Serial Changes in Metabolism and Histology in the Cold-injury Trauma Rat Brain Model
—Proton Magnetic Resonance Imaging and Spectroscopy Study—

Kyousuke KAMADA, Kiyohiro HOUKIN, Kazutoshi HIDA, Yoshinobu IWASAKI, and Hiroshi ABE

Department of Neurosurgery, Hokkaido University School of Medicine, Sapporo

Abstract

The serial changes in metabolism and histology during the first 24 hours in the cold-injury trauma rat brain model were investigated by proton magnetic resonance (MR) imaging and high-resolution proton MR spectroscopy. Edema developed extensively via the corpus callosum in the ipsi- and contralateral hemispheres during observation as shown by gradually increased signal intensity on proton MR images. Proton MR spectroscopy showed increased levels of acetate (Ace), lactate (Lac), and glutamine (Glm) 1 hour after lesion formation. The elevated Glm level slightly decreased, the level of alanine (Ala) increased substantially, and that of N-acetyl-aspartate (NAA) decreased markedly after 24 hours. Increased Lac, Ace, and Ala might reflect anaerobic glycolysis associated with mitochondrial dysfunction, while decreased Glm and NAA reveal brain tissue breakdown. The relationship between brain edema and tissue viability can be analyzed in detail using this simple traumatic model and MR techniques which will be useful in the development of therapeutic agents for brain injury.

Key words: brain edema, cold injury, magnetic resonance imaging, magnetic resonance spectroscopy

Introduction

Severe brain injury causes a mortality of 20% to 50% despite improved diagnostic methods and aggressive therapy. Brain swelling (edema) is an important factor responsible for the dismal outcome of head injury. Many experimental studies of brain injury and edema have used the cold-injury model which can easily form reproducible lesions. This model has been used to investigate water content, concentrations of Na and K ions, pH, staining by horseradish peroxidase and Evans blue, and relaxation time in proton magnetic resonance (1H MR) imaging. However, the relationship between serial changes in the cold injury and the brain metabolism has rarely been studied.

High-resolution analytical MR spectroscopy has renewed interest in quantitative analysis of the complex mixture of compounds found in biological samples. In particular, the proton nucleus has significant advantages compared to other nuclei: highest sensitivity, 100% natural abundance, and general occurrence in metabolites. High-resolution 1H MR spectroscopy was used to investigate changes in brain metabolism after ischemic insult in perchloric acid tissue extracts, a method which can demonstrate numerous metabolites compared to in vivo MR spectroscopy. Ischemic brain utilizes anaerobic metabolism and the accompanying energy failure results in lactic acidosis and changes in the levels of various amino acids (increased lactate [Lac], alanine [Ala], acetate [Ace], and y-aminobutyric acid [GABA], and decreased glutamate [Glm]) which gradually recover after reperfusion.

This study investigated the serial changes in the metabolism of traumatic rat brain caused by cold injury using 1H MR imaging and 1H MR spectroscopy.
Materials and Methods

I. Cold-injury model
Thirty-three female Wistar rats were fasted overnight without physical restraint prior to the experiment. The mean body weight of the rats was 298 g. The rats were anesthetized with 60 mg/kg sodium pentobarbital by intraperitoneal injection. A polyethylene catheter was inserted into the femoral artery for blood pressure monitoring and blood sampling. Body temperatures were continuously recorded with a rectal thermometer and kept at 37°C using a warming blanket. A 5-mm hole was drilled through the skull and the dura mater was carefully exposed above the left hemisphere. The tip of a 4-mm diameter circular copper rod frozen in liquid nitrogen was placed on the dura for 30 seconds.

II. Histological examination
Control (n = 3) and experimental animals were killed 1 (n = 3) and 24 hours (n = 3) after lesion formation by heart perfusion with 200 ml of a 4% buffered formaldehyde solution. Coronal sections were cut through the center of the lesion and stained with hematoxylin and eosin.

III. 1H MR imaging
High-resolution brain MR images were obtained with a 300-MHz imaging spectrometer with a 183-mm horizontal bore magnet (Spectroscopy Imaging Systems, Sunny Vale, Cal., U.S.A.). Immediately after lesion formation, six experimental animals were transferred to the MR imaging unit with a homemade modified saddle-shaped coil 3.5 cm in diameter tuned to the 1H resonance frequency of 297.52 MHz. The imaging field of view was 3.5 cm and the slice thickness 2 mm. To achieve precise repositioning at the beginning of each imaging session, pilot sagittal and coronal T1-weighted images (repetition time [TR]/echo time [TE] 500/12 msec) were obtained. T2-weighted spin-echo images (TR/TE 3500/70 msec) were acquired 12 mm anterior to the medullospinal junction at 1, 3, 6, 12, and 24 hours after lesion formation.

The T2-weighted images were analyzed to assess gross morphological characteristics and signal intensity of the edema fluid using image-processing software. Four regions of interest (ROIs) (2 × 2 mm in size) were selected by a cursor to measure the signal intensity, and the mean intensity (MI) of the ROI was measured. An ROI in the same plane was defined within the right putamen for reference (REF MI) (Fig. 1). The normalized MI (MI_n) was calculated by MI_n = MI/REF MI.

IV. 1H MR spectroscopy
Samples of frozen brain were obtained 1 (n = 6) and 24 hours (n = 6) after lesion formation by Pontén’s method22) using liquid nitrogen. Briefly, the rats underwent tracheostomy and were mechanically ventilated. A plastic funnel with a bottom diameter of 15 mm was fitted to the skin incision and secured by pulling the skin up around it with sutures. This prevents liquid nitrogen from leaking onto the respiratory airways which must stay patent until the brain is frozen solid. After freezing, a part of the damaged hemisphere including the lesion was rapidly removed (Fig. 2). Hemispheres from the control rats without lesions were prepared in the same way (n = 6).

The removed hemispheres were stored in liquid nitrogen until homogenized at 2°C in 0.3 M perchloric acid (10:1 vol:wt) using a digital tissue homogenizer (Iuchi, Tokyo). The homogenate was centrifuged at 15,000 rpm at 2°C for 60 minutes and the supernatant was collected and lyophilized. The dried, powdered supernatant was dissolved in 0.5 ml D2O containing sodium 3-trimethylsilylpropionate (chemical shift reference). 1H nuclear magnetic resonance (NMR) spectra were obtained using a 400-MHz (8.0-T) Bruker AM-400 spectrometer (Karlsruhe, Germany) with a sample temperature of 21°C. For each sample, 300 free induction decays were accumulated into 32,768 (32K) data points using a spectral width of 6756 Hz with an interpulse decay of 4 seconds. A line broadening of 0.3 Hz was used prior to Fourier transformation to give the NMR spectrum.

The peaks in the perchloric acid extracts were assigned to the major metabolites of interests; Lac,
Ala, Ace, N-acetyl-aspartate (NAA), GABA, Glm, glutamine (Glm), aspartate (Asp), phosphocreatine (PCr), creatine (Cr), choline-containing compounds (Cho), taurine (Tau), and inositol (Ino), according to previous studies on perchloric acid extracts of rat brains.\textsuperscript{15,20} The cold-induced changes were evaluated by calculating the ratio of peak height of specific metabolites to the total Cr peak (TCr) which contains the Cr (3.03 ppm) and PCr (3.02 ppm) methyl resonances. TCr was used as an internal reference because the value is relatively stable under various conditions.\textsuperscript{15,20} Metabolite levels were compared between the control and experimental groups by analysis of variance and the Student's t-test.

Results

I. Histological examination

One hour after formation, the lesion was located in the posterior parietal area on the left and was saucer-like in shape. The whole lesion contained microhemorrhage, markedly dilated vessels, neuronal shrinkage, and pyknosis (Fig. 3 upper). Pallor and edema were evident in the surrounding 1 mm of tissue. Extravasated edema fluid had spread along the corpus callosum and dissolution of cortical cells had occurred. After 24 hours, the area of injury which was physically separated from the remainder

Neurol Med Chir (Tokyo) 35, January, 1995
the experiment.

III. Changes in imaging intensity
The serial changes in the signal intensity of the ROIs ($MI_n$) are given in Fig. 5. ROI 1 had the highest $MI_n$. Edema development shown by $^1$H MR imaging was associated with gradually increased $MI_n$ of ROIs 1–3 which reached the maximum at the end of the experiment (24 hrs). However, no changes in the $MI_n$ of ROI 4 occurred.

IV. Metabolic changes
Figure 6 shows a typical $^1$H MR spectra of brain extracts of control and experimental animals 1 and 24 hours postinjury with assigned peaks. The relative concentrations of several metabolites were changed after lesion formation. The levels of Ace, Lac, and Glmi were rapidly elevated shortly after lesion formation (Fig. 7). Changes in the metabolism progressed and became larger at 24 hours. The levels of Ace and Lac continued to increase, but the Glmi level decreased and became slightly lower than the control value. Additionally, the level of Ala rose substantially and that of NAA was markedly decreased.

Discussion
The regional brain water content is linearly related to the signal intensity or prolongation of $T_2$ proton relaxation time and therefore $^1$H MR imaging can be used for quantitative analysis of changes in brain water content in experimental studies of brain edema. Studies including ours have shown that $T_2$-weighted MR imaging can demonstrate the severity and serial changes in lesions and edema exactly consistent with those observed by histological examinations.

Indicators of brain metabolism, such as blood
Fig. 6 ¹H MR spectra of perchloric acid extracts of the hemisphere of control (upper) and experimental rats 1 (middle) and 24 hours (lower) after lesion formation. Peaks were assigned to important metabolites.

Neurol Med Chir (Tokyo) 35, January, 1995
flow and energy reserve levels, decrease in cases of cold injury and vasogenic edema. Our high-resolution $^1$H MR spectroscopy study showed that Lac, Ace, and Glmi of the metabolically most important amino acids were initially affected after lesion formation. Lac is presumably produced by anaerobic glycolysis due to impaired mitochondrial function, and therefore is an indicator of anaerobic glycolysis. The increased Ace level may be caused at least partially by degeneration of fatty acids after ischemic insult, and reflect the susceptibility of brain tissue. The elevation of Lac and Ace levels thus reflect the anaerobic glycolysis associated with deteriorating mitochondrial function due to ischemia. We also found that the Glmi content initially increased. The increased Glmi level subsequently decreased and became slightly lower than the control value. Petito et al. found that brain trauma causes a rapid, transient increase in Glmi synthetase level, but could not clearly explain the mechanism. The glial enzyme Glmi synthetase, which converts Glm to Glmi, is inhibited following ischemic insult, so suppression of this enzyme could result in reduced Glmi accumulation. Perry et al. reported that a decreased Glmi content apparently reflected deamidation of Glmi following postmortem changes. We suggest that the decreased Glmi level reflects focal ischemia and brain tissue breakdown due to cold injury.

The Ala level was elevated and the NAA level markedly decreased 24 hours after lesion formation. The postmortem and postschemia accumulation of Ala is due to the activation of glutamate-pyruvate transaminase and the build up of glycolytic end-products. Therefore, Ala is an indicator of anaerobic glycolysis like Lac and Ace.

NAA, which is found in high concentrations in mammalian central nervous systems, is relatively stable under hypoxic or ischemic conditions. NAA rapidly disappears when the neurotoxin kainic acid is injected into neurons, so is considered to be a neuronal marker. Our histological examination revealed that almost all neurons were initially preserved in the lesions. Subsequently, brain tissue breakdown appeared and the edema fluid extended widely around the lesions. The NAA level did not change at 1 hour after lesion formation, but had decreased significantly after 24 hours. These observations suggest the decrease in NAA level reflects brain tissue breakdown, which indicates "neuronal loss."

Our high-resolution $^1$H MR spectroscopy study has shown the occurrence of characteristic changes in the mitochondrial functions (Lac, Ace, and Ala) and brain tissue viability (Glmi and NAA) within and around brain lesions. Clinically, similar focal abnormalities are likely to occur in patients with head injury or following manipulation of the brain during surgery. This simple trauma model and these MR
techniques could help to find and assess a new therapeutic agent for brain injury.

References


27) Tengvar C: Extensive intraneural spread of horseradish peroxidase from a focus of vasogenic edema into remote areas of central nervous system. *Acta Neuropathol (Berl)* 71: 177-189, 1986


Address reprint requests to: K. Kamada, M.D., Department of Neurosurgery, Hokkaido University School of Medicine, North-15, West-7, Kita-ku, Sapporo 060, Japan.