Assessment of blood-brain barrier integrity by dynamic contrast enhanced MRI in transient middle cerebral artery occlusion model after localized brain cooling

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Assessment of blood-brain barrier integrity by dynamic contrast enhanced MRI in transient middle cerebral artery occlusion model after localized brain cooling

Directed by Professor Seung-Koo Lee

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<ABSTRACT>

Assessment of blood-brain barrier integrity by dynamic contrast enhanced MRI in transient middle cerebral artery occlusion model after localized brain cooling

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Introduction: Localized brain cooling before reperfusion reportedly helps to reduce the inflammatory response and recover the function of brain neurons in stroke therapy. Little is known about the effects of localized brain cooling on permeability changes associated with alterations to the blood-brain barrier (BBB).

Purpose: The purpose of this study was to evaluate the effects of localized brain cooling on BBB permeability following transient middle cerebral artery occlusion (tMCAO) in rats, by using dynamic contrast enhanced- (DCE-) MRI.

Materials and method: Thirty rats were divided into three groups (10 rats each): control group, localized cold-saline (20°C) infusion group, and localized warm-saline (37°C) infusion group. The left middle cerebral artery (MCA) was occluded for 1 h in anesthetized rats, followed by 3 h of reperfusion. In the localized saline infusion group, 6 mL of cold or warm saline was infused through the hollow filament for 10 min after MCA occlusion. DCE-MRI investigations were performed after 3 h and 24 h of reperfusion. Four pharmacokinetic parameters of the Tofts model (wash-in rate [K_{trans}], wash-out rate [K_{ep}], leakage-space volume [V_e], and plasma-space volume [V_p]) were

calculated for each DCE-MRI. In addition, rotarod testing was performed before tMCAO, and on days 1–9 after tMCAO. Myeloperoxidase (MPO) immunohistochemistry was performed to identify infiltrating neutrophils associated with the inflammatory response in the rat brain.

Result: There was a statistically significant decrease in K_{trans} and K_{ep} at the infarction site in the cold-saline group compared with the control group ($P < 0.05$) and a decrease in K_{ep} that approached significance in the cold-saline group compared with the warm-saline group (K_{ep}: cortex, $P = 0.0892$ basal ganglia, $P = 0.0925$). The percentage of MPO-positive cells in the cold-saline group was significantly lower than those in the control and warm-saline groups ($P < 0.05$). However, behavioral testing did not reveal a statistically significant difference among the three groups.

Conclusion: Localized brain cooling can inhibit the increase in BBB permeability that follows transient cerebral ischemia and reperfusion in an animal model.

Keywords: cerebral ischemia, middle cerebral artery, blood–brain barrier, permeability, magnetic resonance imaging

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I. INTRODUCTION

Hypothermia is very effective at preventing ischemia-induced neuronal damage.[1-7] However, the use of whole–body surface cooling for hypothermia therapy has been associated with management problems and complications such as pneumonia in 40% of patients.[8] Recently, localized brain cooling has been reported as a more effective technique than whole-body cooling, and cooling by carotid perfusion, localized low-heat ventricular perfusion, and localized scalp cooling have been shown clinically or experimentally to inhibit or at least delay neuronal damage.[3,9-14] It has been also reported that localized brain cooling before reperfusion significantly reduces the infarct area in multiple animal models of stroke.[15] Localized brain cooling following cerebral ischemia in animal models also markedly reduced inflammatory reactions and endothelial expression of intracellular adhesion molecule-1 (ICAM-1), which has been strongly associated with BBB leakage or breakdown of micro-vessels in ischemic brain tissue.[16-18] To our knowledge, there has been no study to date designed to evaluate the effects of localized brain cooling on BBB permeability following cerebral ischemia in a rat model. Quantitative permeability parameters using DCE-MRI have been assessed in

recent stroke studies related to BBB dysfunction.[19-23] The purpose of the current study was to investigate the effect of localized brain cooling on BBB permeability after transient focal cerebral ischemia–reperfusion in the rat using DCE-MRI.

II. MATERIALS AND METHODS

1. Subjects

A total of 30 Adult Sprague-Dawley rats (280–300 g) were used in the study. This animal study was approved by and performed in accordance with the institutional guidelines by Institutional Animal Care and Use Committee (IACUC) # 2013-0152.

A modified filament technique has been used to produce transient middle cerebral artery occlusion (tMCAO) in a rat model.[24] Rats were anesthetized by intraperitoneal injection of 2:3 mixtures of Rompun and Zoletil (0.3 mg), and the left external carotid artery was exposed. A length of 18.5–19.0 mm modified PE-50 catheter (with 0.2-mm outer diameter and 0.1-mm inner diameter) was inserted via the left external carotid artery (ECA) into the intracranial circulation. The filament was lodged in the narrow proximal anterior cerebral artery (ACA) and blocked the MCA at its origin (Fig 1). After 1 h of MCA occlusion, rats in the control group were re-anesthetized and reperfused for 3 h by withdrawal of the hollow filament from the left MCA.

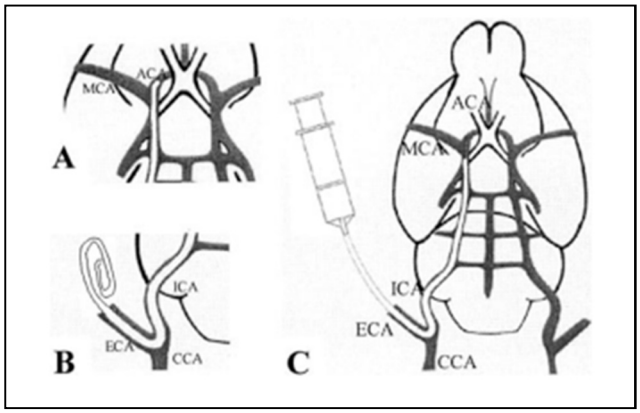

Figure 1: Schematic drawing of the rat model of remote MCA occlusion.
(A) A hollow filament is pushed into the proximal anterior cerebral artery (ACA) and the origin of the MCA is occluded. (B) The catheter is folded under the skin during 1 h

of MCA occlusion. (C) Using a micro-infusion pump, saline is injected posterior to the junction of the MCA and ACA, as the hollow filament is withdrawn 1-2 mm from the origin of the MCA.
ECA, external carotid artery; ICA, internal carotid artery.
(Drawings were used with permission from Ding et al[18] and Zhang et al.[25])

The animals were divided into control, cold-saline infusion, and warm-saline infusion groups, with 10 rats in each group. The rats in the control group did not receive any treatment. The rats in the cold-saline infusion group received an intra-arterial infusion of 6 mL of cold (20°C) saline to the brain after 1 h of MCA occlusion. The rats in the warm-saline infusion group were infused with 6 mL of warm (37°C) saline using the same method as the cold-saline group. The warm-saline infusion group served as another control group, to observe the effects of a localized saline infusion on brain injury resulting from transient ischemia and reperfusion.[16-18] In the rats treated with saline infusion, the catheter was withdrawn 1 mm from the origin of the MCA after 1 h of MCA occlusion. During and after withdrawal of the catheter, 6 mL of cold or warm saline was slowly and continuously injected into the junction of the MCA and ACA, using an infusion pump to maintain a rate of 0.6 mL over 10 min after the catheter was completely withdrawn and reperfusion established (approximately 0.25 mL/g brain tissue per min). Rectal temperature was maintained at 37°C using a warm pad during the surgical procedure. Brain temperature in the rats was monitored in the ipsilateral area supplied by the MCA. Needle thermistor probes (Harvard Apparatus) were placed into the ipsilateral cortex through a hole 3 mm lateral to the bregma and into the striatum through a hole 3 mm posterior and 4 mm lateral to the bregma.

2. MR Imaging Protocol

Animal MRI was performed using a 3.0-Teslar system (Achieva, Philips, Best, The Netherlands) with an 8-channel SENSE wrist coil. The first DCE-MRI was performed immediately after 3 h of MCA reperfusion. The second DCE-MRI was performed after 24 h of reperfusion.

Sequences included axial T2-weighted and T1-weighted images using the following parameters: FOV, 60 mm; matrix, 256 × 192; slice thickness, 2 mm. Acute ischemic lesions were identified on diffusion-weighted images (DWIs). DWI was performed with single-echo diffusion echo-planar imaging using the following parameters: FOV, 60 mm; matrix, 192 × 192; slice thickness, 2 mm; b-values, 0 and 600 along 3 orthogonal directions. DCE-MRI was performed as follows: FOV, 60 mm; matrix, 192 × 192; slice thickness, 4 mm; injection of gadolinium (0.2 mL/kg) through tail vein, 60 dynamic images during 6 min.

3. Post processing and image analysis

Permeability parameters (K_{trans}, K_{ep}, V_e, and V_p) were calculated using off-line PRIDE tools provided by Philips Medical System. This software was based on the pharmacokinetic model of Tofts.[26,27] The two-compartment model of Tofts assumes that the intravascular space and extravascular extracellular space (EES) are divided by the BBB. The degree of contrast leakage from the intravascular space to the EES is referred to as the volume transfer constant (K_{trans}), the reflux leakage of contrast from the EES to the intravascular space (plasma) is referred to as the rate constant (K_{ep}). The volume fractions of EES and plasma space are referred to as V_e and $V_{p,}$ respectively. These permeability parameters were calculated by means of iteration between time-intensity curves of artery and tissue using the assumptions of the Tofts model.[26,27] The arterial input function was measured at the right internal carotid artery and its time concentration curve was verified. MR imaging sequences (DWI/ADC and T2WI) were reviewed for the presence of ischemic lesions at each time point. To measure the permeability changes at the region of interest (ROI) of acute infarction in DCE-MRI, two different ROIs for permeability parameters were firstly placed in an enhancing portion of the infarct area of the cortex and basal ganglia in hemispheres ipsilateral to the MCA occlusion on DCE-MRI using a freehand technique. Secondly, two different ROIs were placed in the normal cortex and basal ganglia of the contralateral hemisphere to determine the baseline permeability parameters. Thirdly, the permeability of infarcted brain tissue was measured in the follow-up DCE-MRI in areas

corresponding to the first MRI. Serial changes in permeability were evaluated for the three ROIs in normal and infarcted areas on DCE-MRI.

4. Rota rod performance test

All rats were subjected to rotarod behavioral testing before tMCAO, and on days 1–9 after tMCAO, by an investigator who was blinded to the group assignments. For the rotarod test, the rat was placed on a rotarod cylinder, and the time the animal remained on the cylinder was measured (in seconds). The speed was slowly increased from 4 to 40 rpm within 5 min.[28] A trial ended if the animal fell off the rungs or gripped the device and spun around for two consecutive revolutions without attempting to walk on the rungs. The animals were trained for 3 days before tMCAO. The mean duration on the device was calculated from 10 trials conducted 1 day before surgery (the baseline value). Motor testing data were recorded for 9 days, and compared with the internal baseline control (before surgery).

5. Immunohistochemistry and histological assay

After the second MRI and rotarod testing were completed, the rats were sacrificed to obtain brain tissue. Rats were deeply anaesthetized and perfused with 0.9% sodium chloride followed by 4% paraformaldehyde. Following decapitation, brains were removed, fixed in 10% formalin, and embedded in paraffin. Coronal sections (5-μm thick) were stained with hematoxylin-eosin (HE). It has been reported that neutrophils are the first leukocyte subpopulation to be recruited to the ischemic brain, and an extensive infiltration of neutrophils has been observed 24 h after transient ischemic changes or infarct in rats, which was associated with BBB breakdown.[29,30] Immunohistochemistry for myeloperoxidase (MPO) was performed to identify infiltrating neutrophils on sections of rat brains subjected to no treatment vs. treatment (cold- or warm-saline infusion) after tMCAO, in order to detect BBB breakdown. Tissue sections were deparaffinized in xylene, rehydrated, and heated at 100 °C in citrate buffer (pH 6.0) for 5 min for antigen retrieval. The sections were incubated with a rabbit polyclonal antibody against myeloperoxidase (1:500 dilution; A0398, Dako, Glostrup, Denmark) for 1 h at room temperature, followed by incubation with

secondary antibody, donkey anti-rabbit IgG (1:500 dilution; Molecular Probes, Eugene, OR, USA) for 1 h at room temperature. Staining was developed by reaction with diaminobenzidine chromogen, and sections were counterstained with hematoxylin. For quantitative analysis of cell numbers in the infarcted regions, the slides were digitally photographed using a confocal microscope at a 400× magnification (BX50, Olympus, Tokyo, Japan). Ten fields of view were randomly chosen and photographed to count the number of MPO-positive cells in each section (Version 4.6, Spot Software, Diagnostic Instruments). All analysis was performed by a pathologist blinded to the treatment conditions.

6. Statistical analysis

The significant differences among permeability parameters between the three groups were firstly assessed using a mixed model. Correlations between permeability parameters (K_{trans}, K_{ep}, V_e, and V_p) for each group in ROIs in the cortex and basal ganglia were analyzed. Difference analysis between baseline control and mean duration (of 10 trials) at each time point was plotted for rotarod tests. The differences in MPO-positive neutrophil infiltration between the three groups were assessed using the Mann-Whitney *U* test. All statistical analyses were performed using the statistical software package, SPSS (version 21, SPSS Inc, IBM Company, Chicago, IL, USA). A *P*-value of less than 0.05 was considered statistically significant.

III. RESULTS

1. Brain temperature

The local brain temperature in the cortex and striatum supplied by the MCA remained unchanged from 37 °C in control and localized warm-saline infusion groups. Brain temperature in the localized cold-saline infusion group was reduced to 33–34 °C after 10 min of cold (20°C) saline infusion. After stopping the cold-saline infusion, the brain temperature gradually increased to 37°C after an average of 8 minutes. The rectal temperature was maintained at 37°C using a circulating heating pad.

2. Permeability parameters

Nineteen rats (5 for control, 8 for cold-saline, and 6 for warm-saline groups) from the original 30 rats successfully underwent surgical procedures, localized brain cooling, and all DCE-MRI, and were included for final analysis. The other 11 rats did not survive until the completion of the experiments.

All rats showed acute infarction in the left basal ganglia or cortex on DWI. The ROIs were placed in contrast-enhancing lesions of the left basal ganglia or cortex.

The control group showed acute infarction in the left basal ganglia and cortex on DWI and apparent diffusion coefficient (ADC) maps, corresponding to strong contrast enhancement (Figure 2 A, B, C, D) on MRIs performed immediately after 3 h of reperfusion. In the same regions, all permeability parameters (K_{trans}, K_{ep}, V_e, V_p) increased (Figure 2). The second MRI showed a mild increase in the extent of the infarcted area and degree of enhancement (Figure 2 I, J, K, L), and the second DCE-MRI also showed a persistent increase in permeability parameters in same areas after 24 h of reperfusion (Figure 2 M, N, O, P).

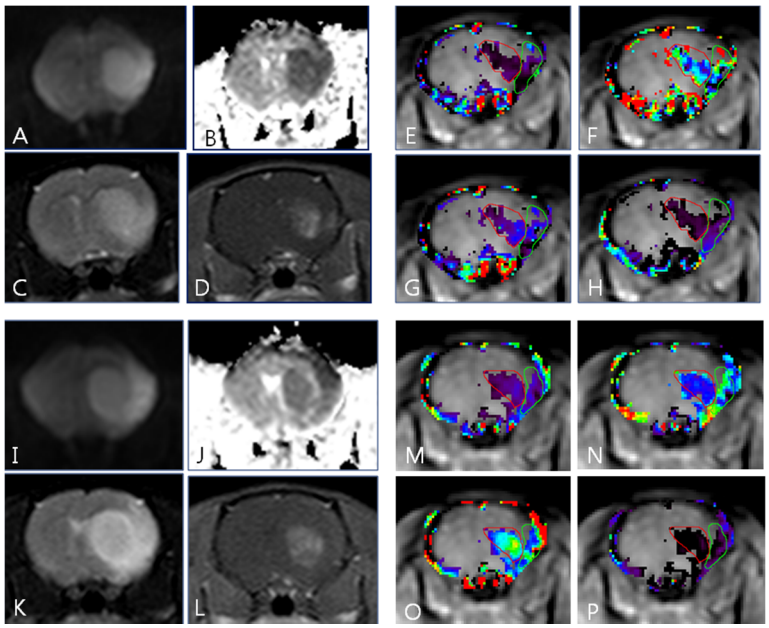

Figure 2: Control group showing DWI, ADC map, T2WI, contrast-enhanced T1-weighted image, and the corresponding permeability imaging.

Increased signal intensity on DWI, decreased ADC value, strong enhancement in the left basal ganglia and cortex, suggesting acute infarction detected on first MRI (A, B, C, D) after 3 h of MCA reperfusion. The first DCE-MRI shows an increase in permeability parameters (K_{trans} = 0.05, K_{ep} = 0.39, V_e = 0.14, and V_p = 0.04 in red-colored ROI; K_{trans} = 0.09, K_{ep} = 0.32, V_e = 0.12, and V_p = 0.09 in green-colored ROI), (E, F, G, H, serially). A further increase in enhancement and diffusion restriction was seen on second MRI (I, J, K, L) after 24 h of reperfusion, and permeability parameters also showed a persistent increase in the same areas on the second MRI (K_{trans} = 0.11, K_{ep} = 0.4, V_e = 0.43, and V_p = 0.05 in red-colored ROI; K_{trans} = 0.14, K_{ep} = 0.39, V_e = 0.61, and V_p = 0.06 in green-colored ROI) (M, N, O, P, serially).

A, I: Diffusion weighted image (DWI)
B, J: Apparent diffusion coefficient (ADC) map
C, K: T2-weighted image
D, L: Contrast-enhanced T1-weighted image
E, M: K_{trans} image
F, N: K_{ep} image
G, O: V_e image
H, P: V_p image

In the cold-saline infusion group, acute infarction was seen in the left basal ganglia and cortex on DWI and ADC maps (Figure 3 A-B). Strong enhancement appeared at sites of acute infarction on contrast-enhanced T1-weighted images and a marked increase in permeability was noted in the corresponding area on DCE-MRI (Figure 3 C-D, E-H). Although the DWI and ADC maps showed no significant interval change (Figure 3 I-J), there were marked decreases in enhancement and permeability parameters on the second MRI after 24 h of reperfusion, compared with the initial MRI study (Figure 3 K-L, M-P).

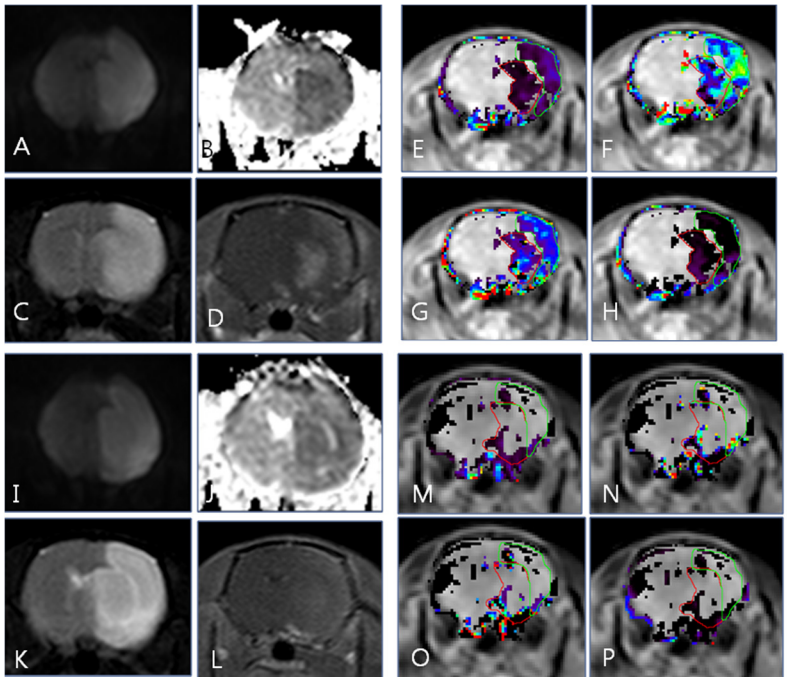

Figure 3: Cold-saline group with diffusion restriction and strong enhancement in the infarct area in the left basal ganglia and cortex on first MRI (A, B, C, D) after localized brain cooling and 3 h of MCA reperfusion. DCE-MRI (E, F, G, H, serially) shows an increase in permeability parameters (K_{trans} = 0.04, K_{ep} = 0.26, V_e = 0.2, and V_p = 0.02 in red-colored ROI; K_{trans} = 0.10, K_{ep} = 0.4, V_e = 0.26, and V_p = 0.02 in green-colored ROI). The second MRI shows a marked decrease in enhancement, compared with the first enhanced image and no significant interval change in diffusion restriction (I, J, K, L). Permeability parameters indicate a marked decrease (K_{trans} = 0.05, K_{ep} = 0.04, V_e = 0.24, and V_p = -0.01 in red-colored ROI; K_{trans} = 0.04, K_{ep} = -0.14, V_e = 0.12, and V_p = -0.01 in green-colored ROI) (M, N, O, P, serially).

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In the warm-saline infusion group, acute infarction was seen in the left basal ganglia and cortex on DWI and ADC maps (Figure 4 A-B). Contrast-enhanced T1-weighted images showed strong enhancement in the infarction areas and DCE-MRI showed increased permeability in the corresponding areas (Figure 4 C-D, E-H). The second MRI showed decreases in enhancement and permeability parameters for same area after

24 h of reperfusion (Figure 4 L-P).

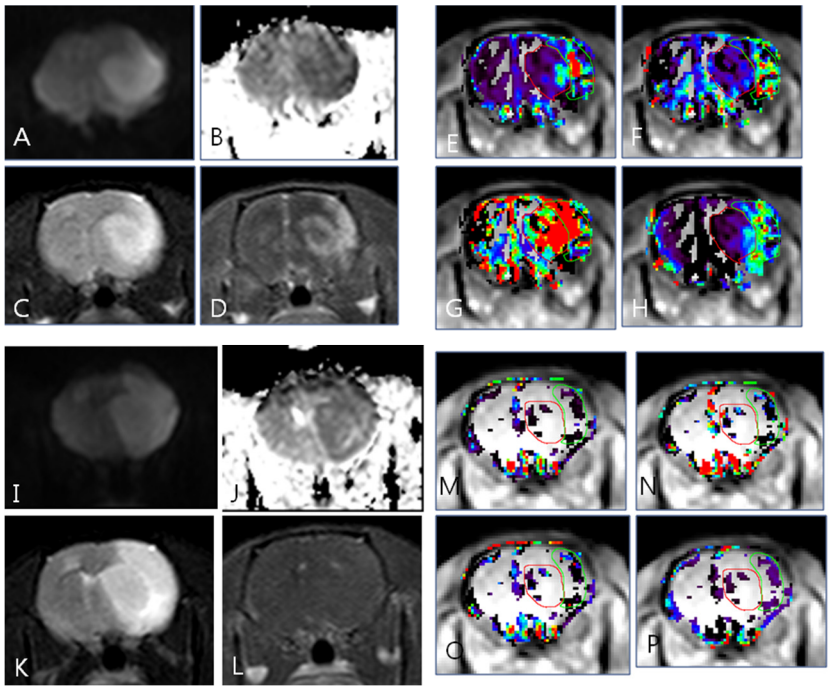

Figure 4: Warm-saline group with diffusion restriction and strong enhancement in the infarct area in the left basal ganglia and cortex on first MRI (A, B, C, D) after localized warm-saline infusion and 3 h of MCA reperfusion. DCE-MRI (E, F, G, H, serially) shows an increase in permeability parameters (K_{trans} = 0.17, K_{ep} = 0.46, V_e = 1.22, and V_p = 0.15 in red-colored ROI; K_{trans} = 0.53, K_{ep} = 0.38, V_e = 0.4, and V_p = 0.43 in green-colored ROI). The second MRI shows a decrease in enhancement and a mild increase in the extent of diffusion restriction in same area (I, J, K, L) after 24 h of reperfusion. Permeability parameters indicate a decrease (K_{trans} = 0.07, K_{ep} = 0.36, V_e = 0.21, and V_p = 0.04 in red-colored ROI; K_{trans} = 0.04, K_{ep} = 0.09, V_e = 0.31, and V_p = 0.1 in green-colored ROI) (M, N, O, P, serially).

DCE-MRI showed an increase in permeability parameters in the hemisphere ipsilateral to the MCA occlusion (Table 1).

Table 1. Permeability parameters after analysis of DCE-MRI

					Ktrans		Kep		Ve		Vp	
					Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
ROI	Cortex	Control	Time	1	0	0	0	0	0	0	0	0
				2	.15	.05	.74	.18	.15	.03	.02	.02
				3	.13	.03	.64	.13	.21	.02	-.01	.04
		Cold	Time	1	0	0	0	0	0	0	0	0
		saline		2	.09	.02	.36	.07	.38	.14	.07	.02
		(20°C)		3	.05	.01	.18	.09	.31	.07	.06	.02
		Warm	Time	1	0	0	0	0	0	0	0	0
		saline		2	.07	.01	.30	.07	.39	.15	.06	.02
		(37°C)		3	.09	.09	.58	.10	.35	.17	.03	.02
	Basal	Control	Time	1	0	0	0	0	0	0	0	0
	ganglia			2	.11	.04	.71	.07	.38	.14	.07	.02
				3	.12	.03	.52	.11	.28	.07	.02	.04
		Cold	Time	1	0	0	0	0	0	0	0	0
		saline		2	.06	.02	.37	.11	.25	.08	.04	.01
		(20°C)		3	.03	.01	.17	.13	.39	0.24	.04	.02
		Warm	Time	1	0	0	0	0	0	0	0	0
		saline		2	.09	.01	.45	.11	.25	.04	.03	.01
		(37°C)		3	.08	.03	.45	.08	.17	.03	.00	.01

Note: ROI refers to region of interest.

Time refers to 1=baseline; 2=after 3hours of MCA reperfusion; 3=after 24 hours of reperfusion

No cases showed an increase in permeability parameters in the contralateral (normal-appearing) hemisphere on DCE-MRI. Mixed model analysis was performed to compare differences in permeability parameters with treatment after 3 h of MCA reperfusion and 24 h of reperfusion. Although the number of samples was small for each group, data for each of the permeability parameters were normally distributed. Areas of contrast enhancement showed variable patterns for each group. Some rats showed dominant enhancement in the basal ganglia and others showed dominant enhancement in both the basal ganglia and cortex. To evaluate the patterns of changes in permeability parameters after reperfusion of the MCA, we used the average values for the basal ganglia and cortex.

In the cold-saline infusion group, serial follow-up of K_{trans} showed that it initially increased but later decreased in the ipsilateral cortex and basal ganglia. In the warm-saline infusion group, K_{trans} initially increased and showed a persistent increase or plateaued. In the control group, K_{trans} showed a marked increase initially and then a slight decrease or a persistent increase. K_{ep} demonstrated a similar pattern to K_{trans} in the cortex and basal ganglia. V_e and V_p showed no consistent pattern of change over follow-up (Figure 5).

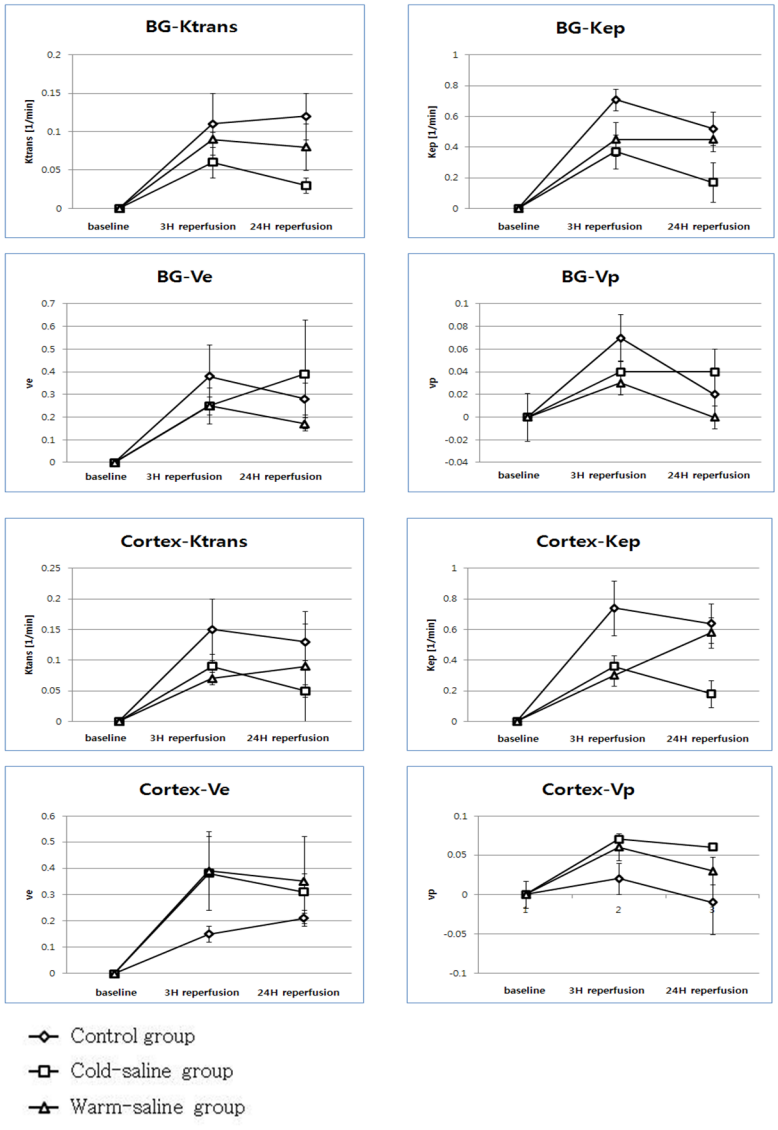

Figure 5: Sequential changes in permeability parameters after reperfusion. (Mixed model)

Among the permeability parameters, K_{trans} showed significant decreases in the ipsilateral cortex and basal ganglia in DCE-MRIs at 3 and 24 h in the cold-saline infusion groups, compared with the control group (0.0095 and 0.0003 respectively, $P < 0.05$) (Table 2). There was also a statistically significant decrease in K_{trans} in the ipsilateral cortex in the warm-saline group compared with the control group at 3 and 24 h (0.0166, $P < 0.05$) (Table 2). K_{ep} showed significant decreases in the ipsilateral cortex and basal ganglia in DCE-MRIs at 3 and 24 h in the cold-saline group (0.0002 and 0.0003 respectively, $P < 0.05$) and warm-saline group (0.0186 and 0.0489 respectively, $P < 0.05$), compared with the control group (Table 2). The K_{ep} of the cold-saline group showed decreases that approached significance in the infarcted areas of the ipsilateral cortex and basal ganglia at 3 h and 24 h follow-up compared with the warm-saline group (cortex: 0.0892, basal ganglia: 0.0925) (Table 2).

Table 2. Mixed model of permeability parameters

		group 1-2	P value <0.05	group 1-3	P value <0.05	group 2-3	P value <0.05
Ktrans	Cortex	0.0095	significant	0.0166	significant	0.6609	
	Basal ganglia	0.0003	significant	0.1054		0.4058	
Kep	Cortex	0.0002	significant	0.0186	significant	0.0892	borderline
	Basal ganglia	0.0003	significant	0.0489	significant	0.0925	borderline
Ve	Cortex	0.2317		0.2232		0.9173	
	Basal ganglia	0.8967		0.5409		0.3943	
Vp	Cortex	0.0648	borderline	0.3042		0.3806	
	Basal ganglia	0.5574		0.6213		0.2276	

Note: Group 1 refers to control; group 2=cold saline; group 3=warm saline

3. Rotarod test

Twenty-four hours after MCA occlusion, the rotarod performance decreased markedly in the three groups, but the changes in the cold-saline and warm-saline groups were insignificant compared with baseline values. Similarly, on days 6 and 9, there was a mild decrease in motor performance in the all groups (Figure 6).

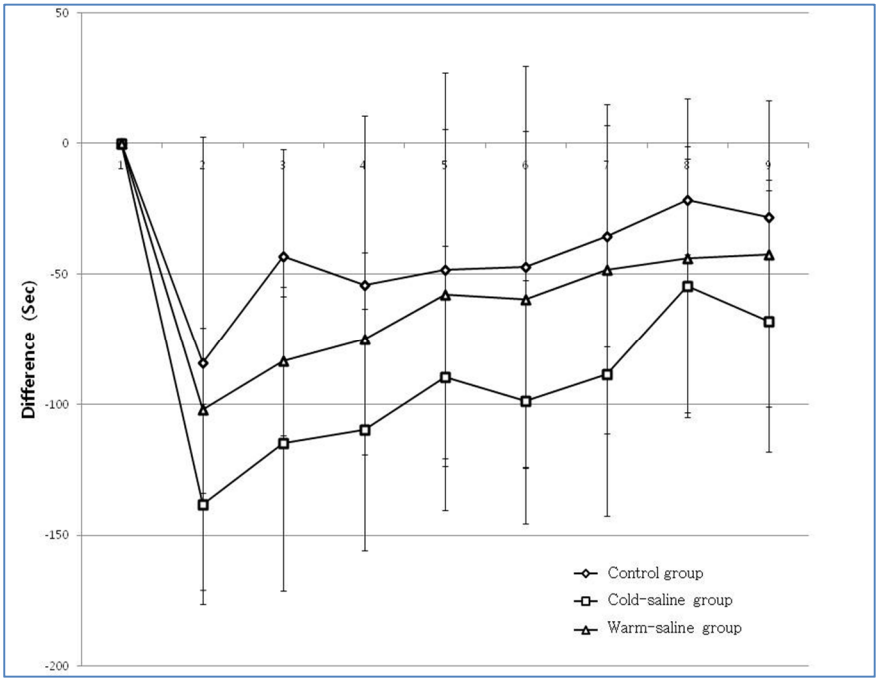

Figure 6: Differences in rotarod testing at each time point (on days 1–9 after tMCAO).

However, there were no significant differences in performance of the rotarod test between the three groups after reperfusion (Table 3).

Table 3.Rotarod test

	P value
Control vs cold saline group	0.2583
Control vs warm saline group	0.784
Cold saline group vs warm saline group	0.378

4. Histopathological and immunohistochemical findings

In the control group, areas of decreased hematoxylin and eosin (H & E) staining were seen in the left basal ganglia and cortex on photographs at low magnification (Figure 7 A). Multiple foamy macrophages and marked increases in the numbers of inflammatory cells were found in the control group (Figure 7 G). In the cold-saline infusion group, decreased H & E staining area was also noted in left basal ganglia and cortex (Figure 7 B). Compared with the control group, however, numbers of foamy macrophages and inflammatory cells were decreased in the cold-saline group (Figure 7 H). In the warm-saline infusion group, decreased H & E staining area was also observed in the left basal ganglia and cortex (Figure 7 C). Compared with the control group, numbers of foamy macrophages and inflammatory cells were also markedly decreased in the warm-saline group (Figure 7 I).

In immunohistochemical staining for MPO, infarction was revealed as areas of reduced staining area, which were consistent with areas showing reduced H & E staining in all groups (Figure 7 D-F). MPO-positive neutrophils infiltrated infracted areas surrounding brain microvessels (Figure 7 J-L). The overall numbers of inflammatory cells were 52.6 ± 27.3 (mean ± *SD*) in the control group, 7.2 ± 2.6 in the cold-saline group, and 29.4 ± 30.9 in the warm-saline group, respectively. The numbers of MPO-positive neutrophils were 37.8 ± 27.3 in the control group, 37.6 ± 11.5 in the cold-saline group, and 65.2 ± 44.5 in the warm-saline group, respectively.

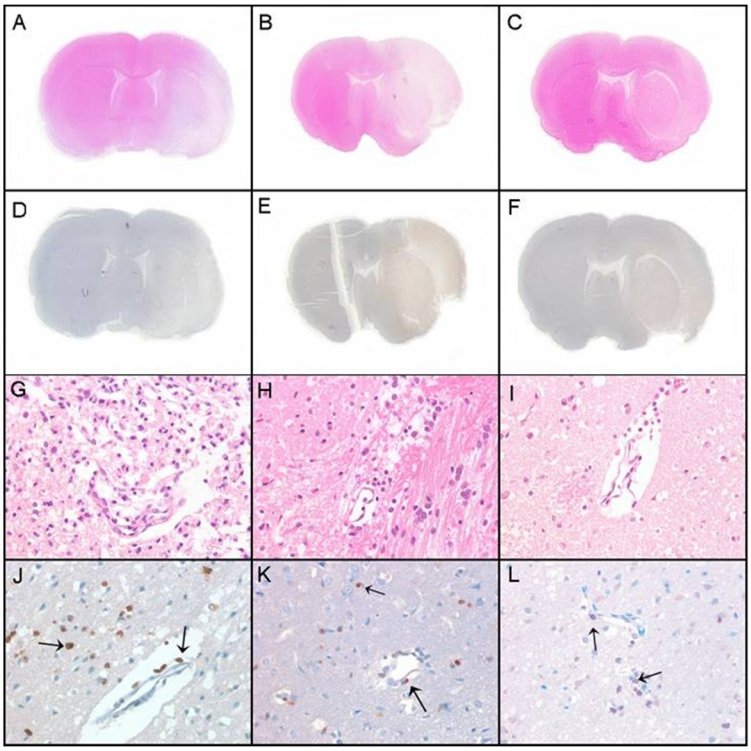

Figure 7. A-C: Scan magnified H&E images of rat brain from control (A), warm-saline (B), and cold-saline (C) groups. Images show reduced H & E staining in the left basal ganglia and cortex of the left MCA territory. D-F: Scan magnified MPO-immunostained images of rat brain in control (D), warm-saline (E), and cold-saline (F) groups. Images show reduced MPO immunostaining in the left basal ganglia and cortex of the left MCA territory. G-I: High-power views (400×) of the H & E-stained sections of the rat brain in control (G), warm-saline (H), and cold-saline (I) groups. (G) In the control group, multiple foamy macrophages and an infiltration of inflammatory cells are seen in the perimicrovascular area. (H) In the warm-saline group, foamy macrophages and inflammatory cells were also decreased compared with the control group. (I) In the cool-saline group, foamy macrophages and inflammatory cells were markedly decreased compared with the control group. J-L: High-power views (400×) of MPO-immunostained sections of the rat brain in control (J), warm-saline (K), and cold-saline (L) groups. (J) In the control group, MPO-positive neutrophils (arrow) infiltrate the perimicrovascular area. There are significant decreases in the number of MPO-

positive cells (arrows) in warm-saline (K) and cold-saline (L) groups compared with the control group.

The percentage of the total number of inflammatory cells that are MPO-positive cells was found to be significantly decreased in the cold- and warm-saline infusion groups compared with the control group ($P = 0.008$ and $P = 0.032$, respectively). Notably, the percentage of MPO-positive cells in the cold-saline group was significantly lower than that of the warm-saline group ($P = 0.009$) (Figure 8).

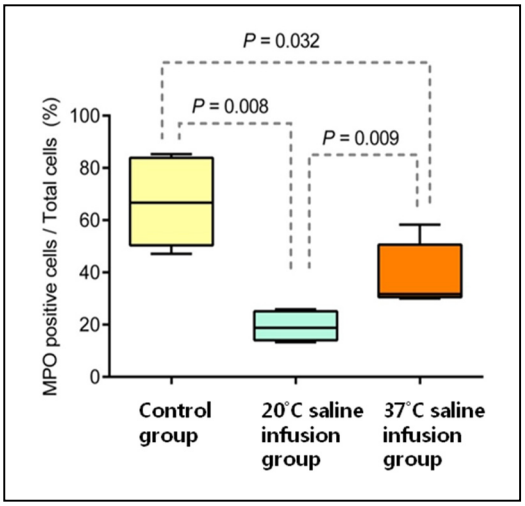

Figure 8. Comparison of the percentage of MPO-positive cells in the total mixed population of inflammatory cells. Each box plot represents the mean and standard deviation and the line through the box plot indicates the range. The percentage of MPO-positive cells is significantly higher in the control group than in the cold-saline and warm-saline groups. In addition, the percentage of MPO-positive cells in the cold-saline group is significantly lower than that of the warm-saline group.

IV. DISCUSSION

Brain hypothermia can reduce the size of the infarct area and damage due to cerebral ischemic after tMCAO in an animal model.[12] Although the exact mechanisms underlying this treatment are not completely understood, it is generally accepted that

brain hypothermia has protective effects against BBB disruption.[31] However, some papers have suggested that the post-ischemic protective effects of brain hypothermia may be related to other mechanisms, such as the reduction of leukotrienes, improvements in glucose utilization and blood flow, and slowing of reactions involving free radicals and the propagation of lipid-peroxidation cascades. [31,32] Physiological parameters of metabolic rate and cerebral blood flow couple over a wide range of temperatures, uncoupling only at <20°C.[33,34] Gadolinium-DTPA does not cross an intact BBB and can therefore be used to detect BBB disruption. After focal cerebral ischemia or infarction, the disturbance of BBB integrity has been confirmed by Gadolinium-DTPA-enhanced T1-weighted image.

Various perfusion MRI techniques have been implemented. Among them, dynamic susceptibility contrast-enhanced MRI (DSC-MRI) using T2 or T2* effects of the contrast medium can produce perfusion imaging with a single contrast injection, as well as effective information on permeability in delayed images.[35-37] However, DSC-MRI exhibits a degree of T1 sensitivity and shows signal changes derived from relaxivity effects, especially from contrast agents leaking from the EES.[38,39] The effects of extravascular leakage of contrast material on the T2 or T2* signal of DSC-MRI are difficult to estimate and, even if it were possible to measure the contrast transfer coefficient, this is less reliable than for DCE-MRI.[40] DCE-MRI can minimize the distortion of the structures around large blood vessels where contrast agents are especially concentrated, because the paramagnetic recruitment effect is small.[38] DCE-MRI can meet the requirements for reliable dynamic imaging of enhancing brain lesions in BBB disruption due to the contrast pre-enhancement techniques available in DCE-MRI and low T1 sensitivity-based sequences. DCE-MRI is primarily recommended for pharmacodynamic assessment of antiangiogenic and antivascular therapies associated with BBB breakdown, and compared with DSC-MRI, this method is ideal for the quantification of permeability parameters.[38-45]

K_{trans} refers to the volume transfer constant of the contrast leakage from blood vessels into the EES. K_{ep} refers to the rate constant for reflux of contrast leakage from the EES

into the intravascular space. These permeability-related parameters were proposed as indicators of the mobility between the two spaces. We have tried to evaluate the changes to BBB permeability-related parameters using DCE-MRI in an animal model following localized brain cooling therapy for ischemia-infarction.

In our study, the contrast enhanced T1-weighted image 24 h after tMCAO showed persistent enhancement in the infarcted areas of rat brains in the control group. On the other hand, contrast enhancement was decreased in both localized saline groups. In addition, we observed a significant decrease in K_{trans} and K_{ep} values, and in the number of inflammatory cells in serial follow-up investigations in the cold- and warm-saline infusion groups compared with the control group. These results suggest that localized saline infusion therapy has exerted protective effects on BBB integrity in the rat brain during ischemia-reperfusion.

Cytotoxic edema is the predominant type of edema at acute infarction.[46] Subsequently, brain ischemia and hypoxia would deteriorate due to brain swelling and intracranial hypertension, which would cause neuron dysfunction and further aggravated the cytotoxic brain edema. At this time, cytotoxic brain edema would predominate, and extracellular extravascular space was to be decreased. However, cytotoxic edema alone is not enough to aggravate the lesion because water content is not increased, When BBB opening occurs, water is driven from the vessels into the extracellular extravascular space and then enters the cells.[47] Finally, cytotoxic edema would diminish due to neuron necrosis and cell collapse. In our study, V_e of control group in ipsilateral cortex showed decreased value compared with cold and warm saline group and serial change of V_e in cold-saline group showed persistent increase at ipsilateral basal ganglia. So BBB stability was estimated to affect the expansion and contraction of the capacity of V_e. Thus, the BBB may be a target for treatments to relieve brain edema and swelling.

In the comparisons between cold-saline and warm-saline groups, a significant decrease in numbers of inflammatory cells was observed in the area affected by ischemia on 3 h and 24 h follow-up in the cold-saline group compared with the warm-saline group. However, there was no statistically significant difference in parameters

related to BBB permeability between the cold- and warm-saline groups. There are several possible explanations for these effects. Firstly, these findings suggest that the contrast leakage was reduced by a more stable BBB in vessels after localized infusion of either cold or warm saline to the ischemic area. We suspect that the warm-saline infusion also had a cooling effect on local brain tissue. Some papers have reported that the infusion of saline into the ischemic territory plays a very important role in the prevention of reperfusion injuries in addition to the effects of hypothermia.[48] Infusion with saline at 37°C can reduce the expression of intercellular adhesion molecule 1 (ICAM-1) and leukocyte infiltration into the ischemic area. ICAM-1 is one of the mediators of the acute inflammatory reaction that causes circulating leukocytes to infiltrate the ischemic brain parenchyma. Secondly, the duration of localized brain cooling (10 min) and the follow-up period (9 days) might have been too short for the specific effects of cooling to be observed.

Most animal experiments have been based on the assumption that the timing of the therapeutic window for mild hypothermia after tMCAO is critical. Several studies in animals and humans investigating systemic hypothermia and localized brain cooling have indicated a cooling duration of only 10 min, because oxygen extraction remains unchanged and investigators need to consider the feasibility and safety of the experiment.[6,33,34,49] Our study was based on a similar hypothesis when planning the animal experiment, and it was difficult to determine the exact time window for localized intra-arterial hypothermia. One report has indicated that localized cold-saline infusion (20 °C) for 2.5 h after brain ischemia significantly reduces infarction volume whereas reperfusion without localized cold-saline infusion does not.[50] The therapeutic time for localized saline infusion could be extended at least to 30 minutes.

In the current study, the results of the rotarod test showed no significant differences among the three groups. A previous study comparing head cooling, systemic whole-body cooling, and vascular cooling reported that vascular cooling led to the most improvements in animal exercise testing.[51] When interpreting the differences between those results and ours, it should be kept in mind that exercise testing in the previous

study was performed 14 and 28 days after the surgical procedure. By contrast, we have tried to compare the difference between our three groups during the first 9 days of the acute period of infarction, and therefore a direct comparison may not be possible. The reduction in exercise capacity after infarction may be related to a variety of reactions such as rapid brain swelling, destruction of axons, and cerebral hemorrhage, as well as BBB breakdown, and therefore there may be no significant correlation between the results of early exercise testing and the stability of the BBB. In our study, rats in the cold-saline infusion group showed a rapid decline in rotarod testing after tMCAO. A possible explanation is that a few rats with good exercise capacity among the randomly selected rats may have suffered greater damage after infarction, but in the recovery time over 9 nine days, they did not show significant differences compared with the other rats.

We acknowledge several limitations to our study. A major weakness is the limited number of animal subjects used in the experiments. Therefore, further investigations with a large number of animals will be necessary in the future. Secondly, we employed a clinical whole-body 3.0-Tesla MRI scanner and 8-channel wrist coil for DCE-MRI analysis of contrast leakage from blood vessels using the pharmacokinetic model in this study, instead of a higher field-strength MRI unit dedicated to animal experiments. Thirdly, the ROI was drawn manually on DCE-MRI images in contrast enhancing areas of the basal ganglia and cortex. It was relatively easy to select ROIs in the contrast enhancing area of the basal ganglia. On the other hand, the cortex was located peripherally and tended to show fine and irregular enhancement, and consequently there was some difficulty in drawing ROIs. Therefore, ROIs were drawn several times in the cortex of each subject, and the average value was calculated. Lastly, we were limited by a general lack of knowledge regarding delayed or secondary results when we selected the temperature of 20 ℃ for the cold saline to make a clear distinction from the warm-saline group. Further investigations will be required in future clinical practice to decide the optimal temperature for localized brain cooling in order to ensure the most viable and efficient results.

V. CONCLUSION

In summary, we demonstrated that localized brain cooling has a protective effect on BBB integrity following transient focal cerebral ischemia and reperfusion in rats. These data raise the intriguing possibility that localized brain cooling may play an important role in reducing ischemic neuronal damage and provide a potential therapeutic approach in patients with cerebral infarction.

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<ABSTRACT (IN KOREAN)>

일과성 중간 대뇌 동맥 폐색 쥐 모델에서 국소 뇌 냉각 후 역동성 조영 증강 자기공명영상을 이용한 혈액 뇌 장벽의 투과성 평가

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김은수

서론: 국소 뇌 냉각 치료는 재 관류 전에 시행하면 뇌졸증 치료에서 뇌염증반응을 감소시키고 뇌신경세포의 기능을 복구하는 것을 돕는다. 그러나 아직까지 국소 뇌 냉각치료가 혈액-뇌 장벽(BBB) 변화와 관련된 투과성 변화에 어떤 영향을 미치는 지에 대해서는 알려진 바가 거의 없다.

목적: 그래서 이 연구의 목적은 역동적 조영 증강 자기공명영상을 이용하여 일시적 중대뇌동맥폐색을 일으킨 쥐들을 대상으로 국소 뇌 냉각치료가 혈액-뇌 장벽 (BBB) 투과율에 어떤 영향을 주는지에 대해서 알아보고자 한다.

재료 및 방법: 서른 마리의 쥐들을 10마리씩 대조군, 국소 냉 식염수 (20 °C) 주입 그룹 그리고 국소 온 식염수(37 °C) 주입 그룹 세 군으로 나누었다. 대조군의 마취된 쥐에서 왼쪽 중대뇌동맥을 1시간 동안 폐색시킨 다음 3시간 동안 재관류을 시행하였다. 국소 식염수주입그룹에서는 중대뇌동맥 폐색 후에 10분 동안 유공 필라멘트를 통해 6ml의 냉 혹은 온 식염수를 주입하

였다. 역동적 조영 증강 자기공명영상을 재관류 3시간과 24시간 후에 시행하였다. Toft 모델의 네 가지 약물 동태학적 매개변수들 (wash-in rate [K_{trans}], wash-out rate [K_{ep}], leakage-space volume [V_e], and plasma-space volume [V_p])을 각각의 역동적 조영 증강 자기공명영상를 찍은 다음 산출하였다. 또한, 로타로드 행동검사를 일시적 중대뇌동맥폐색 전에 시행하고, 일과성 중대뇌동맥폐색 후에 1일에서 9일 동안 계속 시행하였다. 마이엘로퍼옥시다제(MPO)면역 조직 화학 염색은 쥐의 뇌염증반응과 관련된 호중구 침투을 식별하기 위해 수행되었다.

결과: 대조군과 비교하여 국소 냉 식염수 주입그룹에서 뇌 경색부위에서 K_{trans} 와 K_{ep}의 통계적으로 유의한 감소가 있었으며 ($P < 0.05$), 온 식염수 주입그룹과 비교하여 냉 식염수 주입그룹에서 K_{ep}의 유의한 통계학적 접근을 보이는 감소가 있었다 (K_{ep}: cortex, $P = 0.0892$ basal ganglia, $P = 0.0925$).

냉 식염수 주입그룹의 MPO-양성세포의 비율은 대조군과 온 식염수 주입그룹보다 통계학적으로 유의하게 낮았다($P < 0.05$). 그러나 행동실험은 세 집단간에 통계적으로 유의한 차이를 보이지 않았다.

결론: 국소 뇌 냉각 치료는 일과성 대뇌 허혈 및 재관류를 시행한 동물모델에서 BBB투과성의 증가를 억제할 수 있다.

핵심되는 말 : 뇌허혈, 중대대뇌동맥, 혈액 뇌 장벽, 투과성, 자기공명영상.