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MMP-2 and MMP-9 as prognostic factors in ischaemic stroke

MMP-2 i MMP-9 jako czynniki prognostyczne w udarze niedokrwiennym mózgu

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Abstract

Objectives: No widely available, adequately sensitive diagnostic test to establish prognosis in stroke patients has been developed thus far. The aim of this study was to analyse changes in plasma levels of MMP-9 and MMP-2 as potential prognostic factors in patients with ischaemic stroke. **Methods:** The study included 56 patients presenting with the signs of ischaemic stroke for less than 24 hours, and 60 healthy controls without a history of neurological and/or inflammatory disorders. Plasma concentrations of MMP-2 and MMP-9 were determined immunoenzymatically at admission (i.e. within 24 hours of the cerebrovascular episode) and on the 7th day of hospital stay. **Results:** Median concentrations of MMP-9 in stroke patients were significantly lower than in the controls, both at admission and on the 7th day of hospital stay. No significant changes in the concentration of MMP-2 in ischaemic stroke patients were observed during the course of hospital stay. No significant association was found between both MMP concentrations and neurological status of patients with cerebrovascular episodes. **Conclusions:** The lack of significant associations between plasma concentrations of MMP-2/MMP-9 and clinical status suggests that these metalloproteinases should not be used as prognostic factors in patients with ischaemic cerebral episodes.

Key words: metalloproteinase 2, metalloproteinase 9, ischaemic stroke, stroke

Streszczenie

Cel: Jak dotąd nie został opracowany szeroko dostępny, wystarczająco czuły test diagnostyczny oceniający rokowanie u pacjentów po udarze niedokrwiennym mózgu. Celem niniejszego badania była analiza zmian stężenia MMP-9 i MMP-2 w osoczu jako potencjalnych czynników prognostycznych u chorych z udarem niedokrwiennym mózgu. **Metody:** Do badania włączono 56 pacjentów z objawami udaru niedokrwiennego utrzymującymi się nie dłużej niż 24 godziny oraz 60 osób zdrowych, bez historii incydentów neurologicznych i/lub zapalnych. Stężenia MMP-2 i MMP-9 oznaczono immunoenzymatycznie przy przyjęciu (czyli w ciągu 24 godzin od epizodu mózgowego) oraz 7. dnia hospitalizacji. **Wyniki:** Mediana stężenia MMP-9 w udarze mózgu była znacznie niższa niż w grupie kontrolnej, zarówno przy przyjęciu, jak i 7. dnia pobytu w szpitalu. Nie zaobserwowano znamiennych statystycznie zmian stężenia MMP-2 u osób z udarem niedokrwiennym mózgu w trakcie pobytu w szpitalu. Nie stwierdzono również istotnego związku między stężeniami obu MMP i stanem neurologicznym pacjentów z epizodami mózgowo-naczyniowymi. **Wnioski:** Brak istotnych związków pomiędzy stężeniami MMP-2/MMP-9 i obrazem klinicznym sugeruje, że metaloproteinazy nie powinny być stosowane jako czynniki prognostyczne u pacjentów z epizodami udaru niedokrwiennego mózgu.

Słowa kluczowe: metaloproteinaza 2, metaloproteinaza 9, udar niedokrwienny, udar mózgu

INTRODUCTION

Stroke is the third most frequent cause of mortality and the principal reason for permanent disability and loss of independence in adult patients. Every year, 3 million women and 2.5 million men worldwide die from this condition. Approximately every three minutes, one American dies of stroke, the World Health Organization estimates mortality due to stroke in Poland at 43 thousand annually (Lo *et al.*, 2003; Mackay and Mensah, 2004).

Stroke triggers immune response in the central nervous system (CNS) and vascular system, which results in inflammation. Aside from the stimulation of brain cells and vascular system, stroke results also in activation of proinflammatory enzymes, among them metalloproteinases (MMPs) (Zaremba and Losy, 2007). The metalloproteinase family includes metalloproteinase 9 (MMP-9, 92 kD) and metalloproteinase 2 (MMP-2, 72 kD) that are involved in acute and chronic phases of stroke, respectively (Sienkiewicz-Jarosz and Ryglewicz, 2007; Woszczycka-Korczyńska *et al.*, 2005; Zaremba and Losy, 2007). According to some authors, an increase in permeability of the blood–brain barrier (BBB) and haemorrhagic transformation of an ischaemic lesion may be associated with a gradual change in MMP-9 concentration during initial days after stroke (Sienkiewicz-Jarosz and Ryglewicz, 2007; Zaremba and Losy, 2007). Some evidence suggests that an increase in MMP-9 concentration may correlate with a deterioration of neurological status (Zaremba and Losy, 2007); however, this relationship was not observed by all authors (Wen *et al.*, 2014). Changes in MMP-2 concentration, a metalloproteinase involved either in progression of cerebral damage or in reparative processes of the brain, can be detected within a few months after stroke (Lenti *et al.*, 2014; Lucivero *et al.*, 2007; Romi *et al.*, 2012; Sienkiewicz-Jarosz and Ryglewicz, 2007; Zaremba and Losy, 2007).

Diagnosis of a cerebrovascular episode is based mainly on neurological examination and imaging studies, especially computed tomography of the head. However, we lack a widely available, sensitive diagnostic test to establish prognosis and to assess the risk of neurological deterioration in stroke patients. Therefore, the aim of this study was to analyse changes in plasma concentrations of MMP-9 and MMP-2 as potential prognostic factors in patients with ischaemic stroke.

METHODS

The study included patients hospitalised at the Department of Neurology with Stroke Unit, University Clinical Hospital in Bialystok. A total of 56 patients with ischaemic stroke (29 women and 27 men), aged between 47 and 99 years, were examined (Tab. 1), along with 60 healthy controls (30 women and 30 men), aged between 45 and 64 years. We did not present the results of separate analyses for men and women, as no statistically significant differences were found between these groups. The group of patients included exclusively individuals presenting with the signs of ischaemic stroke for no more than 24 hours. The presence of a cerebrovascular episode was confirmed at the Hospital's Emergency Department, based on neurological examination and/or computed tomography (CT) of the head. Follow-up CT of the head was always performed 24 hours and 7 days post-admission. The most common comorbidities found in stroke patients included arterial hypertension (84.8%), hypercholesterolemia (41.3%) and atrial fibrillation (28.2%). Exclusion criteria from the study group were: previous history of a cerebrovascular episode and more than 24 hours elapsed since the onset of cerebrovascular symptoms. The control group was comprised of healthy volunteers without a history of neurological and/or inflammatory disorders, recruited among the hospital personnel.

The neurological status of the patients was determined using the National Institutes of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS). Based on the results of these scales, we divided our patients into two groups: 1) with neurological improvement, and 2) with deterioration (Tab. 2, Fig. 1). Each participant was subjected to CT of the head, 12-lead resting electrocardiography and routine laboratory tests (complete blood count, coagulation parameters, levels of electrolytes, urea, creatinine, glucose, alanine

Parameter	Value	
	Volunteers group	Study group
Age, years (mean ± *SD*)	56.2 ± 5.65	75.3 ± 12.75
Sex (M/F), *n* (%)	30/30 (50/50)	27/29 (47.8/52.2)
Hypertension, *n* (%)	2 (3.3)	39 (84.8)
Smoking, *n* (%)	15 (25)	7 (15.2)
Diabetes mellitus, *n* (%)	0 (0)	9 (19.5)
Hypercholesterolemia, *n* (%)	5 (8.3)	19 (41.3)
Atrial fibrillation, *n* (%)	0 (0)	13 (28.2)
Obesity, *n* (%)	2 (3.3)	3 (6.5)
Coronary artery disease, *n* (%)	0 (0)	8 (17.3)
NIHSS-1, points (mean ± *SD*)	N/A	5.9 ± 4.5
NIHSS-2, points (mean ± *SD*)	N/A	5.96 ± 8.4

Tab. 1. Basic characteristics of patients with cerebrovascular episodes

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aminotransferase, aspartate aminotransferase, creatine kinase and its MB isozyme). Finally, each patient was examined by a specialist in internal medicine from the Hospital's Emergency Department, in order to exclude other comorbidities with potential influence on MMP concentrations; all patients with such comorbidities were not included in the analysis. After obtaining written informed consent from patients or their legal guardians (in the case of individuals who did not maintain a logical contact), a 5-mL blood sample was collected using a peripheral venous catheter (usually placed in one of the veins of the antecubital fossa) to determine MMP concentrations. The blood was sampled twice, at admission (i.e. within 24 h of the cerebrovascular episode) and on the 7th day of hospital stay, as previously described (Kurzepa *et al.*, 2006; Lucivero *et al.*, 2007).

Median concentration	MMP-2, admission	MMP-2, 7th day	MMP-9, admission	MMP-9, 7th day
Deterioration	223.7	209.6	88.6	184.1
p-value	N/S		N/S	
Improvement	227.5	234.1	52.8	89.9
p-value	N/S		N/S	
N/S – non-significant ($p > 0.05$).				

Tab. 2. Median concentrations of MMP-2 and MMP-9 in patients with deterioration and improvement of the neurological status determined using NIHSS and mRS, measured at admission and on the 7th day of hospital stay

The material was centrifuged for 15 minutes at 1000 rpm, plasma was distributed into vials and frozen at –80°C until analysis. Concentrations of MMP-2 and MMP-9 were determined immunoenzymatically (ELISA) using Quantikine kits (R&D Systems, Abingdon, United Kingdom) and FL600 analyser (BioTek Instruments, USA), as previously described (Castellanos *et al.*, 2007; Kelly *et al.*, 2008; Kurzepa *et al.*, 2006; Lucivero *et al.*, 2007; Sotgiu *et al.*, 2006). Duplicate samples from each patient were examined, in line with the standard protocol used at the Department of Biochemical Diagnostics, Medical University of Bialystok. According to the manufacturer, the intra-assay coefficient of variation (CV%) for the MMP-2 test equals 3.8% at a mean concentration of 11.2 ng/mL (*SD* = 0.420), whereas the intra-assay CV% for the MMP-9 test amounts to 1.9% at a mean concentration of 2.04 ng/mL (*SD* = 0.039). Inter-assay CV%s for the MMP-2 and MMP-9 test were 6.6% at a mean concentration of 11.1 ng/mL (*SD* = 0.738) and 7.8% at a mean concentration of 2.35 ng/mL (*SD* = 0.184), respectively, as per the manufacturer's data. No significant cross-reactivity or interference was observed.

Statistics

Statistical calculations and analyses were performed with Statistica 10.0 (StatSoft, USA) software. Power analyses were conducted to determine an adequate sample size. Statistical characteristics of continuous variables are presented as medians and ranges. A preliminary statistical analysis (chi-square test) revealed that the distribution of MMP levels did not follow the normal distribution.

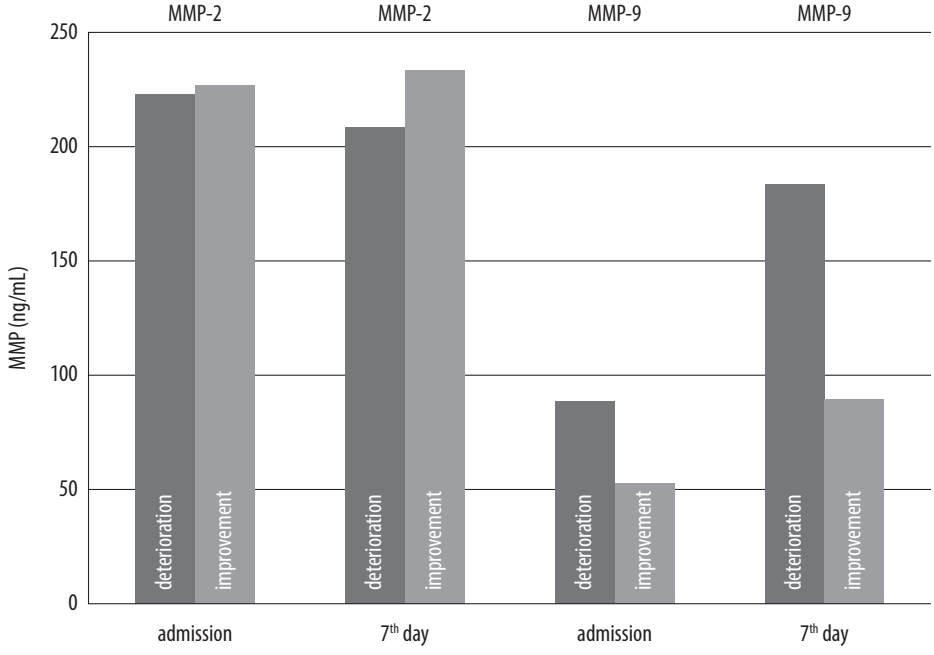

Fig. 1. Median concentrations of MMP-2 and MMP-9 in patients with deterioration and improvement on admission and 7th day

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Consequently, nonparametric tests were used for intergroup comparisons of analysed parameters. Distributions of categorical variables were compared with Mann–Whitney *U* test, and Wilcoxon test was used for intergroup and intragroup comparisons of continuous variable characteristics. The results of all tests were considered significant at $p < 0.05$. Spearman rank correlation coefficients were calculated to study associations between the pairs of variables.

RESULTS

Median concentrations of MMP-2 and MMP-9 in the controls were 224.2 ng/mL and 160.0 ng/mL, respectively. Median concentrations of MMP-2 and MMP-9 in patients with ischaemic stroke amounted to 224.1 ng/mL and 52.9 ng/mL, respectively, at the time of admission, and to 210.3 ng/mL and 66.5 ng/mL, respectively, on the 7th day of hospital stay (Tab. 3, Fig. 2). Median concentrations of MMP-9 at admission turned out to be significantly lower (by 107.1 ng/mL on average, $p < 0.001$) than the respective parameter in the controls. Median concentrations of MMP-9 on the 7th day of hospital stay were significantly higher than at admission (by 13.6 ng/mL on average, $p < 0.001$). In contrast, no statistically significant differences were found in median concentrations of MMP-2 at admission and on the 7th day of hospital stay. Furthermore, median concentrations of MMP-2 in stroke patients, both at admission and on the 7th day of hospital stay, did not differ significantly from the levels of this metalloproteinase in the controls.

We also analysed a relationship between MMP concentrations and the neurological status of stroke patients, expressed with mRS and NIHSS scores. Two groups of patients were identified: with an improvement of neurological status ($n = 36$) and with the lack thereof/deterioration ($n = 20$; Tab. 2, Fig. 1). Median concentrations of MMP-2 in subjects whose neurological status did not improve amounted to 223.7 ng/mL at admission and to 209.6 ng/mL on the 7th day of hospital stay, whereas their median concentrations of MMP-9 equalled 88.6 ng/mL and 184.1 ng/mL, respectively. Median concentrations of MMP-2 in patients with neurological improvement amounted to 227.5 ng/mL at admission and to 234.1 ng/mL on the 7th day of hospitalisation, and their median levels of MMP-9 equalled 52.8 ng/mL and 89.9 ng/mL, respectively. Surprisingly, none of the intergroup differences turned out to be significant on statistical analysis, which suggests that there is no link between MMP concentrations and the neurological status of patients with cerebrovascular episodes.

Median concentration	MMP-2, controls	MMP-2, admission	MMP-2, 7th day	MMP-9, controls	MMP-9, admission	MMP-9, 7th day
Ischaemic stroke	224.2	224.1	210.3	160.0	52.9	66.5
p-value	N/S			* controls *vs.* admission * controls *vs.* 7th day * admission *vs.* 7th day		
* Significant at $p \leq 0.05$; N/S – non-significant ($p > 0.05$).						

Tab. 3. Median concentrations (ng/mL) of MMP-2 and MMP-9 in patients with ischaemic stroke

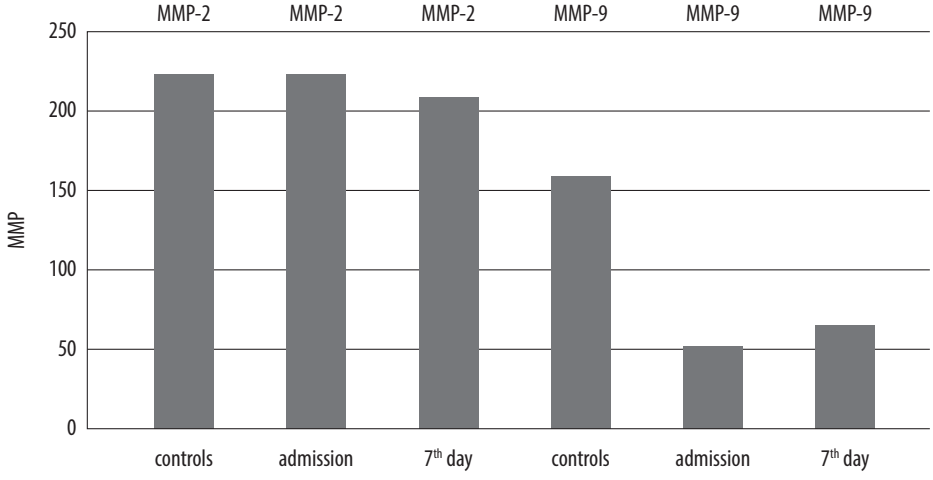

Fig. 2. Median concentrations (ng/mL) of MMP-2 and MMP-9 in patients with ischaemic stroke presented on a bar graph

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DISCUSSION

MMPs belong to the family of zinc endopeptidases. This family includes more than 20 zinc-dependent enzymes with common functional domains. The basic structure of MMPs is made up of: 1) signal peptide which directs these enzymes to a secretory or plasma membrane insertion pathway, 2) prodomain that confers their latency by occupying the active-site zinc and making catalytic MMPs inaccessible to substrates, 3) zinc-containing catalytic domain, 4) hemopexin domain which mediates interactions with substrates and confers specificity of MMPs, and 5) hinge region which links catalytic and hemopexin domains. A plethora of previously published papers have dealt with physiologic and pathologic roles of MMPs. These enzymes were *inter alia* shown to play a role in the pathogenesis of cancer, inflammatory conditions, cardiovascular and pulmonary disorders and diseases of the CNS. Despite a progress in research on MMP biology, the mechanisms due to which these enzymes can interfere with biological functions are still not completely understood. This results *inter alia* from a highly complex biology of MMPs.

Potential biomarkers of stroke reflecting the clinical status of patients with this condition at various stages of treatment and facilitating prognosis, i.e. the likelihood of neurological improvement/deterioration and mortality, have been studied extensively for several years. Recently, also MMP-2 and MMP-9 were recognised as potential biomarkers of stroke due to their documented involvement at both chronic and acute stages of this condition (Castellanos *et al.*, 2007; Corbin *et al.*, 2014; Emsley and Tyrrell, 2002; Hernandez-Guillamon *et al.*, 2012; Horstmann *et al.*, 2003; Jickling *et al.*, 2014; Kelly *et al.*, 2008; Kurzepa *et al.*, 2014, 2006; Lenti *et al.*, 2014; Lo *et al.*, 2003; Lucivero *et al.*, 2007; Mackay and Mensah, 2004; Montaner *et al.*, 2001, 2003; Ott *et al.*, 2014; Rempe *et al.*, 2016; Romi *et al.*, 2012; Rosell and Lo, 2008; Sienkiewicz-Jarosz and Ryglewicz, 2007; Sotgiu *et al.*, 2006; Wen *et al.*, 2014; Woszczycka-Korczyńska *et al.*, 2005; Yong *et al.*, 2001; Zaremba and Losy, 2007). The main source of MMPs in the CNS are resident cells, such as astrocytes, oligodendrocytes, microglial cells, neurons and endothelial cells, as well as infiltrating cells (lymphocytes, granulocytes and macrophages) (Rempe *et al.*, 2016). According to many authors, the acute phase of ischaemic stroke is characterised by overexpression of MMP-9 and brain-derived MMP-2, greater permeability of the BBB and haemorrhagic transformation of ischaemic lesions, which is associated with poor prognosis with regards to the neurological status (Jickling *et al.*, 2014; Montaner *et al.*, 2001). Disruption of the BBB results from enzymatic digestion of proteins in the so-called tight junctions, which facilitates migration of leukocytes across the endothelium (Kurzepa *et al.*, 2014). Lucivero *et al.* (2007) found that an increase in MMP-2 concentration within 12 hours of ischaemic stroke predicts better clinical outcomes, whereas later increase in MMP-9 level (at day 7) is associated with more severe stroke and thus with worse prognosis. In turn, delayed haemorrhagic transformation of ischaemic stroke is postulated to be associated with overactivation of MMP-2, MMP-3, MMP-9 and endogenous tissue plasminogen activator. At admission to hospital, median concentrations of MMP-9 in our stroke patients were significantly lower than in the controls (160 ng/mL *vs.* 52.9 ng/mL), probably due to overexpression of tissue inhibitor of metalloproteinase (TIMP); according to literature, overactivity of TIMP is observed shortly after an ischaemic stroke (Lenti *et al.*, 2014; Lucivero *et al.*, 2007; Woszczycka-Korczyńska *et al.*, 2005). Hernandez-Guillamon *et al.* (2012) reported a slight, statistically insignificant increase in MMP-9 concentration within 24 hours of stroke, and according to other authors (Woszczycka-Korczyńska *et al.*, 2005), overactivity of this enzyme cannot be detected earlier than 4–5 days following the ischaemic episode. Our findings are consistent with this evidence since we observed a significant increase in MMP-9 concentration solely on the 7th day of hospital stay. However, changes in concentration of MMP-2 do not follow a similar pattern; overexpression of this enzyme is detected no earlier than a few months after stroke, which reflects its involvement in regenerative processes (Emsley and Tyrrell, 2002; Hernandez-Guillamon *et al.*, 2012; Horstmann *et al.*, 2003; Montaner *et al.*, 2001, 2003; Rosell and Lo, 2008).

Our study showed that concentrations of MMP-2 and MMP-9 cannot serve as prognostic factors in patients with ischaemic stroke. This observation is consistent with the results of a recently published meta-analysis of 11 studies (Wen *et al.*, 2014). Its authors analysed concentrations of MMP-9 in 540 patients with ischaemic stroke and also concluded that this parameter should not be used as a prognostic marker in this condition (Wen *et al.*, 2014). The results of many previous studies suggest that as proteolytic enzymes, MMPs are involved in various destructive processes in the CNS.

Importantly, recent evidence suggests that MMPs may also exert beneficial effects during the course of reparative and neurodevelopmental processes, including maturation of the nervous system (Corbin *et al.*, 2014; Wen *et al.*, 2014; Yong *et al.*, 2001). Moreover, MMPs have been recently demonstrated to be essential in both acute and chronic phase of ischaemic stroke. While MMPs impair integrity of the BBB and contribute to parenchymal tissue damage in acute stroke, they are also involved in recovery processes during the chronic phase, participating in remodelling of ischaemic and infarct tissue (Rempe *et al.*, 2016). Moreover, these enzymes are involved in angiogenesis, vasculogenesis and neurogenesis (Rempe *et al.*, 2016).

CONCLUSION

To summarise, the lack of significant changes in concentrations of MMP-2 during the acute phase of ischaemic stroke implies that this metalloproteinase may be involved at later

stages of the remodelling process. In turn, the lack of an association between MMP-9 concentration and the clinical status suggests that this enzyme should not be used as a prognostic factor in individuals with ischaemic cerebral episodes.

Ethics

The study was approved by the Local Ethics Committee at the Medical University of Bialystok (R-I-002/164/2011). Written informed consent was sought from the study participants or their next of kin prior to history taking, neurological examination and blood sampling.

Conflict of interest

None of the authors declare any conflicts of interest or competing interests.

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