Thrombosis Research 125 (2010) 549–553

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

Increases in circulating matrix metalloproteinase-9 levels following fibrinolysis for acute pulmonary embolism

Diána Mühl [a,*], Subhamay Ghosh [a], Juliana A. Uzuelli [b], János Lantos [c], Jose E. Tanus-Santos [b,*]

[a] Department of Anaesthesia and Intensive Care, University of Pécs, Ifjúság u.13, 7624 Pécs, Hungary
[b] Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Av. Bandeirantes, 3900; Ribeirao Preto, SP, Brazil, 14049-900
[c] Department of Surgical Research and Techniques, University of Pécs, Kodály Z. u. 20, 7624 Pécs, Hungary

ARTICLE INFO

Article history:
Received 1 October 2009
Received in revised form 11 February 2010
Accepted 17 February 2010
Available online 21 March 2010

Keywords:
Pulmonary embolism
thrombolysis
streptokinase
alteplase
matrix metalloproteinase
tissue inhibitor of metalloproteinase

ABSTRACT

Introduction: Fibrinolyis is one of the first line therapies in high risk pulmonary embolism (PE) according to current guidelines. Previous studies showed that fibrinolytic therapy with tPA (tissue plasminogen activator, or alteplase) upregulates the concentrations of matrix metalloproteinases (MMPs) and contributes to hemorrhagic transformation after cardioembolic stroke. However, no previous study has described the circulating MMPs levels following fibrinolysis for acute PE.
Materials and Methods: We serially measured the circulating levels of MMPs (MMP-9 and MMP-2) and their endogenous inhibitors, the tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 in alteplase and in streptokinase-treated patients with acute PE by gelatin zymography and by enzyme-linked immunosorbent assays, respectively.
Results: We found that therapy of PE streptokinase or with alteplase is associated increased pro-MMP-9, but not MMP-2, concentrations for up to 24 hours, whereas no significant changes were found in TIMP-1 or TIMP-2 concentrations. This alteration returned to normal 3 to 5 days after thrombolysis. This is the first study reporting on MMPs alterations following fibrinolysis for acute PE.
Conclusions: We found transient increases in circulating pro-MMP-9 levels following fibrinolysis for acute PE. Our findings support the hypothesis that increased MMP-9 levels may underlie the risk of intracerebral hemorrhage or other bleeding complication of thrombolysis for acute PE, and the use of MMP inhibitors may decrease such risk.

Introduction

Acute pulmonary embolism (PE) is the third most common cause of cardiovascular mortality, with very high mortality rates, especially in severe cases [1]. Making a rapid and definite diagnosis followed by immediate and appropriate therapy may help to improve the low survival rates of this critical condition. Recent guidelines stratify PE into high-risk and non-high-risk (intermediate and low) PE with basis on clinical assessment [2]. This useful classification helps to define the diagnostic strategy and initial management, which includes fibrinolytic therapy in patients with high-risk PE presenting with cardiogenic shock and/or persistent arterial hypotension [2].

Experimental evidence has indicated that activation of matrix metalloproteinases (MMPs) is implicated in the pathophysiology of PE [3–8]. Indeed, MMPs inhibition attenuates the hemodynamic derangements associated with this condition [3,4]. It is possible that inflammatory activation of neutrophils [9] and rapid release of granules containing large amounts of MMP-9 [10] during PE explains how MMPs, especially MMP-9, are involved in pathophysiology of PE. Interestingly, this context implicating MMP-9 in embolic conditions is very similar to that found during acute ischemic stroke. Of note, MMP-9 levels are rapidly upregulated and plays a delerious role in brain after cerebral ischemia [11]. Enhanced MMP-9 proteolytic activity may attack type IV collagen, laminin, and fibronectin, which are major components of the vascular matrix, thus possibly contributing to hemorrhagic transformation after cardioembolic stroke [12,13]. Moreover, the fibrinolytic drug tPA (tissue plasminogen activator, or alteplase), which is used to induce clot lysis, has been shown to amplify MMP-9 upregulation associated with ischemic brain damage [14–17]. However, although there is evidence indicating that therapies that increase plasmin concentrations activate MMPs, there is no previous study describing the circulating MMPs levels following fibrinolysis for acute PE.

* Corresponding authors. Tanus-Santos is to be contacted at Department of Pharmacology, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo, Av. Bandeirantes, 3900; Ribeirao Preto, SP, Brazil, 14049-900. Tel.: +55 16 3602 3163; fax: +55 16 3633 2301. Muhl, Department of Anaesthesia and Intensive Care, Faculty of Medicine, University of Pécs, Ifjúság u.13, 7624 Pécs, Hungary. Tel.: +36 72 536 440; fax: +36 72 536 441.
E-mail addresses: drdianamuhl@gmail.com (D. Mühl), tanus@fmrp.usp.br, tanussantos@yahoo.com (J.E. Tanus-Santos).

0049-3848/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.thromres.2010.02.015

In the present study, we hypothesized that increased MMP-9 levels would be found following fibrinolysis for acute PE. To investigate this hypothesis, we serially measured the circulating levels of MMPs (MMP-9 and MMP-2) and their endogenous inhibitors, the tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2 in alteplase and in streptokinase-treated patients with acute PE.

Materials and methods

The study protocol was carried out in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and the permission of the Institutional Scientific and Human Research Ethics Committee of the University of Pécs (810/2001). Each patient provided written informed consent and was informed clearly about the details of study and blood sampling.

Study population

Prospectively screened consecutive patients with definite massive and submassive pulmonary embolism (according to the latest ESC guidelines (intermediate risk group) were included in the present study [2]. PE patients were referred to our Intensive Care Unit. Fifteen patients were divided into two therapeutic groups using block randomization: 8 patients for ultra high dose (9 million units/6 hours) streptokinase (UH-SK) and the other 7 patients for alteplase (tPA) 100 mg/2 hours as thrombolysis. We paid attention to homogenous medication during the supportive therapy.

A control group was not selected because withholding thrombolysis from patients suffering from hemodynamic instability due to massive and/or submassive PE would have been a violation against guidelines.

The inclusion criteria were based on the hemodynamic state (high or intermediate risk PE) and on the extension of the unperfused area (>50%). These were verified with spiral CT, perfusion lung scan and echocardiography. Exclusion criteria defined patients who declined to give consent, advanced malignant disease and absolute contraindication to thrombolysis.

Two patients declined to give consent. One patient suffered from hematological disease (lymphoma). Three patients had advanced stage malignant disease. Therefore, six patients were excluded from the study.

Arterial blood samples were collected before thrombolysis as a baseline value then at 8 hours, 1, 3, 5, and 30 days after thrombolysis (time points 1-6, respectively) to measure plasma MMPs and TIMPs concentrations.

Anticoagulant therapy begun with intravenous unfractionated heparin to maintain aPTT between 60-70 seconds for the first 48 hours and was continued with a therapeutic dose of low molecular weight heparin. The effectiveness of thrombolytic treatment was controlled by a second look spiral CT or perfusion lung scan. If any of the examinations did not verify at least 30% decrease in the size of unperfused lung area after the first treatment phase, thrombolysis was repeated after 24 hours elapsed. If the fibrinogen level had been lower than 2 g/l before the second thrombolytic cycle, fresh frozen plasma was administered.

SDS-Polyacrilamide Gel Electrophoresis (PAGE) Gelatin zymography of MMP-9 and MMP-2

Gelatin zymography of MMP-9 and MMP-2 from plasma was performed as previously described [18,19]. Briefly, plasma samples were subjected to electrophoresis on 7% SDS-PAGE co-polymerized with gelatin (1%) as the substrate. After electrophoresis was complete, the gel was incubated for 1 hour at room temperature in a 2% Triton X-100 solution, and incubated at 37 °C for 16 h in Tris–HCl buffer, pH 7.4, containing 10 mmol/L $CaCl_2$. The gels were stained with 0.05% Coomassie Brilliant Blue G-250, and then destained with 30% methanol and 10% acetic acid. Gelatinolytic activities were detected as unstained bands against the background of Coomassie blue-stained gelatin. Enzyme activity was assayed by densitometry using a Kodak Electrophoresis Documentation and Analysis System (EDAS) 290 (Kodak, Rochester, NY)[20]. The pro form of MMP-2 and MMP-9 were identified as bands at 72 and 92 KDa, respectively, by the relation of log Mr to the relative mobility of Sigma SDS-PAGE LMW marker proteins. A representative zymogram of plasma samples is shown in Fig. 1.

Enzyme immunoassays of TIMP-1 and TIMP-2

The plasma concentrations of TIMP-1 and TIMP-2 were measured with commercially available enzyme-linked immunosorbent assay kits according to manufacturer's instructions (R&D Systems, Inc., Minneapolis, USA)[21,22].

Statistical analysis

All the results are expressed as means ± S.E.M. To compare clinical data between groups, we used Student's t or Wilcoxon Signed Ranks Test as appropriate. One-way analysis of variance (ANOVA) for repeated measures was used to determine the changes in the MMPs or TIMPs concentrations in each study group. When the ANOVA was significant, the differences were tested by the Dunnett multiple comparisons test. A probability value <0.05 was considered the minimum level of statistical significance.

Results

Fifteen patients were included in the study, aged between 21 and 84 years, mean of ages: 63 (±16) years. Table 1 shows that both therapeutic groups of patients had similar baseline characteristics.

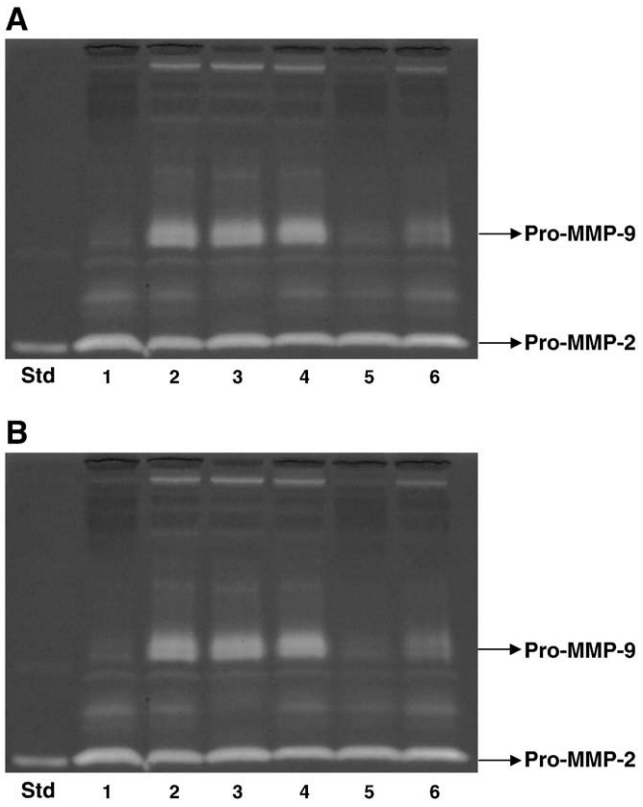

Fig. 1. Representative zymograms of plasma samples. Panel A and B show plasma samples from patients treated with Streptokinase and Alteplase, respectively. The bands corresponding to pro-MMP-2 (72 KDa) and to pro-MMP-9 (92 KDa) are indicated by arrows. Std corresponds to the 72 KDa band (pro-MMP-2) from fetal bovine serum, which was used as a standard to normalize the data from all the gels, thus allowing between gels comparisons.