

Matrix Metalloproteinase

Effect of Brain Hypothermia on Ischemic Injury and Matrix Metalloproteinase Activity in Focal Cerebral Ischemia

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Background: Effects of hypothermia have been demonstrated in experimental animals with cerebral ischemia. Although the exact mechanisms of hypothermic effects are unknown, decrease of the blood-brain barrier (BBB) permeability, inhibition of free radical generation, and anti-inflammatory actions have been suggested. Matrix metalloproteinases (MMPs) may play a role in the hypothermic effect because they are known to mediate neuro-inflammatory responses and BBB breakdown. **Methods:** The rats were subjected to 2 hours of middle cerebral artery occlusion (MCAO) and 18 hours of reperfusion. The normothermia group was maintained at 37°C. Moderate hypothermia was induced by maintaining the temperature at 32°C during MCAO in the intra-ischemic hypothermia (IH) group, and for 2 hours immediately after reperfusion in the postischemic hypothermia (PH) group. The infarction size in coronal slices were measured after 2,3,5-triphenyltetrazolium chloride stain. By gelatin zymography, the activity of MMP-2 and MMP-9 was measured. Evans blue extravasation methods were used to determine the BBB disruption. **Results:** The infarction size was significantly decreased in the IH group compared to the normothermia group. The MMP-2 and MMP-9 activities in the ischemic hemispheres was significantly decreased in the IH group compared to the normothermia group. In the PH group, only the MMP-2 activity was significantly decreased compared to the normothermia group. Infarction size was significantly correlated with MMP-2 ($r=0.688$), and MMP-9 ($r=0.707$) activities. The disruption of BBB was significantly reduced in the IH group compared to the normothermia group. **Conclusions:** Intra-ischemic hypothermia effectively attenuated ischemic injury. Hypothermia-mediated inhibition of MMP-2 and MMP-9 activities may contribute to this protective effect.

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가	2	18	(10 μ) 가	bovine gamma globulin standard Bradford (Bio - Rad Laboratories, Hercules, CA, U.S.A.)
가		20		Gelatin zymography MMP
2.				gelatin - sepharose 4B (Pharmacia Biotech, Uppsala, Sweden) MMP ³² Gelatin - sepharose 4B working buffer 200 μ 3 260 μ 50 μ
(brain temperature)				gelatin - sepharose 4B 1 7000 rpm 5 working buffer 200 μ elusion buffer (10% DMSO 가 working buffer) 70 μ 30
(Precision 4000A Thermometer, YSI, Japan)				80 gelatin zymography
control unit)	(Homeothermic blanket 36.5			5. Gelatin zymography
		32		Gelatin zymography ²⁰ , 70 μg
2				(80 mM Tris - HCl [pH 6.8], 4% sodium dodecyl sulfate [SDS], 10% glycerol, 0.01% bromophenol blue)
3.				recombinant MMP - 2 MMP - 9 (Gelatinase zymography standards, Chemicon International Inc, CA, U.S.A.) 1 μg 1% gelatin
18		가		8% SDS - polyacrylamide 150 ml 2.5% Triton
		brain matrix		X - 100 15 3
		2 mm 7		250 ml 50 mmol/L Tris - HCl (pH 7.5, 10 mM CaCl ₂ , 0.02% NaN ₃) 37 42
		1, 3, 5, 7		
2% 2,3,5 - triphenyltetrazolium chloride (TTC)				
가 37 30			가 1:3:6	0.1% amido black
			1	amido black 130
compound (Miles, Inc., Elkhart, IN, USA)		Tissue - Tek OCT		(Scanmaker 9600XL, 600 dpi
methylbutane		2 -		Microtek, Taiwan)
80				Scion Image program gel plotting macro integrated den -
4.	MMP			sity
		10 μm cryostat		6. 가
45~50 1 mM phenylmethyl sulfonyl fluoride가				TTC 1, 3, 5, 7 1200 dpi Scion Image program
400 μ working buffer (50 mM Tris - HCl [pH 7.5], 150 mM NaCl, 5 mM CaCl ₂ , 0,05% BRIJ - 35, 0.02% NaN ₃ , 1% Triton X - 100)				gelatin zymography 4
4 20 9000 rpm				
		80		

2. TTC

3, 5, 7

4

가

($r=0.934$). 1, 3, 5,

7

($10.2 \pm 1.5\%$) 가

($20.0 \pm 2.0\%$) ($22.7 \pm 2.2\%$)

(Fig. 2). 가

4

($15.7 \pm 2.2\%$) 가

($27.9 \pm 3.4\%$) ($30.9 \pm 3.1\%$)

가

3. MMP - 2 MMP - 9

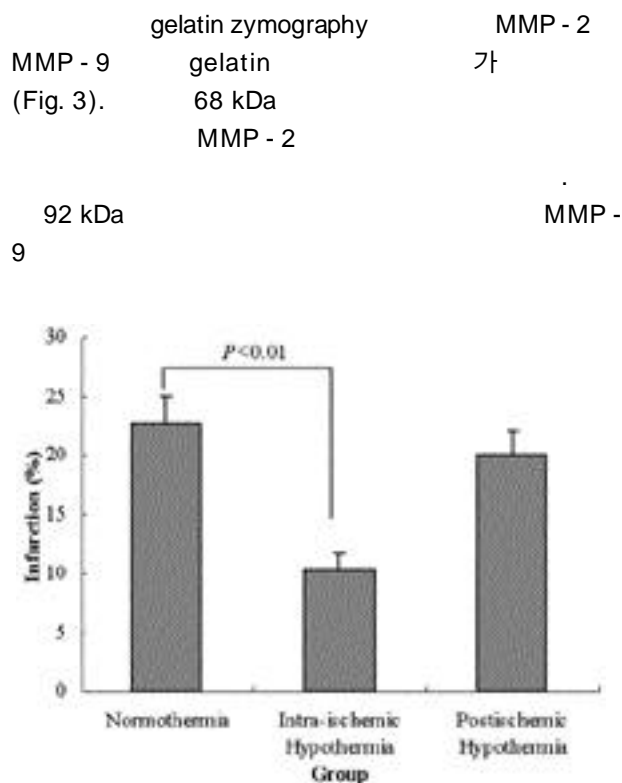


Figure 2. Infarction size of the different study groups. The percent of infarctions in ischemic hemispheres is significantly decreased in intra-ischemic hypothermia group compared to

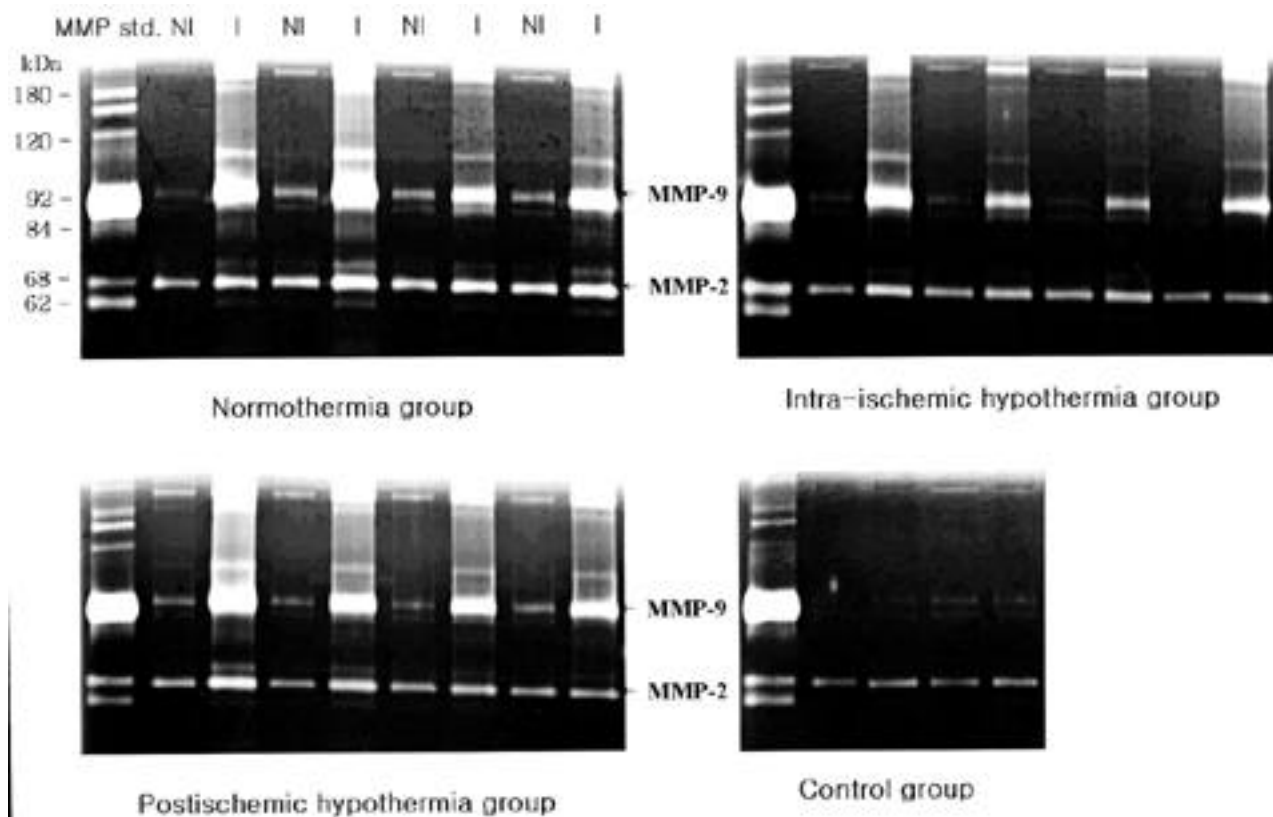


Figure 3. Gelatin zymograms of brain tissues. The MMP-2 and MMP-9 activities are increased in the ischemic hemisphere compared to the non-ischemic hemisphere. MMP std; recombinant MMP-2 and MMP-9 standard, NI; non-ischemic, I; ischemic, MMP; matrix metalloproteinase

(Fig. 5).
 tion type IV collagen, fibronectin, laminin,
 proteoglycans heparan sulfates
 basal lamina . MMP

가
 23,25 - 28
 MMP가 가
 19,22,36
 MMP 가
 가 29
 가
 MMP - 2
 , MMP - 9
 가
 (ischemic
 MMP - 9
 penumbra)
 2,33
 MMP
 MMP
 가 11.9%
 25,27,28
 MMP
 gelatin sepharose
 gelatin zymography
 MMP - 2
 MMP - 9
 가
 MMP가
 21,32
 , MMP
 가 , MMP - 9
 NF - kappa B AP - 1
 가 MMP - 2 AP - 2
 가 NF - kappa B
 AP - 1 가
 MMP - 2 MMP - 9 가
 c - fos, c -
 jun immediate early gene, lipopolysaccharide,
 , tumor necrosis factor - , interleukin - 1
 37 - 39
 MMP 가
 , NF - kappa B, AP - 1
 MMP - 2 MMP - 9
 40 - 42
 MMP 가
 MMP
 MMP가
 가
 MMP가 가
 MMP가 가
 가
 tight junc - 가
 MMP - 2

MMP - 9

가

REFERENCES

1. Kato H, Kogure K. Biochemical and molecular characteristics of the brain with developing cerebral infarction. *Cell Mol Neurobiol* 1999;19:93-108.
2. Mostardini C, Vicenzini E, Altieri M, Di Stani F, Lenzi D, Di Piero V. Neuroprotection and stroke. *Cerebrovasc Dis* 2000;10:21-23.
3. Venti M, Parnetti L, Silvestrelli G, Gallai V. Role of neuroprotective drugs in acute ischemic stroke. *Cerebrovasc Dis* 2000;10:24-26.
4. Lutsep HL, Clark WM. Current status of neuroprotective agents in the treatment of acute ischemic stroke. *Curr Neurol Neurosci Rep* 2001;1:13-18.
5. Martinez-Vila E, Sieira PI. Current status and perspectives of neuroprotection in ischemic stroke treatment. *Cerebrovasc Dis* 2001;11:60-70.
6. Hamann GF, Okada Y, Fitridge R, del Zoppo GJ. Microvascular basal lamina antigens disappear during cerebral ischemia and reperfusion. *Stroke* 1995;26:2120-2126.
7. Hamann GF, Okada Y, del Zoppo GJ. Hemorrhagic transformation and microvascular integrity during focal cerebral ischemia/reperfusion. *J Cereb Blood Flow Metab* 1996;16:1373-1378.
8. Matrisian LM. Metalloproteinases and their inhibitors in matrix remodeling. *Trends Genet* 1990;6:121-125.
9. Murphy G, Willenbrock F, Crabbe T, O'Shea M, Ward R, Atkinson S, et al. Regulation of matrix metalloproteinase activity. *Ann N Y Acad Sci* 1994;732:31-41.
10. Baramova E, Foidart JM. Matrix metalloproteinase family. *Cell Biol Int* 1995;19:239-242.
11. Birkedal-Hansen H. Proteolytic remodeling of extracellular matrix. *Curr Opin Cell Biol* 1995;7:728-735.
12. Ahrens D, Koch AE, Pope RM, Stein-Picarella M, Niedbala MJ. Expression of matrix metalloproteinase 9 (96-kd gelatinase B) in human rheumatoid arthritis. *Arthritis Rheum* 1996;39:1576-1587.
13. Bellosta S, Via D, Canavesi M, Pfister P, Fumagalli R, Paoletti R et al. HMG-CoA reductase inhibitors reduce MMP-9 secretion by macrophages. *Arterioscler Thromb Vasc Biol* 1998;18:1671-1678.
14. Nakagawa T, Kubota T, Kabuto M, Sato K, Kawano H, Hayakawa T, et al. Production of matrix metalloproteinases and tissue inhibitor of metalloproteinases-1 by human brain tumors. *J Neurosurg* 1994;81:69-77.
15. Anthony DC, Ferguson B, Matyzak MK, Miller KM, Esiri MM, Perry VH. Differential matrix metalloproteinase expression in cases of multiple sclerosis and stroke. *Neuropathol Appl Neurobiol* 1997;23:406-415.
16. Friedberg MH, Glantz MJ, Klempner MS, Cole BF, Perides G. Specific matrix metalloproteinase profiles in the cerebrospinal fluid correlated with the presence of malignant astrocytomas, brain metastases, and carcinoma-tous meningitis. *Cancer* 1998;82:923-930.
17. Matsuura E, Umehara F, Hashiguchi T, Fujimoto N, Okada Y, Osame M. Marked increase of matrix metalloproteinase 9 in cerebrospinal fluid of patients with fungal or tuberculous meningoencephalitis. *J Neurol Sci* 2000;173:45-52.
18. Rosenberg GA, Navratil M, Barone F, Feuerstein G. Proteolytic cascade enzymes increase in focal cerebral ischemia in rat. *J Cereb Blood Flow Metab* 1996;16:360-366.
19. Romanic AM, White RF, Arleth AJ, Ohlstein EH, Barone FC. Matrix metalloproteinase expression increases after cerebral focal ischemia in rats: inhibition of matrix metalloproteinase-9 reduces infarct size. *Stroke* 1998;29:1020-1030.
20. Heo JH, Lucero J, Abumiya T, Koziol JA, Copeland BR, del Zoppo GJ. Matrix metalloproteinases increase very early during experimental focal cerebral ischemia. *J Cereb Blood Flow Metab* 1999;19:624-633.
21. Gasche Y, Fujimura M, Morita-Fujimura Y, Copin JC, Kawase M, Massengale J, et al. Early appearance of activated matrix metalloproteinase-9 after focal cerebral ischemia in mice: a possible role in blood-brain barrier dysfunction. *J Cereb Blood Flow Metab* 1999;19:1020-1028.
22. Lapchak PA, Chapman DF, Zivin JA. Metalloproteinase inhibition reduces thrombolytic (tissue plasminogen activator)-induced hemorrhage after thromboembolic stroke. *Stroke* 2000;31:3034-3040.
23. Maier CM, Ahern K, Cheng ML, Lee JE, Yenari MA, Steinberg GK. Optimal depth and duration of mild hypothermia in a focal model of transient cerebral ischemia: effects on neurologic outcome, infarct size, apoptosis, and inflammation. *Stroke* 1998;29:2171-2180.
24. Schwab S, Schwarz S, Spranger M, Keller E, Bertram M, Hacke W. Moderate hypothermia in the treatment of patients with severe middle cerebral artery infarction. *Stroke* 1998;29:2461-2466.
25. Huh PW, Belayev L, Zhao W, Koch S, Busto R, Ginsberg MD. Comparative neuroprotective efficacy of prolonged moderate intraischemic and postischemic hypothermia in focal cerebral ischemia. *J Neurosurg* 2000;92:91-99.
26. Jiang MZ, Koo JS, Yoon BW, Roh JK. Combined effect of hypothermia and hyperglycemia on transient focal cerebral ischemia of the rat. *J Korean Neurol Assoc* 2000;18:748-753.
27. Maier CM, Sun GH, Kunis D, Yenari MA, Steinberg GK. Delayed induction and long-term effects of mild hypothermia in a focal model of transient cerebral ischemia: neurological outcome and infarct size. *J Neurosurg* 2001;94:

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- 90-96.
28. Kollmar R, Schabitz WR, Heiland S, Georgiadis D, Schellinger PD, Bardutzky J, et al. Neuroprotective effect of delayed moderate hypothermia after focal cerebral ischemia: an MRI study. *Stroke* 2002;33:1899-1904.
 29. Kataoka K, Yanase H. Mild hypothermia-a revived countermeasure against ischemic neuronal damages. *Neurosci Res* 1998;32:103-117.
 30. Hayashi N. *Brain hypothermia: pathology, pharmacology, and treatment of severe brain injury*. 2nd ed. Tokyo: Springer-Verlag, 2000;69-82.
 31. Longa EZ, Weinstein PR, Carlson S, Cummins R. Reversible middle cerebral artery occlusion without craniectomy in rats. *Stroke* 1989;20:84-91.
 32. Zhang JW, Gottschall PE. Zymographic measurement of gelatinase activity in brain tissue after detergent extraction and affinity-support purification. *J Neurosci Methods* 1997;76:15-20.
 33. Ginsberg MD, Busto R. Combating hyperthermia in acute stroke: a significant clinical concern. *Stroke* 1998;29:529-534.
 34. Karibe H, Zarow GJ, Graham SH, Weinstein PR. Mild intraischemic hypothermia reduces postischemic hyperperfusion, delayed postischemic hypoperfusion, blood-brain barrier disruption, brain edema, and neuronal damage volume after temporary focal cerebral ischemia in rats. *J Cereb Blood Flow Metab* 1994;14:620-627.
 35. Huang ZG, Xue D, Preston E, Karbalai H, Buchan AM. Biphasic opening of the blood-brain barrier following transient focal ischemia: effects of hypothermia. *Can J Neurol Sci* 1999;26:298-304.
 36. Rosenberg GA, Estrada EY, Dencoff JE. Matrix metalloproteinases and TIMPs are associated with blood-brain barrier opening after reperfusion in rat brain. *Stroke* 1998;29:2189-2195.
 37. Mun-Bryce S, Rosenberg GA. Matrix metalloproteinases in cerebrovascular disease. *J Cereb Blood Flow Metab* 1998;18:1163-1172.
 38. Gabriel C, Justicia C, Camins A, Planas AM. Activation of nuclear factor- κ B in the rat brain after transient focal ischemia. *Brain Res Mol Brain Res* 1999;65:61-69.
 39. Schneider A, Martin-Villalba A, Weih F, Vogel J, Wirth T, Schwaninger M. NF- κ B is activated and promotes cell death in focal cerebral ischemia. *Nat Med* 1999;5:554-559.
 40. Zhao W, Richardson JS, Mombourquette MJ, Weil JA, Ijaz S, Shuaib A. Neuroprotective effects of hypothermia and U-78517F in cerebral ischemia are due to reducing oxygen-based free radicals: an electron paramagnetic resonance study with gerbils. *J Neurosci Res* 1996;45:282-288.
 41. Sutcliffe IT, Smith HA, Stanimirovic D, Hutchison JS. Effects of moderate hypothermia on IL-1 α -induced leukocyte rolling and adhesion in pial microcirculation of mice and on proinflammatory gene expression in human cerebral endothelial cells. *J Cereb Blood Flow Metab* 2001; 21:1310-1319.
 42. Hassoun HT, Kozar RA, Kone BC, Safi HJ, Moore FA. Intraischemic hypothermia differentially modulates oxidative stress proteins during mesenteric ischemia/reperfusion. *Surgery* 2002;132:369-376.