Initial serum MMP-9 activity as a bad prognostic factor after ischemic stroke

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Abstract

An early evaluation of ischemic stroke (IS) biomarkers enhances the chance for proper diagnosis and can predict the clinical outcome after acute phase of the disease. Our purpose was to evaluate the MMP-2 and MMP-9 serum activity as the prognostic factors for patients' disability after IS. Finally, twenty patients with IS confirmed in computed tomography scans were prospectively enrolled into the study. The patients were examined by functional disability scales: Barthel Index (BI) and Modified Rankin Scale (mRS) at the admission and after 3 months from IS onset. The serum MMP-2 and MMP-9 activities were measured on the first day of stroke with use of gelatin zymography. BI scores increased and mRS decreased significantly after 3 months from the stroke onset in comparison with day 1 ($p<0.05$). Significant relationships between serum MMP-9 activity and late BI ($r=-0.63$, $p=0.003$) or mRS ($r=0.67$, $p=0.002$) scores, estimated at three months from the stroke, were noticed. In addition, we observed significant correlation between initial MMP-2 serum activity and late BI ($r=-0.45$, $p=0.05$). Our data indicated on serum MMP-9 rather than MMP-2 measured during acute phase of IS as a predictor of patients' functional status three months after the stroke.

Keywords: stroke scales, MMP-2, MMP-9

Streszczenie

Wczesna analiza biomarkerów wskazujących na udaru mózgu zwiększa szansę postawienia właściwej diagnozy, jak również może przewidzieć stan funkcjonalny pacjentów po okresie ostrej fazy choroby. Celem niniejszej pracy była ocena aktywności MMP-2 i MMP-9 (żelatynaz) w surowicy w pierwszej dobie udaru niedokrwiennego mózgu jako czynników prognostycznych wskazujących na stan funkcjonalny pacjentów po przebyciu udaru. Do badania zakwalifikowano 20 pacjentów z potwierdzonym w badaniu tomografii komputerowej udarem niedokrwiennym mózgu. Ocenę kliniczną pacjentów wykonano przy pomocy zmodyfikowanej Skali Rankina (mSR) oraz Indeksu Barthel (IB) w pierwszej dobie oraz 3 miesiące po udarze. Aktywność żelatynaz w surowicy uzyskanej w pierwszej dobie choroby oceniona została przy użyciu zymografii żelatynowej. Zarówno wartości IB oraz mSR wykazały na poprawę stanu funkcjonalnego pacjentów w 3 miesiącu po udarze w porównaniu do pierwszej doby ($p<0.05$). Wykazano istotą statystycznie zależność pomiędzy aktywnością MMP-9, a wynikiem BI ($r=-0,63$; $p=0,003$) oraz mSR ($r=0,67$; $p=0,002$) ocenianymi w trzecim miesiącu po udarze. Dodatkowo wykazano zależność pomiędzy aktywnością MMP-2 a BI w 3 miesiącu choroby. Uzyskane wyniki wskazują większą wartość oznaczania aktywności MMP-9 w porównaniu do MMP-2, jako wczesnego markera stanu funkcjonalnego pacjentów po przebyciu udaru niedokrwiennego mózgu.

Słowa kluczowe: skale udaru, MMP-2, MMP-9

Introduction

The ischemic stroke (IS) is the third cause of death in the industrialized countries and one of the leading cause of patients' disability. Therefore the rapid diagnosis and applying of proper therapy enhances the chance of numerous patients to come back to normal life after IS. Nowadays computed tomography (CT) scan is the gold standard for IS diagnosis [1]. During the first 24h from the onset of focal symptoms the ischemic lesion is visible at 40% of CT-scans. Therefore the specific biomarkers evaluated from blood can increase the efficacy of accurate IS identification. In addition, some biological compounds measured during the acute phase of IS could have the prognostic value for patients' outcome.

Matrix metalloproteinase (MMP)-2 and MMP-9 (gelatinases) belong to biomarkers that are potentially applicable in the diagnosis of IS. Gelatinases are Zn^{2+} dependent endopeptidases targeting extracellular proteins [2]. These enzymes play an important role in the pathogenesis of numerous inflammatory diseases [3] and cancer [4,5]. The role of gelatinases in the IS has been widely discussed in numerous papers [6-8]. MMP-2 and MMP-9 are involved in blood-brain barrier (BBB) destruction [9], oedema formation [10], the activation of proinflammtory cytokines (tumor necrosis factor α, interleukine-1β) [11], and destruction of myelin proteins [12]. A high serum MMP-9 level in the acute phase of ischemic stroke has been shown to contribute to the increased risk of hemorrhage within an

ischemic focus due to BBB damage [13]. On the other hand, the MMP-2 is suggested to play the beneficial role in the repairing phase of cerebral ischemia [14].

Our purpose was to evaluate the early MMP-2 and MMP-9 serum activity as the prognostic factors for patients' outcome after IS.

Material and Methods

Finally, twenty patients with IS, admitted to the Stroke Unit at the Department of Neurology within the first 24h from the onset of neurological focal symptoms, were prospectively enrolled into the study. The diagnosis of IS was confirmed with CT-scan performed on admission. Written informed consent was obtained from each patient (or from family members when necessary). The local Ethics Committee (Medical University of Lublin) accepted the protocol of the study. Clinical characteristic of the study group was given in Table 1. The patients with regression of neurological symptoms within 24h from the onset (Transient Ischaemic Attack, TIA) were excluded from the study.

Table 1. Characteristic of patients

Men age	70.1 (51-87) years
Gender	12 female, 8 male
Pathogenesis of stroke	embolism, n = 3 arteriosclerosis, n = 11 lacunar, n = 2 unknown etiology, n = 4

The patients were examined by functional disability scales: Barthel Index (BI) and Modified Rankin Scale (mRS) at the admission and after 3 months from IS onset.

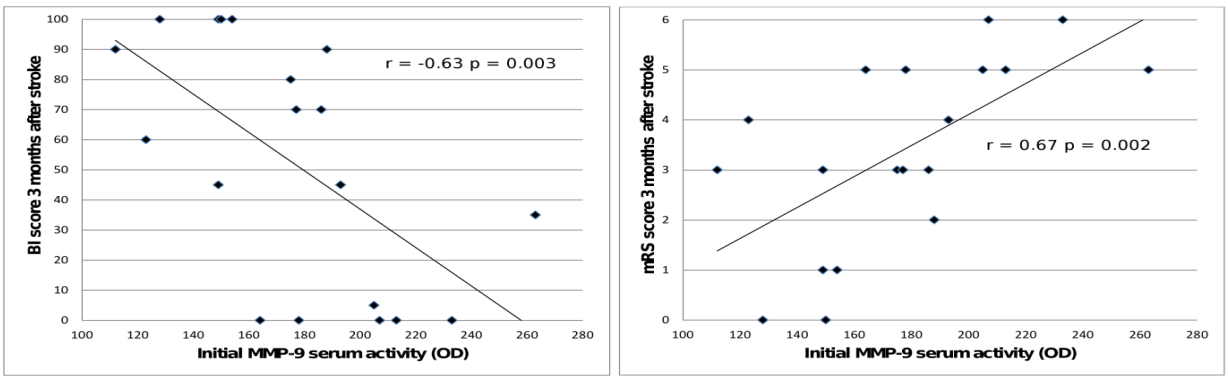
Venous blood samples were obtained during the first 24h of stroke. After centrifugation the serum was stored in -60 ºC. MMP-2 and MMP-9 activity was determined by gelatin zymography according to a previously described method [15]. The samples consisted of 9 µl of diluted serum (1/50 with redistilled water) + 3 µl of sample buffer with 10% sodium dodecyl sulfate (SDS) were separated on a 10% polyacrylamide gel with 0.05% gelatin type A from porcine skin (Sigma-Aldrich, G2500). After electrophoresis washing was carried out for two 30 min periods with buffer 50 mM Tris-HCl, pH 7.2, containing 10 mM $CaCl_2$, 0.02% NaN_3 and 2.5% Triton X-100. The incubation was performed for 18 h at 37°C in the above buffer but with 1% Triton X-100. Gels were stained with 0.1% Coomassie Blue R-250 in 30% ethanol and 10% acetic acid and destained in 30% ethanol and 10% acetic acid. MMP-2 and MMP-9 activities were detected as clear bands on the blue background. Enzymes were identified by comparing of their localization with molecular mass standard (Fermentas, SM0441), as well as with standards of both gelatinases (R&D Systems, 911-MP, 902-MP). Pro-forms of both gelatinases (pro-MMP-2, 72 kDa and pro-MMP-9, 92 kDa) were applied to further analysis. Quantification of zymograms was done using a computer scanner (1200 dpi) and ImageJ 1.42q (Wayne Rasband, NIH, USA). The activity of gelatinases was expressed as the optical density (OD) of the substrate lysis zone.

Spearman rank correlation was applied for calculation of relationship between the MMP-2, MMP-9 activities with BI and mRS scores. Mann-Whitney test was used for comparison of BI and mRS scores estimated on day 1 with month 3 after stroke. Significant values were considered when $p < 0.05$. Statistical analysis was performed with the use of the computer assisted statistical program In Stat v. 3.06. GraphPad Software (La Jolla, CA, USA).

Results

The mean initial BI and mRS amounted 19 and 4.6 respectively (minimum, **median** and maximum: 0, **0**, 85 and 3, **5**, 5 for BI and mRS respectively). BI scores increased and mRS decreased significantly after 3 months from the stroke onset in comparison with day 1 (mean; minimum, **median** and maximum: 33.25; 0, **5**, 100 and3.37; 0, **3**, 6 for BI and mRS respectively, $p<0.05$). We noticed significant relationships between initial MMP-9 serum activity with late BI ($r=-0.63$, $p=0.003$) or with mRS ($r=0.67$, $p=0.002$) scores estimated after three months from stroke (Figure 1). Statistically significant correlation between initial MMP-2

Figure 1. Relationships between initial serum MMP-9 activity and Barthel Index or Modified Rankin Scale at three months after ischemic stroke. Spearman rank correlation



serum activity and late BI was observed (r=-0.45, p=0.05). The relationship between MMP-2 activity and mRS score estimated at three months after the stroke did not reach statistical significance (r=0.45, p>0.05).

Discussion

Our study was aimed at verification of MMP-2 and MMP-9 roles as the early prognostic factors for the prediction of patients' disability after IS. Very often the estimation of patients' disability or dependence in the daily activities after stroke is performed with use of BI and mRS [16]. BI consists of ten items that measure a person's daily functioning, specifically the activities of daily living and mobility. mRS runs from 0 to 6, running from perfect health without symptoms to death. Both of above scales were applied for our study.

We revealed a clear relationship between serum activity of MMP-9 evaluated on day 1 of stroke with BI and mRS. Previous data indicated on plasma MMP-9 measured during acute phase of IS as a predictor of patients' disability expressed in mRS at three months after stroke [14]. Our study confirmed those observations. Analogue relationship between MMP-9 and BI score was found at three months that also indicated on MMP-9 as a predictor of patients' dependence in the daily activities after IS. The correlation of MMP-9 activity during acute phase IS with the volume of ischemic focus was previously described, as well as the relationships between volume of infarcted area vs. BI or mRS at three months [17]. The involvement of MMP-9 in numerous pathological processes during cerebral ischemia causes that elevated MMP-9 activity observed in patients with worse functional patients' status is not a surprise.

However, the significant correlation between initial (day 1) serum MMP-2 activity with BI score at three months, is in opposite to previous studies. Our finding emphasizes MMP-2 as a negative factor for patients' functional status after IS. Lucivero at al. noticed the higher initial MMP-2 activity in patients with stable or recovering symptoms compared to patients who underwent neurological worsening [14]. This result matches to the theory that ranks MMP-2 to the favorable compounds involved into the repairing processes after the stroke. However, the analogue influence of both gelatinases on patients' functional status after stroke that was observed in our study can result from the participation of MMP-2 and MMP-9 in the related pathological processes, their similar structure and substrates.

The role of MMP-2 and MMP-9 in the prediction of patients' outcome after IS has to be confirmed on larger group of individuals. Further studies should include the tissue inhibitors of MMP-2 and -9 to the analysis.

In conclusion, we indicate on serum MMP-9 as a strong predictor of patients disability after ischemic stroke.

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