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Dissociation of brain edema induced by cold injury in rat model: MR imaging and perfusion studies with ^{14}C-iodo-antipyrine

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The purpose of this study is to confirm whether T2-weighted imaging and perfusion imaging, i.e. autoradiogram of ^{14}C-iodoantipyrine, on the course of brain edema correspond to each other or not. Cold injured rat brains were used as a model and were sequentially examined by both methods and compared with each other and with histological specimens. Special focus relies on the time changes in the lesions. High SI of T2-weighted images were observed and the percentages in the high SI area to the total brain area in the same slice were 4.7 ± 0.31, 5.6 ± 0.46 and 3.4 ± 0.42 for 6, 24 and 48 hours, respectively. By contrast, low perfusion areas were indicated in the perfusion study and their percentages were 4.6 ± 0.55, 5.6 ± 0.86 and 2.4 ± 0.35 for 6, 24 and 48 hours, respectively. At 48 hours after cold injury, low perfusion areas were smaller than high SI areas. Moreover, high accumulation areas consisting of macrophages were observed surrounding necrosis. It is concluded that there is dissociation between perfusion and T2-weighted MR imaging, where the collection of macrophages surrounding edema lesions and necrosis had the same appearance on MRI and different accumulations on perfusion studies.

Key words: cold injury, MRI, autoradiography, brain perfusion, rat, edema

INTRODUCTION

BRAIN EDEMA is a pathological status frequently observed in brain diseases and is easily detected by morphological images such as X-ray computed tomography or magnetic resonance imaging (MRI).[1–6] Brain edema can be observed as a high signal intensity (SI) lesion on T2-weighted images. By contrast, regional cerebral blood flow is decreased in regions of brain edema on perfusion studies.[7,8] There are few papers on combined MRI and perfusion studies of brain edema, and some questions are still left unanswered, for example, do both images change coincidentally with each other throughout the course of edema and does any pathological status affect imaging quantity?

In an attempt to answer these questions, a series of experiments with the cold injury method in the rat brain as a model were performed, and later, morphological and functional imaging studies combined with histological examination were conducted. T2-weighted MR imaging (T2WI) and autoradiograms (ARG) of ^{14}C-iodoantipyrine (^{14}C-IAP) of the same rats were performed and compared at 6, 24 and 48 hours after injury and then with the histology.

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MATERIALS AND METHODS

Cold injury induced edema

All animal experiments reported in this paper followed the Guidelines for Animal Experimentation and were approved by the Animal Research Committee of Hirosaki University.

Female Donryu rats weighing 60–120 g were anesthetized by intraperitoneal administration of sodium pentobarbital (5 mg/100 g body weight). After 1 to 2 cm longitudinal skin incisions in the mid-line of the scalp, the skull was exposed and a metal cylinder (ϕ 8 mm) frozen to −70°C with liquid nitrogen was applied on the left parietal region for 20 seconds.[9,10] The skin incision was sutured and the rats were allowed to recover. These techniques were performed under room temperature. The animals were kept in a cage *ad libitum*.

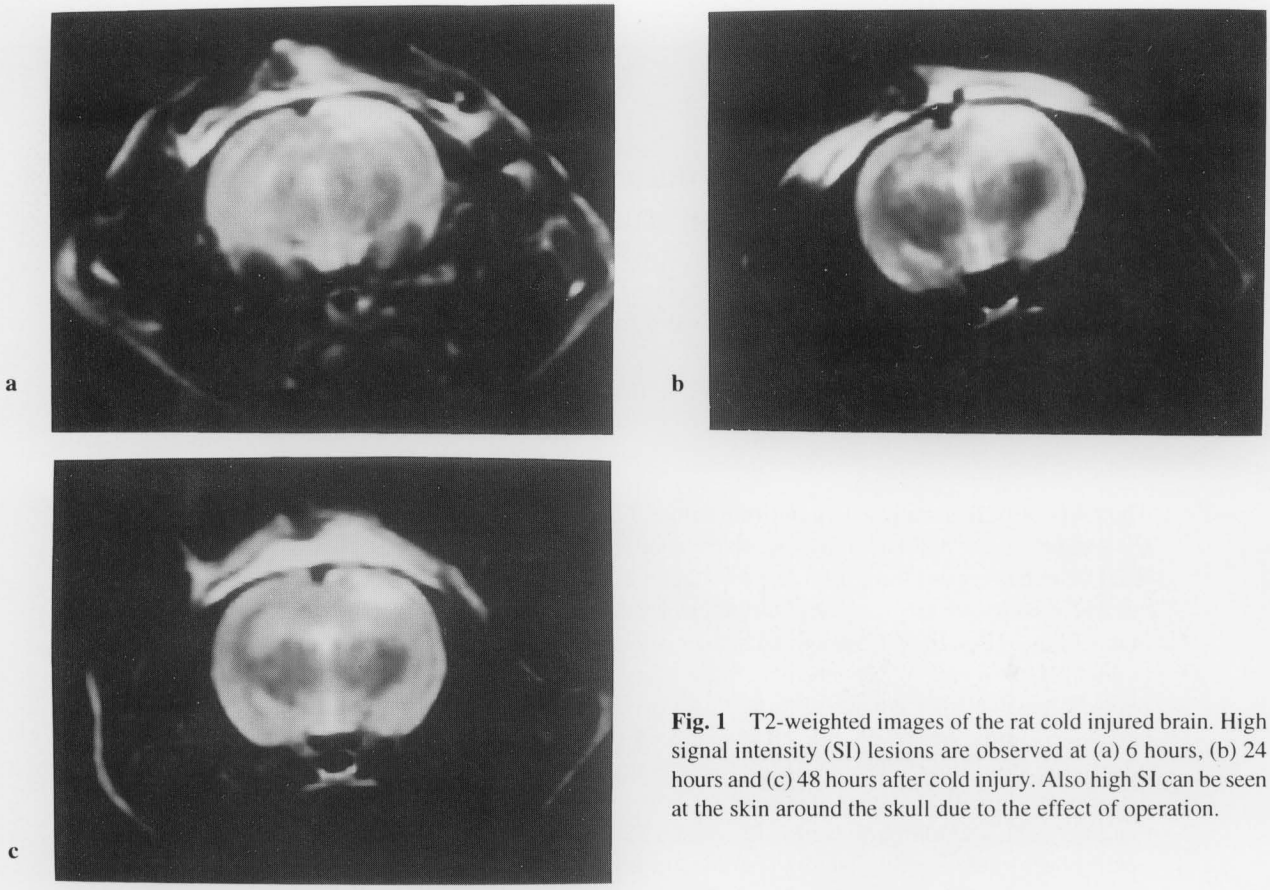

Fig. 1 T2-weighted images of the rat cold injured brain. High signal intensity (SI) lesions are observed at (a) 6 hours, (b) 24 hours and (c) 48 hours after cold injury. Also high SI can be seen at the skin around the skull due to the effect of operation.

Comparison between MRI and perfusion images

The rats were examined at 6 (n = 5), 24 (n = 4) or 48 (n = 4) hours after the cold injury. MR imaging was performed with a high field (1.5 T) unit (Signa; GE Medical Systems, Milwaukee, Wis). During imaging studies, the animals were anesthetized, and placed in the prone position with a wrist coil applied to the head. Coronal T2-weighted images were taken as follows: TR 3000 msec, TE 88 msec, FOV 8 × 8 cm, slice thickness 3 mm, matrix size 256 × 192 and acquisition time 60 min and 6 sec.

Imaging analysis was performed on a Macintosh computer with the public domain NIH Image Program (developed at the U.S. National Institute of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). The ratio of the maximum area representing the high intensity region (one slice) to the total area of the brain in the same slice was obtained and expressed as a percentage. The high SI area was estimated visually.

Perfusion images were obtained from ARG of ^{14}C-iodoantipyrine (^{14}C-IAP: 1.85 GBq/mmol; NEN, Life Science, London). Rats were anesthetized and MRI examined as described above. Approximately 3.7 MBq/kg of ^{14}C-IAP was injected through the tail vein soon after the MRI examination. One minute after injection, the rats were sacrificed by cervical dislocation. The brain was exposed and carefully excised to avoid damage to its surface. The brain was then frozen on powdered dry ice and cut coronally into 20 μm thick slices with an autocryotome. One of every 15 to 20 serial slices was selected, i.e. a slice at every 200–300 μm; dried at 40°C on a hot plate and exposed to X-ray film (Ektascan EC-1, Kodak) for 2 weeks. The film was then processed for image acquisition.[11,12] No corrections were made on the ARG.

For analysis, the ratio of the maximum area representing low perfusion to the total area of the brain in the same slice was obtained and expressed as a percentage by using the NIH image 1.59/ppc as described above. Considering the difference between MRI and ARG slice thickness, 5 sequential low-perfusion areas with a maximum low-perfusion area in the middle were selected and the average area was obtained. After analysis they were stained with Hematoxylin and Eosin.

Histological study

Perfusion fixation of the brain was performed at 6, 24 and 48 hours after cold injury. The number of rats used for each study was 2. The rats were anesthetized, fixed in the supine position and had their hearts exposed. About 100 m*l* of physiologic saline solution at 120 cm H_2O pressure was injected through a 22 gauge catheter inserted into the left ventricle and a cut was made at the right atrium. After confirmation of no blood flow from the right atrium, 10% formaldehyde solution was administered for fixation.

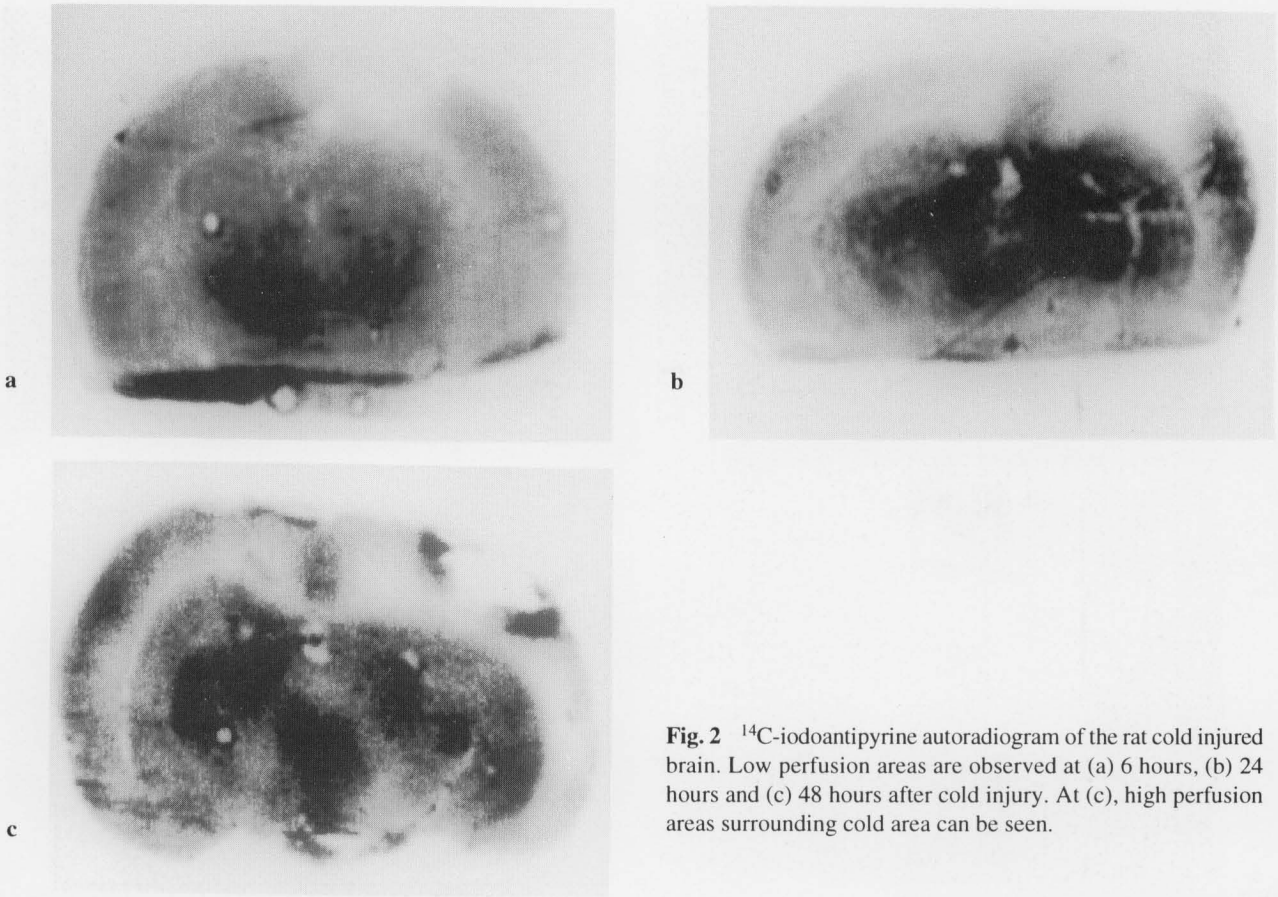

Fig. 2 ^{14}C-iodoantipyrine autoradiogram of the rat cold injured brain. Low perfusion areas are observed at (a) 6 hours, (b) 24 hours and (c) 48 hours after cold injury. At (c), high perfusion areas surrounding cold area can be seen.

Twelve hours after fixation at 4–6°C, the brain was excised and fixed again with a 10% formaldehyde solution. Four-micromillimeter coronal sections were processed for routine histological examination.

Statistical analysis

Experimental data were expressed as means with standard deviation and statistical analysis was done with Student's t-test.

RESULTS

Comparison of MRI and perfusion images

Figure 1 shows the time course of the T2-weighted images. Six hours after cold injury, high SI on MRI was observed at the surface of the cortex of all the rats. At 24 hours, high SI extended deeper into the cortex and, at 48 hours, all the rats had smaller area of high SI than in the 6 and 24-hour examinations. This is the same tendency as for serially observed rats (data not shown).

The percentages of high SI area to the total brain are shown in Table 1. There were significant differences between the 6 and 48-hour examinations ($p = 0.038$) and between the 24 and 48-hour examinations ($p = 0.012$).

Figure 2 shows the time course of the perfusion study. Six hours after cold injury, a low perfusion area was observed at the surface of the cortex of all the rats. At 24 hours, a low perfusion area extended in a pattern similar to the MR image, and at 48-hours all the rats had a smaller low perfusion area surrounded by a small high accumulation area.

The low perfusion areas as percentages of the total brain area are shown in Table 1. There were significant differences between 6 and 48-hour examinations ($p = 0.016$) and between 24 and 48-hour examinations ($p = 0.014$).

Comparing low perfusion areas with high SI areas (Figures 1, 2 and Table 1), there were differences in size at 48 hours. High SI areas on MRI were somewhat larger than the low perfusion areas, excluding the surrounding high accumulation areas. And there were no differences in size between perfusion images and MRI at 6 and 24 hours after cold injury.

Histological studies

At 6 hours after cold injury, histological examination showed necrosis of neurons, a few scattered neutrophils and extravasated erythrocytes with distinct margins of the lesion indicating an initial phase of vascular edema (Figure 3).

In 24-hour specimens, necrosis of neurons was apparent. The lesion extended deeper into the brain and could

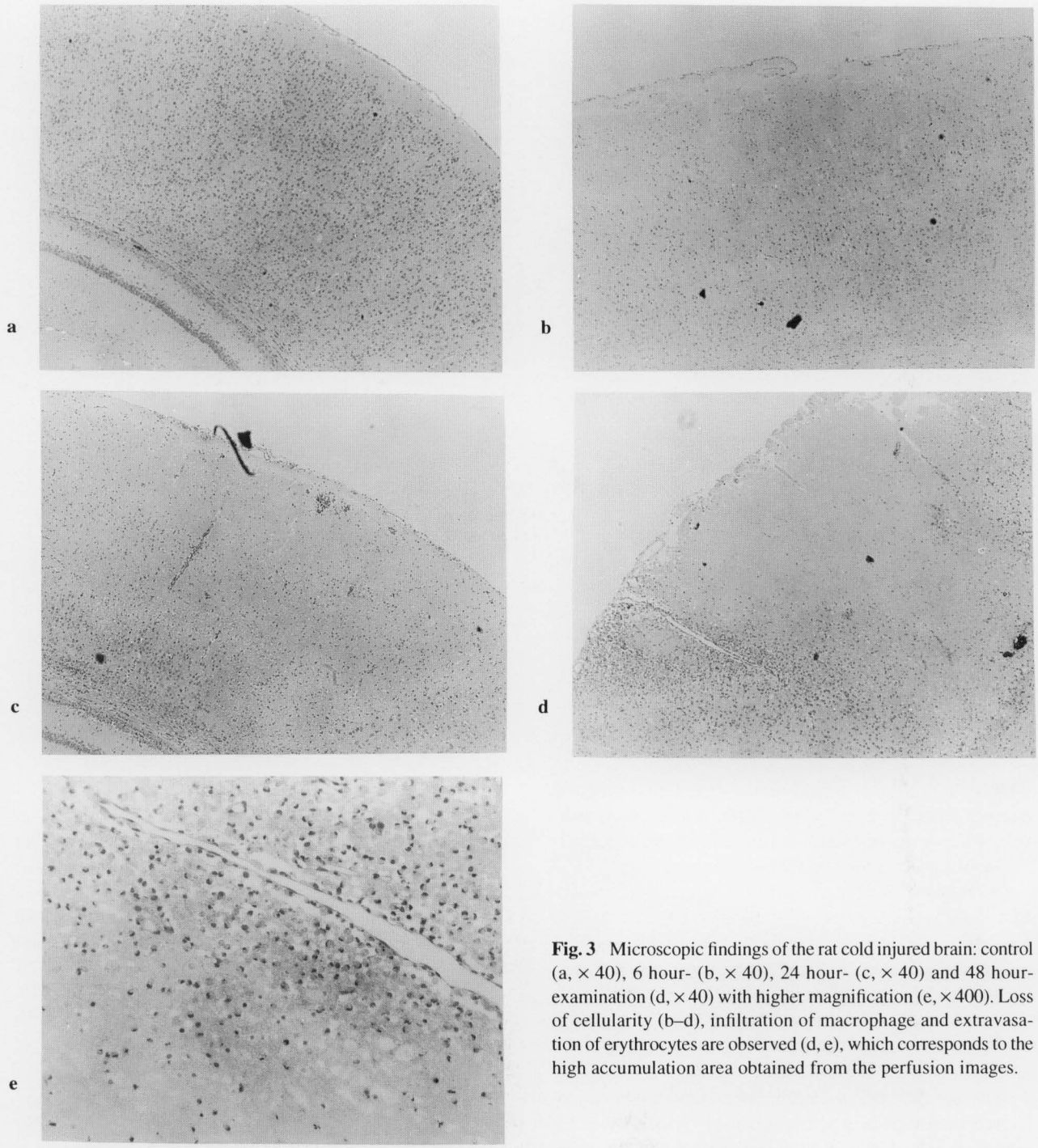

Fig. 3 Microscopic findings of the rat cold injured brain: control (a, × 40), 6 hour- (b, × 40), 24 hour- (c, × 40) and 48 hour-examination (d, × 40) with higher magnification (e, × 400). Loss of cellularity (b–d), infiltration of macrophage and extravasation of erythrocytes are observed (d, e), which corresponds to the high accumulation area obtained from the perfusion images.

be clearly distinguished from the surrounding normal tissues.

In 48-hour specimens, the lesion showed signs of complete necrosis of neurons, and scantily distributed hemorrhagic foci could be seen. The lesion was smaller than in 24-hour specimens. Infiltration foci of macrophages around the lesion were also observed. This finding matches the high accumulation areas observed in perfusion studies as these unclassified cells could be seen in the corresponding ARG specimens.

Table 1 Percentage of high SI areas (MRI) and low perfusion area (ARG)

	Time after cold injury		
	6 hours	24 hours	48 hours
% High SI areas	4.7 ± 0.69*	5.6 ± 0.92**	3.4 ± 0.84*,**
% Low perfusion area	4.6 ± 1.23†	5.6 ± 1.72††	2.4 ± 0.70†,††
	(n = 5)	(n = 4)	(n = 4)

mean ± s.d.

*, **, † and †† indicates p = 0.038, p = 0.012, p = 0.016 and p = 0.014, respectively

DISCUSSION

These experiments revealed that, at 48 hours after cold injury, a perfusion study was able to demonstrate high accumulation surrounding the lesion, corresponding with macrophage infiltration, but MR study failed to detect this change.

MRI is a noninvasive means for ascertaining the characteristics of biological tissues, especially water content and status. The images rely on the signal generated by the proton hydrogen (^{1}H), and, as a simplification, they can reflect the water concentration in the tissues. Most of the brain lesions related to water accumulation such as inflammation, tumor and edema, as well as necrosis have a high SI due to the prolongation of T2 relaxation time,[1–5] but T2-weighted images cannot differentiate inflammation foci from necrotic areas.

In cold injury models, other studies showed abnormal SI after 3–6 hours and reached a maximum volume at 24 hours.[3,10,13] Enhanced study with gadolinium hydrogen $\alpha,\alpha',\alpha'',\alpha'''$-tetramethyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate (DDA-DO3MA-Gd) showed more enhancement at 24 hours than at 3 hours after cold injury.[10] This study detected an accumulation of DDA-DO3MA-Gd even at 3 hours, possibly due to disruption of the blood-brain-barrier (BBB). In our perfusion study, because no obvious uptake was observed until 48 hours, high accumulation of ^{14}C-IAP was mostly in the inflammatory foci, i.e. infiltration of macrophages. Cold injury has been applied for the model of brain edema; it mainly consists of neuronal death and subsequent removal of the necrosis. To the best of our knowledge there have been no reports on perfusion study indicating high accumulation of the tracer around the edema area.

On histological studies of cold injury, it was reported that neuronal necrosis became noticeable after 30 minutes and continued for 2–10 days depending on the extent of the injury.[14,15] Inflammatory cells (neutrophils and macrophages) can be observed at 24 hours.[16] Our findings showed the same tendency as in other study. On the other hand, neovascularization was observed more than 5 days after cold injury.[14,17,18]

In this study, perfusion images were obtained from anesthetized rats immediately after MR imaging. Therefore, in some images, cortex uptake is different from that in the fully awake rats. It might be true, however, for the relative accumulations between necrotic and inflamed tissues because there were pathological consequences which were not controlled by the normal brain function.

It is concluded that there is a dissociation between perfusion and T2WI because the inflammatory tissues and necrotic lesions show the same signal intensity on MRI and high and low accumulations in perfusion studies, respectively.

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REFERENCES

1. Benderson JB, Bartkowski HM, Moon K, Halks-Miller M, Nishimura MC, Brant-Zawadski M, et al. Nuclear magnetic resonance imaging and spectroscopy in experimental brain edema in a rat model. *J Neurosurg* 1986; 64: 795–802.
2. Kamada K. Sequential observations of brain edema with proton magnetic resonance imaging and spectroscopy. *Hokkaido Igaku Zasshi* 1996; 71 (1): 105–122.
3. Ebisu T, Naruse S, Horikawa Y, Ueda S, Tanaka C, Uto M, et al. Discrimination between different types of white matter edema with diffusion weighted MR imaging. *JMRI* 1993; 3: 863–868.
4. Pierpaoli C, Righini A, Linfante I, Tao-Cheng JH, Alger JR, Di Chiro G. Histopathologic correlates of abnormal water diffusion in cerebral ischemia: Diffusion-weighted MR imaging and light and electron micriscopic study. *Radiology* 1993; 189: 439–448.
5. Asato R, Handa H, Hashi T, Hatta J, Komoike M, Yazaki T. Chronological sequences and blood-brain barrier permeability changes in local injury as assessed by nuclear magnetic resonance (NMR) images from sliced rat brain. *Stroke* 1983; 14: 191–197.
6. Hashimoto K, Yamagata S, Minamikawa J, Watanabe Y, Kanesiro M, Kikuchi H. The effect of Gd-DTPA on magnetic resonance imaging: a potential blood-brain barrier indicator. *No Shinkei* 1988; 40 (5): 461–466.
7. Ogawa T. Effect of stimulation of the medullary reticular formation on brain water content in cold induced brain. *No Shinkei* 1990; 42 (3): 261–268.
8. Frei HJ, Wallenfang TH, Poll W, Reulen HJ, Schubert R, Brock M. Regional cerebral blood flow and regional metabolism in cold induced oedema. *Acta Neurochir* 1973; 29: 15–28.
9. Kagawa M, Nagao S. The effect of arginine vasopressin receptor on the rat cold-injured brain. *No Shinkei Geka* 1993; 21 (12): 1103–1107.
10. Houkin K, Abe H, Hashiguchi Y, Seri S. Magnetic resonance imaging of cold-induced brain edema in rats. *Neurol Med Chir (Tokyo)* 1996; 36: 72–77.
11. Sakurada O, Kennedy C, Jehle J, Brown JD, Carbin GL, Sokoloff L. Measurement of local cerebral blood flow with iodo [^{14}C] antipyrine. *Am J Physiol* 1978; 234 (1): H59–H66.
12. Sako K. Strategies for measurement of cerebral blood flow and metabolism using autoradiography. *No Shinkei Geka* 1993; 21 (6): 484–490.
13. Ito J, Marmarou A, Barzo P, Fatouros P, Corwin F. Characterization of edema by diffusion-weighted imaging in experimental traumatic brain injury. *J Neurosurg* 1996; 84: 97–103.

14. Cancilla PA, Frommes SP, Kahn LE, DeBault LE. Regeneration of cerebral microvessels. *Lab Investig* 1979; 40 (1): 74–82.
15. Murakami K, Kondo T, Sato S, Li Y, Chan PH. Occurrence of apoptosis following cold injury-induced brain edema in mice. *Neuroscience* 1997; 81 (1): 231–237.
16. Nag S. Cold-injury of the cerebral cortex: Immunolocalization of cellular proteins and blood-barrier permeability studies. *J Neuropathol Exp Neurol* 1996; 55 (8): 880–888.
17. Olson JJ, Beck DW, Warner DS, Coester H. The role of new vessels and macrophages in the development and resolution of edema following a cortical freeze lesion in the mouse. *J Neuropathol Exp Neurol* 1987; 46 (6): 682–694.
18. Mitchell J, Weller RO, Evans H. Reestablishment of the blood brain barrier to peroxidase following cold injury to mouse cortex. *Acta Neuropathol* 1979; 46: 45–49.