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Naturido Could Modulate Glia-Neuron Interactions

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Abstract

Our groups recently published that a novel cyclic peptide (Naturido) modulates glia-neuron interactions *in vitro* and reverses aging-related deficits in senescence-accelerated mice. This article suggests that Naturido is a promising glia-neuron modulator for the treatment of not only senescence, but also Alzheimer's disease (AD) and other neurodegenerative disorders. We briefly review the main points of this article.

Keywords: Naturido; Glia-neuron interactions; Aging disorders; Neurodegenerative diseases

Introduction

The Alzheimer's Association explains that Alzheimer's disease (AD), which affects 50%-60% of people with dementia and dementia damage nerve cells. More than 50 million people are estimated to living with dementia, and this number is set to increase to 152 million by 2050 [1,2]. Regarding this serious issue, a review of clinical trials over the past 5 years shows that progressive emphasis has been placed on nonamyloid targets, including candidate treatments for inflammation, synapse and neuronal protection, vascular factors, neurogenesis, and epigenetic interventions [3]. In addition to the clinical treatment of AD with the acetylcholinesterase inhibitors (donepezil, galantamine, and rivastigmine) and NMDA receptor antagonist (memantine), immunotherapeutic approaches have been applied to the development of antidementia drugs [4]. However, our group has highlighted the importance of glia-neuron interactions (communications) for the understanding and developing drugs for aging disorders and neurodegenerative diseases [5].

Recently, glia-neuron interactions have become regarded as a clue to elucidate complicated brain mechanisms and develop medicines for brain disease. These interactions are similar in species ranging from *C. elegans* [6] to flies [7] and are potential targets for understanding the pathogenesis of AD and other neurodegenerative disorders [8]. Thus, rather than targeting individual glia or neurons, like in the past, glia-neuron interactions should be targeted for the development of new and effective therapies for central nervous system (CNS) disorders. Our research aimed to isolate a promising bioagent and to assay its function, based on glia-neuron interactions.

Chemical Structure of the Novel Cyclic Peptide Naturido

From the powder of *Isaria japonica* grown on silkworm pupae, we first obtained a final purified product by the combination of reverse-phase (RP) flash chromatography and RP- and hydrophilic interaction liquid chromatography (HILIC)-HPLC. The product was subjected to nuclear magnetic resonance (NMR) and mass spectrometry analyses, and astrocyte proliferation was assessed by measuring the incorporation of BrdU. The resulting novel compound was found to be a water-soluble cyclic peptide derivative with a molecular weight of 566.2588. We named the derivative Naturido (a combination of "Naturo", which means nature, and "id", which is a suffix of progeny in Esperanto) (Figure 1) [1].

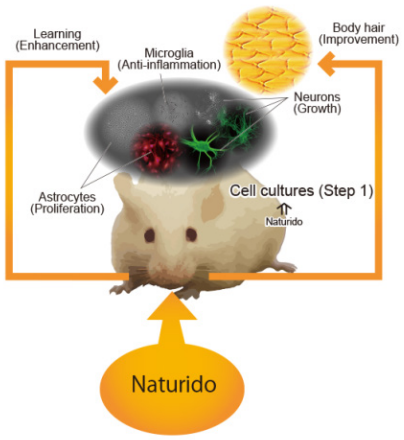

Figure 1: Experimental flowchart and summarized results showing the main evidence of our article [1]. In Step 1, we found that Naturido significantly enhanced astrocyte proliferation and activated the single-copy gene encoding the neuropeptide *VGF* and the neuron-derived *NGF* gene. The addition of the peptide to the primary hippocampal neuron culture medium increased the dendrite length, dendrite number, and axon length. The addition of the peptide to primary microglial cultures also shifted CGA-activated microglia towards anti-inflammatory and neuroprotective phenotypes; In the step 2, *in vivo* analyses revealed that spatial learning ability and hair quality were improved in Naturido-treated mice compared with untreated mice (SAMP8) to the same level observed in the normal aging control (SAMR1).

In vitro Functions of Naturido in Astrocytes, Microglia, and Neurons

Astrocytes

To reproduce physiological reactions to the greatest extent possible

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in vivo, we tried to perform a Naturido assay using each primary culture. We first prepared pure primary cultures from mouse cerebral tissues and compared the expression of GFAP and EAAT-2. The addition of Naturido to the astrocyte culture medium did not change significantly the proportion of GFAP-positive cells (approximately 25.8%-39.8% among EAAT-2 cells), indicating the stability of Naturido for the treatment of GFAP-positive populations, with no loss of EAAT-2 expression and no increase in GFAP expression. GFAP is important for astrocyte-neuron interactions and astrocyte processes play vital roles in modulating synaptic efficacy in the CNS [9]; thus, the stable association between the GFAP level and Naturido suggests that this cyclic peptide can be used to analyze astrocyte-neuron interactions.

We also investigated whether the Naturido-mediated promotion of proliferation was specific to astrocytes or common among the following cell types: human dermal fibroblasts, human hepatocellular carcinoma cells, human myeloid leukemia cells, and glial tumor cells. In contrast to astrocytes, the cell proliferation rates of these other cell types decreased significantly after the addition of Naturido. Given the astrocyte-specific characteristic of Naturido and its potential for use in the treatment of dementia, we compared its astrocyte proliferation ability with those of zonisamide, donepezil, eserine, and galantamine, which are used in the clinical treatment of Parkinson's disease and AD. Naturido modulated astrocyte proliferation, while these other clinical drugs did not have this activity. Next, we investigated the effects of Naturido addition on the expression of five representative genes associated with synaptic strengthening and plasticity in astrocytes, as well as with neuronal modulation: nerve growth factor (*NGF*), glial cell-derived neurotrophic factor (*GDNF*), vascular endothelial growth factor (*VEGF-A*), brain-derived neurotrophic factor (*BDNF*), and nonacronymic neuropeptide (*VGF*). The increases in the mRNA expression of *NGF* and *VGF* induced by Naturido in cultured astrocytes suggest that the induction of axon growth [10] and proliferation are associated with hippocampal neurogenesis [11]. Given the results of these assays, we hypothesized that Naturido targets astrocytes.

Neurons

To test whether Naturido targets neurons as well as astrocytes, we investigated whether the peptide could directly affect neuronal growth using a primary culture of hippocampal neurons. We compared the effects of Naturido with those of the NGF and VGF proteins in cultured hippocampal neurons. In these assays, Naturido had less substantial effects on the dendrite length and number than NGF, while its effects on axon the length were more significant than those of NGF. Naturido also significantly increased the dendrite length and number as well as the axon length relative to those achieved with VGF. These findings suggest that Naturido is a novel neurotrophic peptide in hippocampal neurons.

Glia

Using primary microglial culture systems, we focused on the effects of Naturido on the production of IL-1β, the key proinflammatory mediator and TGF-β1, a prominent anti-inflammatory mediator in microglia [12]. We used chromogranin A (CGA), a neurosecretory acidic glycoprotein, as a microglial stimulator because it localizes in the senile plaques of patients with AD [13] and is thought to activate microglia [12]. In compassion to CGA-treated cells, primary microglia prepared with Naturido exhibited significantly increased. TGF-β1 expression throughout the cultured period (from 24 to 72 hours). In contrast, pretreatment with Naturido significantly decreased the IL-1β expression in the primary microglia throughout the culture period (from 24 to 72 hours). These observations strongly suggest that Naturido shifts CGA-induced proinflammatory microglia to an anti-inflammation phenotype by increasing TGF-β1 expression and suppressing IL-1β expression. Notably, Naturido itself did not affect the expression of TGF-β1 or IL-1β in the primary microglia, suggesting its safety in primary brain cells. Considering the regulating effects of Naturido on CGA-activated microglia, it may improve neurodegenerative diseases, including AD. However, further research is needed to elucidate the cellular mechanisms of Naturido in microglia.

Effect of Oral Naturido Administration on Learning and Memory in Senescence-Accelerated Mice

We aimed to assess the effects of Naturido on a mouse model of aging. We investigated the effects of Naturido on normal aging mice (SAMR1) and on mice with senescence-accelerated aging (SAMP8), which have been used extensively [14]. Four groups were used to evaluate learning and memory: untreated control SAMR1 and SAMP8 mice, SAMP8 mice orally administered 1250 μg/kg/day donepezil (a typical treatment for AD) [15] and SAMP8 mice orally administered 2.5 or 25 μg/kg/day Naturido. Morris water maze tests were performed to evaluate the spatial learning abilities of SAMR1 and SAMP8 mice, and SAMP8 mice spent significantly less time in quadrant 0 (which had the platform) than for SAMR1 mice ($P<0.05$). Additionally, a comparison of the SAMP8 control mice with the SAMP8 mice orally administered Naturido (25 μg/kg/day) revealed that the percentage of time spent in quadrant 0 was significantly recovered in the Naturido-treated mice: the percentage nearly returned to that observed for SAMR1 mice ($P<0.05$). However, the oral administration of donepezil (1250 μg/kg/day) to SAMP8 mice did not recover the spatial learning ability to the same extent as the oral administration of Naturido. Based on these results, we conclude that the oral administration of Naturido improves the spatial learning ability of mice with age-related learning deficits.

Effects of Oral Naturido Administration on Hair Quality in Senescence-Accelerated Mice

In parallel with the effects of the oral administration of Naturido on learning and memory in SAMP8 mice, we investigated the relationship between the deterioration of hair quality in SAMP8 mice as a representative aging-related marker [16] and the effects of Naturido administration. Hair quality was assessed by evaluating the correlation between the coefficient of friction (COF) and the damage area ratio on the mouse body hair surface using a friction tester and a scanning probe microscope (SPM).

The body hairs of SAMP8 mice exhibited high COF and damage area ratio values, while that of SAMP1 mice with normal aging exhibited lower values for both indicators. In SAMP8 mice orally administered donepezil, the damaged hair surface was close to that of normal aging mice (SAMR1). However, the body hair COF of donepezil treated SAMP8 mice was the same as that of untreated SAMP8 mice, and no significant reduction in the COF was observed. In contrast, in SAMP8 mice orally administered Naturido at either concentration (2.5 or 25 μg/kg/day), both the COF and the damage area ratio were lowered to the same levels as those in normal aging mice. Because hair quality was improved following the administration of Naturido, we believe that Naturido may exert an anti-aging effect on hair.

Conclusion

Given the basis of our published article as described above, we propose the following: a novel cyclic peptide named Naturido based on Esperanto, is thought to target glia-neuron interactions in the CNS, and thus has implications for the treatment of the most challenging neurological disorders of AD, Parkinson's disease, schizophrenia, and epileptogenesis. In addition to overcoming pathological effects, Naturido may promote brain improvements in humans, as it potentially has exceptional and

ideal effects, accelerating neuron growth and astrocyte proliferation and inhibiting inflammation in microglia. These important effects could be achieved by the simple oral administration of Naturido in both medicinal and neutraceutical forms.

Competing Interests Statement

KS is a paid employee of DKS Co., Ltd. (the parent company of Biococoon Laboratories, Inc.). There are no marketed products of Naturido itself, but Biococoon Laboratories, Inc. owns the trademark registration of Naturido®.

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