

Matrix Metalloproteinases

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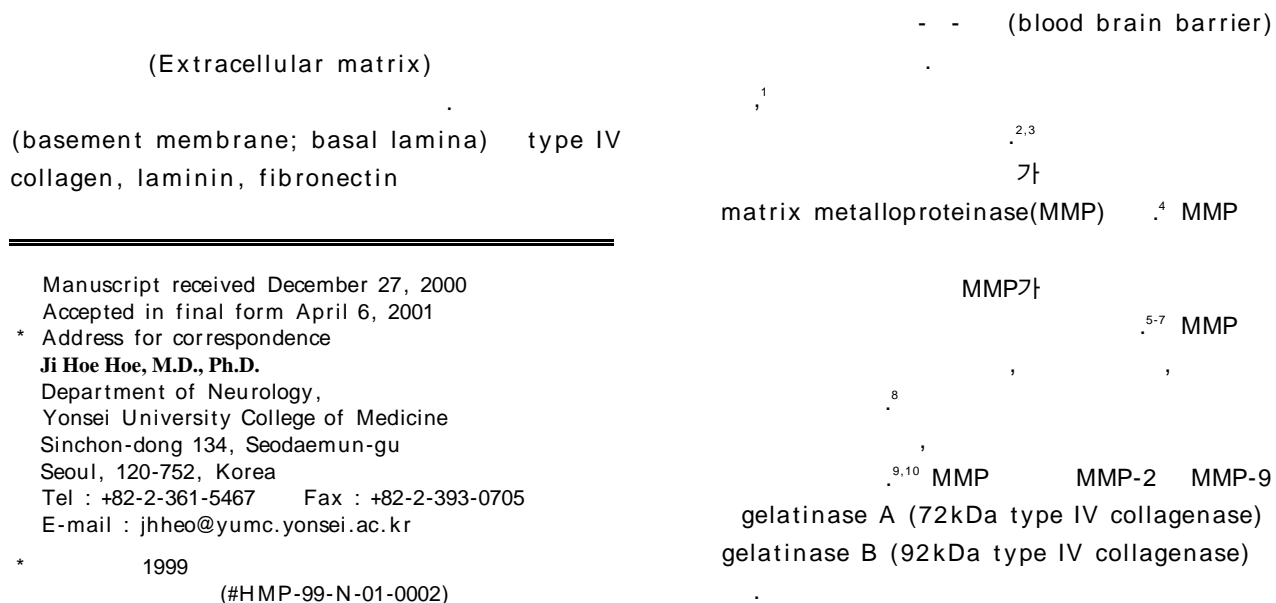
The Temporal Changes of Matrix Metalloproteinases in Experimental Middle Cerebral Artery Occlusion and Reperfusion

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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 µm 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 isoflu-

가 가 가¹¹⁻¹⁶

가 가 가¹⁷⁻¹⁹

가 가 가²⁰⁻²³

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

가 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

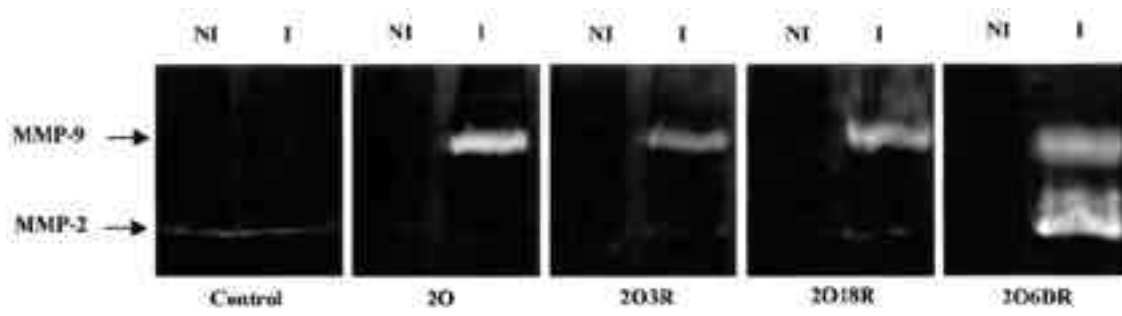


Figure 1. Gelatin zymography of ischemic and non-ischemic rat brain tissues. MMP-9(matrix metalloproteinase-9, 92 kDa) and MMP-2(matrix metalloproteinase-2, 72 kDa) are detected in ischemic (I) brain tissue. In non-ischemic (NI) side, only MMP-2 is detected. 2O; 2 hours occlusion without reperfusion, 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
 -80
 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, 2
 methanol 가 1:3:6 0.1%
 amido black 1 TTC 27,28
 amido black
 130 20 (Table 1).
 MMP 가 2. MMP-2 MMP-9
 (Scanmaker 9600XL, zymography MMP-2 MMP-9
 Microtek, Taiwan) 600 dpi gelatin 가 72
 Scion Image program gel plotting macro kDa MMP-2
 density integrated
 4. MMP-9 92 kDa
 3 5 TTC TTC (Fig. 1). 62 kDa MMP-2
 2 6 3 2
 3 5 4 , 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).

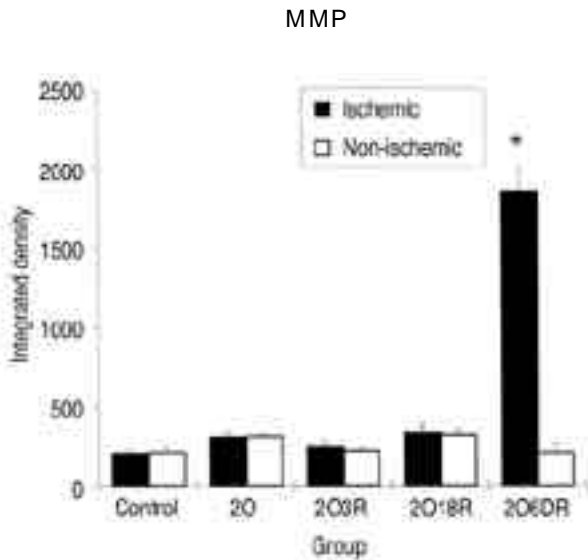


Figure 2. Integrated density of the matrix metalloproteinase-2 (MMP-2) gelatinolytic activity. MMP-2 activity is significantly elevated only in the ischemic hemisphere of a group 2O6DR. 2O; 2 hours occlusion without reperfusion, 2O3R; 2 hours occlusion with 3 hours reperfusion, 2O18R; 2 hours occlusion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

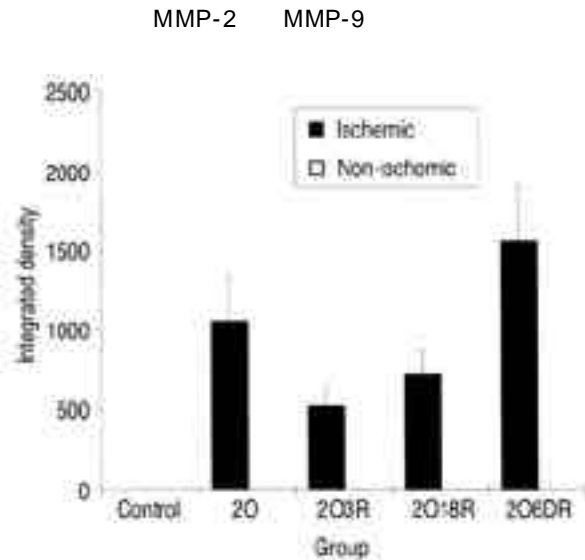


Figure 3. Integrated density of the matrix metalloproteinase-9 (MMP-9) gelatinolytic activity. MMP-9 activities in ischemic hemispheres of all the groups are significantly higher than those in non-ischemic hemispheres. 2O; 2 hours occlusion without reperfusion, 2O3R; 2 hours occlusion with 3 hours reperfusion,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

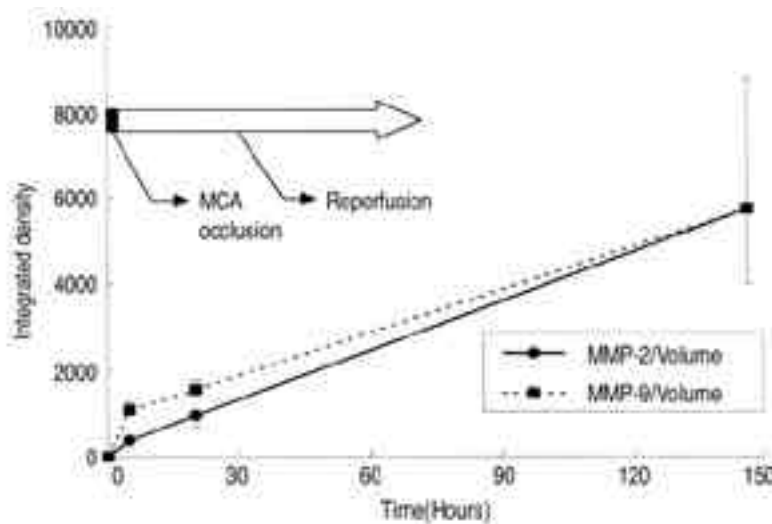


Figure 4. The temporal changes of matrix metalloproteinase-2(MMP-2) and matrix metalloproteinase-9(MMP-9) gelatinolytic activities per unit volume of infarction. MMP activities show a trend of steady increment during the reperfusion period. MCA; middle cerebral artery

. MMP-2 가
 MMP가 ,
 ,
¹⁸
 MMP-2 (angiogenesis)
²⁹ , MMP-9 , MMP가
 가 .
 . MMP-2 MMP-9 . MMP
 . MMP-2 MMP 가
 가 가 6 MMP-9 baboon MMP-9
 가 가 . MMP-9 가 .
 가 가 2 가 MMP t-PA
 가 가 6 가 .¹⁸ MMP t-PA
 MMP-9가 가
 , 가 . Heo .¹⁷
 baboon 1 . (cytotoxic edema)
¹⁸ Rosenberg Wang
 Wistar Kyoto . Rosenberg
 12 ~ 24 가 ,^{22,30} Gasche MMP-9 가
 1 3 가 , MMP-9
²³ zymography 가 ,^{22,32} , Gasche MMP-9
²³ Rosenberg Wang .²³ , MMP
 가
 MMP-9가 가 .³³ vas-
 zymography cular endothelial growth factor(VEGF)가 가 ,³⁴
 MMP VEGF MMP-2 가 .³⁵ ,
¹⁸ MMP-2가 가 , MMP-2가
 10 μm 가 . 가
 , MMP
 MMP-9 가 가 .^{36,37}
 가 MMP-9 MMP-2 .³⁸
 6 가 .²² integrin
 Rosenberg 가 RGD peptides가
 , baboon (apoptosis)
 2 MMP-2가 가
^{18,31} zymography .³⁹
 baboon . Romanic
 가 MMP-9 ,¹⁹ baboon MMP-
 .¹⁸
 MMP-2 MMP-9가 MMP
 , MMP-9가 가
 MMP-
 가 MMP-9가 가
 2 MMP-9 가 .

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

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