Comparison of Effects between Alteplase and Pamiteplase on MMPs Regulation

Jae-Chang Jung¹ and Sun-Ryung Lee*

Department of Life Science, Cheju National University, Jeju, 690-756, Korea,
¹Department of Biology, Kyungook National University, Daegu, 702-701, Korea

Received May 22, 2007 / Accepted June 26, 2007

Thrombolytic therapy with tissue plasminogen activator (tPA) can improve the clinical outcome of ischemic stroke patients. However, its clinical application is limited by narrow therapeutic time windows and elevated risks of cerebral hemorrhage and brain injury. In part, these effects of tPA has been related to matrix metalloproteinase-9 (MMP-9) dysregulation. Here, we investigate that the effects of alteplase (tPA with short half-life) and pamiteplase (a modified tPA with long half-life) on the MMP-9 regulation in neurovascular unit. The total levels of MMP-2 and MMP-9 in neuronal cells are lower than astrocytes. Alteplase (1-10 µg/ml) induced upregulation of MMP-2 and MMP-9 in rat cortical neurons and astrocytes, respectively. Whereas pamiteplase in a wide range of dose did not affect the MMP-2 and MMP-9 responses in both of cells. These results suggest that pamiteplase with long half-life can be provided as a agent that overcome the side effects of alteplase.

Key words – Tissue plasminogen activator, alteplase, pamiteplase, matrix metalloproteinase, hemorrhage, stroke

Introduction

Tissue plasminogen activator (tPA) is used to lyse clots and reperfuse brain in ischemic stroke. Properly titrated use of tPA improves clinical outcomes. Clinical trials have shown that tPA can be effective therapy for stroke if administered within few hours of stroke onset [14]. However, tPA has a short half-life of about 5 min and is cleared rapidly from plasma [12]. The dose of tPA required for effective thrombolysis is substantially higher, and this may contribute to lethal adverse effects such as intracerebral hemorrhage (ICH) and edema. To overcome these shortcomings, several tPA have been developed to lengthen half-life, increase resistance to plasma protease inhibitors or cause more selective binding to fibrin [8,16]. Also, tPA incurs elevated risks of reperfusion injury, which involves edema and cerebral hemorrhage. In part, these phenomena may be related to neurovascular proteolysis mediated by matrix metalloproteinases (MMPs). MMPs can degrade basal lamina and blood brain barrier, thus leading to edema and vascular rupture [1,6,7]. It has been documented that tPA amplified MMP-9 level after stroke and tPA plus MMP inhibitors reduce hemorrhage and improve outcomes in animal models of embolic stroke [4,9,13].

Pamiteplase is a modified tPA with the deletion of the kringle-1 domain and with a point mutation at the site of the kringle-2 domain linkage to the light chain to prolong the plasma half-life. It has been shown in vitro to possess a pronounced affinity for fibrin and to retain the same specific activity as tPA [2]. In a rat model of embolic stroke, pamiteplase induced thrombolysis rapidly within 10-30 min [3] and reduced infarction volume suppressing the incidence of hemorrhage and improve neurological outcomes [11]. Here, we examined the effects of palmitoyl activators on MMP regulation in a neurovascular cells system.

Cortical neurons were prepared from 16 day old rat embryonic cortex. Cell suspensions were seeded onto poly-D-lysine-precoated plates with neurobasal medium supplemented with glutamic acid, glutamine, antibiotic solution and 2% B27 supplement. Cultures were used at 10-12 days and the medium was changed with NBM containing 0.1% B27 for 1 day for experiments. Plasminogen activators, alteplase (1-10 µg/ml) and pamiteplase (0.5-25 µg/ml) were treated for 24 hrs in NBM containing 0.1% B27 and medium was collected for gelatin zymography. The cleared medium was concentrated 10 fold using microcon with a 10 kDa pore diameter cut-off, then electrohoresed on 10 % SDS-PAGE containing 1 mg/ml gelatin as the protease substrate. The gel was placed in 2.7% Triton X-100 for 1 hr and then incubated for 20 hrs in developing buffer. Relative gelatinolytic activity (MMP-2 and -9) was quanti-
Fig. 1. The effects of alteplase and pamiteplase on MMPs regulation in rat neuronal cells. (A) Zymography shows alteplase induced MMP-2 and MMP-9 upregulation. (B) Quantification of changes of MMP-2 and MMP-9 after exposure to alteplase with indicated concentrations. (C) Pamiteplase was not effective on the regulation of MMP-2 and MMP-9. M2 and M9 indicate positive controls loaded with MMP-2 and MMP-9 standards.

Fig. 2. Plasminogen activators-mediated MMPs regulation in rat astrocytes. (A) MMP-2 and MMP-9 levels were measured by gelatin zymogram. All dose of alteplase was effective in the upregulation of MMP-2 and MMP-9. (B) Quantification of changes of MMP-2 and MMP-9 after exposure to alteplase with indicated concentrations. (C) Cells were exposed to pamiteplase with indicated dose for 24 hr. There were no changes in secretion of MMP-2 and MMP-9.
duction of MMP-9. Further, the long-acting characteristics of pamiteplase may permit the dosage to be decreased, thus ameliorating the occurrence of hemorrhagic transformation, especially under conditions where pamiteplase is administered at a delayed time point after the onset of ischemia [10,11]. Further studies are warranted to validate it for possible clinical applications.

In conclusion, the challenge is how to increase the time window for tPA, decrease the risks of cerebral hemorrhage, and increase the efficacy of reperfusion for clinical stroke therapy. Pamiteplase may be provided as a candidate of thrombolytic therapy reducing risks of intracerebral hemorrhage and may allow us to improve its safety and efficacy in stroke.

Acknowledgement

This work was supported by the research grant of the Cheju National University in 2005.

References

초록 : Alteplase와 pamiteplase에 의한 MMPs 조절 효과 비교

정재정1, 이선령2
(제주대학교 생명과학과, 1경북대학교 생명과학)

뇌출혈 환자에 사용되는 tPA 치료법은 혈전을 용해시키고 혈액의 혼란을 유발하게 해 주기 때문에 매우 중요하게 사용되고 있다. 동물 실험모델을 이용한 이전의 실험결과에 따르면 tPA뿐 아니라 tPA의 일종인 pamiteplase 역시 30분 이내 탁월한 혈전 용해의 효과를 보여주었다. 그러나 tPA 치료법은 단시간 치료와 출혈, 부종과 같은 여러 가지 부작용이 심각할 수 있으며 이들은 MMP-9의 활성 조절과 깊은 관련이 있는 것으로 보고되고 있다. 본 연구에서는 실현에 사용되는 tPA의 한 종류인 alteplase와 이러한 부정적인 효과를 극복하기 위해 개발한 pamiteplase의 MMP-9 활성에 미치는 효과를 비교 분석하였다. 몽도의 뇌로부터 추출된 신경세포에서 alteplase의 처리는 뇌피질질대로 MMP-9의 발현을 촉진시켰고 활성화된 형태의 MMP-2 역시 증가되는 양상을 보였다. 반면, pamiteplase의 경우 MMP-2의 MMP-9의 발현양상에 영향을 미치지 않았다. 유사한 효과는 뇌세포와 유전자 조정이 다른 세포인 성장세포에서도 관찰되었다. 즉, 대뇌의 성장세포를 분리, 배양하여 이들의 효과를 확인한 결과 신경세포에서 동장가져로 alteplase의 경우 뇌피질질대로 수용하였고 pamiteplase의 경우 변화를 나타내지 않았다. Pamiteplase는 뇌세포성장세포에서 출혈과 부종을 유도하는 데 관련이 있는 것으로 보고된 MMP-9의 활성에 영향을 미치지 않는 것으로 보아 alteplase에 비해 보다 효과적인 치료제로서의 가능성을 보여주고 있다.