Chronological Sequences and Blood-Brain Barrier Permeability Changes in Local Injury as Assessed by Nuclear Magnetic Resonance (NMR) Images from Sliced Rat Brain

REININ ASATO, M.D., HAJIME HANDA, M.D., TSUNEO HASHI, PH.D.*, JUN’ICHI HATTÀ†, MITSUTAKA KOMOIKE† AND TAKEHITO YAZAKI†

SUMMARY Two experiments were done with a prototype mini-NMR imager to evaluate the potential application of nuclear magnetic resonance (NMR) imaging in neuropathology. Cryo-injury-induced brain edema in brain slices from 22 adult male rats was imaged for observing the chronological sequences. Blood-brain barrier permeability changes were evaluated in 12 other brain slice images. EDTA-2Na-Mn solution was intravenously injected as an indicator of blood-brain barrier permeability. Contrast enhancement was achieved by changing the NMR imaging parameters. High resolution imaging permitted visualization of the corpus callosum, the thickness of which was only 0.2-0.4 mm. The extent of edema in gray matter was clearly shown with a striking contrast; no consistent findings were seen with slight differences in water content between edema and the surrounding normal cortex. As a result, the chronological sequences of brain edema were clearly observed. Mn-EDTA leaking from the circulating blood through the damaged capillary wall had a “paradoxical enhancement” effect on the NMR images; this effect might be suitable for evaluating blood-brain barrier permeability changes in NMR images.

Nuclear Magnetic Resonance (NMR) imaging is very sensitive to physico-chemical changes in tissue characteristics. Images obtained not only possess high spatial resolution but also show striking contrast between normal and pathological tissues. Although several imagers for human application are now available,1 and many clinical investigations have already been published,1-3 there are only a few reports on its application to experimental studies in animals.4-6 A new imaging technique was safely developed for animal experiments. It is of interest to evaluate the characteristics of NMR imaging and its potential application to neuropathology, based on an experimental model such as brain edema induced by cryo-injury.

From the Department of Neurosurgery, Kyoto University Medical School, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606, Japan, *the Department of Physics, Faculty of Science, Kyoto University, Kitashirakawa Oiwake-cho, Sakyo-ku, Kyoto 606, Japan, and †the Research Center, Sanyo Electric Company, 1 Hashiridani, Hirakata, Osaka 573, Japan.

Address correspondence to: Dr. Reinin Asato, Department of Neurosurgery, Kyoto University Medical School, 54 Shogoin Kawahara-cho, Sakyo-ku Kyoto 606, Japan.

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Materials and Methods

1. Animal Preparation

Thirty-four adult male Wistar rats (300-350 g) were used. Edema was produced as described by Klatzo.7 Animals were anesthetized with intraperitoneal pentobarbital and a midline scalp incision was made. The right side of the skull was exposed, and a 2 mm burr hole was made 2 mm posteriorly from the coronary suture and 3 mm laterally from the sagittal suture. The Klatzo procedure often causes macroscopic hemorrhages in rats as a result of direct cooling of the exposed pial brain surface. Thus, in our experiments, the 2 mm burr hole did not penetrate the very thin bone layer, to which a pre-cooled metal plate was applied. A standard lesion time of 30 sec was used. By this modified technique, we could produce a highly uniform local injury in rat brain without any hemorrhages; after the operation the scalp was completely closed. Eleven pairs of rats were sacrificed by intraperitoneal exsanguination at the following time intervals: 10 minutes; 2, 6, 12, 24, 48, 72 hours and 4, 5, 6, 7 days after injury. The brain was rapidly excised in a highly humidified box and a 2-mm coronal slice was made through the cryo-lesion. Then the brain slice was put during ischemia and after restoration of blood flow: Measurement of water, sodium, potassium content and plasma protein permeability. Stroke 10: 542-547, 1979


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into an acrylic cylindrical sample box (17 mm I.D., 3 mm depth), covered with a glass plate, and sealed. The slice was imaged within 60 minutes after exsanguination.

2. Enhancement of the Relaxation Rate with Manganese

Four pairs of edema induced rats were intravenously and intraperitoneally injected with (0.1–0.2 mM/100 g body weight) EDTA-2Na-Mn salt (Analytical grade; Nakarai Chemical LTD, Japan) solution 2 hours before sacrificing, at intervals of 2, 6, 24, and 48 hours after injury. Another 4 rats, injected at the same time as the first pairs, were also sacrificed without administering the manganese compound at the same time intervals as the previous pairs. Brain slices were obtained and imaged with two different NMR parameters.

3. NMR Imaging

A prototype mini-NMR imager was developed by the Research Center of Sanyo Electric Company and improved at the Department of Physics, Kyoto University. The main static magnetic field along the brain slice was produced by an iron-core resistive magnet (JEOL, Japan) operating at a field strength of 0.47 tesla (4700 gauss) with a proton resonance frequency of approximately 19.8 MHz. The brain slice was surrounded by a RF (radio frequency) coil, which was used for both excitation and detection of proton NMR. NMR signals were obtained by using the spin-echo technique with Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence (fig. 1). This method, using spin echoes, gives images dependent on both $T_1$, the spin-spin relaxation time, and $T_2$, the spin-lattice relaxation time, of the nuclei.

Protons in the static magnetic field $\vec{H}_0$ line up preferentially in the direction of the field, producing a net magnetization $\vec{M}_0$ in the $+Z$ direction. A short RF pulse $\vec{H}_1$ (the excitation pulse) is then applied at a time

$$t = 0$$

to rotate the net magnetization through 90° around $\vec{H}_0$ into the X-Y plane. The precessional motion of the magnetization in the X-Y plane generates a signal which is detected by the receiver coil. The transverse magnetization in the X-Y plane decays because of spin-spin relaxation and dephasing due to inhomogeneous broadening. After a time interval designated $\alpha$ (fig. 1) from the excitation pulse $\vec{H}_1$, a second RF pulse $\vec{H}_2$ (the observation pulse) is applied to rotate the magnetization through 180° around $\vec{H}_2$. The decay of the transverse magnetization due to the inhomogeneous broadening is recovered and the echo is formed at $t = 2\alpha$. The echo can be formed repeatedly by applying a series of the observation pulses (fig. 1), but the echo amplitude decays with time constant $T_2$. In this study the observation pulse was repeated eight times with the same interpulse spacing $2\alpha$, and eight similar echoes were obtained in one pulse sequence. The magnetization along $\vec{H}_0$ becomes zero just after the 90° pulse. However, it returns exponentially to its original value with a time constant $T_1$ (fig. 2). Therefore the signal intensity changes with repetition of the pulse sequence designated $b$ (fig. 1). This fact can be used to examine the effect of $T_1$, $T_2$ is one-tenth to one-twentieth times shorter than $T_1$ in mammalian tissues.

Spatial labelling of the protons in the brain slice being examined was achieved by applying a carefully controlled gradient magnetic field in the X, Z axis. Slice selection in the Y direction has not been achieved yet. Echo signals, accumulated for twelve CPMG sequences, provided the data for one projection of the slice image. For successive projections, the gradient axis in the ZX plane was rotated 4° at a time through a total of 180°. Image reconstruction using Fourier transformation from the frequency to the spatial domain.
followed by filtered back projection was analogous to that used in early translation-rotation CT scanning systems. Routine images were produced with an interpulse spacing $2a$ ($a = 90°-180°$ interpulse interval) of 18 msec and an interval $b$ ($90°-90°$ interpulse interval) of the CPMG sequence of 1.6 sec. The time taken to complete the 45 projections for each slice was 15 min. For contrast enhancement, images were produced with the $T_1$ parameter $b$ of 0.3 sec or 0.6 sec, and with the $T_2$ parameter $a$ of 9 msec; the time to complete 45 projections was 3–5 min. The resulting picture was displayed on a $170 \times 170$ matrix with a 16 variety color scale. The highest NMR signal intensities appeared white as shown in figure 3. Pixel dimensions were less than 0.2 mm$^2$.

**Results**

1. **Chronological Sequences of Edema**

The images reflect the distribution of hydrogen nuclei, thus characterizing the tissue state of hydration in the slice. Increases in water concentration correspond to changes in picture color from blue to white. Thus the edematous region, in which water concentration is high, is indicated by a white zone (fig. 3). Round swelling (about 2–3 mm in diameter) could be seen in the cortical surface, cooled through the thin bone layer, 10 min after injury (in the two rats sacrificed at this time); in cross section, the zone of edema extended into the superficial cortical layer. The NMR images at this stage clearly showed the edematous changes, and the white zone, indicating strong signal intensity, appeared to be slightly larger than the original area of cold application (fig. 3). The white zone gradually extended into the underlying cortex, reaching the corpus callosum after 12 h, but there was only slight lateral extension (fig. 4). After 24 to 48 h, the widening of the corpus callosum was pronounced and the edematous region extended into it laterally, and also into the underlying hippocampal region in some cases but edema did not extend to the basal ganglia. These changes were clearer in the images of Mn-EDTA injected rats. On the other hand, the white zone in the cortex was less pronounced and was slightly reduced in extent at this stage, while its intensity was unchanged (fig. 4). After 3 days, the edematous region in the corpus callosum was not seen in the NMR images. With time the white zone in the cortex was gradually reduced in extent, disappearing on the 7th day. In the images with higher window levels, the concentric intensity gradient was clearly shown within the white zone (fig. 5). Although the extent of the white zone varied according to the interval after injury, the relative signal intensity of the central part, which corresponded to the region immediately beneath the original area of cold application, (compared to that of the con-
tralateral normal cortex), changed only slightly throughout the course.

2. Relaxation Rate Enhancement with Manganese

In general, the shortening of the $T_1$ parameter $b$ caused a decrease in signal intensity, which was more prominent in the edematous region than in the contralateral cortex when the animals were not injected with Mn-EDTA solution (fig. 6).

In 8 animals injected with the solution, relative signal intensity in the edematous region was markedly decreased, and in three cases, the signal intensity in this region was lower than in the contralateral normal cortex. But, when parameter $b$ was changed to 0.3 or 0.6 sec, the relative signal intensity in the edematous region was so enhanced that the extent of edema could be clearly delineated (figs. 7, 8).

Discussion

1. Characteristics of NMR Imaging

Proton-nuclear magnetic resonance imaging (‘H – NMR · CT) is a noninvasive technique for obtaining cross-sectional images of biological objects. The images obtained depend primarily on proton density, and they can reflect water concentration in tissue. The principle of the NMR imaging technique is that hydrogen nuclei in the static magnetic field absorb and transmit the known RF waves; the frequency of which are determined by the strength of the magnetic field. Thus, the spatial domain of hydrogen nuclei can be transformed into the frequency domain by applying a field gradient in which the field strength changes in a linear fashion according to the position of the hydrogen nuclei in the field. The intensity of NMR signals depends not only on the density but also the spin-lattice relaxation time ($T_1$), the spin-spin relaxation time ($T_2$), and the movement of nuclei. In this study, the Carr-Purcell-Meiboom-Gill pulse sequence was used in the spin-echo experiments. The pulse sequence was sequentially repeated 12 times for one projection, and consequently, 96 similar echoes were obtained. As previously described, the signal intensity of the first echo is defined as:

$$I = A \cdot f(v) \cdot [1 - \exp (-b/T_1)] \cdot \exp (-2a/T_2), \ [1]$$

where $I$ is the NMR intensity in a particular region of the image, $A$ is the constant determined by the local hydrogen density, $a = 90^\circ-180^\circ$ interpulse interval, $b = 90^\circ-90^\circ$ interpulse interval, respectively, of the experiment, and $f(v)$ is a function of both the speed with which hydrogen nuclei move through the region being imaged and of the fraction of the total number of nuclei that are moving. As brain slices were imaged in our study, $f(v)$ was negligible.

Equation [1] shows that, when parameters $a$ and $b$ remain unchanged, the signal intensity becomes higher for shorter values of $T_1$ and for longer values of $T_2$. The echo envelope represents exponential decay with time constant $T_2$. Upon summation of the echoes, $T_2$ dependence of the signal is found to be not exactly the same as that shown by Eq. [1]. However the signal intensity also becomes high when $T_1$ values become longer. The longitudinal magnetization recovery curve in fig. 2 represents the recovery process of a net magnetization $M_0$ to its thermal equilibrium after a $90^\circ$ pulse. If parameter $b$ is not long enough for the once excited magnetization to recover completely its equilibrium state, intensity of the signal obtained by the following pulse sequence might become lower than that obtained by the preceding one. This results in a decrease in the signal intensity identical to the effects of a decrease in hydrogen density. In routine programs, interval $b$ between two pulse sequences was set 60–70% shorter than the optimal value. Consequently, the signal intensity of the second pulse sequence becomes weaker than that of the first pulse sequence, and finally has a steady state value determined by parameter $b$. This effect appears more prominently in nuclear spins of longer $T_1$ relaxation time.

It might be emphasized that signal intensity depends not only on the density but also on the relaxation times ($T_1$, $T_2$) of hydrogen nuclei. In pathological tissues,
changes in the local molecular environment of the hydrogen nuclei influence their physical properties and result in a change of both T₁ and T₂. It is probable that the effect of these relaxation times on NMR images enables specific identification of pathological tissue. On the other hand, X-ray computed tomography (CT) produces images which reflect tissue specific gravity and it cannot indicate chemical changes resulting from pathological processes. Furthermore, contrast enhancement of NMR images can be easily achieved by changing the NMR imaging parameters, and thus enhancing the differences in signal intensities between normal and pathological tissues based on their different relaxation times (fig. 7). The opposite effect ("reversed enhancement") may be produced (fig. 6).

2. Chronological Sequences in Brain Edema

By carefully controlling the linearity and stability of the gradient magnetic field, highly stable echoes were obtained which yielded excellent images of brain edema in rat, although the pixel size was a mere 1/700 of that obtained by prototype human imaging devices. With high resolution, the corpus callosum of 0.2–0.4 mm thickness could be delineated in the images. Inherent soft-tissue contrast was adequate enough to differentiate gray and white matter, the difference in water content of which was only 7%. Although water content of gray matter increases only slightly in edema, the extent of the edematous lesion was imaged in high contrast compared to the normal cortex. It is the small increase in hydrogen density and H₂O physico-chemical changes that are reflected in prolongations of relaxation times and thus in increases in image intensity. Therefore, the intensity gradient inside a small region can be easily evaluated in NMR images (fig. 5).

Through chronological sequences, the relative signal intensity in the central area of edema, compared to the contralateral normal cortex, changed only slightly, though the extent of the lesion clearly depended on the time after injury. This might indicate that the edematous area concentrically extended from the central locus, which was just under the cooled superficial cortical layer, and contracted toward this central locus at later times in its evolution. This finding supports the hypothesis that edema fluid can extravasate only from capillaries in cryo-lesions and it diffuses peripherally according to the tissue pressure gradient. In cryoinjury-induced edema of cats, marked changes in white matter were reported, with a slight increase in water content of the gray matter. From these results, some authors have speculated that edema in rats reaches a peak 48–72 h after injury. But precise measurements of water content in a small volume such as rat brain is very difficult, especially when attempting to evaluate separately this content in individual internal compartments. In NMR images, edema in gray matter was most prominent 12 h after injury, and it was already reduced after 24 h. As the white matter in rats is not developed, these changes in gray matter appear to represent the main features of edema produced by local cryo-lesion, thus indicating that edema reaches a peak after 12 h in our model. This speculation agrees well with the histological studies by Klatzo. In his results, on the other hand, white matter showed striking histological changes and an increase in edema fluid 24 to 48 h after injury. These changes gradually recovered thereafter, but swelling of astrocytes still continued after 72 h. On the contrary, although edematous changes in the corpus callosum were prominent in NMR images after 24 to 48 h, they disappeared after 72 h. This discrepancy between our results and Klatzo’s may be due to the limitation of spatial resolution. Further histological evaluations are now being undertaken.

3. Evaluation of Blood-Brain Barrier Permeability Changes

Blood-brain barrier permeability changes are common features in a variety of pathological processes in the brain, and it is important to evaluate them in neuro-

![Figure 7. Contrast enhancement with NMR parameters. Changing parameter b from 1.6 to 0.6 sec caused the strong enhancement of edematous tissue. The rat was injected intravenously with EDTA-2Na-Mn solution 2 h before sacrificing.](http://stroke.ahajournals.org/)

![Figure 8. Paradoxical enhancement with EDTA-2Na-Mn. Both images were obtained from the same slice 2 h after injury. EDTA-2Na-Mn solution was injected before sacrificing. Image with b = 1.6 sec shows no region of high signal intensity (Left). On the other hand, image with b = 0.3 sec visualized approximately the same extent of lesion as in figure 4 (Right).](http://stroke.ahajournals.org/)
surgical patients. In X ray CT there exists a possible linear relationship between the plasma concentration of the contrast material and the attenuation coefficient of plasma. Thus, permeability changes can be evaluated by “contrast enhancement” due to extravasation of the contrast material. However, because of inconsistencies in enhancement with fixed doses of the contrast material and also because of the effect of the expanded vascular bed on enhancement, contrast enhancement in X ray CT images can not clearly evaluate brain edema. The extent of extravasated $^{82}\text{Rb}$ is apparently visualized in $^{82}\text{Rb}$-PET, but PET cannot produce brain images of suitable spatial resolution for adequate anatomical evaluation.

Paramagnetic ions, such as $\text{Mn}^{++} \text{Co}^{++}$, show remarkable relaxation rate enhancement on water protons in their solution. Although many water-soluble salts of $\text{Mn}^{++}$ have strong acidity in solutions and cannot be injected into veins, the solution of Mn-EDTA is neutral and it rapidly disappears from the circulating blood, finally being excreted through the kidney and liver (Asato R, Ueda T: unpublished data). We have evaluated the permeability changes in the blood-brain barrier based on the relaxation rate enhancement effect of EDTA-2Na-Mn salt in conjunction with contrast enhancement by NMR parameters.

In images of untreated rats brain, the changing of $T_1$ parameter from 1.6 sec to 0.6 sec resulted in a marked decrease in contrast between edematous and normal cortex (“reversed enhancement”) (fig. 6). In edematous tissues, both $T_1$ and $T_2$ relaxation times were prolonged. $T_2$ prolongation increased the signal intensity, and $T_1$ prolongation weakened it. It appears that the effect of $T_1$ prolongation was greater than that of $T_2$, prolongation with NMR parameters $a = 9$ msec, and $b = 1.6$ sec, which resulted in strong enhancement of the edematous tissue. By contrast, with NMR parameters $a = 9$ msec, and $b = 0.6$ sec, the effect of $T_1$ prolongation on the decrease in the signal intensity was greater than that of $T_2$ prolongation, thus the “reversed enhancement” effect could be seen. In the rats administered Mn-EDTA, both $T_1$ and $T_2$ relaxation times were thought to be shortened, and the image contrast between edematous and normal cortex was decreased because of the greater effect of the shortened $T_1$ on signal intensity with NMR parameters $a = 9$ msec, and $b = 1.6$ sec (“reversed enhancement”) (fig. 7). In the most prominent cases edema was not visualized (fig. 8). On the other hand, with parameter $b = 0.6$ sec, the effect of shortened $T_1$ appeared to be greater than $T_2$, clearly visualizing the extent of edema (fig. 8). Such effects were not shown in the non-treated rats. These particular effects of Mn-EDTA are designated “paradoxical enhancement”, which means the reversed effect of $\text{Mn}^{++}$ on both contrast enhancement and “reversed enhancement” with NMR parameters. Thus it may be concluded that “paradoxical enhancement” with Mn-EDTA represents the blood-brain barrier permeability changes in NMR images. In this study, “paradoxical enhancement” was still observed in images 24 h after injury but this enhancement was not consistently seen 48 h after injury. Blood-brain barrier is a functional concept concerning the ability of brain capillaries to impede extravasation of plasma constituents to some extent, dependent on each material. By using EDTA-2Na-Mn (mol. wt. 429.16) in NMR imaging, we could evaluate the function of this barrier without using electrolytes or macromolecules in situ.

Conclusions

$^1\text{H}$-NMR images of cryo-injury-induced edema from sliced rat brain clearly showed chronological evolution. Edematous changes in cortex were most extensive 12 h after injury and thereafter gradually decreased in extent. The images 24–48 h after injury showed a widening and a striking increase in signal intensity of the corpus callosum, which was not observed in the images after 72 h. Edematous changes did not extend to the basal ganglia. Contrast enhancement was achieved by changing the NMR parameters. Finally, blood-brain barrier permeability changes were estimated by the “paradoxical enhancement” effect of EDTA-2Na-Mn.

References

1. Abstracts of the First Annual Meeting of the Society of Magnetic Resonance in Medicine, Boston, Massachusetts, August 16–18, 1982
Treatment of Ischaemic Stroke with Prostacyclin


SUMMARY Ten patients with ischaemic stroke were treated with prostacyclin (2.5-5.0 ng/kg/min i.v. in 6 h courses 4-10 times during 1-2.5 days). In all patients a dramatic regression of hemiplegia, or hemiparesis, or aphasia occurred in the first few hours of prostacyclin infusion. Four to eight weeks later 6 patients left the clinic without neurological deficit; 3 patients had minor residual hemiparesis in upper limbs. In one patient, the occlusion of the contralateral carotid artery led to his death. It is considered that an antagonism may exist between endogenous cerebral prostanoids and prostacyclin and may have been responsible for the beneficial effects of prostacyclin therapy.

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THE BRAIN LEVEL OF PROSTANOIDS in vivo is rather low, however, cerebral ischaemia leads to a rapid accumulation of free arachidonic acid, prostanooids and lipid peroxides in ischaemic regions. Products of arachidonic acid cyclooxygenation (prostaglandins and thromboxane A2) and lipoxygenation (lipid peroxides and leukotriens) are believed to play a major role in cerebral vasoconstriction, brain oedema and neurotoxic phenomena which are characteristic of the postischaemic reperfusion period.

Among dienoic prostanoids prostacyclin (PGI2) is the only one that relaxes cerebral arteries of humans, baboons and dogs whereas prostaglandins A2, B2, D2, E2, F2a and thromboxane A2 (TXA2) contract cerebral arteries or have little effect on their tone.

The physiological role of prostanoids in the regulation of cerebral blood flow (CBF) is debatable. However in ischaemic canine brain and in infarcted rat brain prostanooids are released and are responsible for cerebral vasoconstriction, brain oedema and neuronal hypermetabolism. Those phenomena are abolished by the treatment of cerebral ischaemia with PGI2 with cyclooxygenase inhibitors or with thromboxane synthetase inhibitors.

In summary, cerebral ischaemia is associated with a local release of free arachidonic acid and its lipoxydation products. Lipid peroxides cause a further damage to cell membranes and inhibit PGI2 synthetase. Thereby a subsequent cyclooxygenation of arachidonic acid yields only vasoconstrictor prostanooids which contribute to postischaemic hypoperfusion. Brain oedema and neuronal hypermetabolism. Prostacyclin deficiency also promotes formation of platelet microthrombi in the cerebral circulation and it facilitates the release of vasoconstrictory TXA2, PGF2a and 5-HT from aggregating platelets. Here, we report on the administration of prostacyclin therapy to ten patients with ischaemic stroke.

Patients and Methods

Ten patients with ischaemic stroke (tables 1 and 2) were treated with prostacyclin (PGI2) not earlier than 1 day and not later than 5 days after hospital admission. Consent for this clinical trial was signed by the closest family member or by the patients themselves when this was possible. Patients with transient ischaemic attacks (TIA), cerebral or subarachnoid haemorrhages were excluded by neurological examination, by cerebrospinal fluid (CSF) analysis and by angiography.

Unilateral cerebral angiography, by direct carotid artery puncture was performed on all patients, of whom eight had partial or complete occlusion of the internal carotid artery or of a major cerebral artery and two patients showed diffuse atherosclerosis of the internal carotid and intracranial arteries (table 2). In all patients, diffuse or focal pathological discharges from the affected hemisphere were recorded by EEG examination. In one patient focal temporal and parietal discharges were associated with unilateral epileptic-like twitches.
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