

Carnosine: A Possible Drug for Vascular Dementia

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Abstract

Carnosine (β -alanyl histidine) is a small dipeptide with numerous beneficial effects, including the maintenance of the acid-base balance, antioxidant, chelating, anti-crosslinking, and anti-glycation activities in the living organism. High levels of carnosine are found in the skeletal muscles and in the brain. We have found that carnosine inhibits Zn²⁺-induced neuronal death, which plays a crucial role in the pathogenesis of vascular dementia. Our previous research demonstrated that carnosine participates in the Endoplasmic Reticulum (ER)-stress pathway in Zn-induced neurotoxicity and we have applied for a patent related to drugs for Vascular Dementia (VD). Here, we review the roles of carnosine in VD and other neurodegenerative diseases and discuss perspectives about the future therapeutic use of this dipeptide.

Keywords: Carnosine; Vascular-type dementia; Zinc
Abbreviations: A β P: Amyloid β Protein; AD: Alzheimer's Disease; AGE: Advanced Glycation End-Product; AMPA: Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic acid; Arc: Activity-Related Cytoskeleton Protein; BDNF: Brain-Derived Neurotrophic Factor; Ca: Calcium; EDTA: Ethylenediaminetetra Acetic Acid; GADD: Growth-Arrest DNA Damage; His: Histidine; MT: Metallothionein; NMDA: N-Methyl-D-Aspartate; VD: Vascular-Type Dementia; VGLG: Voltage-Gated Ca²⁺ Channels; Zn: Zinc; Znt: Zn Transporter

Introduction

Carnosine, which is composed of β -alanine and L-histidine (His), is synthesized by the enzyme carnosine synthase, in a reaction that also requires ATP. Carnosine and its related compounds, homocarnosine and anserine (Figure 1A), are found in birds, fish, and mammals, including humans [1-4]. Carnosine is synthesized by muscle cells, glial cells, and oligodendrocytes [5], and the biggest concentration is observed in skeletal muscle tissue, the stomach, the kidney, cardiac muscle, and the brain [6-8]. In the brain, carnosine accumulates in neurons in the olfactory bulbs and in glial cells in other areas. The structures of carnosine and its related compounds, and the possible roles of carnosine, are shown in Figure 1A. Carnosine reportedly has numerous beneficial characteristics, such as maintaining pH balance [1], anti-glycation [9], antioxidant [7], hydroxyl radical scavenger [10], and as a chelator of metal ions [11,12] as shown in Figure 1B. Considering that these properties are related to aging and that the olfactory bulb which acts as a gateway to the external environment, carnosine is believed to act as an endogenous anti-aging or neuroprotective agent.

Kanayama et al. have shown that intranasal administered metal ions were transported from the nasal cavity to the olfactory bulb via the olfactory nerve pathway, and then, metal ions are received by other brain regions such as the hypothalamus and the hippocampus [13]. These influxes may lead to excessive accumulation of metal ions that cause a neuronal injury as described below. We have previously investigated the molecular mechanism of zinc-induced neuronal

death. After transient ischemia condition, an excess amount of glutamate causes the calcium (Ca) dyshomeostasis and induces neuronal death, which finally leads to the pathogenesis of a Vascular type of Dementia (VD). Increasing evidence suggests that zinc (Zn) which is released with glutamate, enhances the neurotoxicity of the latter and plays crucial roles in the pathogenesis of VD. During the search for protective substances against Zn-induced neurotoxicity, we found that carnosine exhibits marked inhibitory effects on Zn-induced neurotoxicity and proposed its use as a possible therapeutic agent for VD. The level of carnosine varies during development and is low in the aged animals [3]. Therefore, it is highly possible that carnosine protects against external toxins and acts as an endogenous protective substance against neuronal injury and aging. In this article, we review the newest findings about the properties of carnosine, based on our own and other previous studies, and discuss possible drugs for VD and other neurodegenerative diseases.

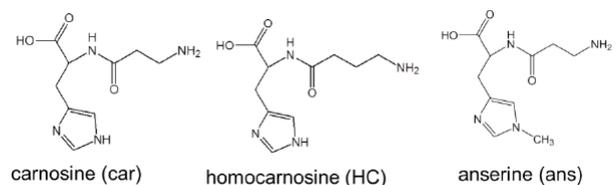


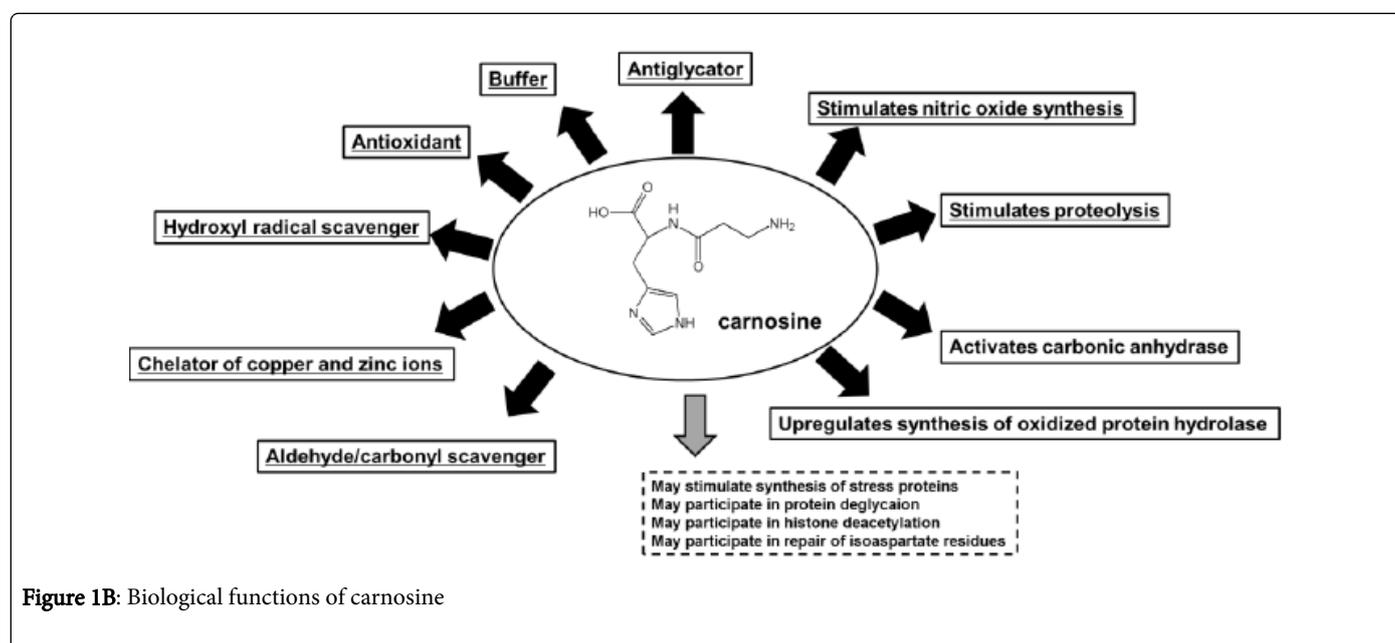
Figure 1A: Structures of carnosine and its related compound

Expression and Distribution of Carnosine

Carnosine contributes as much as 0.2-0.5 % to the net weight of some muscles [14]. Studies performed on the gastrocnemius muscle showed that the concentration of carnosine in the human body is higher in males, decreases with age, and is dependent on diet (a vegetarian diet reduces the level of carnosine in skeletal muscle) [15,16]. In animals, factors such as trauma, shock, starvation, or injection negatively affect the level of carnosine in muscle tissue.

Infection and trauma may be associated with cellular Ca dysregulation and myocardial depression. Carnosine administration may also play a role in the contractility of cardiac cells and the regulation of intracellular Ca levels [7]. In the brain, carnosine is also present in the olfactory bulb [2]. We have developed a high-performance liquid chromatography system for analyzing and quantifying carnosine [17] and have confirmed that carnosine is abundant in the olfactory bulb in the rat brain. Biffo et al. also showed that carnosine is located in the olfactory receptor neurons, more specifically in the perikarya and cell processes, including the axons and synaptic terminals in the olfactory bulb [18]. Carnosine is rapidly formed and directed, via axoplasmic transport, to the olfactory bulb [19]. In the primary olfactory neurons, carnosine synthase activity is decreased upon denervation and is recovered upon regeneration [20,21]. The sensory neurons may be primarily responsible for the presence of carnosine in the olfactory bulb [3]. Carnosine and its related compounds are not degraded by

regular dipeptidases, but are metabolized by specific hydrolytic enzymes, named carnosinases. The activity of carnosinase is very low shortly after birth and gradually increases to adult levels during adolescence, and attains higher levels in males than in females [15, 22]. Two types of carnosinases (CN1 and CN2) have been identified in humans and mice [4]. CN1 is highly present in human serum, but absent from non-primate mammals, except for the Syrian golden hamster [23]. Human CN1 is also expressed in the brain, liver, and kidney [4]. Most likely, these tissues are involved in the delivery of L-His or β -alanine. Recently, the gene for an enzyme, CN1 (EC3.4.13.20), which is expressed specifically in human brain, was characterized [24]. This enzyme may be involved in controlling the content of carnosine in the human brain. Under physiological conditions, the catalytic rate of CN2 is markedly lower than that of CN1. It remains to be determined whether CN2 is involved in the degradation of carnosine in tissues that are abundant in carnosine.



Biological and Physiological Roles of Carnosine

Buffering activity

Carnosine has many of possible biological functions, as shown in Figure 1B [25]. The most convincing proposal is that carnosine plays one or more roles in the control of the intracellular hydrogen ion concentration [1,26]. During high-intensity anaerobic exercise, proton accumulation causes a decrease in intracellular pH, which influences various metabolic functions. The pKa value of carnosine is 7.01, which is close to the intracellular pH. Therefore, carnosine contributes to physicochemical non-bicarbonate buffering in skeletal muscles, and the administration of carnosine has been reported to induce hyperactivity in animals. This property may explain its predominant association with white, glycolytic, muscles which possess relatively few mitochondria and thereby generate lactic acid. These properties may help explain the protective action of carnosine in conditions associated with severe intracellular acidosis, such as ischemia.

Metal ion-chelating activity

Carnosine is a chelator of metal ions and forms complexes with Ca, copper, and Zn ions [11,12]. Therefore, carnosine may exert some sort of control of Ca metabolism in muscle tissue (heart or skeletal). It is also likely that the dipeptide controls the availability of Zn²⁺ in neuronal tissue, particularly in the olfactory lobe where both carnosine and Zn are enriched [2,27,28]. Pharmacological Zn-carnosine complexes are called polaprezinc and are also effective in the repair of ulcers and other lesions in the alimentary tract [29].

Antioxidant activity

When provided to humans as a supplement, carnosine has antioxidant properties, acting against free radicals, which contribute to aging in human. Carnosine scavenges both reactive oxygen and nitrogen, which contain unpaired electrons [30,31]. Carnosine may inhibit lipid oxidation through a combination of free radical scavenging and metal chelation. It also provides cells with an antioxidant system that functions in the cytosolic environment, where water soluble oxidation mediators are often present in high concentrations [7]. It has been demonstrated that oral ingestion of

carnosine for 3 months improves the overall appearance of skin and reduces the wrinkles that appear with age [32]. In mice, carnosine administration prolongs life expectancy and improves their physical appearance and behavior [33].

Carnosine in cardiovascular function

Carnosine transiently decreases the systemic blood pressure in various mammalian species [34,35], most likely by causing systemic arterial vasodilation. The direct vaso-relaxing effect of carnosine was demonstrated on isolated rat aortic rings [36]. This dipeptide may regulate the vascular tone. The *in vivo* formation of a Zn/carnosine complex and histamine H1 receptors appear to be involved in vascular smooth muscle contraction/relaxation [37]. Carnosine modulates Ca-regulated proteins in cardiac muscle cells, and can potentiate cardiac contractility [38]. In chemically skinned cardiac cells, carnosine releases Ca, produces contracture, and alters the tension response of the contractile proteins to Ca. Carnosine also acts directly on the ryanodine receptor, a Ca-release channel, producing large increases in the open state probability and dwell time. A side from the modulation of intracellular Ca, carnosine may play a role in pH regulation and may contribute to the mobile buffering system in cardiac cells [19].

Carnosine in the sense organs

Carnosine may affect the sense organs. The dipeptide can have a protective effect on retinal capillary cells. Oral administration of carnosine to rats that had remained hyperglycemic for 6 months reduced the blood vessels of the retina [39,40]. It has been shown that the use of eye drops containing N-acetyl carnosine prevented development of cataracts and lens turbidity and contributed to prevention of the development of blurred vision and even blindness in the elderly [40]. Carnosine may also protect the hearing system. In rats, intraperitoneal injections of carnosine were shown to reduce the severity

Carnosine in the brain

A number of roles of carnosine in the brain have been proposed. On the basis of the high concentrations of carnosine found in olfactory neurons, this dipeptide was hypothesized to be involved in sensory neurotransmission, either as a neurotransmitter or a neuromodulator. Immunohistochemical studies have shown colocalization of carnosine and glutamate in the synaptic terminals of mouse olfactory bulbs [28]. As for most other sensory pathways, glutamate is the main excitatory neurotransmitter involved in the synapses between olfactory neurons and target cells (mitral and periglomerular cells) in the olfactory bulb. This supports the hypothesis of a role for carnosine in neuromodulation of glutamatergic sensory neurons.

Carnosine as a Protective Substance against Neurotoxicity

Carnosine inhibits the Maillard reaction that involves reduction of sugar and proteins, providing a multitude of end-products, most notably advanced glycation end-products (AGEs) [9]. AGEs can contribute to the pathogenesis of various senile diseases, such as Alzheimer's disease (AD), vascular stiffening, atherosclerosis, osteoarthritis, inflammatory arthritis, and cataracts [42]. Furthermore, carnosine is reported to have anti-crosslinking properties [43]. Carnosine has been reported to inhibit alpha-crystallin fibrillation [44]. It is widely recognized that crosslinking and conformational

changes in disease-related proteins (e.g., amyloid β protein (A β P), prion protein) are central to the pathogenesis of various neurodegenerative diseases, termed "conformational diseases", including AD and prion diseases [45,46]. We previously demonstrated that carnosine attenuates neuronal death induced by prion protein fragment peptide (PrP106-126), by changing its conformation [47]. It has also been demonstrated that carnosine inhibits the aggregation and subsequent neurotoxicity of A β P [48]. Corona et al. showed that dietary supplementation of carnosine attenuates the accumulation of A β P and mitochondrial dysfunction in Alzheimer's model mice [49]. Carnosine levels are also significantly reduced in the serum of AD patients [50]. These results suggest possible beneficial effects of carnosine as a treatment for AD and prion diseases. Analogues of carnosine, such as anserine or homocarnosine, may also prove to be useful owing to their similar antioxidant activities. Furthermore, as described below, we also demonstrated that carnosine inhibited Zn $^{2+}$ -induced neuronal death, which is involved in the pathogenic mechanisms of VD [51].

Carnosine as a Possible Therapeutic Agent for Vascular Type Dementia

Zinc induced neurodegeneration after Ischemia

VD is a degenerative cerebrovascular disease, and its risk factors include age, male gender, diabetes, and high blood pressure. The most common type of VD is caused by a series of small strokes or ischemia [52]. Following transient global ischemia or stroke, the interruption of blood flow and the resulting oxygen-glucose deprivation induce long-lasting membrane depolarization and an excessive release of glutamate into synaptic clefts. Thereafter, the excess glutamate causes an overstimulation of the relevant receptors, namely N-methyl-D-aspartate (NMDA)-type receptors, amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type receptors, and kainate-type receptors. Finally, Ca $^{2+}$ dyshomeostasis, which involves the entry of large quantities of Ca $^{2+}$ into glutamate-responsive neurons, triggers the delayed death of vulnerable populations of neurons, such as pyramidal neurons in the hippocampus—an area associated with learning and memory. Thereafter, the development of an infarct and subsequent cognitive dysfunction mark the pathogenesis of VD in elderly people. Approximately 30% of stroke patients show symptoms of dementia within 3 months of the initial stroke [53]. Furthermore, chelatable Zn reportedly moves from presynaptic terminals into postsynaptic neuronal cell bodies. An increase in intracellular Zn $^{2+}$ levels ([Zn $^{2+}$]_i), i.e., Zn translocation, occurs in vulnerable neurons in the CA1 or CA3 regions of the hippocampus prior to the onset of delayed neuronal death after transient global ischemia [50]. This Zn translocation has been reported to enhance the appearance of infarcts. Administration of Ca-ethylene diamine tetra acetic acid (Ca-EDTA), a membrane-impermeable chelator that chelates cations other than Ca, has been shown to block the translocation of Zn, protect hippocampal neurons after transient global ischemia, and reduce infarct volume [54]. Thus, Zn translocation has been recognized as the primary event in the pathway of Zn-induced neuronal death. Sensi et al. have observed temporal changes in [Zn $^{2+}$]_i in cultured cortical neurons, using a Zn-sensitive fluorescent dye; their results revealed at least 3 major routes for Zn $^{2+}$ entry: voltage-gated Ca $^{2+}$ channels (VGLG), NMDA-type glutamate receptors, and AMPA/kainate-type glutamate receptors (A/K-R). Although the NMDA-type glutamate receptors are present in most neurons, the permeability of AMPA/kainate channels for Zn $^{2+}$ and Ca $^{2+}$ is greater than that of NMDA-

type glutamate receptor channels [55]. Under normal conditions, most hippocampal neurons express AMPA receptors with GluR2 subunits, which are poorly permeable to divalent cations, including Ca^{2+} and Zn^{2+} (A/K-R). However, under ischemic conditions, there is an acute reduction in the expression of the GluR2 subunit, and neurons express specific types of AMPA receptors in which the channels are directly Ca^{2+} -permeable (Ca-AMPA/kainate channels; Ca-A/K-R) [56]. The appearance of Ca-A/K-R causes increased Ca^{2+} permeability and enhances cytotoxicity. Therefore, the expression of Zn^{2+} -permeable Ca-A/K-R and the entry of Ca^{2+} and/or Zn^{2+} through the channels are involved in delayed neuronal death after ischemia. Considering that Ca-EDTA, a Zn chelator, attenuates the ischemia-induced down regulation of the GluR2 gene [55], Zn has also been implicated in the transcriptional regulation of Ca-A/K-R-encoding genes. These results strongly implicate Zn as a key player in delayed neuronal death after transient global ischemia, a process that may be involved in the pathogenesis of VD [57,58]. The accumulation of Zn has also been observed following head trauma and epilepsy [59], implying that Zn neurotoxicity may underlie the pathological mechanisms of various injuries. Moreover, the disruption of Zn homeostasis has also been implicated in other neurodegenerative diseases, including AD [60–62], prion disease [63], amyotrophic lateral sclerosis [64], and Wilson’s disease [65]. Thus, Zn may play a role like that of Janus, the ancient Roman god of doorways who has 2 different faces, in the brain: both Zn depletion and excess Zn cause severe damage to neurons.

Carnosine inhibits zinc-induced neurodegeneration

Considering the implication of Zn in transient global ischemia, substances that protect against Zn-induced neuronal death could be potential candidates for the prevention or treatment of neurodegeneration following ischemia and could ultimately provide a clue to treatments for VD. We examined the potential inhibitory effects of various agricultural products, such as vegetable extracts, fruit extracts, and fish extracts, and found that extracts from eel muscles significantly protected against Zn-induced neurotoxicity [66]. Finally, we determined that carnosine, a small hydrophilic peptide abundant in eel muscles, protected GT1-7 cells from Zn-induced neurotoxicity in a dose-dependent manner (Figure 2). GT1-7 cells, which are immortalized hypothalamic neurons, are more vulnerable to Zn than other neuronal cells are [67,68].

Zn causes the apoptotic death of GT1-7 cells in a dose-dependent and time-dependent manner. The cells possess neuronal characteristics, such as the extension of neurites and the secretion of the expression of several neuron-specific proteins or receptors. Additionally, GT1-7 cells either lack, or possess low levels of, ionotropic glutamate receptors and do not exhibit glutamate toxicity [69]. These properties make the GT1-7 cell line an excellent model system for the investigation of Zn-induced neurotoxicity. We recently suggested that Ca dyshomeostasis may be involved in the mechanisms underlying Zn-induced neurotoxicity [51]. Zn-induced neuronal death revealed an upregulation of several genes, including metal-related genes (metallothionein (*MT*)-1, *MT*-2, and the Zn transporter 1 (*ZnT*-1)), ER stress-related genes (growth-arrest DNA damage (*GADD*)34, *GADD*45, and *p8*), and the Ca^{2+} -related gene *Arc* (activity-related cytoskeleton protein) [70]. These findings are significant, given the involvement of Ca^{2+} homeostasis in Zn-induced neurotoxicity. It is widely accepted that the ER Ca^{2+} *GADD34* *GADD45* *p8* *Arc* *Arc*

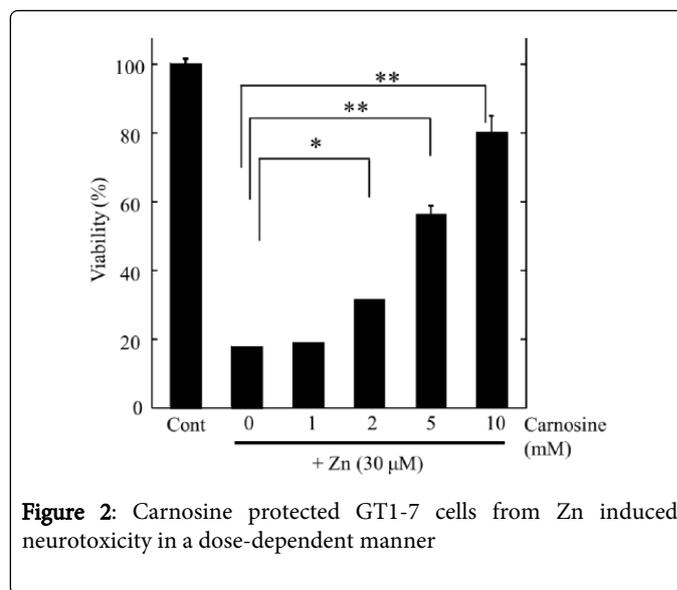


Figure 2: Carnosine protected GT1-7 cells from Zn induced neurotoxicity in a dose-dependent manner

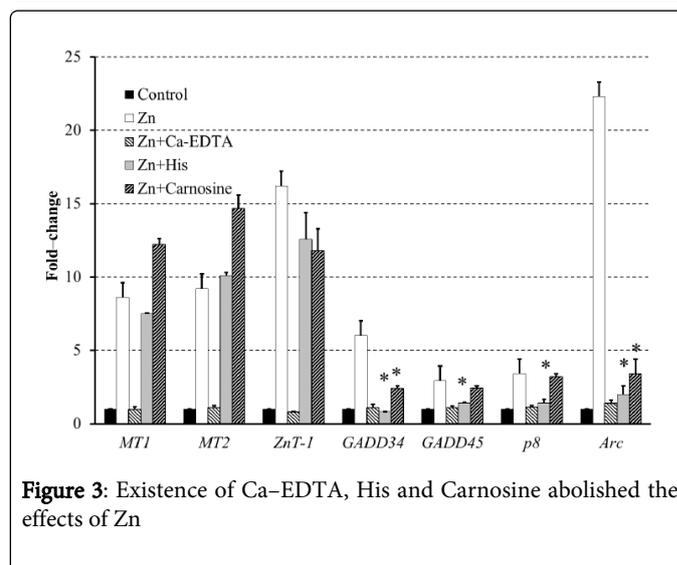


Figure 3: Existence of Ca-EDTA, His and Carnosine abolished the effects of Zn

MT1, MT2, or ZnT-1 *GADD34, GADD45, p8* *Arc* *Arc*

Conclusion

We have the relevance of carnosine as a possible therapeutic agent for VD. Carnosine, which is a naturally occurring dipeptide, is commonly present in vertebrate tissues, particularly within the skeletal muscles and nervous tissues [31]. It is found in high concentrations in the muscles of animals and fish with high levels of physical exertion, such as horses, chickens, and whales. Carnosine reportedly has various functions, including anti-oxidant, anti-glycation, and anti-crosslinking functions, and it is considered to be an endogenous neuroprotective and anti-aging substance. Considering the advantageous properties of carnosine (relatively nontoxic, heat-stable, and water-soluble), dietary supplementation of carnosine may be an effective strategy for the prevention or treatment of neurodegenerative diseases, such as ischemia, VD, AD, and prion diseases. Corona et al. have reported that the supplementation of carnosine improved the learning abilities of Alzheimer’s model mice [49]. We have demonstrated that the neurotoxicity of the prion protein fragment is

attenuated by Zn and carnosine [47]. Therefore, we have applied for patents for carnosine (application No.2006-145857; publication No. 2007-314467 in Japan) [77] and for His (application No.2008-098675; publication No. 2009-249335 in Japan) as drugs for the treatment of VD or for slowing the progress of cognitive decline after ischemia. Alimentary absorbed carnosine elevates the blood level of His via digestion in small intestinal cells [78].

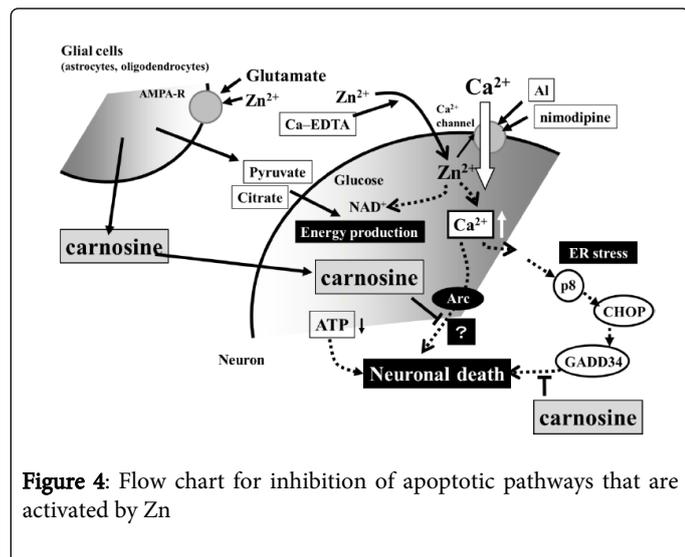


Figure 4: Flow chart for inhibition of apoptotic pathways that are activated by Zn

The elevation in circulating levels of His raises the concentration of carnosine in the skeletal muscle [79,80]. Therefore, dietary intake of carnosine may accumulate carnosine in the body including the brain, and may be effective for the prevention of VD. Szczeniak et al. reported that conducted carnosine and anserine supplementation in the elderly brings promising effects on cognitive functioning of participants [81]. Carnosine may also be useful for the drugs for the treatment of established VD. However, further research into the role of Zn in neuronal injury and the significance of Zn and Ca homeostasis are need to the development of new treatments for VD. In the brain, carnosine is also present in the olfactory bulb. Although the physiological roles of carnosine in the olfactory bulb are still unclear, olfactory bulb neurons are less sensitive to damage after ischemia compared to hippocampal neurons, despite the accumulation of Zn. Furthermore, carnosine levels have been shown to vary during development [3], and the content of carnosine in muscle is decreased in aged animals [82]. Therefore, carnosine may play protective roles in Zn-induced neuro-degeneration after ischemia in the olfactory bulb. It is plausible that carnosine may be transported into cell bodies, where it can inhibit several apoptotic pathways that are activated by Zn (Figure 4). In conclusion, we hope that our approaches about carnosine may benefit the development of drugs for the treatment of VD and other neurodegenerative diseases.

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Conflicts of interest

The authors declare no conflicts of interest.

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