, Ministry of Agriculture and Forestry, Republic of Korea (grant 203022-3).

* Corresponding reviewer. Tel.: +82 2 8804853; fax: +82 2 8735095.
E-mail address: leehyjo@snu.ac.kr (H.J. Lee).

0955-2863/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2005.06.010

diseases, little is known about the influence of soy isoflavones on cognitive function. Various types of neural damage, including that resulting from aging, may induce neurodegenerative diseases associated in cognitive decline. Several studies have found that soy isoflavones can improve cognitive function in both humans and rats [13,14], but the underlying mechanisms are not known.

Cognitive function is generally divided into four classes: reception, learning and memory, thinking and expression. Information is acquired, processed and classified by the receptive function, and subsequently organized, stored and retrieved by learning and memory function. Then, the thinking function organizes and reorganizes information retrieved from memory, and the expressive function is used in expressing the information as verbal or physical acts based upon thinking. Although all of these functions are important and interwoven in cognitive processes, the learning and memory function is pivotal, and, therefore, many studies into cognitive function have focused on learning and memory.

Soy isoflavones have an estrogenic effect, and it was reported that soy isoflavones may improve cognitive functions by mimicking the effects of estrogen in the brain [15]. However, the estrogen agonist properties of soy isoflavones may not explain all of their effects on the brain. In this report, we discuss the effects of soy isoflavones on cognitive function and nervous systems, and the possible mechanisms underlying these effects.

2. Effects of soy isoflavones on cognitive function

2.1. Human studies

Soy isoflavones appear to improve cognitive function in postmenopausal women. The SOPHIA study [16] examined the effects of soy isoflavone supplementation (110 mg/day) on cognitive function of postmenopausal women. That study showed that category fluency was higher in women receiving soy isoflavone supplement than in controls. It was also observed that postmenopausal women receiving 60 mg of soy isoflavones per day for 12 weeks showed significantly higher performance in the recall of pictures and in sustained-attention and planning tasks [17], and that soy isoflavone supplementation had no effect on menopausal symptoms, self-ratings of mood, bodily symptoms or sleepiness. However, a double-blind, randomized, placebo-controlled trial of 202 healthy postmenopausal women aged 60 to 75 years conducted in the Netherlands [18] showed no improvement of cognitive function in postmenopausal women. The women were randomly assigned into two groups, with one receiving 25.6 g of soy protein containing 99 mg of isoflavones and the other receiving only milk protein on a daily basis, and cognitive function did not differ significantly between the groups after 1 year.

Although some studies have shown that soy isoflavones have beneficial effects on the cognitive function of women, very few such studies have involved men, and one of these even demonstrated that soy isoflavones appear to be harmful to cognitive function in men [19]. That longitudinal study found that aged men with higher midlife tofu consumption had lower brain weight and increased cognitive impairment compared to those consuming less tofu. Although that study was not a controlled trial and information on the consumption of selected foods was acquired only from interviews, these results suggest that soy isoflavones have a negative influence on cognitive function in men. However, a case-control study found that soy isoflavone supplementation improved cognitive function in men [13]. Young healthy adults receiving high dosages of soy (100 mg soy isoflavones/day) showed significant improvements in short- and long-term memory and in mental flexibility; these improvements were found in both males and females. Although clinical data for investigating the effects of soy isoflavones on the cognitive function are few and do not show the same results, clinical trials indicate that soy isoflavones might improve cognitive function not only in postmenopausal and young adult women but also in young adult men.

2.2. Animal studies

Several studies have shown that soy isoflavones improve cognitive function in female rats. In one study, OVX rats consumed soy-isoflavone-containing diets for 10 months and showed a dose-dependent improvement in their performance in radial arm maze tests [14]. It is suggested that soy isoflavones act as estrogen agonists in improving working memory and do not block the beneficial effects of estradiol on the working memory in the OVX rats. Lund et al. [20] reported that female rats consuming a lifelong high-isoflavone diet showed the acquisition of the radial arm maze test faster than female rats consuming an isoflavone-free diet. In that study, when the rats were at 80 days of age, some of those consuming a high-isoflavone diet lifelong were changed to the isoflavone-free diet until they were 120 days of age, and these rats performed worse than those who continued to consume a high-isoflavone diet.

Soy isoflavones can affect the synthesis of acetylcholine, and neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in the brain of the female rat. Pan et al. [15] showed the mRNA levels of BDNF in the frontal cortex were significantly higher in rats receiving soy isoflavones than in OVX rats. That group also reported [21] the effects of soy isoflavones on choline acetyltransferase (ChAT) and NGF mRNAs in the frontal cortex and hippocampus of OVX young and retired breeder rats. Nerve growth factor mRNA levels in the soy isoflavone group were intermediate between the estradiol and OVX control groups in the hippocampus of young rats. Choline acetyltransferase mRNA levels in the frontal cortex were significantly higher in retired breeder rats consuming soy isoflavones than in OVX retired breeder rats.

It is not yet clear whether the effects of soy isoflavones on cognitive function in male rats are beneficial or detrimental. Lund et al. [20] reported that the performance

of a visual spatial memory test was worse in male rats consuming a high-isoflavone diet than in rats consuming an isoflavone-free diet. In addition, male rats consuming a lifelong high-isoflavone diet showed poorer performance in the radial arm maze test than male rats changed to the isoflavone-free diet at 80 days of age and continued on the same diet until 120 days of age. However, it was recently reported that male rats consuming the soy isoflavone diet significantly outperformed male rats consuming the isoflavone-free diet in the spatial delayed matching-to-place test [22]. In that study, 10-month-old male Sprague–Dawley (SD) rats consuming a diet containing 0.3 g/kg isoflavone (ISO 0.3) for 16 weeks significantly outperformed rats consuming an isoflavone-free diet in the spatial DMP water maze test. Moreover, ChAT immunoreactivity was significantly higher in the ISO 0.3 rats than in controls in the medial septum and hippocampal CA1 region. The discrepancy between the two studies may be attributable to difference in the age of rats, dietary regimens and maze tests, and it is also possible that soy isoflavones exert a beneficial or detrimental effect on cognitive function in male rats depending on treatment duration and/or dosage.

3. Effects of soy isoflavones on the nervous system

3.1. In vitro neuroprotection of soy isoflavones

Several studies have shown that soy isoflavones have neuroprotective effects in various insults (Table 1). Genistein showed an antioxidative effect on neurons exposed to radical damage. Incubation of primary cortical cells from rats with 100 μmol/L genistein in the presence of iron for 24 h significantly reduced the level of iron-induced thiobarbituric-acid-reactive species [23], which indicates that genistein has an inhibitory role on iron-induced lipid peroxidation. In human cortical cell lines HCN1-A and HCN2, it was observed that pretreatment with 10 or 50 μmol/L genistein prevented cell death due to tertiary butylhydroperoxide [24]; in HCN2 cells, this was due to genistein preventing morphological disruption. That study showed that the down-regulation of the antiapoptotic protein bcl-2 was prevented by genistein treatment. β-Amyloid protein (Aβ) elevates the intracellular concentration of free calcium and generates free radicals. In hippocampal cells from rats, $A\beta_{25–35}$ treatment induces loss of cell viability, DNA fragmentation, an intracellular increase in free calcium, the accumulation of reactive oxygen species and caspase-3 activation, all of which are reversed by genistein [25].

Table 1
Neuroprotection by genistein in cultured neurons

Neuron type	Species	Treatment	Ref.
Primary cortical	Rat	Iron	[23]
HCN1-A HCN2	Human	Tertiary butylhydroperoxide	[24]
Hippocampal	Rat	β-Amyloid	[25]
Primary cortical	Rat	Thapsigargin	[26]
SH-SY5Y	Human	β-Amyloid	[27]
Hippocampal	Rat	β-Amyloid Glutamate	[28]

Genistein attenuates neuronal apoptosis induced by the endoplasmic reticulum calcium-ATPase inhibitor thapsigargin [26]. Thapsigargin-induced apoptosis is associated with loss of mitochondrial function, DNA laddering, nuclear condensation and fragmentation, and caspase activation. Genistein decreases the number of apoptotic neurons and the number of neurons containing active caspase-3. That study showed that 50 nmol/L genistein significantly reduced neuronal apoptosis and that 50 μmol/L genistein completely reversed the protection of neuronal apoptosis.

Genistein protects SH-SY5Y human neuroblastoma cells from Aβ-induced death [27] and attenuates Aβ-induced DNA fragmentation and suppresses Aβ-induced apoptosis. In addition, genistein protects SH-SY5Y cells from H_2O_2-induced toxicity, and this effect is not reversed by an ER antagonist. One study investigated six phytoestrogens (genistein, genistin, daidzein, daidzin, formononetin and equol) for their neuroprotective efficacy against two toxic insults: glutamate excitotoxicity and $A\beta_{25–35}$ [28]. That study showed that in hippocampal neurons, all phytoestrogens induced a modest but significant reduction in lactate dehydrogenase (LDH) release following exposure to glutamate and $A\beta_{25–35}$.

The effective concentrations for genistein-mediated protection appear to range from approximately 0.1 to 50 μmol/L. However, some investigators have reported that high concentrations of genistein induce neuronal apoptosis in primary cultures of cortical neurons from SD rats [29]. This apoptosis was suggested to be dependent on caspase activity, but not ER.

3.2. In vivo neuroprotection of soy isoflavones

Soy isoflavones are also potent neuroprotective agents in animal models of neuronal death. Trieu et al. [30] evaluated the antioxidant activity of genistein in an in vivo mouse model of singlet-oxygen-induced cerebral stroke. The cerebral lesions in mice treated with genistein were 44% smaller than those in control mice, and this neuroprotective effect of genistein was attributed to the inhibition of tyrosine kinase. Radiation-induced apoptotic cell death is mediated by tyrosine kinases, and genistein may prevent apoptosis of cells by their inhibition.

In familial amyotrophic lateral sclerosis (FALS) mice, treatment with genistein retarded disease onset and decreased mortality in males but not in females [31]. In that study, the disease onset and mortality were significantly lower in untreated female FALS mice than in untreated male FALS mice. These findings suggest that estrogen protects neurons from FALS and that genistein acts as estrogen agonist. In the FALS model, treatment with a specific inhibitor of Janus kinase 3 (JNK3), WHI-P131,

increased the survival of neurons by more than 2 months [32]. In that study, genistein also inhibited the activity of JNK3. In another in vivo study, genistein ameliorated retinal degeneration after ischemia-reperfusion injury in rats [33]. The treatment with higher doses of genistein (3.4 mg) suppressed the increase in tyrosine phosphorylation and significantly protected the eyes from the induced ischemic retinal degeneration. However, treatments with lower doses of genistein (0.034 mg and 0.34 mg) did not significantly protect the retina from ischemic damage.

Although the above studies have revealed the neuroprotective effects of genistein, there is also a report that genistein treatment induces apoptosis in neurons. Rats consuming large amounts of genistein (20 mg/day) showed a significant increase of LDH in rat brain tissue homogenates, which did not occur in rats consuming genistein at 2 mg/day [34]. However, DNA fragmentation was detected in homogenates of brain tissue from rats receiving either dose of genistein.

3.3. Regulation of cerebrovascular blood flow

Relaxation of blood vessels can be mediated by several mechanisms, including K^+ channels. Traumatic brain injury is thought to contribute to cerebrovascular dysfunction. Fluid percussion brain injury (FPI) treatment impairs pial artery dilation, and two studies have shown that such impairment was partially restored by genistein. Pretreatment with genistein partially protected dilation induced by three K^+ channel agonists (cromakalim, CGRP and NS1619) following FPI in newborn pigs [35]. It has also been observed in adult rats [36] that genistein pretreatment partially protected dilation induced by cromakalim and CGRP following FPI. Pial artery dilation induced by K^+ channel agonists was blunted within 1 h post-FRI. Pretreatment with genistein partially prevented impaired vasodilation in pigs. Genistein had no effect on vascular responses to K^+ channel agonist in sham-operated control animals.

The *N*-methyl-D-aspartate (NMDA) receptor is an ionotropic receptor that binds to glutamate. Activation of this receptor induces cerebrovascular dilation and may increase local cerebral blood flow [37]. Glutamate is released following hypoxia/ischemia and traumatic brain injury, and NMDA-induced pial dilation is attenuated after hypoxic/ischemic impairment in the piglet [38]. After cerebral hypoxia/ischemia or FPI, cerebrovascular dysfunction during hypotension is associated with activation of NMDA receptors [39]. Tyrosine kinase activation by oxygen free radicals, generated after FPI, reverses NMDA- and glutamate-induced pial artery dilation to vasoconstriction and impairs pial artery dilation. However, pretreatment with genistein and subsequent administration of NMDA or glutamate in the presence of a xanthine-based activated oxygen-generating system partially prevented dilation impairment [40]. These effects of genistein are caused by inhibition of the activity of tyrosine kinase. Genistein also has recently been observed to inhibit superoxide generation resulting from exposure of cerebral vessels to whole blood [41].

Nitric oxide (NO) reportedly functions as a neurotransmitter of the vasodilator nerve [42]. Neuronal excitation induces calcium influx through N-type Ca^{2+} channels, and this activates neuronal NO synthase that produces NO, which subsequently triggers relaxation of vessels via a cyclic cGMP-dependent mechanism. Short-term treatment with soy isoflavones also reportedly increases the basal NO activity [43]. Treatment with daidzein for 7 days also enhanced basilar artery contraction in response to the NO synthase inhibitor, N^G-nitro-L-arginine. Thus, daidzein augments NO synthesis in the basilar artery and increases contraction activity in the basilar arteries of male rats.

3.4. Effects of soy isoflavones on neurotransmitter systems

3.4.1. Dopaminergic system

Genistein can modulate the dopaminergic system. The pups of SD rats that consumed isoflavone-free diets containing 500 ppm genistein from conception showed significant increases in amphetamine-stimulated dopamine release in males but not in OVX females, who only showed a similar but nonsignificant trend [44]. Genistein also inhibits dopamine uptake into mouse striatal homogenates [45]. Genistein dose-dependently suppresses dopamine transport over a concentration range of 5–50 μmol/L.

3.4.2. Cholinergic system

Soy isoflavones affect ChAT expression and activity. Choline acetyltransferase mRNA levels in soy isoflavone-treated retired breeder rats were significantly higher than those in the OVX retired breeder rats in the frontal cortex [21]. In elderly male rats, ChAT immunoreactivity was significantly higher in the hippocampal CA1 region and basal forebrain in rats consuming soy isoflavones than in controls, and ChAT activities were significantly greater in the cortex and basal forebrain [22].

3.4.3. GABAergic system

Genistein decreases the expression of $GABA_A$ receptors in the plasma membrane of rat cerebellar granule cell bodies and dendrites [46]. It also inhibits GABA-activated currents in HEK293 cells [47]. However, this inhibitory activity of $GABA_A$ receptors is not attributable to the inhibition of tyrosine kinase by genistein.

3.4.4. Glycinergic system

It has also been reported that glycine receptors are inhibited by genistein [48,49]. Genistein is inhibitory only when glycine is bound to the receptor and cotreatment of genistein with glycine reversibly blocks the glycine-activated current recorded from hypothalamic neurons. However, these inhibitory effects were also not mediated by inhibition of tyrosine kinase.

4. Putative mechanisms of soy isoflavone actions on the nervous system

4.1. Estrogen receptor-mediated regulation

17β-Estradiol, a mammalian estrogen, has been shown to protect neurons and affect cognitive function. This effect was reported to be associated with ER in the brain [50], and it was also suggested that soy isoflavones (phytoestrogens) had neuroprotective and cognitive functions in the brain. The actions of estrogen on neuron and brain functions have been comprehensively reviewed elsewhere [51–53]. These reviews suggest that estrogen affects neuron survival and growth, synaptic plasticity and brain function via an ER-mediated pathway and/or antioxidant properties. The effects of estrogen on nuclear and nonnuclear ERs can modulate brain functions by regulating gene transcription and second messenger systems. Genistein and other soy isoflavones may act in a similar way because they also exhibit estrogenic activity; estrogenic effects of soy isoflavones on neurons have been observed in various studies [24–26] (Fig. 1). Estrogen prevents the down-regulation of bcl-2, and in human cortical cells, genistein acts as a neuroprotective agent by preventing the down-regulation of bcl-2 [24]. Estrogen also increases neurotrophic factor and ChAT. Likewise, soy isoflavones increase the mRNA levels of BDNF in the frontal cortex in OVX rats [15], and ChAT mRNA levels in the frontal cortex are significantly higher in soy isoflavone-treated retired breeder rats than in OVX rats [21].

The estrogenic effects of soy isoflavones appear to be dose dependent. Zeng et al. [25] reported the neuroprotective effects of genistein against $A\beta_{25-35}$ insult and suggested that they were mediated by two mechanisms that operate at different concentrations: (1) at the nanomolar level, genistein protects neurons via an ER-mediated pathway, and (2) at the micromolar level, genistein behaves as an antioxidant. On the other hand, another study found that high concentrations of genistein are toxic to neurons [26]. That study showed that treatment with 50 μmol/L genistein did not attenuate neuronal apoptosis induced by the endoplasmic reticulum calcium-ATPase inhibitor thapsigargin and enhanced apoptosis of cells, whereas suggested that low concentrations of genistein ameliorate apoptosis of neurons. However, it was also reported that pretreatment with 50 μmol/L genistein protected HCN1-A and HCN2 cells from death [24].

The abovementioned studies indicate that soy isoflavones can affect the viability of neurons and cognitive function by acting as an estrogenic agonist, and they can also utilize differential distribution and regulation of ERα and ERβ in the brain. The binding affinity of soy isoflavones for ERβ is higher than that for ERα by a factor of 20-fold in vitro [54]. Genistein also affects ERβ- but not ERα-dependent gene expression in the hypothalamus [55].

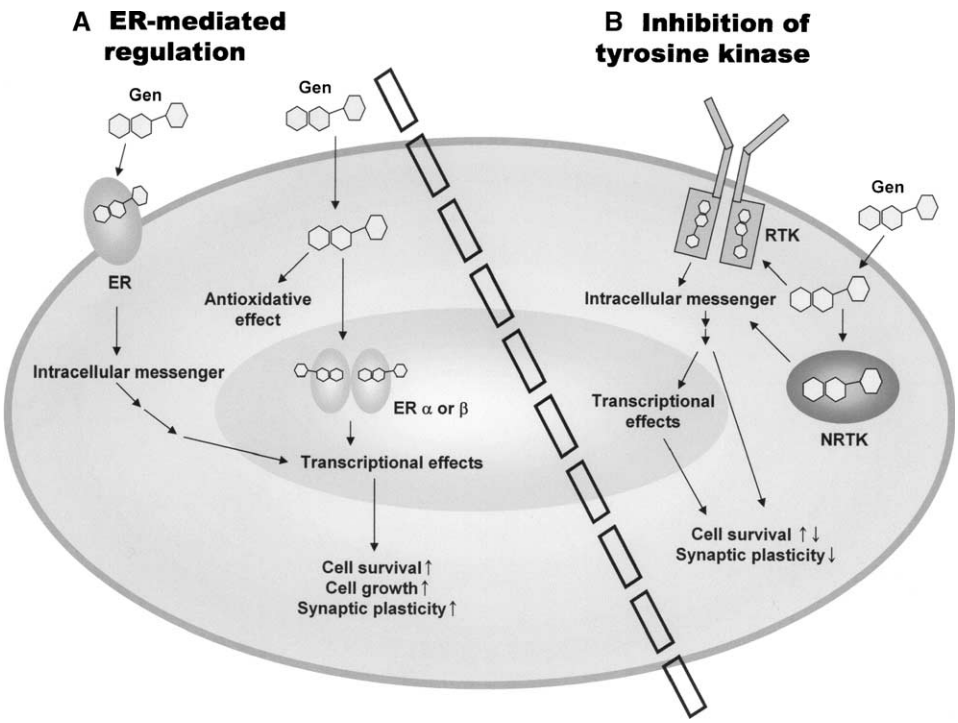

Fig. 1. Summary of soy isoflavone actions on the nervous system. (A) ER-mediated regulation represents that genistein and other isoflavones increase the survival and growth of a neuron, and synaptic plasticity via an ER-mediated pathway. (B) Inhibition of tyrosine kinase represents that genistein suppresses synaptic plasticity and modulates neuron survival by inhibition of tyrosine kinase. Gen, genistein; NRTK, nonreceptor tyrosine kinase; RTK, receptor tyrosine kinase.

However, it is still unclear which ER subtype mediates the neuroprotective efficacy of estrogen/phytoestrogen. Nilsen et al. [56] reported that estrogen regulates developmental neuronal apoptosis, and that this is determined by the ER subtype and the Fas/Fas ligand system. They proposed that ERα mediates neuroprotection, while ERβ mediates the induction of apoptosis in neurons. It was also shown that estrogen induced ERβ-mediated apoptosis following the expression of Fas/Fas ligand proteins. In another study, treatment with 17β-estradiol prevented apoptotic neuron death induced by AF64A, and resulted in the strong expression of ERα and increased bcl-2 in primary cultures from rat hippocampus and septum [57].

There are several lines of evidence that ERβ is involved in neuroprotection. Sawada et al. [58] reported that estradiol exerted neuroprotective effects on nigral dopaminergic neurons by the suppression of proapoptotic gene transcription through the AP-1 site via activation of ERβ. In experimental stroke models, ERα deficiency did not enhance brain damage in female mice, suggesting that in this model, the prevention of brain injury does not require activation of ERα [59]. Moreover, it was recently reported that in a transient global ischemia model, pretreatment with an ERβ-specific agonist reduced ischemic damage by 70% in the caudate nucleus and 55% in the CA1 region, whereas pretreatment with an ERα-specific agonist did not affect the extent of neuronal damage [60]. Genistein also attenuates neuronal apoptosis via an ERβ-mediated pathway [26]. In that study, only ERβ (and not ERα) was detected in a neuron culture; hence, it was suggested that the neuroprotective effect of isoflavone is mediated through ERβ. However, there is a report that both subtypes of ER are involved in neuroprotection from ischemic damage, since both ERα and ERβ agonists were found to protect hippocampal neurons from glutamate-induced neurotoxicity [61]. Furthermore, these agonists significantly increased the expression of bcl-2 in primary hippocampal neurons.

There have been studies on the efficacy of ER subtypes on cognitive function. 17β-Estradiol treatment ameliorated the impaired performance of OVX ERα-knockout (ERαKO) mice in the inhibitory avoidance task, whereas OVX ERβ-knockout (ERβKO) mice were not rescued by 17β-estradiol [62]. In addition, ERαKO female mice had no failure in spatial discrimination in the Morris water maze test, and ERβKO female mice showed impairment in spatial learning [63,64]. However, ERαKO and ERαβKO male mice showed decrease or absence of sexual behavior and reduced aggressive behavior [65]. These evidences suggested that ERα is involved in mediating sexual and aggressive behavior, whereas ERβ modulates emotional and cognitive behavior [66], but further research is needed.

4.2. Inhibition of tyrosine kinase

Protein tyrosine phosphorylation is a regulatory mechanism involved in various responses in the brain, including neuroregeneration [67], synaptic plasticity [68] and neuronal injury [69]. Genistein is known to inhibit tyrosine kinase [70], which is generally considered to be detrimental to a neuron. However, those effects appear to occur only when the concentration of genistein in a cell is quite high [26]. In a neuronal apoptosis model induced by the endoplasmic reticulum calcium-ATPase inhibitor, low concentrations of genistein prevent apoptosis, but high concentrations reverse all the phenotypes. It was also reported that high concentrations of genistein induced neuronal apoptosis in primary cortical neurons [29], blocked tyrosine kinase activity and contributed to H_2O_2-induced apoptosis in SH-SY5Y human neuroblastoma cells [27].

However, the tyrosine-kinase-inhibiting activity of genistein is also likely to exert neuroprotective effects. Genistein treatment protects against singlet-oxygen-induced cerebral damage in vivo [30] via estrogenic and tyrosine-kinase-inhibiting activities. Moreover, genistein can restore impairment of cerebrovascular dilation induced by traumatic brain injury. Genistein prevents bluntness of partial dilation induced by cromakalim and CGRP following FPI [35,36], and the restoration of vasodilation may be due to inhibition of tyrosine kinase phosphorylation.

Protein tyrosine kinases are highly expressed in several brain regions, including the hippocampus, and are reported to be involved in the induction of long-term potentiation (LTP) in the hippocampus [71], which is crucial to learning and memory. Long-term potentiation is also reportedly associated with increased calcium influx and glutamate release. Protein tyrosine phosphorylation contributes to LTP and synaptic plasticity. There are three families of tyrosine kinases implicated in memory: the Trk (topomycin-related kinase) family of receptor kinase, the Src family of nonreceptor kinase and the Eph receptor kinase. Their roles in memory and effects in neurons have already been reviewed comprehensively [72–74]. Because genistein is a tyrosine kinase inhibitor, it can inhibit LTP, which will also suppress an increase in phosphorylation of the α-subunit of calcium channels and extracellular-signal-related kinase in synaptosomes prepared from dentate gyrus [75]. In addition, genistein inhibits protein synthesis and phosphorylation of cAMP response-element binding protein in entorhinal cortex.

This inhibitory activity of LTP by genistein is associated with the inhibition of high-threshold voltage-activated calcium currents. One study has demonstrated a 30–40% reduction of P-/Q-type voltage-gated Ca^{2+} channels (VGCCs), but not L- or N-type VGCCs, by genistein in CA1 pyramidal cells in rat hippocampal slices [76]. In addition, it was reported that genistein inhibits the Ca^{2+}-dependent glutamate release by partially inhibiting P-/Q-type VGCCs, and by inhibiting N-type and unidentified VGCCs in rat hippocampal synaptosomes [77].

It can be postulated that the inhibition of VGCCs and the suppression of LTP by genistein are dose dependent, similar to its effects in neuroprotection. The abovementioned studies indicate that at micromolar concentrations, genistein inhibits LTP, and several in vivo studies have shown that

rats consuming soy isoflavones exhibit improved spatial memory [14,22]. The induction of LTP in the hippocampus is involved in memory and learning, with the hippocampus playing a crucial role in spatial memory. If the concentrations of genistein used for spatial memory tests are sufficient to inhibit LTP, it is reasonable to assume that rats consuming soy isoflavones have impaired spatial memory. However, although studies into the effects of soy isoflavones on the spatial memory of male rats have not produced consistent results, most experimental studies have shown that dietary soy isoflavones (at various dosages) improve the performance in the spatial maze task and indicate that large amounts of genistein are needed to inhibit tyrosine kinases in the brain and suppress cognitive function in vivo. Thus, although soy isoflavones — especially genistein as tyrosine kinase inhibitor — can theoretically inhibit LTP and induce impairment of memory processing and cognitive function, these effects appear to occur only when the concentration of genistein in vivo is excessive.

5. Conclusions

This review focuses on the effects of soy isoflavones on cognitive function and the nervous system, and potential underlying mechanisms. There have been only a few studies into the effects of soy isoflavones on the cognitive function in humans and animals, but these studies have suggested that soy isoflavones improve the cognitive function of females irrespective of age. In contrast, the results for the effects of soy isoflavones on the cognitive function of males have been inconsistent in both human and animal studies. Soy isoflavones can affect brain function by ER-mediated processes and by inhibiting tyrosine kinase. Various neuronal culture and animal studies indicate that soy isoflavones prevent neuronal injury and cognitive decline, and human studies show comparable results. However, genistein (one of soy isoflavones) can have negative influences on cognitive function when it is present at a high level due to its action as a tyrosine kinase inhibitor, which enables it to block LTP and cognitive function. These biphasic properties of soy isoflavones make it difficult to determine whether they have an overall positive or negative effect on cognitive function. Future studies should attempt to elucidate their effects on cognitive function by considering differences due to the sex and age of subjects, and treatment duration.

References

[1] Anderson RL, Wolf WJ. Compositional changes in trypsin inhibitors, phytic acid, saponins and isoflavones related to soybean processing. J Nutr 1995;125:581S–8S.

[2] Molteni A, Brizio-Molteni L, Persky V. In vitro hormonal effects of soybean isoflavones. J Nutr 1995;125:751S–6S.

[3] Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson J-A. Cloning of a novel estrogen receptor expressed in rat prostate and ovary. Proc Natl Acad Sci U S A 1996;93:5925–30.

[4] Arts J, Kuiper GGJM, Janssen JMMF, Gustafsson JA, Lowik CW, Pols HA, et al. Differential expression of estrogen receptors α and β mRNA during differentiation of human osteoblast SV-HFO cells. Endocrinology 1997;138:5067–70.

[5] Mäkela S, Savolainen H, Aavik E, Myllarniemi M, Strauss L, Taskinen E, et al. Differentiation between vasculoprotective and uterotrophic effects of ligands with different binding affinities to estrogen receptors α and β. Proc Natl Acad Sci U S A 1999;96:7077–82.

[6] Shughrue PJ, Lane MV, Merchenthaler I. Comparative distribution of estrogen receptor-α and -β mRNA in the rat central nervous system. J Comp Neurol 1997;388:507–25.

[7] Kuiper GGJM, Carlsson B, Grandien K, Enmark E, Haggblad J, Nilsson S, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 1997;138:863–70.

[8] Miksicek RJ. Estrogenic flavonoids: structural requirements for biological activity. Proc Soc Exp Biol Med 1995;208:44–50.

[9] Adlercreutz H, Mazur W. Phyto-oestrogens and Western diseases. Ann Med 1997;29:95–120.

[10] Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. N Engl J Med 1995;333:276–82.

[11] Alekel DL, Germain AS, Peterson CT, Hanson KB, Stewart JW, Toda T. Isoflavone-rich soy protein isolate attenuate bone loss in the lumbar spine of perimenopausal women. Am J Clin Nutr 2000;72:844–52.

[12] Lee YB, Lee HJ, Kim KS, Lee JY, Nam SY, Cheon SH, et al. Evaluation of the preventive effect of isoflavone extract on bone loss in ovariectomized rats. Biosci Biotechnol Biochem 2004;68:1040–5.

[13] File SE, Jarrett N, Fluck E, Duffy R, Casey K, Wiseman H. Eating soya improves human memory. Psychopharmacology (Berl) 2001;157:430–6.

[14] Pan Y, Anthony M, Watson S, Clarkson TB. Soy phytoestrogens improve radial arm maze performance in ovariectomized retired breeder rats and do not attenuate benefits of 17β-estradiol treatment. Menopause 2000;7:230–5.

[15] Pan Y, Anthony M, Clarkson TB. Evidence for up-regulation of brain-derived neurotrophic factor mRNA by soy phytoestrogens in the frontal cortex of retired breeder female rats. Neurosci Lett 1999;261:17–20.

[16] Kritz-Silverstein D, Von Muhlen D, Barrett-Connor E, Bressel MA. Isoflavones and cognitive function in older women: the SOy and Postmenopausal Health In Aging (SOPHIA) Study. Menopause 2003;10:196–202.

[17] Duffy R, Wiseman H, File SE. Improved cognitive function in postmenopausal women after 12 weeks of consumption of a soya extract containing isoflavones. Pharmacol Biochem Behav 2003;75:721–9.

[18] Kreijkamp-Kaspers S, Kok L, Grobbee DE, de Haan EH, Aleman A, Lampe JW, et al. Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. JAMA 2004;292:65–74.

[19] White LR, Petrovitch H, Ross GW, Masaki K, Hardman J, Nelson J, et al. Brain aging and midlife tofu consumption. J Am Coll Nutr 2000;19:242–55.

[20] Lund TD, West TW, Tian LY, Bu LH, Simmons DL, Setchell KD, et al. Visual spatial memory is enhanced in female rats (but inhibited in males) by dietary soy phytoestrogens. BMC Neurosci 2001;2:20.

[21] Pan Y, Anthony M, Clarkson TB. Effect of estradiol and soy phytoestrogens on choline acetyltransferase and nerve growth factor mRNAs in the frontal cortex and hippocampus of female rats. Proc Soc Exp Biol Med 1999;221:118–25.

[22] Lee YB, Lee HJ, Won MH, Hwang IK, Kang TC, Lee JY, et al. Soy isoflavones improve spatial delayed matching-to-place performance and reduce cholinergic neuron loss in elderly male rats. J Nutr 2004;134:1827–31.

[23] Ho KP, Li L, Zhao L, Qian ZM. Genistein protects primary cortical neurons from iron-induced lipid peroxidation. Mol Cell Biochem 2003;247:219–22.
[24] Sonee M, Sum T, Wang C, Mukherjee SK. The soy isoflavone, genistein, protects human cortical neuronal cells from oxidative stress. Neurotoxicology 2004;25:885–91.
[25] Zeng H, Chen Q, Zhao B. Genistein ameliorates β-amyloid peptide (25–35)-induced hippocampal neuronal apoptosis. Free Radic Biol Med 2004;36:180–8.
[26] Linford NJ, Dorsa DM. 17β-Estradiol and the phytoestrogen genistein attenuate neuronal apoptosis induced by the endoplasmic reticulum calcium-ATPase inhibitor thapsigargin. Steroids 2002;67: 1029–40.
[27] Bang OY, Hong HS, Kim DH, Kim H, Boo JH, Huh K, et al. Neuroprotective effect of genistein against β amyloid-induced neurotoxicity. Neurobiol Dis 2004;16:21–8.
[28] Zhao L, Chen Q, Diaz Brinton R. Neuroprotective and neurotrophic efficacy of phytoestrogens in cultured hippocampal neurons. Exp Biol Med (Maywood) 2002;227:509–19.
[29] Linford NJ, Yang Y, Cook DG, Dorsa DM. Neuronal apoptosis resulting from high doses of the isoflavone genistein: role for calcium and p42/44 mitogen-activated protein kinase. J Pharmacol Exp Ther 2001;299:67–75.
[30] Trieu VN, Dong Y, Zheng Y, Uckun FM. In vivo antioxidant activity of genistein in a murine model of singlet oxygen-induced cerebral stroke. Radiat Res 1999;152:508–16.
[31] Trieu VN, Uckun FM. Genistein is neuroprotective in murine models of familial amyotrophic lateral sclerosis and stroke. Biochem Biophys Res Commun 1999;258:685–8.
[32] Trieu VN, Liu R, Liu XP, Uckun FM. A specific inhibitor of janus kinase-3 increases survival in a transgenic mouse model of amyotrophic lateral sclerosis. Biochem Biophys Res Commun 2000; 267:22–5.
[33] Hayashi A, Weinberger AW, Kim HC, de Juan Jr E. Genistein, a protein tyrosine kinase inhibitor, ameliorates retinal degeneration after ischemia-reperfusion injury in rat. Invest Ophthalmol Vis Sci 1999;38:1193–202.
[34] Choi EJ, Lee BH. Evidence for genistein mediated cytotoxicity and apoptosis in rat brain. Life Sci 2004;75:499–509.
[35] Armstead WM. Protein tyrosine kinase and mitogen-activated protein kinase activation contribute to K_{ATP} and K_{Ca} channel impairment after brain injury. Brain Res 2002;943:276–82.
[36] Hong KW, Shin HK, Kim CD, Lee WS, Rhim BY. Restoration of vasodilation and CBF autoregulation by genistein in rat pial artery after brain injury. Am J Physiol Heart Circ Physiol 2001;281:H308–15.
[37] Faraci FM, Breese KR. Nitric oxide mediates vasodilatation in response to activation of *N*-methyl-D-aspartate receptors in brain. Circ Res 1993;72:476–80.
[38] Armstead WM. NOC/oFQ PKC-dependent superoxide generation contributes to hypoxic–ischemic impairment of NMDA cerebrovasodilation. Am J Physiol Heart Circ Physiol 2000;279:H2678–84.
[39] Armstead WM. Age dependent NMDA contribution to impaired hypotensive cerebral hemodynamics following brain injury. Brain Res Dev Brain Res 2002;139:19–28.
[40] Philip S, Armstead WM. Differential role of PTK, ERK and p38 MAPK in superoxide impairment of NMDA cerebrovasodilation. Brain Res 2003;979:98–103.
[41] Shin HK, Lee JH, Kim KY, Kim CD, Lee WS, Rhim BY, et al. Impairment of autoregulatory vasodilation by NAD(P)H oxidase-dependent superoxide generation during acute stage of subarachnoid hemorrhage in rat pial artery. J Cereb Blood Flow Metab 2002;22:869–77.
[42] Okamura T, Ayajiki K, Fujioka H, Shinozaki K, Toda N. Neurogenic cerebral vasodilation mediated by nitric oxide. Jpn J Pharmacol 2002;88:32–8.
[43] Sobey CG, Weiler JM, Boujaoude M, Woodman OL. Effect of short-term phytoestrogen treatment in male rats on nitric oxide-mediated responses of carotid and cerebral arteries: comparison with 17β-estradiol. J Pharmacol Exp Ther 2004;310:135–40.
[44] Ferguson SA, Flynn KM, Delclos KB, Newbold RR, Gough BJ. Effects of lifelong dietary exposure to genistein or nonylphenol on amphetamine-stimulated striatal dopamine release in male and female rats. Neurotoxicol Teratol 2002;24:37–45.
[45] Simon JR, Bare DJ, Ghetti B, Richter JA. A possible role for tyrosine kinases in the regulation of the neuronal dopamine transporter in mouse striatum. Neurosci Lett 1997;224:201–5.
[46] Balduzzi R, Cupello A, Robello M. Modulation of the expression of $GABA_A$ receptors in rat cerebellar granule cells by protein tyrosine kinases and protein kinase C. Biochim Biophys Acta 2002;1564: 263–70.
[47] Huang RQ, Fang MJ, Dillon GH. The tyrosine kinase inhibitor genistein directly inhibits GABAA receptors. Brain Res Mol Brain Res 1999;67:177–83.
[48] Zhu L, Jiang ZL, Krnjevic K, Wang FS, Ye JH. Genistein directly blocks glycine receptors of rat neurons freshly isolated from the ventral tegmental area. Neuropharmacology 2003;45:270–80.
[49] Huang RQ, Dillon GH. Direct inhibition of glycine receptors by genistein, a tyrosine kinase inhibitor. Neuropharmacology 2000;39: 2195–204.
[50] Shughrue PJ, Scrimo PJ, Merchenthaler I. Estrogen binding and estrogen receptor characterization (ERα and ERβ) in the cholinergic neurons of the rat basal forebrain. Neuroscience 2000;96:41–9.
[51] McEwen BS. Invited review: estrogens effects on the brain: multiple sites and molecular mechanisms. J Appl Physiol 2001;91:2785–801.
[52] Garcia-Segura LM, Azcoitia I, DonCarlos LL. Neuroprotection by estradiol. Prog Neurobiol 2001;63:29–60.
[53] Norbury R, Cutter WJ, Compton J, Robertson DM, Craig M, Whitehead M, et al. The neuroprotective effects of estrogen on the aging brain. Exp Gerontol 2003;38:109–17.
[54] Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 1998;139:4252–63.
[55] Patisaul HB, Melby M, Whitten PL, Young LJ. Genistein affects ERβ- but not ERα-dependent gene expression in the hypothalamus. Endocrinology 2002;143:2189–97.
[56] Nilsen J, Mor G, Naftolin F. Estrogen-regulated developmental neuronal apoptosis is determined by estrogen receptor subtype and the Fas/Fas ligand system. J Neurobiol 2000;43:64–78.
[57] Harms C, Lautenschlager M, Bergk A, Katchanov J, Freyer D, Kapinya K, et al. Differential mechanisms of neuroprotection by 17 β-estradiol in apoptotic versus necrotic neurodegeneration. J Neurosci 2001;21:2600–9.
[58] Sawada H, Ibi M, Kihara T, Urushitani M, Akaike A, Shimohama S. Estradiol protects mesencephalic dopaminergic neurons from oxidative stress-induced neuronal death. J Neurosci Res 1998;54:707–19.
[59] Sampei K, Goto S, Alkayed NJ, Crain BJ, Korach KS, Traystman RJ, et al. Stroke in estrogen receptor-α-deficient mice. Stroke 2000;31: 738–44.
[60] Carswell HV, Macrae IM, Gallagher L, Harrop E, Horsburgh KJ. Neuroprotection by a selective estrogen receptor β agonist in a mouse model of global ischemia. Am J Physiol Heart Circ Physiol 2004; 287:H1501–4.
[61] Zhao L, Wu TW, Brinton RD. Estrogen receptor subtypes α and β contribute to neuroprotection and increased Bcl-2 expression in primary hippocampal neurons. Brain Res 2004;1010:22–34.
[62] Fugger HN, Foster TC, Gustafsson J, Rissman EF. Novel effects of estradiol and estrogen receptor α and β on cognitive function. Brain Res 2000;883:258–64.
[63] Fugger HN, Cunningham SG, Rissman EF, Foster TC. Sex differences in the activational effect of ERα on spatial learning. Horm Behav 1998;34:163–70.
[64] Rissman EF, Heck AL, Leonard JE, Shupnik MA, Gustafsson JA. Disruption of estrogen receptor β gene impairs spatial learning in female mice. Proc Natl Acad Sci U S A 2002;99:3996–4001.

[65] Ogawa S, Chester AE, Hewitt SC, Walker VR, Gustafsson JA, Smithies O, et al. Abolition of male sexual behaviors in mice lacking estrogen receptors α and β (αβERKO). Proc Natl Acad Sci U S A 2000;97:14737–41.
[66] Gustafsson JA. What pharmacologists can learn from recent advances in estrogen signalling. Trends Pharmacol Sci 2003;24: 479–85.
[67] Bregman BS, Coumans JV, Dai HN, Kuhn PL, Lynskey J, McAtee M, et al. Transplants and neurotrophic factors increase regeneration and recovery of function after spinal cord injury. Prog Brain Res 2002;137:257–73.
[68] Gurd JW. Protein tyrosine phosphorylation: implications for synaptic function. Neurochem Int 1997;31:635–49.
[69] Phillis JW, Song D, O'Regan MH. Inhibition of tyrosine phosphorylation attenuates amino acid neurotransmitter release from the ischemic/reperfused rat cerebral cortex. Neurosci Lett 1996;207: 151–4.
[70] Akiyama T, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, et al. Genistein, a specific inhibitor of tyrosine-specific protein kinases. J Biol Chem 1987;262:5592–5.
[71] O'Dell TJ, Kandel ER, Grant SG. Long-term potentiation in the hippocampus is blocked by tyrosine kinase inhibitors. Nature 1991;353:558–60.
[72] Huang EJ, Reichardt LF. Trk receptors: roles in neuronal signal transduction. Annu Rev Biochem 2003;72:609–42.
[73] Purcell AL, Carew TJ. Tyrosine kinases, synaptic plasticity and memory: insights from vertebrates and invertebrates. Trends Neurosci 2003;26:625–30.
[74] Ernfors P, Bramham CR. The coupling of a trkB tyrosine residue to LTP. Trends Neurosci 2003;26:171–3.
[75] Casey M, Maguire C, Kelly A, Gooney MA, Lynch MA. Analysis of the presynaptic signaling mechanisms underlying the inhibition of LTP in rat dentate gyrus by the tyrosine kinase inhibitor, genistein. Hippocampus 2002;12:377–85.
[76] Potier B, Rovira C. Protein tyrosine kinase inhibitors reduce high-voltage activating calcium currents in CA1 pyramidal neurones from rat hippocampal slices. Brain Res 1999;816:587–97.
[77] Pereira DB, Carvalho AP, Duarte CB. Genistein inhibits Ca^{2+} influx and glutamate release from hippocampal synaptosomes: putative non-specific effects. Neurochem Int 2003;42:179–88.