

Matrix Metalloproteinases

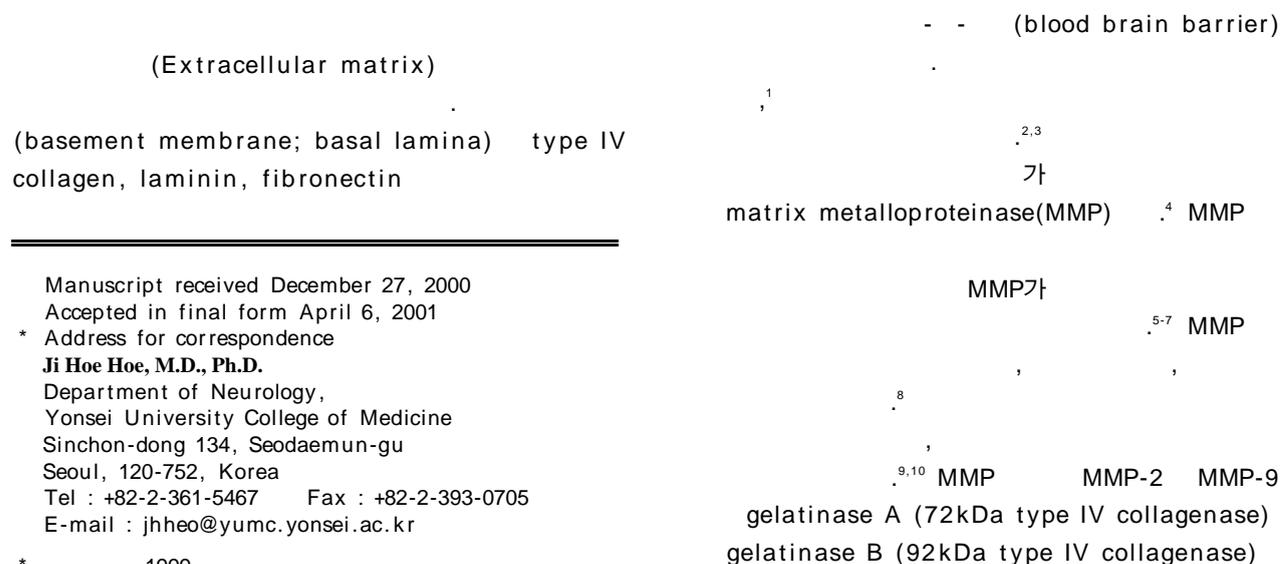
The Temporal Changes of Matrix Metalloproteinases in Experimental Middle Cerebral Artery Occlusion and Reperfusion

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Background : Matrix metalloproteinases (MMPs) can degrade a wide range of extracellular matrix components. The degradation of microvascular basal lamina by MMPs may be, in part, responsible for the hemorrhagic transformation, brain edema, and accentuation of ischemic injury in cerebral ischemia. Although MMP-2 and MMP-9 were reported to increase in cerebral ischemia, the temporal patterns of their increase are uncertain. **Methods** : By using gelatin zymography, we investigated the activity of MMP-2 and MMP-9 in 10 μ m frozen sections of ischemic and non-ischemic hemispheres in spontaneous hypertensive rats (SHRs) after variable time of reperfusion following 2 hours of middle cerebral artery occlusion (MCA:O). Adjacent 2mm-thick slices were stained with 2,3,5-triphenyltetrazolium chloride (TTC) solution to define the area of ischemic damage. **Results** : The infarcted zone could be visualized well by TTC staining after 3 hours of reperfusion. MMP-2 was observed in all samples examined, while MMP-9 was observed only in the ischemic hemispheres. In the ischemic hemispheres when comparing with non-ischemic sides, MMP-9 was increased in all groups undergoing MCA:O, as early as in 2 hours of MCA:O group, while MMP-2 was increased only after 6 days in the reperfusion group. MMP-2 and MMP-9 activities per unit volume of infarction increased during the reperfusion period and were highest after 6 days. **Conclusions** : MMP-9 increased early after MCA:O in the SHR and both MMP-2 and MMP-9 increased during the reperfusion period. These findings highlight the early potential role of MMP-9 in cerebral ischemia. J Korean Neurol Assoc 19(3):278~284, 2001

Key Words : Matrix metalloproteinase, Cerebral ischemia, Middle cerebral artery occlusion



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type IV collagen laminin L-lysine 4-0 (Ethicon, Edinburg, UK)²⁶

MMP-9가 가 MMP-2

MMP 가 , MMP rane isoflu-

가 가 가 가¹¹⁻¹⁶

가 가 가¹⁷⁻¹⁹

가 가²⁰⁻²³ 18

가 2. Urethane peri-

가 staltic pump 가

가 brain matrix

MMP-2 MMP-9가 2 mm

가 4 (6~7 mm)

1. Tissue-Tek OCT compound (Miles, Inc., Elkhart, IN, USA) 2-methylbutane -80 zymography

250~350 g (spontaneous hypertensive rat) 가 2% 2,3,5-triphenyltetrazolium chloride(TTC) 가 37 30

MMP 2 3, 18 6

가 4 , 20

6 가 19 가

가^{24,25} , isoflurane

3. Modified zymography 10 μm cryostat 120 μ (homogenizing buffer) (1% Triton X-100, 50 mmol/L Tris-HCl[pH 7.5], 75 mmol/L NaCl, 1 mmol/L phenylmethyl sulfonyl fluoride[PMSF; Sigma]) 4 20 9000 rpm -80 zymography bovine gamma globulin standard Bradford (Bio-Rad Laboratories, herculus, CA, U.S.A.)

poly-

Table 1. Size of infarctions according to the duration of experimental middle cerebral artery occlusion/reperfusion of the rat

Group		Number of rats	Size of infarction(%)
MCA occlusion	Reperfusion		
0 hours	18 hours	3	0.0
2 hours	none	4	not measured
2 hours	3 hours	4	32.4 ± 0.70
2 hours	18 hours	5	32.8 ± 3.54
2 hours	6 days	3	29.2 ± 8.17

MCA : middle cerebral artery

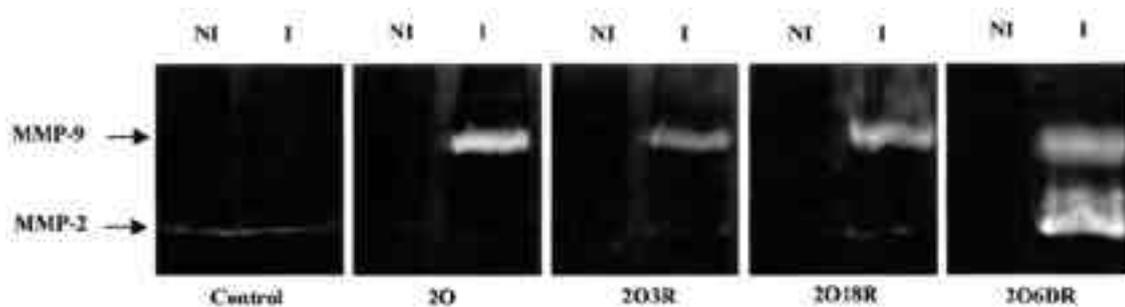


Figure 1. Gelatin zymography of ischemic and non-ischemic rat brain tissues. MMP-9(matrix metalloproteinase-9, 92 kDa) and MMP-2(matrix metalloproteinase-2, 72 kDa) are detected in ischemic (I) brain tissue. In non-ischemic (NI) side, only MMP-2 is detected. 2O; 2 hours occlusion without reperfusion, 2O3R; 2 hours occlusion with 3 hours reperfusion, 2O18R; 2 hours occlusion with 18 hours reperfusion, 2O6DR; 2 hours occlusion with 6 days reperfusion

Gelatin zymography
¹⁸, 70 μg
 (80 mmol/L Tris-HCl[pH 6.8], 4% sodium dodecyl sulfate[SDS], 10% glycerol, 0.01% bromphenol blue)

recombinant MMP-2 MMP-9
 MMP-2 MMP-9 가 Vogt
 Koyanagi Harada 2 μg MMP

1200 dpi
 Scion Image program
 zymography
 paired t-test
 Kruskal-Wallis test
 ANOVA test
 ±

1% gelatin 8% SDS-polyacrylamide
 150 ml 2.5% Triton X-100 가
 shaker 15 3
 250 ml 50 mmol/L Tris-HCl (pH 7.5,
 10 mmol/L CaCl₂, 0.02% NaN₃) 37
 66 acetic acid,
 methanol 가 1:3:6 0.1%
 amido black 1
 amido black 130 20
 MMP 가
 (Scanmaker 9600XL,
 Microtek, Taiwan) 600 dpi
 Scion Image program gel plotting macro
 integrated
 density
 4.
 3 5 TTC TTC
 (Fig. 1). 62 kDa
 2 6 3 2
 3 5 4 kDa
 MMP-9 92 kDa
 MMP-2 72 kDa
 MMP-9 84 kDa

27,28
 (Table 1).
 2. MMP-2 MMP-9
 zymography MMP-2 MMP-9
 gelatin 가 72
 kDa MMP-2
 92 kDa
 MMP-9
 62 kDa
 2 6 3 2
 MMP-9 84
 kDa

MMP-9
가
MMP-2
2
MMP-9
2
p=0.0152).
2
3
18
6
(Fig. 3).

MMP-2
MMP-9
2
(MMP-
2; p=0.0063, MMP-9; p=0.0119).
MMP-2
MMP-9
가
(p<0.01)
6
가
(Fig. 4).

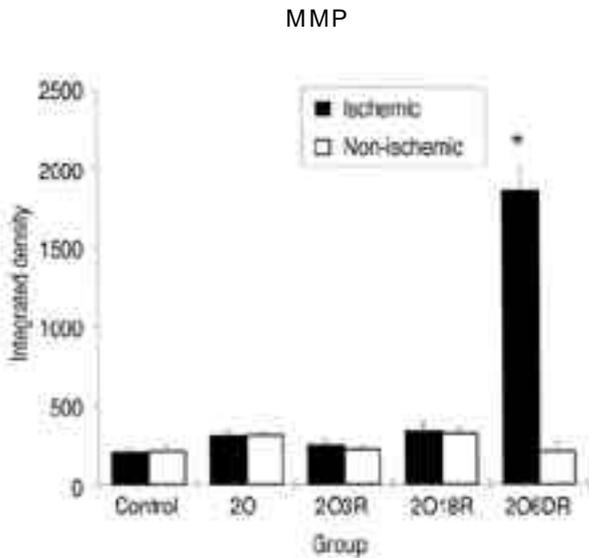


Figure 2. Integrated density of the matrix metalloproteinase-2 (MMP-2) gelatinolytic activity. MMP-2 activity is significantly elevated only in the ischemic hemisphere of a group 2O6DR. 2O; 2 hours occlusion without reperfusion, 2O3R; 2 hours occlusion with 3 hours reperfusion, 2O18R; 2 hours occlusion with 18 hours reperfusion, 2O6DR; 2 hours occlusion with 6 days reperfusion, * p=0.0152

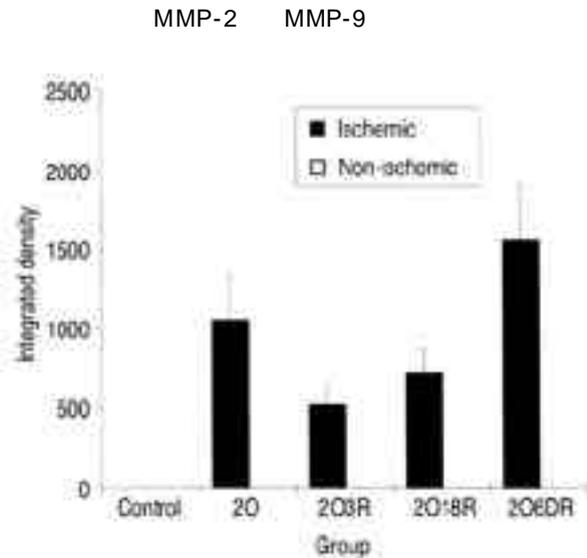


Figure 3. Integrated density of the matrix metalloproteinase-9 (MMP-9) gelatinolytic activity. MMP-9 activities in ischemic hemispheres of all the groups are significantly higher than those in non-ischemic hemispheres. 2O; 2 hours occlusion without reperfusion, 2O3R; 2 hours occlusion with 3 hours reperfusion, 2O18R; 2 hours; occlusion with 18 hours reperfusion, 2O6DR; 2 hours occlusion with 6 days reperfusion

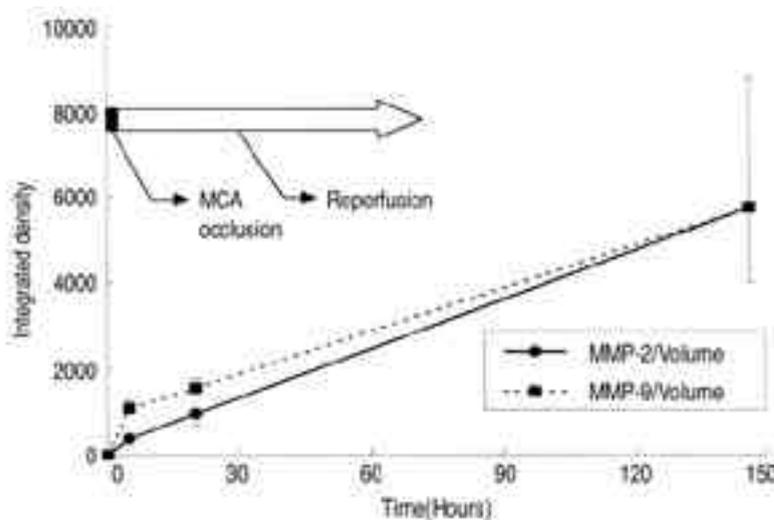


Figure 4. The temporal changes of matrix metalloproteinase-2(MMP-2) and matrix metalloproteinase-9(MMP-9) gelatinolytic activities per unit volume of infarction. MMP activities show a trend of steady increment during the reperfusion period. MCA; middle cerebral artery

가 , MMP-2 MMP-9가
가
가
MMP-9가
가
가
MMP-9
가
가
MMP-9

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