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Review Article

Experimental Neurobiology

Naringin: A Protector of the Nigrostriatal Dopaminergic Projection

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Parkinson's disease is the second most common neurodegenerative disorder characterized by the progressive degeneration of dopaminergic neurons and a biochemical reduction of striatal dopamine levels. Despite the lack of fully understanding of the etiology of Parkinson's disease, accumulating evidences suggest that Parkinson's disease may be caused by the insufficient support of neurotrophic factors, and by microglial activation, resident immune cells in the brain. Naringin, a major flavonone glycoside in grapefruits and citrus fruits, is considered as a protective agent against neurodegenerative diseases because it can induce not only anti-oxidant effects but also neuroprotective effects by the activation of anti-apoptotic pathways and the induction of neurotrophic factors such as brain-derived neurotrophic factor and vascular endothelial growth factor. We have recently reported that naringin has neuroprotective effects in a neurotoxin model of Parkinson's disease. Our observations show that intraperitoneal injection of naringin induces increases in glial cell line-derived neurotrophic factor expression and mammalian target of rapamycin complex 1 activity in dopaminergic neurons of rat brains with anti-inflammatory effects. Moreover, the production of glial cell line-derived neurotrophic factor by naringin treatment contributes to the protection of the nigrostriatal dopaminergic projection in a neurotoxin model of Parkinson's disease. Although the effects of naringin on the nigrostriatal dopaminergic system in human brains are largely unknown, these results suggest that naringin may be a beneficial natural product for the prevention of dopaminergic degeneration in the adult brain.

Key words: Parkinson's disease, naringin, mTORC1, GDNF, neuroprotection

TWO STRATEGIES FOR PARKINSON'S DISEASE THERAPY

Parkinson's disease is the second common neurodegenerative disorder and is characterized by the progressive degeneration of dopaminergic (DA) neurons and biochemical reduction in striatal dopamine levels, associated with major clinical symptoms including tremor at rest, rigidity, bradykinesia and postural instability [1, 2]. Although the developments of knowledge-based therapeutics have been extensively studied, fully understanding of the etiology of Parkinson's disease still remains lack. However, one treatment area that has gained significant momentum is the use of various growth factors such as glial cell line-derived neurotrophic factor (GDNF) [3-6]. GDNF induces trophic effects by the activation the Akt/mammalian target of rapamycin (mTOR) signaling pathway in neurons [7]; in DA neurons of Parkinson's disease brains, GDNF levels are reduced more than any neurotrophic factor [8].

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Although the etiology of Parkinson's disease is unknown as described above, another accumulating evidence suggests that Parkinson's disease is partly caused by activation of microglia, resident immune cells in the brain. Under neuropathological conditions, microglia are activated in response to DA neuronal damages, and activated microglia could produce various potentially neurotoxic molecules, including inducible nitric oxide synthase (iNOS) and proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β) [9-13]. iNOS and proinflammatory cytokines may be involved in nigrostriatal DA neuronal death [12, 13]. Moreover, increasing evidence suggests that activated microglia generate reactive oxygen species, resulting in oxidative stress to DA neurons in the substantia nigra of Parkinson's disease patients and animal models of Parkinson's disease generated by administration of 1-methyl-4-phenylpyridinium (MPP^+) [9]. These results suggest that the control of microglial activation can be useful to prevent the degeneration of the nigrostriatal DA projection.

GDNF FOR PARKINSON'S DISEASE THERAPY

GDNF is a member of the transforming growth factor-β family of trophic factors, and has been identified in many types of neurons including DA neurons [14]. Treatment with GDNF has consistently demonstrated neurotrophic and protective effects in animal models of Parkinson's disease [3-6], and conditional ablation of GDNF in adult mice results in a delayed and progressive loss of DA neurons [15]. These results suggest that GDNF is an indispensable neurotrophic factor for the survival and protection of DA neurons. However, GDNF has a critical problem to treat Parkinson's disease patients. GDNF must be directly treated in the brain to apply to Parkinson's disease patients because it does not cross the blood-brain barrier which is the brain's protective membrane. To treat Parkinson's disease patients, the first clinical trial utilizing the direct delivery of GDNF into the brain was actually initiated in 1996. However, clinical trial by intracerebroventricular injection and intraputaminal infusion of GDNF failed to treat Parkinson's disease patients [16, 17], probably because of the limited penetration and distribution to the target brain areas. These clinical results suggest that the therapeutic potential of GDNF for Parkinson's disease is likely to depend on sustained delivery of the appropriate amount to the target areas.

IMPORTANCE OF MTORC1 ACTIVATION IN THE ANIMAL MODELS OF PARKINSON'S DISEASE

An alternative to delivering neurotrophic protein molecules within brain extracellular space is to directly activate the intracellular signaling pathways responsible for their effects. This activation is possible by viral vector approaches to transduction of neurons [18]. Many of the cellular effects of GDNF are initiated by binding to GNDF family receptor alpha-1 (GFRα1) [19], and the stimulation of the receptors by treatment with GDNF activates the Akt/mTOR signaling pathway, resulting in the downstream activation of prosurvival pathway in neurons [7]. In addition, many research groups have reported that the activation of Akt/mTOR signaling pathway enhances the activity of intracellular cell survival pathways under a variety of conditions, such as ischemic shock, oxidative stress and the withdrawal of trophic factors [20-22]. Consistent with these results, we have recently reported that the activation of mammalian target of rapamycin complex 1 (mTORC1) by adeno-associated virus 1 transduction with a gene encoding the constitutively active form of Akt or ras homolog enriched in brain (Rheb) with a mutation of the serine to histidine at the 16 position [Rheb(S16H)] in the substantia nigra induces neurotrophic effects, resulting in the protection and restoration of the nigrostriatal DA projection in a neurotoxin model of Parkinson's disease [23-25]. Moreover, MPP^+ treatment as a neurotoxin model of Parkinson's disease decreases Akt phosphorylation resulting in loss of mTORC1 activation, and the decreased level of Akt phosphorylation is also observed in the substantia nigra of Parkinson's disease patients [26]. These results indicate that the neurotrophic effects by mTORC1 activation are necessary for the survival of DA neurons and functional maintenance of DA system in the adult brain.

NARINGIN AS A NOVEL DRUG FOR PARKINSON'S DISEASE THERAPY

As described above, however, brain surgery is necessarily needed to apply the delivery of GDNF and viral vectors inducing its effects such as mTORC1 activation to Parkinson's disease patients. Although gene therapy by viral vectors is still ongoing to treat Parkinson's disease patients, the issues for brain surgery suggest that another of alternatives such as natural compounds or chemical drugs to the induction of neurotrophic effects through internal medication could be beneficial to prevent the degeneration of the nigrostriatal DA projection in the adult brain. However, although Parkinson's disease-induced motor manifestations can be treated successfully for a limited period by treatment with chemical drugs, which restore dopaminergic function, there is still no treatment that forestalls deterioration attributable to progressive neurodegeneration [18].

Naringin is a major flavonone glycoside in grapefruits and

citrus fruits [27], and it is considered as a protective agent against neurodegenerative diseases because it can induce not only anti-oxidant effects [27, 28] but also neuroprotective effects by the induction of neurotrophic factors such as brain-derived neurotrophic factor and vascular endothelial growth factor, and by the activation of anti-apoptotic pathways [29-31]. However, it is still unknown whether naringin has neuroprotective effects against the degeneration of the nigrostriatal DA projection in the adult brain, which is associated with Parkinson's disease. To investigate the possibility of naringin-mediated neuroprotection on the nigrostriatal DA projection, we have recently examined whether daily intraperitoneal injection of naringin can induce neuroprotective effects in the MPP^+-treated rat model of Parkinson's disease [32]. Our observations showed that intraperitoneal injection of naringin could significantly increases the level of GDNF with activation of mTORC1 in nigral DA neurons, and naringin-induced the production of GDNF contributed to the neuroprotection of the nigrostriatal DA projection in a neurotoxin model of Parkinson's disease [32]. In addition to the neurotrophic and protective effects, we found that naringin could attenuate the level of TNF-α in microglia increased by MPP^+-induced neurotoxicity, indicating the anti-inflammatory activity of naringin against inflammation in the substantia nigra [32].

mTORC1 ACTIVATION AND GDNF REPRODUCTION IN DA NEURONS

It is largely unknown whether activated mTORC1 can activate production of neurotrophic factors, contributing to the protection of the nigrostriatal DA projection, by intracellular signaling pathways in neurons of the adult brain. The answer for this

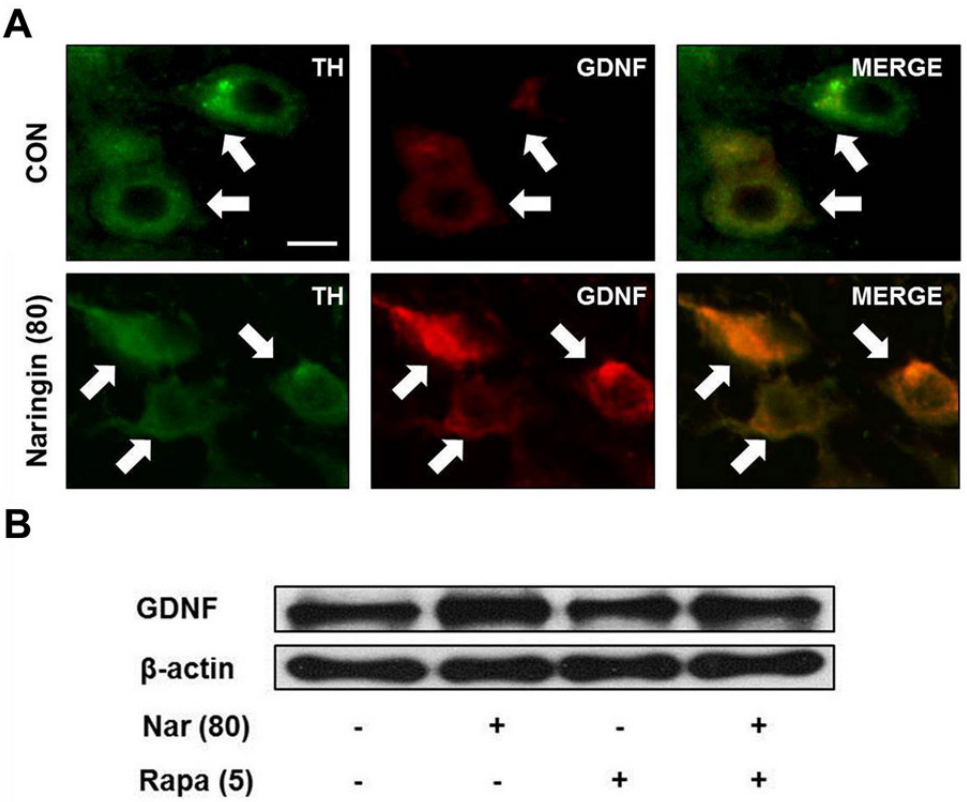

Fig. 1. Intraperitoneal injection of naringin induces an increase in the expression of GDNF in DA neurons in the rat substantia nigra. (A) Immunofluorescence double staining for tyrosine hydroxylase (TH, green) and GDNF (red) shows that naringin-increased expression of GDNF is identifiable within DA neurons. Scale bar, 10 μm. (B) Representative Western blots of GDNF expression in the rat substantia nigra. To investigate whether the production of GDNF by treatment with naringin is dependent on the activation of mTORC1, rats received daily intraperitoneal injection of rapamycin [5 mg/kg, Rapa (5)] or naringin [80 mg/kg, Nar (80)], or co-injection of rapamycin and naringin for 4 days. The results show that treatment with 5 mg/kg rapamycin alone indicates no alteration on the basic level of BDNF compared to the intact control ($p=0.120$, compared with CON). However, its treatment attenuated the level of naringin-increased GDNF [$p<0.05$, compared with Nar (80)]. All values on the optical density of each band represent the mean±SEM of three pooled samples (one-way ANOVA and Student-Newman-Keuls analysis).

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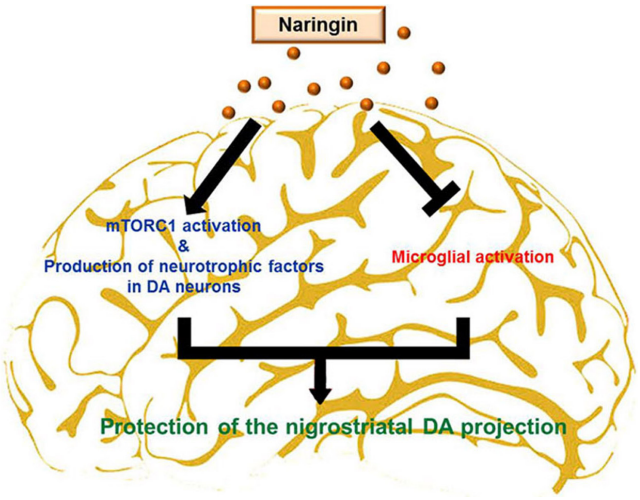

Fig. 2. Schematic representation of the protective effects of naringin on the nigrostriatal DA projection. Intraperitoneal injection of naringin induces an increase in GDNF and mTORC1 activity in DA neurons, and attenuates microglial activation to protect the nigrostriatal DA projection in the adult brain.

question describes the relations between GDNF reproduction and mTORC1 activation. We have recently found that Rheb (S16H) expression by a viral vector induces a robust ability to induce GDNF in adult DA neurons *in vivo*, which is dependent on mTORC1 activity and contributed to the protection of the nigrostriatal DA projection [33]. To ascertain whether mTORC1 activated by treatment with naringin mediates the induction of GDNF, we further examined the effects of rapamycin, a specific inhibitor of mTORC1 [34], on the level of GDNF (Fig. 1). Rats received daily intraperitoneal injection of rapamycin (5 mg/kg) or naringin (80 mg/kg), or co-injection of rapamycin and naringin for 4 days. Similar to the previous results [32, 33], GDNF expression was increased in the substantia nigra of rat brain by treatment with naringin, and the naringin-increased levels were obvious in DA neurons as demonstrated by immunofluorescence double-labeling for tyrosine hydroxylase, indicating DA neurons, and GDNF (Fig. 1A). Although rapamycin treatment did not alter the basic level of GDNF in the substantia nigra, indicating a modest inhibitory effect, it attenuated the expression of GDNF induced by naringin as demonstrated by Western blot analysis (Fig. 1B). Similar to Rheb(S16H)-induced effects, these results show that naringin-activated mTORC1 stimulates the production of GDNF in DA neurons of the adult brain.

CONCLUSIONS

Naringin is a major flavonone glycoside in grapefruits and citrus fruits [27], which is expectable to be harmless to health. Our present observations on the effects of naringin in a neurotoxin model of Parkinson's disease show that treatment with naringin can impart to adult DA neurons the important ability to reproduce GDNF as a therapeutic agent against Parkinson's disease with additional anti-inflammatory effects on brain inflammation (Fig. 2). These results suggest that naringin may be a beneficial natural product offering promise for the prevention of neurodegeneration involved in Parkinson's disease. However, it is still unclear whether naringin can induce the activation of another prosurvival pathway except GDNF-mediated pathway, and post-treatment with naringin can restore the function of DA neurons in adult brains. Therefore, to make the possibility to treat Parkinson's disease patients clear, further study is needed to determine the effects of post-treatment with naringin such as the induction of dopamine and the regeneration of axons after damage in DA system of adult brain as well as the study on the mechanisms of naringin-induced effects in the adult brain.

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