Imaging of the Inflammatory Response in Reperfusion Injury After Transient Cerebral Ischemia in Rats: Correlation of SPIO-enhanced MR Images with Histopathology

Jinna Kim

Department of Medicine

The Graduate School, Yonsei University

Imaging of the Inflammatory Response in Reperfusion Injury After Transient Cerebral Ischemia in Rats: Correlation of SPIO-enhanced MR Images with Histopathology

Directed by Professor Dong Ik Kim

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Jinna Kim

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This certifies that the
Doctoral Dissertation of Jinna Kim
is approved.

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Thesis Supervisor : Dong Ik Kim

_____

Tae Seung Kim

_____

Dong Goo Kim

_____

Jong Doo Lee

_____

Ji Hoe Heo

The Graduate School

Yonsei University

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저 자 씀

Table of contents

List of figures

Abstract

Imaging of the Inflammatory Response in Reperfusion Injury After Transient Cerebral Ischemia in Rats: Correlation of SPIO-enhanced MR Images with Histopathology

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Reperfusion by recanalization of the occluded artery after acute ischemic stroke may result in microscopic cellular damage, and acute inflammatory responses have been thought to play a central role in ischemia-reperfusion injury. The purpose of this study was to determine if the accumulation of macrophages could be seen *in vivo* in a reperfusion animal model after focal cerebral ischemia using superparamagnetic iron oxide (SPIO)-enhanced magnetic resonance (MR) imaging, and to correlate the spatial distribution of SPIO-induced MR signal alterations with histopathologic

findings.

One-hour transient occlusion of the middle cerebral artery was produced in adult male Sprague-Dawley rats. We injected SPIO particles at different time points after reperfusion and performed three-dimensional (3-D) T2*-weighted MR images with gradient-echo sequence 24 hours later. After the acquisition of MR imaging, a specimen was obtained. Macrophages were detected with Prussian blue and immunohistochemical staining. Histochemical iron detection was compared with T2* signal abnormalities.

At days 3 and 4 post-reperfusion, focal areas of signal loss indicating local accumulation of SPIO particles appeared at the center of the damaged brain. Areas of signal loss corresponded to local accumulation of iron-laden macrophages in histologic sections, and SPIO-induced signal loss indicated active macrophage transmigration into the reperfused brain.

SPIO-enhanced MR imaging demonstrated through *in vivo* monitoring that macrophages participate in reperfusion injury at early stages of injury development. SPIO-enhanced MR imaging can be a useful tool to examine the inflammatory mechanisms involved in reperfusion brain injury.

Key words: acute ischemic stroke, reperfusion, inflammation, macrophage, magnetic resonance imaging

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I. INTRODUCTION

Recently, reperfusion by recanalization of occluded arteries has proven the most effective therapy for acute ischemic stroke in humans. Aggressive therapeutic trials have studied the effectiveness of intravenous, intra-arterial, mechanical, or combined thrombolysis for the treatment of hyperacute cerebral infarcts.[1-3] In light of these

advances, elucidation of the pathophysiological events that complicate early cerebral reperfusion is essential.

Although reperfusion can save ischemic tissue by rapid restoration of cerebral blood flow, microscopic cellular injury after reperfusion may occur by various mechanisms. The adverse effects of reperfusion diminish the net positive effect on clinical outcome and independently increase the risk of decline in stroke patients.[4-6] Reperfusion injury has been defined in numerous ways that implicate secondary hemodynamic disturbances, enhancement of inflammatory responses, excess production of oxygen free radicals, glutamatergic excitotoxicity, apoptosis, and breakdown of the blood-brain barrier (BBB) as factors that contribute to reperfusion injury.[4,7-13] In all of them, it has been well documented that profound inflammatory responses are evoked and that immune mediators produced by the inflammatory cells contribute to reperfusion cellular injury.[7-9]

Recent studies have demonstrated that superparamagnetic iron oxide (SPIO) particles taken up by macrophages in the mononuclear phagocyte system enable *in vivo* monitoring of macrophage infiltration by magnetic resonance (MR) imaging in the experimental ischemic rat brain.[14-19]

The purpose of the present study was to visualize early activation and accumulation of macrophages in an experimental reperfusion animal model by means of SPIO-enhanced MR imaging, to evaluate the clinical usefulness of SPIO-enhanced MR

imaging for the assessment of inflammatory responses in reperfusion brain injury, and to correlate the spatial distribution of SPIO-induced MR signal alterations with histopathologic findings.

II. MATERIALS & METHODS

1. Animal preparation

Animal experiments were approved by the Committee for the Care & Use of Laboratory Animals at the Yonsei University College of Medicine. Adult male Sprague-Dawley rats weighing 300 to 350 g were subjected to transient middle cerebral artery occlusion (MCAO). Animals were anesthetized with 100 mg/kg body weight ketamine chloride (Ketalar; Yuhan yanghang, Seoul, Korea) and 10 mg/kg body weight xylazine hydrochloride (Rompun; Bayer Korea, Seoul, Korea) administered intraperitoneally. During anesthesia, rectal temperature was maintained at 36.5 ^{o}C to 37.5 ^{o}C by a heating pad. Ischemia was induced using an occluding intraluminal suture as previously described.[20] In brief, an uncoated 15-mm segment of 3–0 nylon monofilament suture with the tip rounded by a flame was inserted into the arteriotomy and advanced under direct visualization into the internal carotid artery 21 mm from the bifurcation to occlude the ostium of the middle cerebral artery. After 1 hour, the suture was withdrawn and surgical incisions were closed.

2. Administration of contrast material and MR imaging acquisition

0.2 mmol Fe/kg body weight SPIO particles (Resovist; Schering, Berlin, Germany) were applied systemically into the circulatory system 24 hours before every MR examination. Ferucarbotran (Resovist®) is composed of a dextran-coated iron oxide core of 4 nm, and its hydrodynamic diameter is about 60 nm. All measurements were performed using a clinical 3.0 Tesla MR system (Intera Achieva, Philips Medical Systems, the Netherlands) and a wrist coil to improve resolution. Rats were anesthetized with intraperitoneal injections of 100 mg/kg body weight ketamine chloride (Ketalar) and 10 mg/kg body weight xylazine hydrochloride (Rompun) and MR examinations were performed at days 1 (n = 3), 2 (n = 3), 3 (n = 4), 4 (n = 4), 5 (n = 3), 6 (n = 2), 8 (n = 2), 10 (n = 2) and 14 (n = 2) after reperfusion.

Three control animals that had been operated on received sodium chloride at day 2 and were scanned by MR on day 3. In addition, three animals underwent MR examinations without SPIO application at day 3 after reperfusion to obtain T2*-weighted images and then subsequently received systemic 0.2 mmol/kg body weight Gadolinium-DTPA (Magnevist; Schering, Berlin, Germany).

To specifically assess the relation between SPIO-induced signal loss and disruption of the BBB, groups of rats received SPIO particles on day 2 (n = 3). They were scanned by MR on day 3 for iron deposition on three-dimensional (3-D) T2*-

weighted MR images and immediately thereafter received additional systemic Gadolinium-DTPA injections as a marker for BBB integrity. Animals were then scanned a second time to assess Gadolinium-DTPA enhancement on T1-weighted images. Thereafter, T2*-weighted and T1-weighted images were compared in individual animals.

For all MR measurements, animals were lying in a prone position with their heads fixed in a wrist coil. The T1-weighted (TR/TE = 625/18 ms) and T2-weighted (TR/TE = 1700/80 ms) images were acquired in the coronal planes to characterize the extent and location of the ischemic injury in all of the animals. The field of view = 8 x 8 cm^2, matrix = 256 x 256, excitations = 4, and slice thickness = 2 mm. Moreover, 3-D data sets of T2*-weighted sequence were acquired using a gradient-echo pulse sequence in the coronal plane (TR/TE = 40/16 ms, flip angle = 108°), with a slice thickness of 1 mm.

3. Image analysis

For postprocessing MR data, region of interest (ROI) measurements were performed using Image J (NIH, Bethesda, MD, USA). We determined the overall lesion volume (mm^3) in each animal by manually delineating the lesion borders in subsequent slices. Moreover, in animals 3 to 4 days after reperfusion, the extent of the

hypointense area caused by iron-accumulation was calculated and related to the overall lesion volume. In addition, the mean signal intensities of the hypointense and hyperintense areas on T2*-weighted images were determined and normalized to cerebrospinal fluid signals to allow for interindividual comparison. Mean values were statistically compared using Kruskal Wallis and Mann-Whitney U tests.

4. Histopathology

All rats were sacrificed for histologic analysis directly after MR imaging acquisition. The brains were rapidly removed, immersion-fixed overnight in 4% paraformaldehyde in phosphate-buffer, and embedded in paraffin. Subsequently, coronal sections (4 μm thick) were cut at multiple levels through the infarcts by microtome.

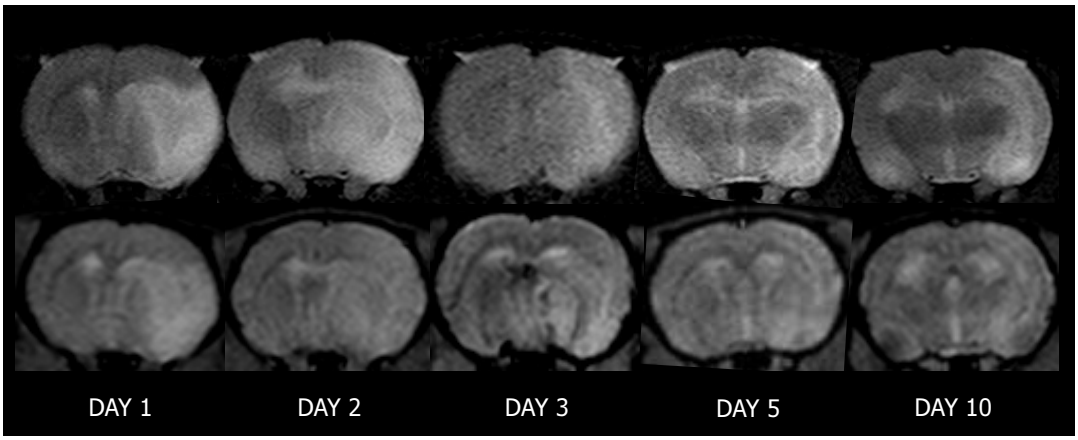
For iron detection, tissue sections were deparaffinized, rinsed in deionized water and immersed in Perl's solution containing 2% potassium ferrocyanide and 2% HCl at a 1:1 concentration for 30 minutes, then counterstained with nuclear fast red. Additional sections were then rinsed in deionized water and stained by immunocytochemistry using the antibody ED-1 at a dilution of 1:200 as a marker for monocytes/macrophages and phagocytic microglia (Serotec, Oxford, UK). Binding of ED-1 antibodies to cells was visualized by the avidin-biotin-peroxidase method with

3,3' -diaminobenzidine using the ABC kit (Vector Labs, Burlinghame, CA, USA).[21] For equivocal identification of iron-labeled cells, we performed Perl's stain and ED-1 immunocytochemistry upon the next sections. Results were recorded with a DP-70 digital camera (Olympus, Melville, NY, USA). For comparison of T2* signal abnormalities and histochemical iron detection, sections through the center of the infarctions stained for iron were compared with the corresponding MR imagings. Premounted sections were also stained with hematoxylin-eosin (H&E).

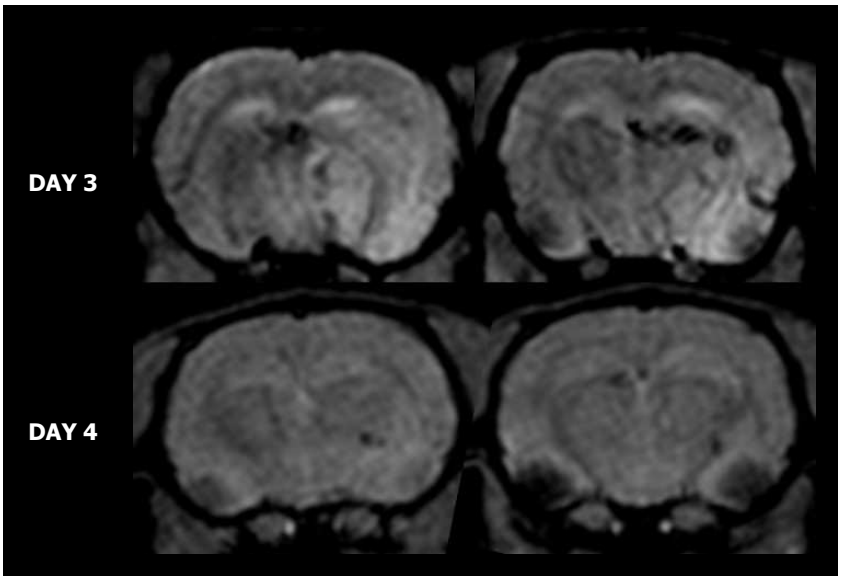
III. RESULTS

1. MR imaging findings after reperfusion

All animals received systemic SPIO particles injections 24 hours before MR imaging acquisition. Ischemic lesions appeared as hyperintensities on T2-weighted and T2*-weighted MR images during all stages of infarct development (Fig. 1A). No areas of signal loss were present in ischemic lesions at 1 to 2 days after reperfusion and SPIO injection the day before. By day 3, focal areas of signal loss with a patchy appearance, indicative of local accumulation of SPIO particles, emerged at the center of the hyperintense ischemic territory, especially in the ischemic dorsolateral striatum around striatal blood vessels, on T2-weighted and T2*-weighted images at days 3 (Fig. 1B). The extent of the signal loss could best be demonstrated on high resolution 3-D T2*-weighted images. At later stages of infarct development after day 5, lesions were shrunken and appeared entirely hyperintense on T2-weighted images. Focal areas of signal loss were no longer seen, indicating that SPIO particle-induced signal loss in ischemic brain lesions is a transient event.



A



B

Fig. 1. ***In vivo*** **assessment of macrophage infiltration by SPIO-enhanced MR imaging at various stages of reperfusion.** All animals received systemic SPIO particle injections 24 hours before MR imaging. Ischemic lesions appeared as hyperintensities on T2-weighted and T2*-weighted MR images during all stages of infarct development (A). Whereas no areas of

signal loss were present in ischemic lesions at 1 to 2 days after reperfusion, focal areas of signal loss were seen in the ischemic striatum on 3-D T2*-weighted images at day 3 and 4 after reperfusion (B). At later stages of infarct development after day 5, lesions were shrunken and appeared entirely hyperintense on T2-weighted images. Focal areas of signal loss were no longer seen.

Lesion volume (Fig. 2) and signal intensity of the hyperintense lesions (Fig. 3) significantly differed between days 1 to 14 days after reperfusion (Kruskal Wallis test, $P = 0.024$, $P = 0.023$, respectively). The proportion of the hypointense area in relation to total infarct volume between days 3 and 4 after reperfusion did not significantly differ ($P = 0.243$, Mann-Whitney U test). The hypointense signal reached lowest values at day 3, indicating peak iron deposition, and was significantly different from the values at days 4 ($P = 0.021$, Mann-Whitney U test).

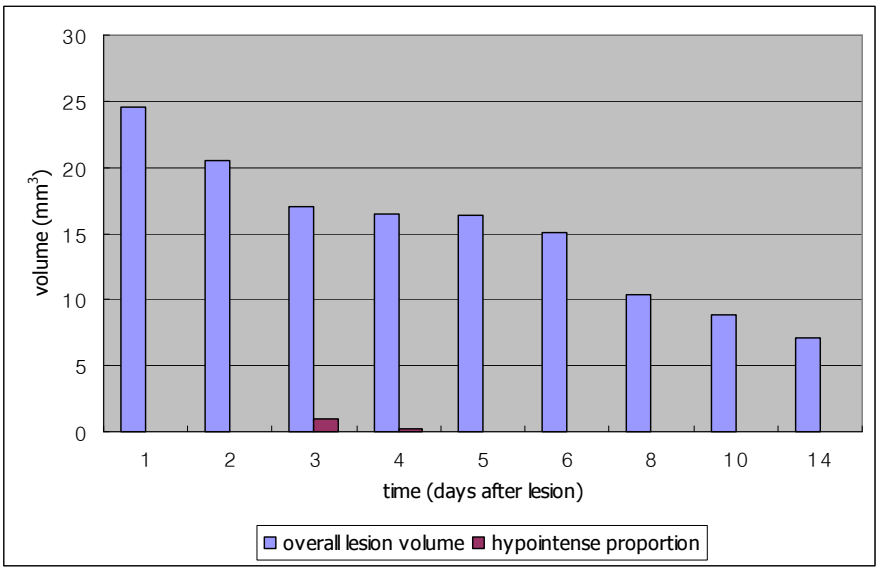


Fig. 2. Overall lesion volume and the volume of hypointense area on T2*-weighted MR images at various stages of reperfusion. Proportion of the hypointense area in relation to total infarct volume did not significantly differ between days 3 and 4 after reperfusion.

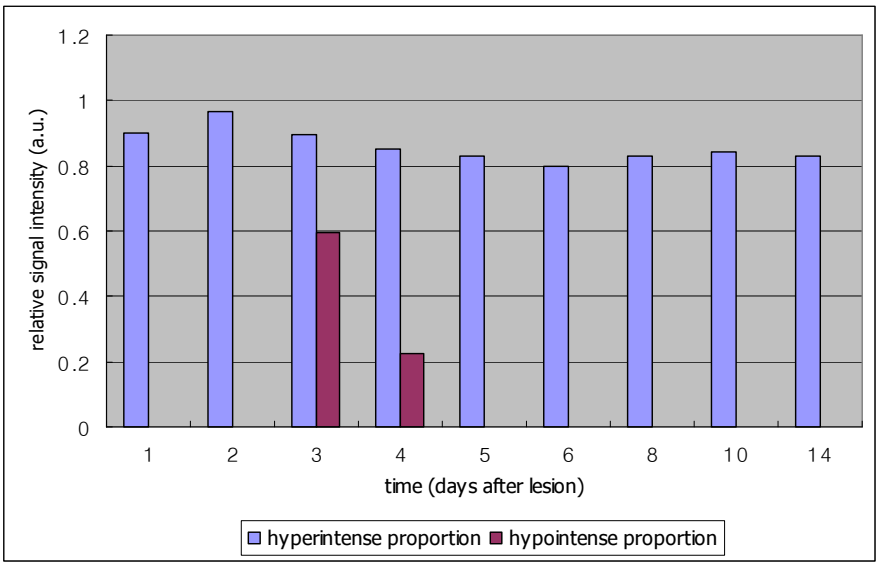
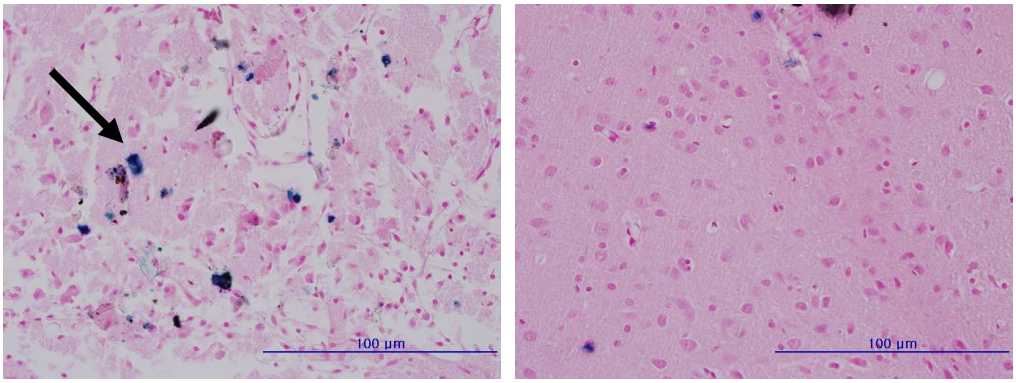


Fig. 3. Mean signal intensities of the hyperintense and hypointense areas on T2*-weighted MR images at various stages of reperfusion. Signal intensity measurements of the hypointense lesions exhibited a significant difference between days 3 and 4 after reperfusion.

2. Histopathologic findings

H&E staining revealed ischemic tissue in the cortex and striatum in all animals, regardless of the day post-reperfusion. This ischemic territory was in good spatial agreement with the hyperintense area on T2-weighted MR images. There were no hemorrhages in the ischemic lesions after reperfusion, either associated with surgery or not.

We confirmed our MR imaging findings by demonstrating focal iron deposition in histologic sections stained with Perl's Prussian blue solution. In the ischemic lesions up to day 2, only occasional cells on the pial surface were iron-positive, whereas 3 and 4 days after reperfusion a large number of iron-laden cells were located at the center of ischemic brain lesions (Fig. 4). Iron-positive macrophages were predominantly found in the ischemic striatum, near blood vessels. Regions with iron-positive macrophages showed an excellent spatial correlation with the hypointense areas on T2*-weighted images in all animals. At later stages of infarct development, i.e. beyond day 5, iron-positive cells were virtually absent in ischemic lesions. Thus, our histologic findings corresponded to the MR imaging results and confirmed the transient nature of the iron-induced signal loss.



A **B**

Fig. 4. Histologic section with Prussian blue stain 3 days after reperfusion. Histological section with Prussian blue stain corresponding to hypointense lesions on T2*-weighted MR imaging revealed many macrophages with blue iron inclusions (arrow) at the center of the ischemic brain, next to a blood vessel (A). Normal brain (B). Magnification x400.

In corresponding sections stained with ED-1, a marker for phagocytic monocytes/macrophages and activated microglia, an abundant number of ED-1-positive macrophages were present in both the ischemic cortex and striatum 3 to 4 days after reperfusion (Fig. 5). ED-1-positive cells were present in higher numbers than cells stained with Perl's Prussian blue solution, and comparison of stained sections revealed that most Prussian blue-stained cells were also ED-1-positive (Fig. 6). Prussian blue- or ED-1-positive cells were not detected in the contralateral brain.

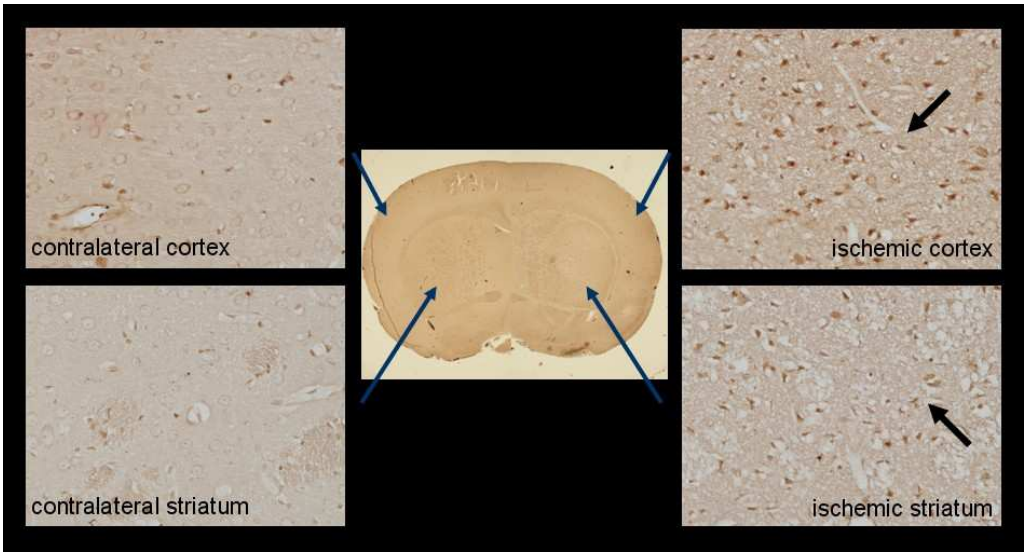
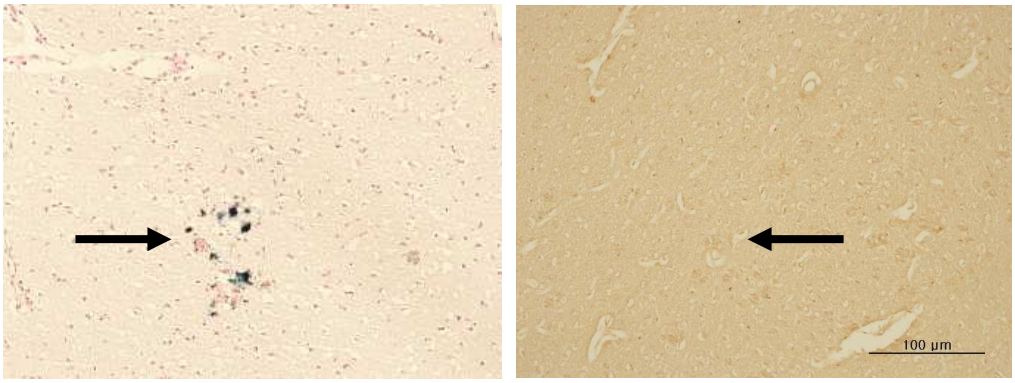


Fig. 5. Histologic section stained for ED-1 3 days after reperfusion. An abundant number of ED-1-positive macrophages (both activated microglia and monocytes) were present in the ischemic cortex as well as in the ischemic striatum (arrows) at days 3 to 4 after reperfusion.



A **B**

Fig. 6. Comparison of stained sections with Prussian blue solution and ED-1 3 days after reperfusion. ED-1-positive cells (B, arrow) in the ischemic striatum were present in higher numbers than cells stained with Prussian blue solution (A, arrow).

3. Correlation with BBB disruption

In control animals with transient MCAO but no SPIO injections, ischemic lesions did not exhibit signal loss on T2-weighted or T2*-weighted images on day 3, but did show Gadolinum-DTPA enhancement in the ischemic brain (Fig. 7A and B). When we further assessed the relation between SPIO-induced signal loss and disruption of the BBB, we found that Gadolinum-DTPA enhancement on T1-weighted images uniformly covered the entire ischemic lesion on day 3 after reperfusion. SPIO-induced signal loss was restricted to the center of the ischemic lesions on T1-weighted images after immediate injections of additional systemic Gadolinium-DTPA (Fig. 7C and D). This finding indicates that breakdown of the BBB and macrophage infiltration are unrelated events.

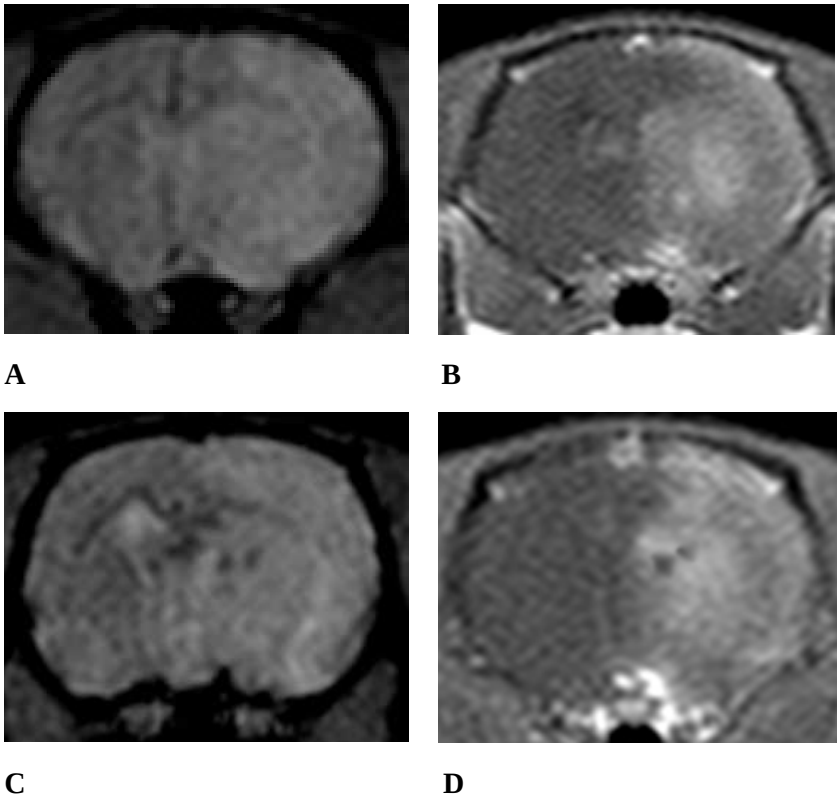


Fig. 7. Relationship between SPIO-induced signal loss and breakdown of the BBB 3 days after reperfusion. In control animals with sodium chloride injections, there was no signal loss area within the ischemic lesion on T2*-weighted images (A), and Gadolinum-DTPA enhancement on T1-weighted images uniformly covered the entire ischemic lesion (B). Gadolinum-DTPA enhancement on T1-weighted images uniformly covered the entire ischemic lesion on day 3 after reperfusion, and SPIO-induced signal loss was restricted to the center of the ischemic lesion after immediate injections of additional systemic Gadolinium-DTPA (C, D), suggesting that breakdown of the BBB and macrophage infiltration are unrelated events.

Ⅳ. DISCUSSION

The development of cerebral infarcts can be monitored with modern MR imaging techniques.[22,23] However, various MR parameters such as the apparent diffusion coefficient (ADC) and T2 relaxation time only reflect consecutive changes in the amount and mobility of protons, failing to delineate areas of specific cellular responses such as astrogliosis, microglial activation, and macrophage recruitment.[24] SPIO particles are taken up by macrophages in the mononuclear phagocyte system and stored in lysosomes, and the iron-laden macrophages produce a strong contrast on T2*-weighted MR imaging.[14] Such cell-type specific imaging using SPIO particles enables the detection of macrophages in animal models of various central nervous system (CNS) pathologies, including tumor, nerve trauma, and autoimmune neuritis, as well as inflammatory CNS lesions.[25-29] In this study, we sought to investigate the feasibility of SPIO-enhanced MR imaging in the evaluation of reperfusion cellular injury.

Ischemic brain injury not only leads to cell death through energy depletion, but also to a post-ischemic inflammatory process that contributes to a delayed progression of the injury.[7,30,31] It is well documented that focal ischemia in the experimental MCAO model induces a time-dependent activation of various inflammatory cells and that many cytokines secreted by the inflammatory cells contribute to reperfusion

cellular injury. Granulocytes are the first cells to invade the ischemic tissue, after which they disappear rapidly, followed by an infiltration of T cells.[32,33] Macrophage activity through the activation of resident microglia and the infiltration of hematogenous monocytes starts after a delay of 1-5 days and persists for a longer time period.[24, 30,34]

There have been previous attempts to specifically monitor these cellular responses in models of cerebral ischemia by use of cell-specific contrast agents.[15-19] Kleinschnitz C et al.[15] have shown that SPIO particle-labeled macrophages accumulate at the borderzone of a focal photothrombotic cerebral infarction as early as 5.5 days after stroke induction. Using a transient MCAO model, we could detect signal loss on T2*-weighted 3-D MR images in evolving ischemic lesions and the presence of numerous iron-laden cells in corresponding locations through Prussian blue staining of tissue sections. Macrophage recruitment occurred 3 or 4 days after reperfusion within a very tight time frame, which again confirmed the fact that transient MCAO results in an earlier influx of inflammatory cells than permanent MCAO.[35] If early recanalization is applied clinically, anti-inflammatory treatment might attenuate pathogenic processes responsible for delayed reperfusion cellular injury, and timing of neuroprotective anti-inflammatory therapy might be critical to prevent injury progression.

The previous study showed the spatiotemporal profile of SPIO distribution over 7

days in the ischemic rat brain after a single injection of SPIO particles, but SPIO cleared from the blood plasma after day 2.[16,17] To visualize macrophage infiltration *in vivo*, we systemically injected SPIO particles 24 hours before MR scans at various stages of infarct development, rather than applying iron particles at a fixed time point after reperfusion induction. Since iron particles are almost completely eliminated from the blood pool by macrophages after 24 hours, the time lapse between intravenous injection of SPIO particles and scanning is an important technical parameter. It is important to exceed the blood phase of the free contrast agent molecule and to allow sufficient time for the iron particles to accumulate within the lysosomes of the macrophages.[16,36] As a result, we conclude that SPIO distribution in the brains we studied reflects active recruitment and migration of blood-borne macrophages, suggesting successful *in vivo* monitoring of the inflammatory response dynamics of the reperfused brain.

Histopathologic analysis of serial sections at earlier stages of reperfusion revealed a larger number of ED-1-positive macrophages than iron-positive cells with Prussian blue stain in the ischemic brain. These findings indicate that SPIO-enhanced MR imaging allows the detection of a subpopulation of ED-1 positive infiltrating macrophages and depicts the actual migration of iron-laden monocytes from the circulation into the brain, probably arguing against passive diffusion of SPIO particles through a disrupted BBB and consecutive uptake by local phagocytes.[17]

We also sought to clarify the relationship between BBB breakdown and macrophage infiltration in transient cerebral ischemia, and our study demonstrates that these are unrelated events. SPIO particles did not passively diffuse through the defective BBB, but must have been phagocytosed by monocytes in the circulation and transported cell-bound into the brain during phases of active infiltration.[15,18] Reperfusion injury and hemorrhagic transformation have been known to share the common substrate of an abnormally permeable capillary bed resulting from a disruption of the BBB.[4] Although early BBB disruption has been associated with hemorrhagic transformation and poor clinical outcome, reperfusion injury does not directly mean BBB disruption; it is actually associated with inflammatory reactions under the influence of various cytokines. An experimental study revealed that reperfusion after short periods of ischemia (30 to 60 minutes) appears to be mainly complicated by secondary ischemic damage, whereas BBB damage associated with vasogenic edema becomes a dominant factor with longer occlusion times (2.5 hours).[37] Accordingly, the ischemic time of the brain and the timing of anti-inflammatory therapy are important considerations in the protection of the reperfused brain in acute stroke patients.

As a pathogenic mechanism of ischemic brain damage, neurons die via two different pathways under the influence of various neurotoxic factors: necrosis and apoptosis.[38,39] While necrosis represents passive degeneration of cells, apoptosis is an

active form of programmed suicidal cell death which is energy dependent. Previous neuropathologic studies have reported that apoptotic cell death after ischemia is spatially related to the presence of inflammatory cells in the cerebral ischemia, and it is assumed that inflammatory mechanisms might play an important role in the delayed increase of infarct volume.[19,40] Activated microglia cells, blood-derived monocytes, and T-lymphocytes are major sources of the cytotoxic molecules which promote delayed apoptosis of neurons and hence a spatial increase of infarct volume, despite restoration of cerebral perfusion.[30,34] In the transient ischemic model, it has also been reported that reperfused brain tissue expresses various degrees of positivity for apoptosis.[5] Further studies of the spatiotemporal relationship of apoptotic cell death to inflammatory responses in reperfusion brain injury are necessary.

Finally, there are a number of limitations to our study. One of most important technical limitations is that cellular damage results not only from reperfusion injury but also from the initial ischemic insult. Actually, it is very difficult to make a pure reperfusion model of transient ischemia *in vivo*. In addition, whereas recanalization with either spontaneous or drug-induced thrombolysis in humans is a comparatively slow process, there is a sudden restoration of blood flow in the rat suture model and the resulting early postischemic hyperperfusion may exacerbate the effects of ischemic-reperfusion brain injury. Dose of SPIO contrast agent is another important parameter requiring further consideration, because the blood half-life of the

compound increases with the dose administered.[25,36] Although we used the dose recommended for human MR imaging, the increase of blood half-life would increase the probability of SPIO uptake by cells from the mononucelar phagocytic system.

Ⅴ. CONCLUSION

To visualize macrophage infiltration in an experimental transient MCAO model, we systemically injected SPIO particles 24 hours before MR imaging. We showed that macrophages are involved in reperfusion brain injury after transient cerebral ischemia at early stages of infarct progress, and that SPIO-enhanced MR imaging could help to further elucidate the role of inflammatory cells in reperfusion cellular injury.

1. The accumulation of macrophages was visualized *in vivo* with SPIO-enhanced MR imaging in an experimental rat model of transient cerebral ischemia.

2. At early stages of infarct progress, macrophages seem to be transiently involved in reperfusion brain injury after transient cerebral ischemia. The macrophages showed a nonspecific spatial distribution at the center of the ischemic lesion, especially around striatal blood vessels.

3. Signal loss on T2*-weighted MR images in transient cerebral ischemia was caused by the presence of numerous iron-laden cells in corresponding locations as revealed by Prussian blue staining of tissue sections.

4. Although a high number of iron-containing cells were visible, the majority of ED-1-positive macrophages were not iron-positive.

5. SPIO-induced signal loss on MR imaging was independent from disturbance of the BBB.

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Abstract (in Korean)

일시적 뇌허혈 쥐모델에서 재관류 손상부위의 염증성 반응에 대한 영상 : SPIO 조영증강후 자기공명영상과 조직병리학적 소견의 비교

<지도교수 김동익>

연세대학교 대학원 의학과

김 진 아

급성 허혈성 뇌졸중 치료에 있어서 막힌 혈관을 재개통시켜 재관류를 유도하는 것은 허혈 조직의 세포 손상을 초래할 수 있으며 급성 염증성 반응이 이러한 재관류 조직 손상에 기여하는 것으로 알려져 있다. 본 연구에서는 일시적 뇌허혈 동물 모델에서 초상자성 철산화물 조영제를 이용한 자기공명영상을 통하여 생체 내에서의 대식세포 축적을 영상화할 수 있는지를 규명하며 초상자성 철산화물을 사용하여 얻은 자기공명영상에서 관찰되는 신호강도 변화 부위와 조직병리학적 소견을

비교하고자 하였다.

성인 쥐을 이용하여 중뇌동맥을 한 시간동안 일시적으로 폐색하였고 재관류 유도 후 각각 다른 시점에서 초상자성 철산화물 조영제을 주입하였으며 24시간 후에 경사 자장을 이용하여 삼차원 T2* 강조영상을 얻었다. 자기공명영상을 시행한 후에 표본을 얻었으며 대식세포를 발견하기 위해 Prussian blue 염색 및 면역조직화학염색을 시행하였다. 또한 조직학적 검사상 관찰된 철 성분을 T2* 신호강도 변화와 비교하였다.

재관류 후 3일과 4일째에 초상자성 철산화물 입자에 의한 신호강도 소실이 뇌손상 부위의 중앙에서 부분적으로 관찰되었으며 이러한 소견이 1일과 2일째에는 관찰되지 않았다. 신호강도 소실 부위는 조직학적 검사에서 철 성분이 적재된 대식세포의 분포 부위와 일치하였으며 이러한 초상자성 철산화물에 의한 신호강도 소실은 활성화된 대식세포가 재관류 뇌손상 부위로 이동했음을 시사한다.

결론적으로 초상자성 철산화물 조영증강 후 자기공명영상을 이용하여 조직 손상 초기에 대식세포가 재관류 뇌손상에 참여하는 것을 생체 내에서 관찰할 수 있었으며 이러한 연구가 재관류 뇌손상 부위에 관여하는 염증성 반응을 이해하는데 도움이 되리라 생각한다.

핵심되는 말: 급성 허혈성 뇌졸중, 재관류, 염증성 반응, 대식세포, 자기공명영상