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<Review>

How Do Oroxylin A and Spinosin Exert Their Activities on Cognitive Function?

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Abstract – Flavonoids are mainly contained in the vegetables and medicinal herbs. Until now, over 5,000 kinds of flavonoid have been identified and their biological activities have been reported. Among them, we are interested in oroxylin A and spinosin because of their specific structures having bulky group at C-6 of ring A. Oroxylin A is contained in the *Scutellaria baicalensis* and exhibits cognitive enhancing activity as a $GABA_A$ receptor antagonist, which is different from those of mainly contained in the *S. baicalenis*, baicalein or wogonin. Spinosin is isolated from *Zizyphus jujuba* var. *spinosa* and mainly studied as a hypnotic or anxiolytic agent because of traditional knowledge about its original herb. As far as we know, the cognitive function of spinosin was first identified by our group. In this review, we discuss how such flavonoids exert their pharmacological activities associated with cognitive function based on the receptor binding study and behavioral studies. Traditional knowledge and reverse pharmacology may be addressed in the research field of phytochemical pharmacology and useful to unveil the secret of phytochemicals.
Keywords – flavonoid, oroxylin A, spinosin, cognitive function, Gabaergic neurotransmitter system, serotonergic neurotransmitter system

Introduction

Cognitive function is core activity of our general life and its impairment is observed in neurodegenerative disease, especially in Alzheimer's disease (AD), or several neuropsychiatric disorders including schizophrenia, autism, or attention deficit hyperactivity disorder (ADHD). Especially, schizophrenia is regarded as an early form of dementia.[1,2] However, the mechanism of action of anti-dementia drugs might be different from those of anti-psychotics. Since the employment of donepezil, several acetylcholinesterase (AChE) inhibitors and NMDA receptor antagonist have been prescribed in clinic for AD. In addition, typical or atypical antipsychotic agents have been used for treating schizophrenia. Until yet, however, anti-dementia agents including donepezil could not modify AD, and antipsychotics also could not attenuate cognitive decline observed in neuropsychiatric disorders. Therefore, disease modifying therapy is urgently needed to reverse cognitive decline in the neurodegenerative or neuropsychiatric disorders. One of such efforts in schizophrenia field has led to establish the Measurement and Treatment Research to Improve Cognition in Schizophrenia (MATRICS) initiative.[3]

Herbal material-derived flavonoids are hot topics in biomedical sciences because of their beneficial activities, such as anti-inflammatory, anti-dementia, anti-rheumatoid, or neuroprotective activities. Basically, flavonoid is composed of benzopyran and benzene rings. There are several types of flavonoid including flavones, isoflavones, flavonones, flavonols, flavanols and anthocyanidins. Flavonoids are immensely studied on their brain functions related with cognitive activities. Anti-inflammatory, anti-oxidative, or neurotrophin-producing activities are likely to contribute to cognitive function of flavonoids.[4] Although flavonoids have strong anti-inflammatory and anti-oxidative activities, some flavonoids bind to $GABA_A$ receptor as agonist, suggesting that they might induce amnesia or dementia associated with cognitive function.[5] For example, baicalein which is contained in *Scutellaria baicalensis* Gorge (Lamiaceae) as baicalin, glycoside form of baicalein,

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is reported to be a $GABA_A$ receptor agonist, but it ameliorated cognitive dysfunction induced by neuroinflammation or transgenic AD model.[6,7] However, it could not reverse memory impairment induced by cholinergic blockade in mouse (unpublished data), suggesting that anti-oxidative property is more prominent than agonistic activity of $GABA_A$ receptor in the neurodegenerative states. Similar phenomenon was also reported in ursolic acid which attenuates cognitive dysfunction in lipopolysaccharide-induced cognitive deficits in mouse but not in the cholinergic blockade-induced amnesic model.[8,9] In addition, brain-derived neurotrophic factor (BDNF) receptor or other receptors including $GABA_A$ receptor are also involved in cognitive function of flavonoids.[10] Does flavone have common rules in structure to exert their pharmacological activity? However, it is unclear whether structural similarity in flavone is attributed to the similar cognitive properties or which functional group(s) in the flavonoid backbone is crucial role in its cognitive function.

In the present review, we focus on flavones, especially oroxylin A and spinosin, and their action mechanism related with cognitive function. Oroxylin A is a flavone contained in the *S. baicalensis* with baicalein and wogonin. As mentioned, baicalein is a $GABA_A$ receptor agonist and oroxylin A is an antagonist of $GABA_A$ receptor.[11] The difference between oroxylin A and bacalein in structure is hydroxyl or methoxyl group at C-6 of ring A. In addition, spinosin is another flavone isolated from *Zizyphus jujuba* Miller var. *spinosa* Hu ex H. F. Chou (Rhamnaceae). Spinosin is one of C-glycoside flavonoids and metabolized into swertisin or other metabolites. The common feature of orxylin A and spinosin or swertisin is that bulky group is attached at C-6 of ring A, which led us to have interest on.

Oroxylin A and cognition function

The origin of oroxylin A – Oroxylin A (Fig. 1) was firstly isolated from *Oroxylum indicum* (L.) Kurz on 1936 by Shah et al.[12] In addition to *O. indicum*, oroxylin A is also contained in the *S. baicalensis* with baicalin, baicalein, or wogonin. The amount of oroxylin A in the *S. baicalensis* is not greater than that of baicalein or wogonin. Therefore, the total amount of baicalin, baicalein and wogonin without oroxylin A in the *S. baicalensis* is used for its specification in Korean Pharmacopeia. Recently, oroxylin A has been also identified in the *Capparis spinosa* L., *Ardisia crispa* (Thunb.) A. DC., *Stachys geobombycis* C. Y. Wu, and *Eucommia ulmoides* Oliver.[13]

Oroxylin A as a $GABA_A$ receptor antagonist – Several research groups including us have been working on the effects of oroxylin A on the brain functions related with neuroprotection and cognition. Based on the idea that the content of flavonoids is high in *S. baicalensis*, we tried to investigate whether the water extract of *S. baicalensis* exerts anxiolytic activity and found that the water extract of *S. baicalenis* at 200 or 400 mg/kg (p.o.) showed an anxiolytic-like activity through $GABA_A$ receptor activation.[14] Recently, another group reported that standardized extract of *S. baicalenis* did not exhibit anxiolytic behavior at 600 or 1200 mg/kg (p.o.) in rat.[15] It is unclear why the different results were obtained by two groups, but it can be speculated. Different doses or probably different extract solution (Jung's group, water extract; Fong's group, unclear) might affect the results. However, Fong's group suggested that the reason why anxiolytic behavior was not observed in *S. baicalenis* treatment group may be the highest brain uptake of oroxylin A, a $GABA_A$ receptor antagonist. Further researches are needed to clarify this issue.

Before the results that oroxylin A was found to be a $GABA_A$ receptor antagonist, oroxylin A might be assumed as a $GABA_A$ receptor modulator because it interacted with the benzodiazepine binding site of $GABA_A$ receptors with a Ki value of 14.6 μmol/L.[16] In 2003, Xue's group from Hong Kong conducted binding study using synaptosomal fraction from rat. And they examined the antagonistic activity of oroxylin A to the $GABA_A$ receptor using several behavioral tests and antagonism studies. They first reported that oroxylin A as a naturally occurring flavonoid has selective antagonistic actions through the benzodiazepine binding site of $GABA_A$ receptor.[17] However, they did not examine whether oroxylin A ameliorates cognitive dysfunction.

Oroxylin A and cognition function – It is well acknowledged that $GABA_A$ receptor agonist cannot attenuate cognitive dysfunction in cholinergic blockade state. As expected, we observed that baicalein or wogonin from *S. baicalensis* did not reverse cognitive dysfunction induced by cholinergic blocking in mice (unpublished data). Surprisingly, oroxylin A recovered the decreased latency in the passive avoidance test.[11] Similar results were also observed in the Morris water maze and the Y maze tests. Thereafter, we conducted receptor binding assay and electrophysiological study to explore the exact receptor(s) involved in cognitive ameliorating activities of oroxylin A. As reported by Huen et al.,[17] the binding affinity of oroxylin A to $GABA_A$ receptor was significantly inhibited by respective specific ligand by 97% inhibition. In addition, oroxylin A also showed binding affinity to adenosine A_{2A} receptor and the inhibition rate was 54%.[18]

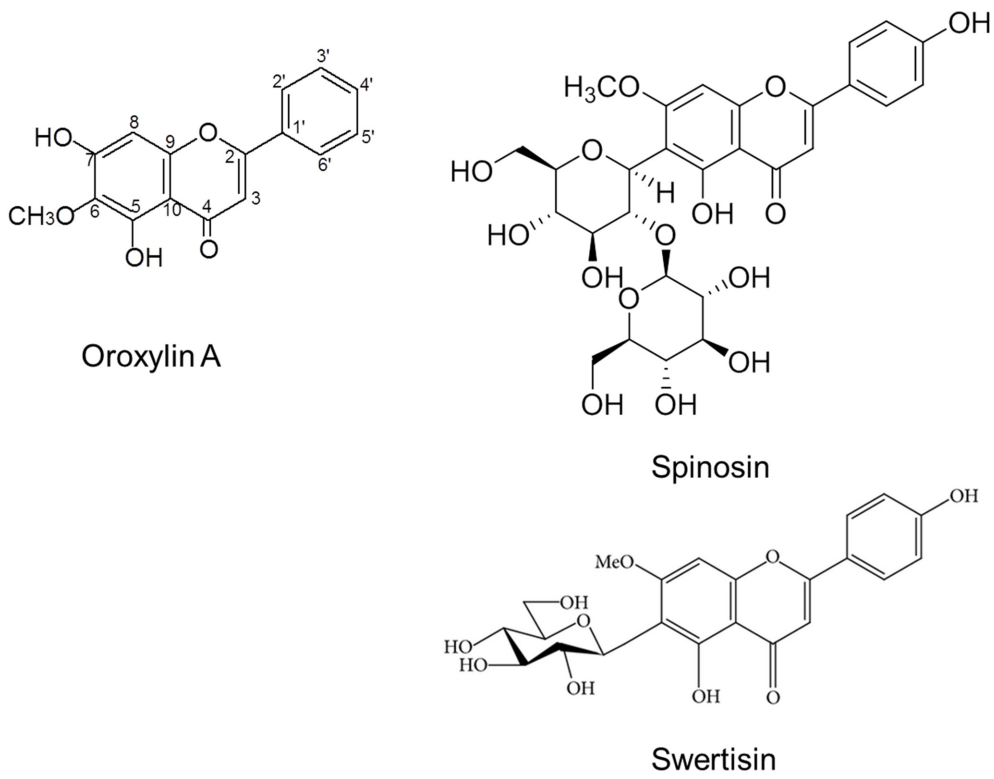

Fig. 1. The structures of oroxylin A, spinosin and swertisin focused on this review.

These results persuaded us to confirm the effect of oroxylin A on $GABA_A$ receptor-activated Cl^- current in CA1 neurons or on adenosine A_{2A} receptor. If oroxylin A is $GABA_A$ receptor antagonist, the GABA-induced inward Cl^- current should be blocked by oroxylin A treatment. By the patch-clamp recording technique, we observed that GABA-induced Cl^- current was significantly inhibited to $62 \pm 6\%$ of the control level.[11] Thus, our study suggests that oroxylin A rescues cognitive deterioration induced by cholinergic dysfunction via its $GABA_A$ receptor antagonistic properties.

In addition, based on the receptor binding study, Shin's group investigated that oroxylin A regulates BDNF production via the activation of adenosine A_{2A} receptor in vitro. Their researches are originated from the idea that adenosine A_{2A} receptor is functionally interacted with BDNF.[19] Adenosine A_{2A} receptor is coupled with adenylate cyclase, and its activation consequently activates protein kinase A (PKA) and phosphoinositide 3-kinase (PI3K).[20] Therefore, it can be possible that oroxylin A promotes cell survival, neurite extension, or synaptic plasticity through adenosine A_{2A} receptor activation, as suggested by Shin's group.[19] Similarly, we also observed that oroxylin A promotes neurogenesis in the subgranular region of the hippocampus, with stimulation of the progenitor cell proliferation and new born cell survival.[21] Although we did not examine whether adenosine A_{2A} receptor plays a role in the neurogenesis in vivo, adenosinergic and/or GABAergic neurotransmitter system would be involved in the enhancement of neurogenesis after the administration of oroxylin A. The hitherto findings that adult neurogenesis contributes to the enhancement of cognitive functions support the ideas that oroxylin A may enhance learning and memory activities.

In summary, oroxylin A could reverse cognitive impairment induced by hypo-cholinergic state through $GABA_A$ receptor blockade and adenosine A_{2A} receptor activation (Fig. 2).

The effects of oroxylin A on memory processes – Memory is preceded by a process consisted of the acquisition or perception of new experiences about the world.[22] After an acquisition of the information, it should be stored in the brain as a concrete form from labile one for retrieval behavior, which is mentioned as a consolidation process. Recalling the concrete information stored in the brain is called as retrieval process. To measure the effects of any compound on cognitive processes, the passive avoidance test may be usually employed by

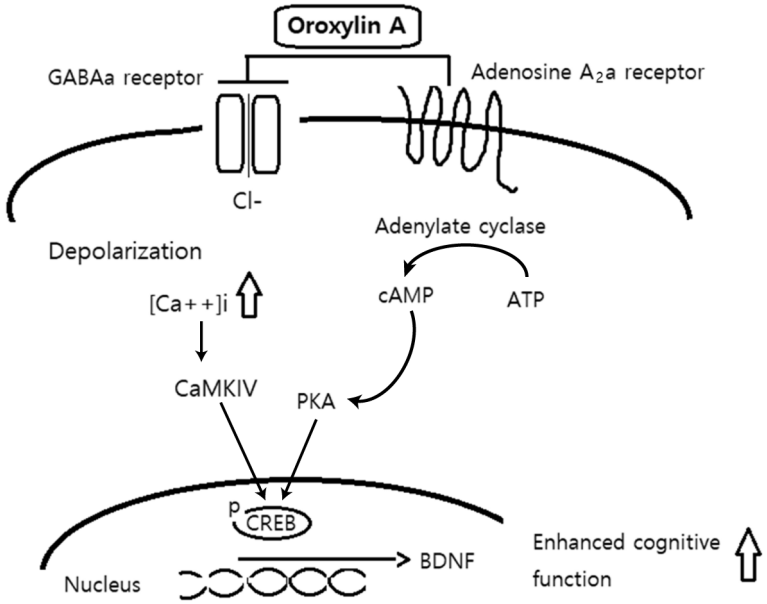

Fig. 2. Schematic expression of a proposed mechanism underlying memory enhancement and memory improvement of oroxylin A. The administration of oroxylin A enhances cognitive performance and ameliorates cognitive impairment in mice through the activation of adenosine A_{2A} receptor and inhibition of $GABA_A$ receptor, resulting in the activation of various signaling molecules, such as CaMKIV, PKA, CREB, and BDNF. Brain-derived neurotrophic factor, BDNF; Calcium/calmodulin-dependent protein kinase IV, CaMKIV; cAMP response element-binding protein, CREB; Protein kinase A, PKA.

controlling the treatment schedules.[23,24]

Previously, we and others reported that the expression levels of mBDNF at specific time point(s) after a training in the step-through or step-down passive avoidance is important for memory consolidation.[22,25] Similarly, exogenous human recombinant BDNF treatment at 9 h after the training trial also enhanced memory consolidation.[25] Therefore, it would be valuable to examine whether oroxylin A enhances memory consolidation process because it increases the expression levels of BDNF in the hippocampus. If oroxylin A enhances memory consolidation, it would be a potential agent for treating amnesia or AD because it reverses cognitive dysfunctions observed in the chemically induced amnesia or dementia mouse models.[26]

After an acquisition trial, mice were treated with oroxylin A at immediately, 1 h, 3 h, 6 h or 12 h to examine the effects of oroxylin A on memory consolidation. A longer latency in the retention trial was observed in the group treated with oroxylin A up to 3 h after the acquisition trial, suggesting that oroxylin A enhances memory consolidation.[11] Based on the previous findings for memory consolidation and mBDNF,[25] the temporal profiles of mBDNF were investigated in the hippocampus after the administration of oroxylin A. Similarly with the previous results,[18] oroxylin A also enhanced mBDNF and its downstream signaling pathway from 6 to 12 h after the acquisition trial in the passive avoidance test,[18] suggesting that the increase of mBDNF would play a role in the facilitation of memory consolidation after the oroxylin A administration. Expectedly, oroxylin A-induced increase of memory consolidation was completely reversed by blocking the BDNF receptors using its antagonists, K252a or TrkB-Fc. In addition, we also observed that the inhibition of protein synthesis by using cycloheximide blocked the memory consolidation effects induced by oroxylin A (unpublished data). Given that BDNF signaling is regulated by the $GABA_A$ receptor or adenosine A_{2A} receptor, the effects of oroxylin A on the memory consolidation should be exerted by the activation of adenosinergic and/or the inhibition of GABAergic neurotransmitter system. Moreover, protein synthetic process would also be involved in the memory consolidation process of oroxylin A because cycloheximide inhibited such effects of oroxylin A.

Miscellaneous – Oroxylin A has anti-oxidative and anti-inflammatory activities as shown as other flavonoids. Due to such activities, neuroprotective activity of oroxylin A has been investigated using hypoperfusion- or amyloid-β protein-induced neuroinflammatory model. Global brain ischemia caused by hypoperfusion induces neuronal injury, especially in the hippocampal CA1 region,[27] which

could induce cognitive impairment in mice. Neuronal damages after the hypoperfusion-induced ischemia might be resulted from the activated microglia which releases inflammatory cytokines, nitric oxide or hydrogen peroxides.[28] Intracerebroventricular (ICV) injection of amyloid-β protein also activates microglia, as shown in the hypoperfused ischemia model.[29] Marked memory impairment was observed in both models, and oroxylin A ameliorated cognitive dysfunction induced by hypoperfused ischemia or ICV amyloid-β protein injection with the attenuation of microglial activation or enhanced BDNF signaling pathway.[26] In an earlier review, it has also been suggested that Scullcap flavones including oroxylin A exert neuroprotective activities through free radical-scavenging and antioxidant actions.[30]

Oroxylin A is reported to be active against the attention-deficit hyperactivity disorder (ADHD).[31] Core symptoms of ADHD are hyperactivity, impulsivity and inattention. Generally, it is common that oroxylin A could not exhibit anti-ADHD activities because of its $GABA_A$ receptor antagonistic activity. However, Cheong's group found that oroxylin A reduces sleeping time and relieves ADHD-like behaviors in the spontaneously hypertensive rat, an ADHD animal model.[32] Thereafter, over 100 compounds based on the structure activity relationship were synthesized, and their anti-ADHD activities were evaluated via the inhibitory activity against to dopamine transporter (Korean patent, KR 10-1172153, 2012). Some of compounds also ameliorated sensorimotor gating deficits and cognitive deficits in mouse model.[32] However, unfortunately, preclinical studies including toxicology studies had not been further proceeded.

Although much amounts of results on the usefulness of oroxylin A has been accumulated, it had not been clear whether oroxylin A penetrates the blood-brain barrier (BBB) and could be detected in the brain tissue. Recently, the Chinese University of Hong Kong group reported the pharmacokinetic profiles of oroxylin A.[15] According to that report, brain-to-plasma ratio was 4 times higher in oroxylin A with comparing to baicalein or wogonin. However, the amount of oroxylin A in the *S. baicalensis* was lower compared to that of baicalein or wogonin. In addition, they also explained that the absence of anxiolytic properties of *S. baicalensis* is owing to the presence of high amount of oroxylin A in the brain, which is different with our data (*vide supra*).[14]

More recently, it has been cleared how oroxyin A is distributed or metabolized in the body. Oroxylin A and its metabolites have been found in almost body regions including plasma, brain, heart, liver, kidney, and urine.[33] In addition, it goes into phase II metabolism and is metabolized into oroxylin A-5-*O*-glucuronide, oroxylin A-5-*O*-glucoside, oroxylin A-7-*O*- glucuronide, and oroxylin A-7-*O*-glucoside.[33] Now, the full story of oroxylin A is becoming clearer although the proceeding is slow.

Spinosin and cognition function

The origin of spinosin – In 1978, Woo and Kang with Wagner' group from Germany firstly reported the spinosin (Fig. 1), as new flavone C-glycoside from *Z. jujuba* var. *spinosa*.[34] Spinosin is mainly contained in the fruits of *Z. jujuba* var. *spinosa* (Rhamnaceae) and also in the *Ziziphus mauritiana* Lamarck (Rhamnoceae). *Z. mauritiana* is called as Indian jujube and found in the tropical regions. Although the fruits from both trees contain spinosin as much amounts, the fruits of *Z. jujuba* var. *spinosa* are different from those of *Z. mauritiana* and Zizyphi Semen indicates *Z. jujuba* var. *spinosa* but not *Z. mauritiana*. Woo's group reported that spinosin as well as jujuboside A and magnoflorine can be used for marker compounds to differentiate *Z. jujuba* var. *spinosa* and *Z. mauritiana*.[35] The content of spinosin in the *Z. jujuba* var. *spinosa* is described to contain less than 0.08% in the Chinese Pharmacopoeia, which is supported by the above report.[35] Spinosin is one of C-glycoside flavonoids and metabolized into swertisin or other metabolites (Fig. 1). As described, spinosin has also the bulky group at C-6 of ring A, similarly with oroxylin A.

Spinosin as an anxiolytic agent – In the traditional medicine, *Z. jujuba* var. *spinosa* has been used as a hypnotic agent. On the contrary to this, the *Z. jujuba* has been used as tonic agent. Therefore, the two traditional medicines are different in their usages. Based on the traditional knowledge, *Z. jujuba* var. *spinosa* and its constituents, such as spinosin, jujuboside A or magnoflorine have been studied as hypnotic or anxiolytic agent.

After isolation of spinosin, Shin's group examined the pharmacological activity of spinosin as a hypnotic agent, based on the traditional knowledge.[36] As expected, they observed that spinosin potentiated the hexobarbital-induced sleeping time and antagonized caffeine-induced hyperactive behavior in mice although they were treated with very high dose of spinosin, 500 mg/kg.[36] About 30 years later, Chinese research group had interested on the mode of action of spinosin. They observed that spinosin potentiated pentobarbital-induced sleep time, which was augmented with 5-hydroxytryptamine (serotonin, 5-HT) precursor, 5-hydroxytryptophan, and antagonized by the inhibitor of tryptophan hydroxylase, p-chlorphenylalanine.[37]

They proposed that serotonergic neurotransmitter system might be involved in the hypnotic activity of spinosin. Thereafter, they also wanted to elucidate which serotonin receptor(s) is involved in sleeping behavior of spinosin and observed that postsynaptic and presynaptic 5-HT_{1A} receptors were involved in the sleeping behavior of spinosin.[38,39] Others also reported that spinosin exhibited anxiolytic-like behavior through the modulation of $GABA_A$ and 5-HT_{1A} receptors.[40] Thus, spinosin would be one of active components of the *Z. jujuba* var. *spinosa* and have hypnotic and anxiolytic-like behaviors. If spinosin activates $GABA_A$ receptors, it should exert anxiolytic, hypnotic and amnesic behavior. Strikingly, however, we found that spinosin reversed scopolamine-induced memory impairment in mouse model, which means that spinosin may be not a $GABA_A$ receptor agonist but a 5-HT_{1A} receptor antagonist.[41]

Spinsoin and cognition function – We expected spinosin as a $GABA_A$ receptor antagonist because it has glycoside group at C-6 of ring A, similarly with that of oroxylin A. However, as described above, spinosin may exert its pharmacological activity through 5-HT_{1A} receptor antagonist.

5-HT_{1A} receptor is negatively coupled to the adenylate cyclase and inhibits neuronal firing. It is well known that 5-HT_{1A} receptor antagonists reverse memory impairment caused by cholinergic or glutamatergic blocking.[42-44] If spinosin has an antagonistic activity against postsynaptic 5-HT_{1A} receptor as suggested by Wang et al.,[38] it can be speculated that spinosin could attenuate cognitive dysfunction. We confirmed such speculation through classical behavioral studies including the passive avoidance task, the Y-maze task, and the Morris water maze task. Especially, we observed that spinosin-treated attenuation of memory impairment was completely antagonized by 5-HT_{1A} receptor agonist.[41] These results suggested that spinosin would have memory ameliorating activity through an antagonistic activity against postsynaptic 5-HT_{1A} receptor in the cholinergic dysfunction-induced memory impairment model.

Although 5-HT_{1A} receptor antagonist had not been proceeded for the development of new drug for treating AD after the lecozotan, 5-HT_{1A} receptor is still interesting one for cognitive function and its antagonist would be a promising agent for AD.[45] It has been reported that spinosin is distributed into most tissues including the brain, especially in the hippocampus and corpus striatum. The level of spinosin in the brain after intravenous administration is low but sustains within 20 min and disappeared thereafter.[46] In addition, spinosin is detected in the plasma after oral administration but the absolute bioavailability was low in rat (2.2%).[47] These findings suggest that spinosin penetrates into the BBB and affects brain function including cognitive and hypnotic activities. Therefore, we tried to clarify the receptor binding profiles of spinosin, including 5-HT_{1A} receptor. Receptor binding study was performed. However, unfortunately, spinosin did not exhibit any binding sites including G protein-coupled receptors and channels we tested. It is still unclear which receptor, channel, or any protein is involved in the cognitive enhancing activities of spinosin (Fig. 3).

Is spinosin a prodrug of swertisin? – It has been reported that spinosin is found in plasma, urine and feces as intact form after oral treatment.[48] In addition, swertisin (Fig. 1), one of the spinosin metabolites, is also detected in plasma, urine and feces, as similarly with spinosin.[49] Jiao et al. reported that 6”-*p*-coumaroylspinosin, a spinosin congener, was metabolized into spinosin and swertisin by the intestinal microflora.[50] Especially, swertisin was mainly produced by the incubation with the low concentration of 6”-*p*-coumaroylspinosin, suggesting that swertisin would be an active form of spinosin for learning and memory. Therefore, we explored whether swertisin reverses short-term or long-term memory impairment in mice. As shown in the spinosin, swertisin also ameliorated cognitive dysfunction measured by several behavioral tests.[51,52] If swertisin is responsible for the pharmacological activity of spinosin, swertisin might be expected to have affinity to 5-HT_{1A} receptor. We conducted receptor binding study to confirm whether swertisin has binding affinity to 5-HT_{1A} receptor. However, swertisin did show significant affinity to adenosine A1 receptor but not to 5-HT_{1A} receptor. In addition, swertisin was found to be an antagonist to adenosine A1 receptor by our in vitro and in vivo studies,[50] which should be confirmed by other research groups. Taken together, the idea that swertisin is the prodrug of spinosin should be further studied because the exact mechanism of action of spinosin and swertisin might be different from each other and spinosin is also found in the brain as an intact form.

Miscellaneous – In addition to the model of hypofunction of cholinergic neurotransmitter system, Aβ protein-induced AD mouse models were also introduced to investigate the effects of spinosin on cognitive functions. We and others confirmed that spinosin reversed memory declines in such mouse models,[41,51,52] suggesting that spinosin would be a potential for treating cognitive deficits observed in AD patients. Spinosin also increases adult neurogenesis through the proliferation of neural stem cells and survival of new born cells,[53] which might

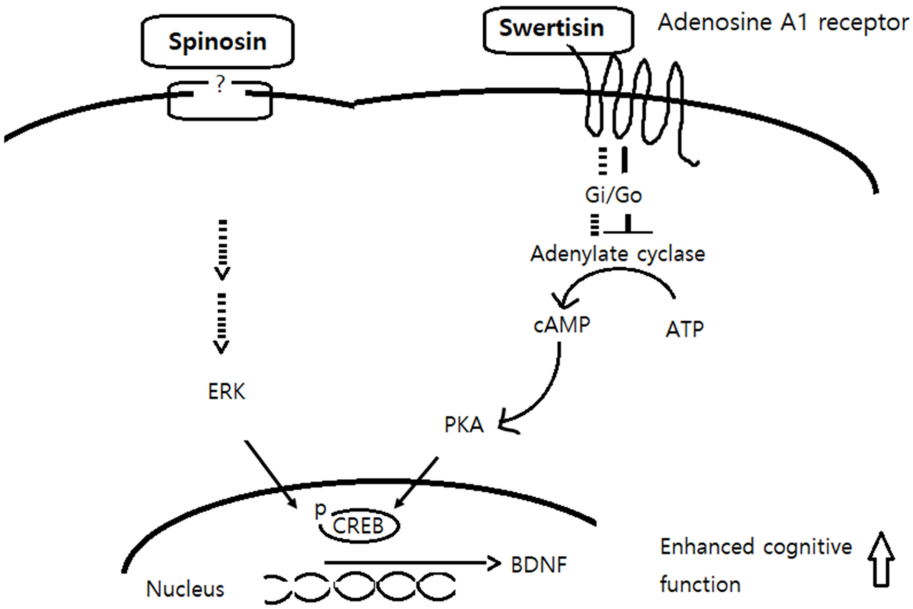

Fig. 3. Proposed mechanism of spinosin or swertisin on the memory enhancement and memory improvement. The administration of spinosin enhances cognitive performance and ameliorates cognitive impairment through the activation of ERK-CREB-BDNF signaling pathway in mice, but the exact binding site(s) is still unclear. Swertisin, a metabolite of spinosin, also enhances cognitive performance and ameliorates cognitive impairment via the inhibition of adenosine A1 receptor, resulting in the activation of various signaling molecules, such as PKA, CREB, and BDNF. Extracellular signal-regulated kinase, ERK; Brain-derived neurotrophic factor, BDNF; cAMP response element-binding protein, CREB; Protein kinase A, PKA.

result in cognitive enhancement. Now, the ethanolic extract of *Z. jujuba* var. *spinosa* standardized by spinosin (DHP1401) is under clinical trial (Phase II) in Korea (NCT03055741).

Extracellular signal-regulated kinase (ERK) and cAMP response element-binding protein (CREB) as well as BDNF signaling pathway is an important one in the learning and memory process. Although the exact mechanism of spinosin on cognitive function is unclear as described above, ERK-CREB-BDNF signaling pathway is reported to be involved in the pharmacological function of spinosin, especially in learning and memory or neurogenesis.[41] More recently, we found that spinosin regulated plasmin activity and synaptic plasticity, which would be one of the mechanism of spinosin on the cognitive function (unpublished data). Further researches are expected to clear the mode of action of spinosin and swertisin and expand the usefulness of such active flavonoids.

Conclusion

In this review, we summarized the pharmacological activities of oroxylin A and spinosin which have methoxyl group or C-glycoside group at C-6 of ring A. Others also took noticed of the value of bulky group at C-6 of ring A.[54] They did lots of works to unveil the importance of C-6 substituents. Unfortunately, they did not suggest that there is a common rule but only suggested that binding affinity to $GABA_A$ receptor depends on the size of first atom on the C-6 substituents and is also affected by 2'-substituent. Oroxylin A is likely to exhibit a characteristic, as suggested by previous report, but spinosin or it metabolite, swertisin, is much different from their suggestion because they did not have any binding affinity to the $GABA_A$ receptor. Small molecules or medicines exhibit their biological activities after binding to the receptor, channel, enzyme, or other proteins. Although the exact binding site(s) of spinosin is unclear, reverse pharmacology may contribute to understand the biological activity of spinosin. Beside the description in this review, oroxylin A and spinosin or swertisin have lots of pharmacological activities, such as anti-inflammatory or antidiabetic activities.[55,56] Further expanded pharmacological studies would open the road to develop new drug using above flavonoids.

Acknowledgements

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Conflict of interest

RJH is one of inventor of a PCT patent (WO2013 081419A1) entitled "Pharmaceutical composition for prevention or treatment of cognitive function disorders comprising spinosin". None of the other authors have any conflict of interest.

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