Original Article

Early Biomarkers in 1H Nuclear Magnetic Resonance Spectroscopy of Striatal Pathological Mechanisms after Acute Carbon Monoxide Poisoning in Rats*

GUAN Li*, LI Zong Yang, ZHANG Yan Lin, CONG Cui Cui, and ZHAO Jin Yuan

Research Center of Occupational Medicine, Peking University Third Hospital, Beijing 100191, China

Abstract
Objective In vivo Proton Magnetic Resonance Spectroscopy (1H-MRS) can be used to evaluate the levels of specific neurochemical biomarkers of pathological mechanisms in the brain.

Methods We conducted T2-Weighted Magnetic Resonance Imaging (MRI) and 1H-MRS with a 3.0-Tesla animal MRI system to investigate the early microstructural and metabolic profiles in vivo in the striatum of rats following carbon monoxide (CO) poisoning.

Results Compared to baseline, we found significant cortical surface deformation, cerebral edema changes, which were indicated by the unclear gray/white matter border, and lateral ventricular volume changes in the brain. A significant reduction in the metabolite to total creatine (Cr) ratios of N-acetylaspartate (NAA) was observed as early as 1 h after the last CO administration, while the lactate (Lac) levels increased marginally. Both the Lac/Cr and NAA/Cr ratios leveled off at 6 h and showed no subsequent significant changes. In addition, compared to the control, the choline (Cho)/Cr ratio was slightly reduced in the early stages and significantly increased after 6 h. In addition, a pathological examination revealed mild cerebral edema on cessation of the insult and more severe cerebral injury after additional CO poisoning.

Conclusion The present study demonstrated that 1H-MRS of the brain identified early metabolic changes after CO poisoning. Notably, the relationship between the increased Cho/Cr ratio in the striatum and delayed neuropsychologic sequelae requires further research.

Key words: Carbon monoxide poisoning; Magnetic resonance spectroscopy; Delayed neuropsychologic sequelae; Choline


INTRODUCTION

Carbon monoxide (CO), which is a colorless, odorless, nonirritating, and tasteless gas that is ubiquitous in the atmosphere, is produced by both natural and anthropogenic sources. The health effects of acute CO poisoning have been extensively documented[1]. Endogenously produced CO is not associated with toxicity; CO toxicity occurs following exposure to exogenous carbon dioxide. The toxicity of CO results from its effects on cell metabolism through hypoxic and nonhypoxic modes of action, including inflammation, oxidative stress, mitochondrial dysfunction, excitotoxicity, edema, and hypoxia[2]. Both the hypoxic and nonhypoxic modes of action

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Correspondence should be addressed to GUAN Li, Tel: 86-10-82266268, E-mail: guanlisf@bjmu.edu.cn

Biographical note of the first author GUAN Li, female, associate professor, research direction is to study on the neural pathology of chemical poisoning.
are thought to result from the ability of CO to bind to heme and alter the function and/or metabolism of heme proteins. The formation of carboxyhemoglobin (COHb) results in a decrease in the O_2 carrying capacity of blood and impairs the release of O_2 from Hb for its utilization in tissues. Current toxicological and epidemiological research has revealed that the heart and cardiovascular system and the brain and developing nervous system are particularly sensitive to CO\textsuperscript{[3]}. The effects of severe CO poisoning may result in life-threatening complications, including cardiac arrhythmias, myocardial ischemia, cardiac arrest, hypotension, respiratory arrest, noncardiogenic pulmonary edema, seizures, and coma. In addition to the immediate-onset effects of exposure, the delayed-onset development of neuropsychiatric impairment typically occurs from several days to approximately 3-4 weeks after exposure. It manifests with a variety of symptoms, including inappropriate euphoria, impaired judgment, poor concentration, memory loss, cognitive and personality changes, psychosis, and Parkinsonism symptoms; the symptoms of acute CO poisoning in children are the same as those in adults\textsuperscript{[4]}.

Despite the major public health implications of CO-hypoxic brain injury, methods that prevent and treat the delayed neuropsychologic sequelae (DNS) that occur after CO poisoning and that limit brain damage and promote recovery, remain elusive. Imaging studies have suggested that the damage to the basal ganglia, pallidorecticular pathway, and prefrontal lobe contributes to the parkinsonian features and behavioral changes in patients with DNS\textsuperscript{[5]}. The failure of clinical prevention and treatment for DNS is due in part to the complexity of the CO poisoning pathology and to the absence of biomarkers that reflect specific pathophysiological mechanisms in individual patients\textsuperscript{[6]}.

Recent advances in magnetic resonance (MR) technology have resulted in the use of newer techniques of neuroimaging for research and clinical diagnostic imaging. In acute metabolic disease, MR spectroscopy (MRS) is helpful for characterizing the microstructural and metabolic responses of the disease\textsuperscript{[7]}. Mounting evidence has indicated that the specific neurochemicals measured with \textit{in vivo} proton-MRS (1H-MRS), which is a non-invasive analytical technique, may serve as biomarkers of injury mechanisms. This technique measures metabolite levels that reflect the degree of pathology in the brain. For example, several studies of traumatic brain injury (TBI) have found decreased levels of N-acetylaspartate (NAA), which suggests neuronal mitochondrial dysfunction and/or neurodegeneration, increased levels of lactate (Lac), which suggests hypoxia, and increased levels of choline (Cho) and myo-inositol (Ins), which implicate membrane breakdown and/or inflammation\textsuperscript{[8-9]}. Moreover, the alterations in certain neurochemicals that are measured with 1H-MRS have been shown to correlate with neurodegeneration\textsuperscript{[10-11]} and cognitive impairment in patients with Alzheimer's disease and Parkinson's disease\textsuperscript{[12-14]}. More recently, 1H-MRS studies of patients with DNS after CO poisoning have found altered brain neurochemistry, including increased levels of Cho and decreased levels of NAA ratios to creatine (Cr) predominantly in the white matter\textsuperscript{[15-17]}. These results suggest that MRS would be useful for the clinical management of patients after CO poisoning.

To date, 1H-MRS investigations of animal models of acute CO poisoning have been limited to examinations of brain chemicals. Nonetheless, animal studies provide an opportunity to link spectroscopic findings with specific pathological mechanisms. Therefore, our goal in the current study was to assess the evolution of a comprehensive profile of neurochemicals after CO poisoning and to determine whether 1H-MRS detects any changes in putative biomarkers of injury mechanisms in the predilection site of CO poisoning. We created a rat model of CO exposure and used high-field \textit{in vivo} 1H-MRS to examine the effects of acute CO poisoning and to evaluate whether CO exposure causes dynamic alterations in the striatum.

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley rats weighing 250-300 g obtained from the Animal Research Committee of Peking University were used in this study. All of the animals were maintained on a normal 12-h light cycle (lights on at 07:00 h; lights off at 19:00 h) with food and water available \textit{ad libitum} at a temperature of 25±2 °C). All of the experimental procedures involving animals were approved by the ethical animal committee of Peking University, Beijing, China. CO was purchased from Beijing Hengju Oilfield Chemical Agent Co., Ltd. (Beijing, China).
Protocol Design and CO Treatment

The animals were divided into two groups. For the control group, the animals (n=10) were treated with 40 mL/kg of air (intraperitoneal, i.p.) first and then 20 mL/kg of air (i.p.) 3 times at 4 h intervals. For the CO poisoning group, the animals (n=10) were treated with 40 mL/kg of CO (i.p.) first and then 20 mL/kg of CO 3 times at 3-4 h intervals, which increased the levels of carboxyhemoglobin (HbCO) in the plasma to above 50% for at least 12 h[18]. After the last CO administration and while they were under continuous anesthesia, the animals were transferred immediately to the adjacent magnetic resonance imaging (MRI) suite.

For the striatal histochemical staining, the animals were subjected to more CO poisoning. The rats were sacrificed at each defined time point (0-24 h), and the striatal tissue was collected and stored as before.

Concentration of Blood HbCO

Samples (100 μL) of blood collected from the auricular marginal vein were added to 20 mL of 0.4 mol/L ammonium hydroxide and then mixed with 20 mg of sodium dithionite. The absorbances at 535 nmol/L and 578 nmol/L were measured with a spectrophotometer within 10 min of mixture. The levels of HbCO were calculated as follows: HbCO (%) = (2.44×A538/A578×100%[19]). The changes in the HbCO levels in the blood were measured over the 24 h after the last injection.

In vivo MRI and 1H-MRS

MRI and 1H-MRS scans were performed within 2 weeks before the CO poisoning and 1 h, 3 h, 6 h, 12 h, and 24 h after CO administration. All of the MR assessments were performed with an Achieva 3T scanner (Philips Healthcare, Best, The Netherlands) that was equipped with a 12-cm-diameter gradient coil (40 G/cm, 250 ms) and interfaced with a Varian INOVA console. During the imaging, the anesthesia was maintained with 1.5% to 3% isoflurane in a 70/30 mixture of air/O₂ delivered through a nose cone in order to maintain a respiration rate of 40-80 cycles/min. The animals were placed on a heating pad in the scanning cradle, and their body temperature, monitored rectally, was maintained at 37 °C through feedback control (Cole-Parmer, Vernon Hills, IL, USA).

Coronal and sagittal gradient-echo multislice images were acquired in order to check the animal’s positioning in the magnet [repetition time (TR)=100 ms, echo time (TE)=2.8 ms, number of slices=10, slice thickness=2 mm]. Next, coronal and sagittal T2-weighted images were acquired with rapid acquisition and relaxation enhancement [TR=4,000 ms, TE=18 ms, echo train length=8; average=2; field of view=2.56x2.56 cm²; resolution=256x256 pixels; number of slices=10; slice thickness=2 mm] and used to position the 1H-MRS voxels. The 1H-MRS voxel was 10 mm×20 mm×10 mm in the striatum. The voxel positioning was based on anatomic landmarks, and care was taken to ensure reproducibility of the voxel position across all of the scans. 1H-MRS was performed with a water-suppressed stimulated echo acquisition mode sequence (TE=2 ms, TR=4000 ms). If repeated shimming attempts could not achieve a line width < 20 Hz, then the spectra were not collected. The spectra were analyzed with LCModel in the frequency domain[20]. LCModel uses a basic set of spectra acquired from in vitro samples of pure chemicals in order to estimate the in vivo levels of the neurochemicals, and the unsuppressed water signal from the prescribed voxel was used as a reference for each scan to correct for small variations in the coil sensitivity[21]. The peak values for the individual metabolites in the neurochemical profiles were based on validated reports[8,21].

Histological Analysis

Immediately after the MR imaging, the experimental rats were removed from the MR imaging unit and killed by anesthetic overdose. The animals were then decapitated, and their brains were removed and soaked in 10% formalin for 24 h. The striatal tissue was sectioned into sequential 2-mm-thick axial slices that corresponded to the MR images. The tissue was stained with hematoxylin and eosin. Five control piglets were also sacrificed immediately after the last MRI examination. Images of the striatum were captured and digitized with a microscope (Eclipse E-200; Nikon Corporation, Tokyo, Japan) coupled to a high-performance CCD camera and processed with Image-Pro Plus software, version 6.0 (Media Cybernetics, Inc., Rockville, MD, USA). The cerebral damage, including brain edema and cell death, was scored from 0 to 6 as described in Table 1. Mild, moderate, and severe damage were the terms used to depict the severity of the cerebral edema and cellular necrosis. The brain edema included cytotoxic and vasogenic edema. Cell death included necrosis in individual cells, groups of cells, and all of the cells within a certain area.
Statistical Analysis

The 1H-MRS data were fitted with the LCModel package (Provencher, 2001), and only the metabolites with standard deviations ≤20% were included in the subsequent analysis. Comparisons of the MRS parameters were performed for both of the hemispheres and at each time point with one-way repeated analysis of variance, which was followed by paired t-tests that were adjusted for multiple comparisons with a Bonferroni correction. P values less than 0.05 were considered statistically significant.

RESULTS

Blood HbCO Levels after CO Poisoning

In order to evaluate the effectiveness of the CO poisoning, we monitored the changes in the levels of HbCO in the plasma before the CO injection and during the first 12 h after the last CO injection. As shown in Figure 1, the HbCO levels rapidly increased during the first 15 min, reached a peak at 30 min, and then decreased 4-6 h later. The HbCO levels were maintained above 50% for 12 h. During this same time, we observed that the animals became agitated after the exposure. They were calmed down with forced breathing about 30 min later. Some of the animals lost consciousness and died.

Histopathologic Results

No histological abnormalities were observed in the control rats. After the last CO administration, astrocytes were mildly swollen at 1 h, and the perivascular space was slightly enlarged. In addition, neurons showed no significant anomalies. Three hours after the last CO administration, moderate astrocytic swelling and a more enlarged perivascular space were observed, while the neurons showed no obvious anomalies. Six hours after the last CO administration, the astrocytes were severely swollen, and the perivascular space showed marked enlargement. Many neurons were pale and swollen with lightly stained cytoplasms. Twelve hours after the last CO exposure, the astrocytic swelling and perivascular space enlargement were much more severe, and the plasma membranes of some astrocytes were disrupted. In addition, the neurons showed edematous swelling. No neuronal necrosis was seen. Twenty-four hours after the last CO administration, the astrocytes were extremely swollen. Many of the astrocytes were dead, and the plasma membranes were all inosculated into cribriform necrotic foci. The perivascular space was exceedingly enlarged. Neuropilar microvacuolation and microglia proliferation were seen. The histopathologic results are shown in Table 2 and Figure 2. The pathological scores differed significantly among all six of the experimental groups (P<0.01).

Lesion Characteristics on T2-Weighted MRI

T2-weighted MRI verified the tissue effects of CO poisoning and allowed us to follow the longitudinal development of the brain contusion in vivo (Figure 3). Tissue disruption is visible in Figure 3B-3D (1, 3, and 6 h after the last CO administration), and these alterations include cortical surface deformation, cerebral edema changes, which were indicated by the unclear gray/white matter border, and lateral ventricular volume changes. Twelve hours after the last CO administration, the tissue swelling had subsided, giving way to ventricular enlargement. After 24 h, the lateral ventricular boundaries were visible, and a somewhat clear border could be seen between the gray matter and white matter.
Table 2. Histopathologic Investigation of Effects of Carbon Monoxide (CO) Poisoning in the Striatum of the Control and Experimental Rats

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>After the Last CO Administration</th>
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<tr>
<td></td>
<td>1 h</td>
<td>3 h</td>
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<td>Pathological Score</td>
<td>0</td>
<td>1.6±0.8</td>
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Figure 2. Pathological examination of striatal tissue sections from CO-poisoned rats (original magnification, 400 X). (A) Striatum of a control rat. No histological abnormalities are seen, the neurons and astrocytes are normal, and the cell density is well preserved. (B) Striatum of rats 1 h after CO administration. All of the astrocytes are mildly swollen, and the perivascular space is slightly enlarged. The neurons exhibit no anomalies. (C) Striatum of rats 3 h after CO administration. Light microscopy revealed moderate astrocytic swelling and an increasingly enlarged perivascular space. The neurons exhibit no obvious anomalies. (D) Striatum of rats 6 h after CO administration. All of the astrocytes are severely swollen, and the perivascular space is markedly enlarged. The neurons with pale nuclei were swollen with lightly stained cytoplasm. (E) Striatum of rats 12 h after CO administration. The astrocytic swelling and perivascular space enlargement are much more severe. The cytoplasmic membranes of some astrocytes are disrupted, and neuronal necrosis is seen. At 12 h, the striatum shows massive vacuolation of the neuropil and extremely shrunken, scalloped, and pyknotic neurons. A cell with apparent apoptotic bodies is shown in the center. (F) Striatum of rats 24 h after CO administration. Light microscopy revealed extreme astrocytic swelling. In addition, many astrocytes are dead, and all of the cytoplasmic membranes inosculated into cribriform necrotic foci. Neuropilar microvacuolation, microglia proliferation, and severe neuronal necrosis are seen.

Figure 3. T2-weighted magnetic resonance imaging (MRI) of a rat brain. Representative coronal images (bregma, -0.5 mm) show the development of the cortical contusion from A to F (A, control, B-F, 1 h, 3 h, 6 h, 12 h, and 24 h, respectively, after the last CO administration). Soon after the last CO administration, tissue disruption, cortical surface deformation, cerebral edema (unclear border between gray matter and white matter, white arrows in B, C, and D), and lateral ventricular volume changes (black arrows in B, C, and D) are visible. The tissue swelling subsided with ventricular enlargement (black arrows in B, C, and D). A clear border is seen between the gray matter and white matter (white arrows in E and F).
The MRS Measurements of Neurochemical Changes in the Striatum of Rats after CO Poisoning

The coronal images, spectroscopic voxel locations in the striatal region, and the corresponding spectra from an animal are shown in Figure 4. In vivo 1H-MRS revealed alterations in various metabolites as a function of time after the acute administration of CO. Figure 4 shows representative serial changes in 1H-MRS in a volume of interest that was selected at the level of the striatum before and at the indicated time points after CO administration. The control spectra showed the usual characteristics of normal brain tissue, with three predominant resonances: one from NAA at 2.02 ppm, one from Cr at 3.04 ppm, and one from Cho-containing compounds at 3.20 ppm. No Lac resonance was observed in the control. The in vivo 1H spectra demonstrated excellent spectral resolution and sensitivity in control and CO-administered rats. One

Figure 4. Representative proton-magnetic resonance spectroscopy (1H-MRS) in the striatum (black square in A) at different time points before (control) and after the administration of CO. (A) Voxel location in the striatum. (B) In the series of cortical spectra, neurochemical changes were apparent as early as 1 h after the last CO administration. Four predominant resonances were observed: one from N-acetylaspartate (NAA) at 2.02 ppm, one from creatine (Cr) at 3.04 ppm, one from choline (Cho)-containing compounds at 3.20 ppm, and one lactate (Lac) doublet at 1.33 ppm. (C-E) The observations included increased Lac/Cr, decreased NAA/Cr, and first increased and then decreased Cho/Cr. The changes in the selected neurochemicals were measured with 1H-MRS in the striatum of rats after CO poisoning (control indicates before CO poisoning, *P<0.05 vs. before CO poisoning; **P<0.01 vs. before CO poisoning). The data are expressed as mean±standard error.
hour after the last CO injection, an increase was observed in Lac/Cr (a prominent Lac doublet was demonstrated at 1.33 ppm), and it became significant at 1 h \((P=0.0006)\). Compared with the control, the NAA/Cr ratio reduced from 1.813 at 1 h to 1.124 at 3 h \((P=0.004)\). Although the NAA/Cr ratio began to recover at 6 h, it was still lower than that of the control until 24 h after the last CO administration. The Lac/Cr ratios leveled off at 6 h (Figure 3) and showed no significant changes after that point. In addition, we found a slight reduction in the Cho/Cr ratio in the early stage after the last CO administration \((P>0.05)\). However, after 6 h, the Cho/Cr ratio began to increase, with significance \((P=0.02)\) from 12 h after the last CO administration compared to the control.

**DISCUSSION**

The majority of studies on the brain physiology and pathology of CO poisoning, including a purely morphological companion study, have involved rats, and these studies have found that the greatest CO-induced damage to the myelin occurred to the nodes of Ranvier after 7-10 days\(^{[22-25]}\). Grunnet has reported that the recovery of function was discontinuous: myelin repair was noted 14 and 21 days after the CO poisoning, and this was accompanied by a resumption of the maximal conduction velocity. However, some nodes remained abnormal after 60 days\(^{[24]}\). Recently, clinical research on the DNS after CO poisoning has shown significant abnormal changes in the Cho/Cr and NAA/Cr ratios in the subacute phase after CO intoxication; these changes represent early demyelination in the centrum semiovale and could predict delayed neurological symptoms\(^{[7,16]}\). However, whether the pathological demyelination and metabolic changes are seen in the early stage after CO poisoning remains unknown.

In the present study, the histological examinations performed during this phase revealed that the astrocytes were mildly swollen, and the perivascular space was slightly enlarged. A lack of oxygen impairs oxidative phosphorylation, which results in ion pump impairments and cytotoxic cerebral edema. Disturbances in cellular function and intracellular energy failures cause lactate accumulation and NAA and Cho decreases in the early stage after acute CO exposure. Both of these findings were consistent with the MRI findings. These results indicated that changes in T2-weighted images and intracerebral metabolites can be used to sensibly detect acute CO poisoning that is always negative on a conventional computed tomography scan.

Many human and experimental studies have shown the metabolic vulnerabilities related to hypoxic ischemic encephalopathy and CO poisoning\(^{[7,16]}\). The MRS results showed persistent biochemical alterations even though the MRI showed normalization of the morphological changes. However, these experiments were based on different times: several hours to days after CO exposure. Some clinical studies have found that patients with delayed encephalopathy from acute CO poisoning have abnormalities, usually decreased NAA/Cr and increased Cho/Cr in the white matter but also in the gray matter, that are visible on MRS scans\(^{[16,26-27]}\). However, the dynamic changes of these metabolic alterations in the early stage were still not clear. Therefore, in order to further examine the short-term and long-term effects of exogenous CO on the central nervous system, we assessed rats with CO poisoning with striatum MRS and MRI.

In the present study, 1H MRS, which covered all of the layers of the striatal structures, revealed that a considerably dynamic decrease in the NAA/Cr ratio during the first 12 h after CO administration, and the ratio recovered to the control level at 24 h. We found that the most severe drop in NAA in the striatum occurred 1-3 h after the exposure. Compared with the HbCO levels in the control, the NAA/Cr ratio was still obviously lower than the control group 12 and 24 h after CO administration. These results were consistent with the striatal pathological changes of astrocytic swelling and neuronal necrosis after acute CO poisoning. Schuhmann has reported that the most severe drop in NAA occurred 2-4 h after TBI. However, the alterations in NAA immediately after the TBI are not yet fully understood, which may be due to impaired NAA synthesis in the mitochondria\(^{[28-30]}\). The nervous system-specific metabolite NAA is synthesized from aspartate and acetyl-Coenzyme A by L-aspartate N-acetyltransferase in the mitochondria or through the cleaving of N-acetyl-aspartyl-glutamate by N-acetylated-a-linked-amino dipeptidase, along with glutamate\(^{[32]}\). Therefore, the synthesis and catabolism of NAA are related to the mitochondrial integrity and normal oxidation respiratory chain. An electron transport chain couples electron transfer between an electron donor, such as NADH, and an electron acceptor, such as \(O_2\), with the transfer of \(H^+\).
ions (protons) across the membrane. After CO poisoning, CO specifically inhibits cytochrome C oxidase in the mitochondrial respiratory chain. Because NAA is primarily located in neuronal tissue, the initial NAA reduction reflects dysfunctional neurons that suffer from energetic impairments after the CO poisoning. In addition to being a neuronal marker, recent reports have shown that NAA is present in the oligodendrocyte Type 2 progenitors and immature oligodendrocytes\[^{[33]}\]. Oligodendrocytes are an important component of the white matter, which is also vulnerable to ischemia. Thus, NAA might be a marker of astrogliosis, mitochondrial dysfunction, and neuronal loss after CO poisoning. However, the HbCO levels recovered to the control level 24 h after CO administration, and the mechanisms underlying the decrease in the NAA/Cr ratio might not be related to the direct toxicity of CO in the CNS after CO poisoning and subacute phase after CO intoxication.

The Lac resonances suggest a conversion of aerobic metabolism to anaerobic energy production; however, whether this is due to mitochondrial impairment secondary to hypoxia, or macrophage invasion, or a direct consequence of CO-induced neurotoxicity is not clear. Parsons et al. have found that, during the acute stage after stroke, Lac is a marker that predicts which tissue ultimately progresses to infarction\[^{[34]}\]. In the current study, the levels of Lac were significantly increased in the CO poisoning model, and they decreased rapidly at 12 h when the HbCO recovered after the last CO administration. In addition, the Lac levels progressively decreased because it might have been an alternative fuel, in addition to glucose, for oxidative phosphorylation to utilize. In addition to being used as a fuel, Lac may be washed out by perfusion. In this MRS study, the striatal volume of interest partially overlapped with regions that were hyperintense on T2-weighted images (Figure 3), which suggested temporary compromise of aerobic metabolism due to cytotoxic or vasogenic edema. A similar transient occurrence of Lac combined with hyperintense signals on diffusion-weighted MRI has also been found in pericontusional edematous brain regions after mild head injury\[^{[35]}\].

The current study found a significant increase in the levels of Cho/Cr in the striatum 6 h after the last CO administration. As a metabolic marker of myelin and cellular membrane density and integrity (i.e., phospholipid synthesis and degradation), the decrease in Cho in the later stages of CO poisoning is possibly a result of membrane degradation in the striatal area. A corresponding pathological study described only progressive astrogliosis without neuronal death.

Miyagishi et al. have conducted electron microscopy studies of experimental acute carbon monoxide poisoning and found that more marked and widespread pathological changes in the brain were seen in the white matter compared with the gray matter. The nerve cells with slightly dilated, and fragmented endoplasmic reticulums and Golgi complexes were infrequently found in the gray matter. However, most of the mitochondria did not show any kinds of abnormalities in those cells. In the white matter, the normal constituents of the myelin sheath were partially lost, and the myelin lamellae showed moderate exfoliation or a change into a homogenous or amorphous substance. The myelinated axons were partially contracted, and they exhibited an irregular shape in transverse sections. Enlargements of the occasional axonal mitochondria and the destruction of their internal parallel structures were observed\[^{[25]}\]. CO poisoning is well known to cause necrosis. In our study, the histological evidence of damage in the striatum was heterogeneous, and the histological injuries were most extensive 24 h after CO administration. Little is known about the mechanisms of neuronal damage induced by CO poisoning. Some studies have mentioned the occurrence of inflammation in association with demyelination of the CNS in the acute phase in CO-poisoned patients\[^{[36]}\]. In our study, the Cho/Cr ratio was significantly higher from 6 h after the last CO administration compared with the controls. These results suggested that early changes in membrane metabolism had already occurred after CO exposure. These results are consistent with the findings of electron microscopic studies on cerebral lesions of rats with experimental CO poisoning.

Recently, the metabolic changes of the delayed encephalopathy that were detected with MRS after acute CO poisoning can be interpreted as follows\[^{[15,37-40]}\]. An increased Cho signal reflects active membrane metabolism associated with pathological conditions, such as degenerative changes to the white matter and gliosis due to progressive demyelination. Beppu has suggested that the Cho/Cr ratio in the subacute phase after CO intoxication represents early demyelination in the centrum semiovale and can predict chronic neurological symptoms. In the chronic phase after
CO exposure, previous studies have reported increased Cho/Cr ratios in patients both with and without chronic neuropsychiatric symptoms. However, the slightly higher Cho/Cr ratio might represent reversible membrane metabolism that was not associated with demyelination-causing chronic symptoms, as the MBP levels were normal in patients for whom the acute neurological symptoms improved completely and who showed no neurological symptoms at 6 weeks\textsuperscript{[16]}. However, in the present study, we found a significant increase in the Cho/Cr levels in the early stage after acute CO poisoning in rats with MRS. These results suggested that it is too early to conclude that the Cho/Cr ratio in the later stage after CO poisoning can act as a predictor of the subsequent occurrence of DNS. It is very important to understand the changing processes of brain metabolism and pathological processes after CO poisoning in order to forecast DNS. In addition, the injury-induced alterations in the concentrations of the excitatory neurotransmitter glutamate and the inhibitory neurotransmitter GABA may indicate an imbalance in excitatory and inhibitory activity in the hippocampal region at a very early stage of TBI and ischemia and therefore may further contribute to the neurological dysfunction caused by TBI and ischemia. Xu suggested that the neurotransmission process is completed through the Glu-Gln cycle. The cycle begins with the release of Glu from presynaptic terminals to transport primarily to astrocytes, where it is converted to Gln via the Gln synthetase pathway\textsuperscript{[31,41-42]}. Further studies with the excitatory neurotransmitter and the inhibitory neurotransmitter may provide more insight into the disruption after CO poisoning.

This study demonstrated for the first time that the combination of information from 1H-MRS detected \textit{in vivo} changes in metabolism and microstructural changes in the early stage following CO poisoning in rat striatum. All previous reports on delayed encephalopathy with MRS were on patients with DNS. In this study, we observed the metabolic changes in the striatum after acute carbon monoxide poisoning for 24 h. These findings might provide noninvasive imaging evidence for early diagnosis of DNS. The present study demonstrated that MRS of the brain can sensitively identify early metabolic changes after CO poisoning. Notably, the relationship between increased Cho/Cr for the striatum and DNS might still need further research.

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