Protective Effect of Carnosine on Subcortical Ischemic Vascular Dementia in Mice

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Keywords

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SUMMARY

Aims: Recently, we found carnosine protects against N-Methyl-D-Aspartate (NMDA) induced excitotoxicity through a histaminergic pathway. The aim of this study was to determine whether the carnosine-histidine-histamine pathway also played a protective role in subcortical ischemic vascular dementia (SIVD). **Methods:** Adult male mice (C57BL/6 strain) were subjected to right unilateral common carotid arteries occlusion (rUCCAO) and treated with carnosine or histidine. Object recognition test, passive avoidance task, Morris water maze, and immunohistochemical analyses were performed after rUCCAO. **Results:** We found that carnosine (200, 500 mg/kg) ameliorated white matter lesion and cognitive impairment evaluated by object recognition test, passive avoidance task, and Morris water maze test after rUCCAO in both wide-type mice and histidine decarboxylase knockout mice, which are lack of endogenous histamine. However, administration of histidine did not show the same effect. The myelin basic protein in the corpus callosum decreased obviously at day 37 after rUCCAO, which was largely reversed by carnosine (200, 500 mg/kg). Carnosine (200, 500 mg/kg) suppressed the activation of microglia and astrocyte as attenuating the elevation of glial fibrillary acidic protein (GFAP) and Iba-1 fluorescent intensity. Moreover, carnosine (200, 500 mg/kg) significantly attenuated the increase in reactive oxygen species generation after rUCCAO. **Conclusion:** These data suggest that the neuroprotective effect of carnosine on rUCCAO in mice is not dependent on the histaminergic pathway, but may be due to a suppression of reactive oxygen species generation, glia activation, and myelin degeneration.

Introduction

Vascular dementia (VaD) is recognized as the second most prevalent type of dementia. Subcortical ischemic vascular dementia (SIVD) caused by chronic hypoperfusion because of small-artery disease seems to be a common cause of VaD and its pathological changes are the development of ischemic white matter (WM) lesion, glia activation, and cognitive impairment [1,2]. The therapy of SIVD has received extensive attention [3–6]. It appears that there are several compounds with different mechanisms showing mild efficacy in SIVD patients [7,8]. However, so far no drug has been approved to potentially prevent the progress of SIVD [9,10].

Histamine is a neurotransmitter or neuromodulator in the central nervous system and shows regulative action in arousal, circadian and feeding rhythms, food intake, learning, and memory [11,12]. Accumulating experiments by histamine-deficient and histamine receptor-deficient mice suggest that histamine reinforces episodic memory and learned behaviors

[13,14]. In our previous studies, we found that hippocampal histamine plays an important role in the ameliorating effect on MK-801 or scopolamine-induced spatial memory deficits, and its action is mediated through postsynaptic H1 or H2 receptor [15–17]. Dere et al. [14] reported that the histidine decarboxylase knockout (HDC-KO) mice, which are deficient in histamine, show improved negatively reinforced performance in a water-maze and retention of contextual fear memory. Furthermore, histamine can also protect against cerebral ischemia induced by acute blockade of global or focal cerebral perfusion [18–20]. Histamine improves the delayed ischemic damage in hippocampal CA1 pyramidal cells induced by 3 min of transient forebrain ischemia [20]. And histidine, a precursor of histamine, given immediately and 6 h after reperfusion, also markedly alleviates the infarction after middle cerebral artery occlusion (MCAO) [18], while the H2 antagonists, cimetidine, and ranitidine aggravate the neuronal damage [19]. Some data suggest that H3 antagonists had potential use in SIVD [21]. So it is proposed that histamine might protect against SIVD.

However, the nonpenetration of histamine across the blood-brain barrier and its involvement in inflammation limit its direct application.

Carnosine (β -alanine-L-histidine) is a natural dipeptide that is highly expressed in the central nervous system and can easily enter the brain from the periphery. Carnosine can converse to histidine and then histamine and serves as a nonmast-cell reservoir for histamine [22,23]. It ameliorates acute renal failure induced by ischemia/reperfusion in rats and N-Methyl D-Aspartate (NMDA)-induced excitotoxic injury in differentiated PC12 cells through its conversion to histidine and histamine [24]. However, we recently found that the neuroprotective effect of carnosine on permanent MCAO in mice is not dependent on the histaminergic pathway [25]. Carnosine has also been assigned many other putative roles, such as anti-inflammatory agent, free radical scavenger, and mobile organic pH buffer. Meanwhile, carnosine is found to protect rats and mice against hypoxia or ischemia-induced brain damage by antioxidation and attenuation of apoptosis in transient cerebral ischemia [25–27]. So, carnosine is hypothesized to have therapeutic value in SIVD. In this study, we investigated the effect of carnosine on SIVD induced by permanent occlusion of the right unilateral common carotid arteries (rUCCAO) and whether its action involves the histaminergic pathway or other mechanisms.

Materials and Methods

Animal Preparation

All experiments using animals were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Eight-week-old wild-type (WT, C57BL/6 strain) and HDC-KO male mice weighing 22–30 g were used [28].

Experimental Design

After administered with sodium pentobarbital (60 mg/kg) for anesthesia, the right common carotid artery was isolated from the adjacent vagus nerve and double-ligated with 6-0 silk sutures to perform rUCCAO. Sham-operated mice were subjected to the same procedure, except for carotid ligation.

Carnosine and histidine (Sigma, St. Louis, MO, USA) were dissolved in sterile saline and administered by intraperitoneal injection. Adult male WT mice received rUCCAO and were administered with saline, carnosine (100, 200, or 500 mg/kg), or histidine (200 or 500 mg/kg), 30 min before surgery and every other day until the mice were killed (Figure S1). HDC-KO mice received rUCCAO and were administered with saline or carnosine 200 mg/kg at the same way. On day 27 after rUCCAO, the mice were subjected to object recognition test for pretest, and on day 28 for test procedure. On day 29, mice started 2-day passive avoidance task, and then on day 31 started 7-day Morris water maze test. During the behavioral evaluation, carnosine or histidine was administered after the tests every other day. On day 37 after rUCCAO, the mice were killed and brain tissues were removed for immunohistochemical staining, and neurotransmitter and reactive oxygen species (ROS) determination.

Object Recognition Test

An object recognition test was performed to evaluate nonspatial working memory related to frontal-subcortical circuits [29]. A glass box (30 × 45 × 30 cm) was used in the test. The objects to be discriminated were made of plastic and in three different shapes and colors: cubes (green), pyramids (blue), and cylinders (red) of 5.8 cm height. On the first day of the test, the mice were allowed to explore the box without any objects for 10 min. On the second day of the test, there were two trials, and the inter-trial interval was 1 h. In the first trial, two identical objects were presented on two opposite sides of the box, and the mouse was placed in the box and allowed to explore for 10 min. During the second trial, one of the objects presented in the first trial was replaced with a new object and the mice were allowed to explore for 3 min. Exploration was considered as directing the nose at a distance <1 cm from the object and/or touching it with the nose. The exploration time spent on each of the familiar (F) object and the new (N) object was recorded manually. Discrimination index was calculated by $(N - F/N + F) \times 100\%$ for intergroup comparison.

Passive Avoidance Task

Passive avoidance [30] typically began by placing the mouse in the lighted chamber for 10 s and then opened the door between the chambers. Most mice were highly exploratory and preferred the dark over the lighted chamber, so the mice would quickly enter the dark chamber. The door was closed, and a single footshock (0.3 mA, 2 s) was delivered through the grid floor. The mice remained in the dark chamber for an additional 10 s, a period designed to allow strengthening of the association between the properties of the chamber and the footshock. The mice were then returned to the home cages. Twenty-four hours later, the mice were placed in the lighted chamber, with the door open between chambers. Then, the latency for the mice to enter the dark chamber was recorded. Normal mice would be very slow to enter the dark chamber, often not enter at all, up to a 300 s cutoff latency, presumably because the mice remembered that a shock was delivered in the dark chamber the day before.

Morris Water Maze test

The Morris water maze [31] is currently the most frequently used paradigm to evaluate learning and memory abilities in transgenic and knockout mice. A circular pool, with a diameter 150 cm and a height 50 cm, was filled with water at a depth of 30 cm, so that the mice could neither escape over the edge of the tank nor balance its tail on the bottom of the tank. The hidden platform was submerged 1.5 cm below the surface of the water, and the mice were placed randomly between quadrants in the water. Our experimental design was four trials for the first day and six trials for the next 2 days for the acquisition phase, and the interval between the trials was 10 min. During each trial, the escape latency as the time to reach the platform and climb up out of the water was recorded, but limited to 60 s. And then, the average escape latency for each day was calculated. On the fourth day, each mouse was tested in a probe trial, by removing the platform

from the pool, and the time they stayed in the quadrant of the platform was recorded. Then, we changed the platform to the opposite quadrant. The mouse was trained for 2 days and tested on a probe trial again for the reversal phase.

Histochemical Staining of WM and Glia Cells

Mice were deeply anesthetized with sodium pentobarbital (60 mg/kg), and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brains were separated and stored in 4% paraformaldehyde at 4°C for 24 h, and then in 30% sucrose for 3 days. Frozen brain sections (10 µm thick) were made by a cryostat (SM2000R, LEICA, Wetzlar, Germany).

The severity of WM lesions was evaluated by the fiber density of Klüver–Barrera staining as in Wakita's report [32]. Individual brain sections were incubated with PBS containing 3% normal donkey serum, 0.3% Triton X-100 for 2 h, and then with the appropriate primary antibodies overnight as following: rabbit polyclonal anti-ionized calcium-binding adapter -1 (anti-Iba1; 1:1000; Osaka, Japan) to label microglia; mouse monoclonal anti-gial fibrillary acidic protein (anti-GFAP; 1:500; Sigma-Aldrich, St. Louis, MO, USA) to label astrocytes; and rat monoclonal anti-myelin basic protein (anti-MBP; 1:250; Millipore, Billerica, MA, USA). Then, the sections were washed in PBS and incubated with FITC-conjugated anti-rat IgG antibody for MBP; Cy3-conjugated anti-rabbit IgG antibody for Iba-1; Cy3-conjugated anti-mouse IgG antibody for GFAP (1:400) for 2 h at room temperature. Finally, the sections were observed under a fluorescence microscope (Olympus BX51; Olympus, Tokyo, Japan).

ROS Measurement

Mice were killed by decapitation, and the brain were removed and dissected on ice into three regions of interest: cortex and corpus callosum, striatum, and hippocampus. Each region was weighed and homogenized (50 mg tissue/ml buffer) in ice-cold Locke's buffer by homogenizer (Bertin Precellys 24, Montigny, France). Homogenates were diluted 1:10 in ice-cold Locke's buffer to obtain a concentration of 5 mg tissue/ml. Then, the homogenates were pipetted into 24-well plates (0.45 ml/well) and allowed to warm to room temperature for 5 min. At that time, 5 µl of DCFH-DA (10 µM final concentration) was added to each well and the plates were preincubated for 30 min at room temperature to allow the DCFH-DA to be incorporated into any membrane-bound vesicles and the diacetate group cleaved by esterases. The conversion of DCFH to the fluorescent product DCF was measured by the (FLUOSTAR OPTIMA, Ortenberg, Germany) with excitation at 485 nm and emission at 530 nm. Background fluorescence was corrected by the inclusion of parallel blanks. ROS production was quantified from a DCF standard curve and normalized by the protein concentration in homogenates, which was determined by BCA protein assay kit (Pierce, Rockford, IL, USA).

Statistical Analysis

Data are presented as mean ± standard error of the mean (SEM). All data from *in vitro* experiments represent three or more

independent experiments. Statistical analyses were performed with SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Escape latency in Morris water maze test was analyzed by two-way analysis of variance (ANOVA) for repeated measures followed by the LSD test. Other analyses used one-way ANOVA followed by the LSD or Dunnett's T3 *post hoc* test (where equal variances were not assumed) for multiple comparisons.

Results

Effect of carnosine on learning and memory impairment induced by the rUCCAO

In this experiment, the learning and memory ability were evaluated by object recognition test, passive avoidance task, and Morris water maze test. After rUCCAO, mice showed a significant decrease in discriminative ability in the object recognition test (discrimination index: -18% vs. sham group: 46%, $P < 0.01$, Figure 1A), while carnosine (100, 200, 500 mg/kg) significantly elevated the discrimination index ($P < 0.01$). In passive avoidance task, rUCCAO induced a significant reduction in escape latency, which was completely reversed by treatment of carnosine (500 mg/kg) (Figure 1B, $P < 0.05$). In Morris water maze training trials, rUCCAO-treated mice showed prolonged escape latency in acquisition phase and reversal phase, carnosine 200 mg/kg markedly shortened the escape latency both during acquisition and reversal phase, while the higher dose has an effect only during the reversal phase (Figure 1C). However, in probe trials of Morris water maze, rUCCAO did not induce change in escape latency either in acquisition phase or in reversal phase (data not shown).

Protective Effect of Carnosine on rUCCAO is Independent on the Histaminergic Pathway

Histidine decarboxylase (HDC) synthesizes histamine from histidine in mammals, and the HDC-KO mice show a histamine deficiency and lack histamine-synthesizing activity from histidine or carnosine. So, the effect of histidine in WT mice and carnosine in HDC-KO mice on learning and memory after rUCCAO was evaluated, to define the involvement of carnosine-histidine-histamine metabolic pathway in the protection against rUCCAO. Histidine (200, 500 mg/kg) neither improved the discrimination index after rUCCAO in object recognition test, nor changed the escape latency in passive avoidance task and Morris water maze (Figure 2). HDC-KO sham group showed a significant lower level of discrimination index in the object recognition test and longer escape latency in Morris water maze than the WT sham group. Although HDC-KO mice showed a slight but not significant reduction in discrimination index in the object recognition test after rUCCAO (Figure 3A), carnosine 200 mg/kg greatly reversed the reduction in discrimination index. Moreover, rUCCAO reduced escape latency in passive avoidance and prolonged it in acquisition phase in Morris water maze but only with significance on the third day, compared with the sham group in HDC-KO mice (Figure 3B,C). Carnosine (200 mg/kg) still significantly improved the learning and memory in passive avoidance task and Morris water maze in HDC-KO mice (Figure 3).

Figure 1 Effect of carnosine on learning and memory in object recognition test (A), passive avoidance task (B), and Morris water maze test (C) after right unilateral common carotid arteries occlusion (rUCCAO). $n = 8-14$. # $P < 0.05$, ## $P < 0.01$, versus the sham group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, versus the rUCCAO group.

Figure 2 Effect of histidine on learning and memory in object recognition test (A), passive avoidance task (B), and Morris water maze test (C) after right unilateral common carotid arteries occlusion in wild-type mice. $n = 6-12$. # $P < 0.05$, ## $P < 0.01$, versus the sham group.

The Protective Effect of Carnosine on WM Damage is Independent of the Histaminergic Pathway

WM damage in the corpus callosum was examined by Klüver-Barrera staining. At day 1 after rUCCAO, fiber density in the

corpus callosum increased and then consistently declined and was greatly lower than the sham group at day 37 after rUCCAO (Figure 4A). Carnosine dose-dependently delayed the progress of the decline of fiber density (Figure 4A,C). On day 37 after rUCCAO, carnosine 500 mg/kg markedly recovered the fiber density to the level in the sham group, while histidine (200, 500 mg/kg)

Figure 3 Effect of carnosine on learning and memory in object recognition test (A), passive avoidance task (B), and Morris water maze test (C) after right unilateral common carotid arteries occlusion (rUCCAO) in HDC-KO mice. $n = 7-12$. # $P < 0.05$, ## $P < 0.01$, ### $P < 0.001$, versus the sham group; & $P < 0.05$, && $P < 0.001$, versus the sham group in wild-type mice; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, versus the rUCCAO group in HDC-KO mice.

did not alleviate the WM rarefaction after rUCCAO (Figure 4B,C). In HDC-KO mice, administration of carnosine (200 mg/kg) obviously inhibited the WM rarefaction, although rUCCAO did not induce a significant reduction in the fiber density (Figure 4D,E).

Effects of Carnosine on Glia Activation and MBP After rUCCAO

In the corpus callosum, the mean fluorescent intensity of GFAP immunopositive astrocytes and Iba-1 immunopositive microglia greatly increased at day 37 after rUCCAO, indicating the activation of astrocyte and microglia (Figure 5A–C). Carnosine (200, 500 mg/kg) inhibited their activation as attenuating the elevation of GFAP and Iba-1 fluorescent intensities. MBP is a protein believed to be important in the process of myelination of nerves in the central nervous system. The MBP protein in the corpus callosum decreased obviously at day 37 after rUCCAO, which was largely reversed by carnosine (200, 500 mg/kg; Figure 5A,D). By toluidine blue staining, no obvious neuronal loss and apparent morphological change were observed in the cortex, striatum, and hippocampus after rUCCAO and carnosine treatment (data not shown).

Alleviation of ROS Injury by Carnosine After rUCCAO

The ROS production increased in the cortex and corpus callosum, and striatum but not in the hippocampus at day 1 after rUCCAO and increased only in the cortex and corpus callosum at day 37 after rUCCAO (Figure 6), which suggest ROS may be involved in WM damage. Carnosine (200, 500 mg/kg) significantly attenuated

the increase in ROS to the level of sham group in the cortex and corpus callosum, and striatum at day 1 (Figure 6A,B) and in the cortex and corpus callosum at day 37 (Figure 6D) after rUCCAO.

Discussion

In this study, we found that under chronic cerebral hypoperfusion induced by rUCCAO, carnosine ameliorated WM lesions, and cognitive impairment both in HDC-KO mice, which are lack of histamine, and WT mice almost to the same extent. Histidine did not show the same effect. It is likely that the neuroprotective effect of carnosine on rUCCAO in mice is not dependent on the histaminergic pathway.

In this study, we utilized a mouse model of chronic cerebral hypoperfusion induced by rUCCAO [33] to study the effect of carnosine on SIVD. A model of transient ligation of bilateral common carotid arteries (BCCAs) is often performed to rats to induce SIVD, but it cannot be applicable to transgenic or knockout mice because of the poor development of the circle of Willis in C57BL/6 strains [34]. Later, Shibata et al. developed a mouse model induced by the stenosis of the BCCAs using microcoils, which also shows WM rarefaction and delayed memory impairment, but the degree of stenosis is controlled by adjusting the internal diameter of the microcoils, which seems a little complicated [35,36]. Recently, Yoshizaki et al. [33] reported a novel rUCCAO model, which is more simple and convenient with low mortality rate and can induce impairment of WM and nonspatial memory in C57BL/6 mice. In the present study, we further found that after rUCCAO mice also showed a significant damage in learning and memory evaluated by passive avoidance task and in Morris water maze, which reflect the fear memory and spatial working memory,

Figure 4 Effect of carnosine on fiber density in corpus callosum is independent of the histaminergic pathway. The fiber density in corpus callosum in wild-type mice was analyzed by Klüver-Barrera staining after carnosine (A) and histidine (B) treatment in right unilateral common carotid arteries occlusion (rUCCAO) or sham groups. In histidine decarboxylase knockout (HDC-KO) mice, the fiber density in corpus callosum was also analyzed after carnosine treatment (E). The representative photomicrographs of fiber density in corpus callosum at day 37 after rUCCAO are shown in C and D. Scale bar, 50 μ m. $n = 8$. # $P < 0.05$, ## $P < 0.01$, versus the sham group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, versus the rUCCAO group.

respectively. The corpus callosum was associated with the behavioral effects pertaining to emotionality, spatial working memory, and motivated exploration [37]. Taken together, it is likely that chronic cerebral ischemia induced by rUCCAO impairs both the spatial and nonspatial working memory, as well as fear memory,

which better simulates the clinical cognitive impairment because of WM damage in SIVD [38]. In addition, we confirmed that the pathological lesions after rUCCAO share common features with those in SIVD involving the progressive WM rarefaction and glia activation [33]. Therefore, such novel model benefits the study of

Figure 5 Effect of carnosine on glia activation and myelin basic protein (MBP) at day 37 after right unilateral common carotid arteries occlusion (rUCCAO). Astrocyte and microglia activation in the corpus callosum was analyzed by immunohistochemical staining of glial fibrillary acidic protein (A, B) and Iba-1 (A, C), respectively. MBP immunohistochemical results are shown in (A, D). Scale bar, 50 μ m. $n = 8$. ### $P < 0.001$, versus the sham group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, versus the rUCCAO group.

Figure 6 Attenuation of reactive oxygen species (ROS) production by carnosine after right unilateral common carotid arteries occlusion (rUCCAO). At days 1 (A–C) and 37 (D–F) after rUCCAO, the ROS production in the cortex and corpus callosum (A, D), striatum (B, E), and hippocampus (C, F) was measured after carnosine (200, 500 mg/kg) treatment. $n = 6$. # $P < 0.05$, ## $P < 0.01$, versus the sham group; * $P < 0.05$, ** $P < 0.01$, versus the rUCCAO group.

the mechanism and therapy of SIVD. But at the early phase after rUCCAO, the fiber density in the corpus callosum unexpectedly increased. It may be due to the changes in the structure of myelin and the expression of myelin protein [39].

Carnosine significantly improved the learning and memory in the object recognition test, passive avoidance task, and Morris water maze and significantly delayed the progression of WM rarefaction after rUCCAO. These results suggest carnosine may have

potent clinical value to treat SIVD. It was proposed that carnosine may protect against rUCCAO through the histaminergic pathway, because carnosine serves as a nonmast-cell reservoir for histidine, utilized for the synthesis of histamine [12, 24–26]. However, surprisingly, we found that carnosine significantly attenuated the cognitive impairment and WM injury after rUCCAO in both HDC-KO and WT mice almost to the same extent, which indicates that carnosine has the same protective effect against chronic cerebral ischemic whether or not mice lack histamine. Meanwhile, histidine, which will be transformed to histamine, did not show the protective action in WT mice after rUCCAO, although histamine is considered to participate in normal learning and memory, indicated by the impaired learning and memory in object recognition test and Morris water maze in HDC-KO mice without rUCCAO. These data revealed that the protective action of carnosine on rUCCAO may not involve the carnosine-histidine-histamine metabolic pathway.

Previous data showed that histamine provides beneficial effects against acute ischemic damage possibly through alleviation of NMDA-induced excitotoxicity via H₂ receptors in neurons [24], and carnosine causes a time-dependent synthesis of histamine and exerts protection against NMDA-induced excitotoxic injury [40]. However, in our study, no obvious neuronal loss and apparent morphological change were observed in the cortex, striatum, and hippocampus after rUCCAO and carnosine treatment. Our chemical analysis also found that there was no significant difference in the glutamate and GABA level between the rUCCAO and sham group (Figure S2), although carnosine elevated the level of GABA in the striatum at day 1 and in the cortex and corpus callosum at day 37 after rUCCAO. Moreover, our data suggest that histamine may not be involved in SIVD, because there is no significant difference between WT and HDC-KO mice after rUCCAO, and histidine has no protection against WM lesion and the impairment of learning and memory after rUCCAO, but histamine is found to participate in acute cerebral ischemia from a lot of previous studies [18–20]. So, it seems that pathological mechanism of SIVD is so different from the acute cerebral ischemia which causes excitotoxicity because of excessive glutamate release and involves histamine. This discrepancy of pathological change may result in the different mechanism of a protection of carnosine against acute and chronic cerebral ischemia.

We also found that glia activation and myelin degeneration, indicated by MBP expression reduction, are the major changes in WM other than rarefaction. Although the exact cause of white matter damage after SIVD has not been conclusively established, white matter lesions have frequently been suggested to have the relationship with glia activation [35,41]. Activated

microglia and astrocyte are the major sources of proinflammatory cytokines, including TNF- α , IL-1 β , and IL-6, which may result in the degeneration of myelin and apoptosis of oligodendrocyte [42]. We found that carnosine (200, 500 mg/kg) markedly inhibited the activation of astrocyte and microglia as attenuating the elevation of GFAP and Iba-1 fluorescent intensities. The MBP protein in the corpus callosum decreased obviously at day 37 after rUCCAO, which was largely reversed by carnosine. Carnosine is reported significantly inhibit lipopolysaccharide-induced inflammatory mediators in cultured BV2 microglia [43]. So, the protection of carnosine on SIVD may be related to the inhibition of the glia activation and then myelin degeneration in the corpus callosum. Meanwhile, we found that carnosine significantly attenuated the increase in ROS to the level in the sham group at days 1 and 37 after rUCCAO. ROS often participates in the pathogenesis of WM injury [44–47], and it initiates lesion formation by inducing activation of astrocyte and microglia and myelin phagocytosis [48]. Carnosine is considered to be a free radical scavenger [49], and we previously found that it also improves mitochondrial function to reduce the ROS generation and recover glutamate transporter expression in astrocytes and then to ameliorate the neurological dysfunction after permanent focal cerebral ischemia [25]. Therefore, our results suggest that the protection of carnosine against WM damage after rUCCAO may be through inhibiting ROS generation to reduce glia activation and myelin degeneration, but not the carnosine-histidine-histamine metabolic pathway, although other action such as the direct promotion of myelin regeneration cannot be ruled out.

In conclusion, we found that carnosine had a protective effect in SIVD induced by rUCCAO, which did not involve the carnosine-histidine-histamine metabolic pathway, but may be due to an inhibition of ROS generation, glia activation, and myelin degeneration. These data suggest that carnosine may have potential value for the therapeutic treatment of SIVD.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

The following supplementary material is available for this article:

Figure S1. The schematic figure for the order of experiments.

Figure S2. Glutamate and GABA levels after rUCCAO and carnosine treatment.

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