Simvastatin Could Prevent Increase of the Serum MMP-9/TIMP-1 Ratio in Acute Ischaemic Stroke

(simvastatin / MMP-9 / TIMP-1 / stroke)

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Abstract. MMP-9 plays an important role in the pathogenesis of AIS and predicts haemorrhagic transformation of the ischaemic focus. The aim of our study was to analyse both serum MMP-9 and its most specific endogenous inhibitor (TIMP-1) levels in AIS and to check whether HMG-CoA reductase inhibitor (simvastatin) affects the MMP-9/TIMP-1 ratio value. Fifty patients with AIS were randomly divided into two groups: Group I (N = 25) treated with 40 mg/day with simvastatin within 24 hours after the onset of stroke and Group II (N = 25) non-treated with statin. To evaluate MMP-9 and TIMP-1 serum levels, the ELISA method was used. The serum MMP-9 level was significantly elevated on the 7th day of stroke in both groups (from 668 to 862 ng/ml and 670 to 855 ng/ml, respectively, in Group I and II). The serum TIMP-1 level was also elevated on the 7th day of stroke in both groups but the results were not significant. The MMP-9/TIMP-1 ratio was elevated on the 7th day of stroke in both groups, but the result was significant only in the Group II ($P < 0.01$). These findings indicate that simvastatin given during 24 hours after the onset of stroke could have an influence on the MMP-9/TIMP-1 ratio during AIS.

Metalloproteinase-9 (MMP-9) belongs to Zn^{2+}-dependent inducible endopeptidases targeting extracellular proteins such as different types of collagen (mainly type IV), elastin, laminin, and moreover gelatin (Chandler et al., 1997). All MMPs are blocked by specific tissue inhibitors of matrix metalloproteinases (TIMPs) in the extracellular fluid (ECF). The most specific inhibitor of MMP-9 is TIMP-1 (Brew, 2000). The serum MMP-9/TIMP-1 concentration ratio indirectly defines the serum MMP-9 activity *in vivo* (Avolio et al., 2003). MMP-9 plays an important role in numerous neurological diseases especially with the inflammatory pathogenesis (Hartung and Kieseier, 2000) as well as in the acute ischaemic stroke (AIS) (Planas et al., 2001; Castillo and Rodriguez, 2004). MMP-9 is involved in many pathological processes: blood-brain barrier (BBB) destruction, oedema formation (Petty and Wettstein, 2001), facilitation of leukocyte migration within the brain tissue, activating proinflammatory cytokines such as tumour necrosis factor α (TNF-α), interleukin-1β (IL-1β) (Opdenakker et al., 2001), and destruction of myelin proteins (Chandler et al., 1995). High serum MMP-9 activity in the acute phase of ischaemic stroke contributes to the increased risk of haemorrhage within an ischaemic focus (Castellanos et al., 2003). Clinical studies with 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) suggest significant benefits of treatment with statins in acute phase of stroke (Montaner et al., 2004). Review papers about statins indicate reduced risk of stroke by both stabilization of the carotid artery atherosclerotic plaque and a variety of pleiotropic mechanisms including decrease of the MMP expression and activation (Montaner, 2005). The general mechanism of statin action on the inhibition of MMP activity is known, but this effect has not been clearly explained in AIS. Our purpose was to analyse the influence of simvastatin, an HMG-CoA reductase inhibitor, on serum MMP-9 and TIMP-1 concentrations in patients with AIS.

Material and Methods

Patients

The study group finally consisted of 50 patients with AIS diagnosed by CT-scan and randomly assigned to two groups:

Group I (N = 25) patients treated with 40 mg/day of simvastatin *p.o.* Treatment was started within the first 24 h of stroke onset; mean age was 75.7 years (SD 8.3; range 59-94 years), male/female: 10/15;

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Abbreviations: AIS – acute ischaemic stroke, BBB – blood-brain barrier, ECF – extracellular fluid, HMG-CoA – 3-hydroxy-3-methylglutaryl coenzyme A, MMP-9 – matrix metalloproteinase-9, TIMP-1 – tissue inhibitor of matrix metalloproteinase-1.

Group II (N = 25) patients non-treated with statin; mean age was 72.1 years (SD 7.2; range 53-91 years), male/female: 12/13.

All patients were untreated with statins within the last 6 months before entering the study, without the history of autoimmunological diseases and cancer, had a normal level of serum total protein and renal function parameters. Patients with inflammatory symptoms and body temperature above 37°C during the first 7 days of observation were not included into the study. 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.

Biochemical procedures

Blood samples were obtained at two time-points after the stroke: during the first 24 hours of stroke (before statin administration) and 7 days after the stroke onset. After centrifugation the serum was stored at – 30°C no longer than 6 months. A commercially available ELISA kit was used to evaluate the total serum MMP-9 (R&D System, Minneapolis, MN) and TIMP-1 (R&D System) levels according to the producer's instructions. The optical density was determined by using a microplate reader set to 450 nm (correction 540 nm). All measurements were performed in duplicate. Results were expressed in ng/ml.

Statistics

Paired t-test was performed to compare differences (MMP-9, TIMP-1 concentrations, MMP-9/TIMP-1 ratio) between the 1st and 7th day of the stroke. Differences between both groups in MMP-9, TIMP-1 concentrations and MMP-9/TIMP-1 ratio measured on the 1st day and 7th day of stroke were calculated by using Mann-Whitney's U test. Statistically significant values were considered when $P < 0.05$. Statistical analysis was performed with the use of the computer-assisted statistical program GraphPad InStat v. 3.06.

Results

The total serum MMP-9 level on the 7th day of stroke was significantly higher compared to the level at 24 h in both groups. In Group I mean MMP-9 concentration was increased from 668 ng/ml [SD 473] at 24 h to 869 ng/ml [SD 504] on the 7th day of stroke ($P < 0.05$). In Group II serum MMP-9 concentrations were 670 ng/ml [SD 335] and 855 ng/ml [SD 381] at 24 h and the 7th day, respectively ($P < 0.05$) (Fig. 1). The serum TIMP-1 level was also elevated on the 7th day of stroke, but the results were not significant in both groups (from 304 ng/ml [SD 113] to 341 ng/ml [SD 146] in Group I and from 313 ng/ml [SD 73] to 348 ng/ml [SD 119] in Group II).

The MMP-9/TIMP-1 concentration ratio was elevated on the 7th day of stroke compared to 24 h in both groups, but the result was statistically relevant only in Group II. In Group I, the MMP-9/TIMP-1 ratio was increased from 2.23 [SD 1.39] to 2.54 [SD 1.37] ($P > 0.05$), but in Group II the ratio was increased from 2.19 [SD 1.13] to 2.62 [SD 1.27] ($P < 0.01$) (Fig. 2).

There were no statistical differences between both groups in MMP-9, TIMP-1 levels and MMP-9/TIMP-1 ratio measured on the 1st day and 7th day of stroke ($P > 0.05$).

Discussion

In our previous study we noticed an inhibiting influence of simvastatin on the serum MMP-9 (92 kDa) activity during the first week of ischaemic stroke (Kurzepa et al., 2006). The present study shows that early treatment with simvastatin (40 mg/day) started at

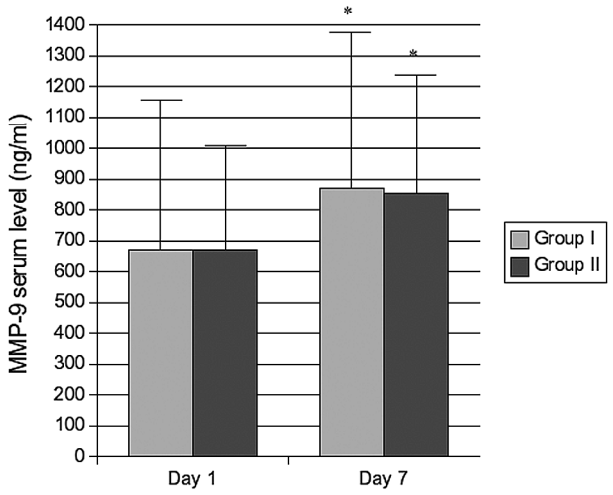

Fig. 1. Total MMP-9 serum level on the 1st and 7th day of ischaemic stroke in Group I (treated with simvastatin) and Group II (non-treated with statin). * $P < 0.05$, paired t-test.

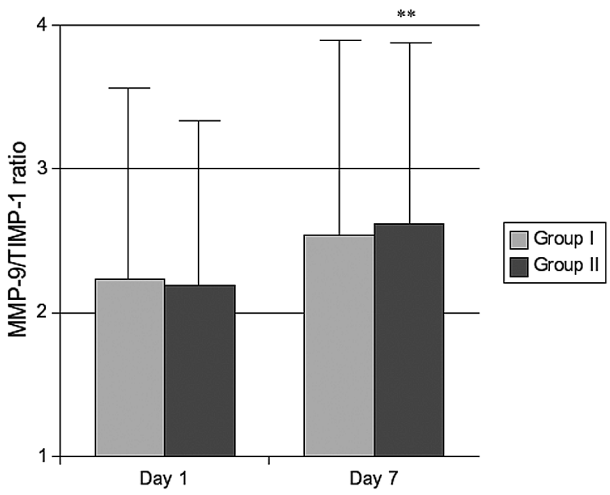

Fig. 2. MMP-9/TIMP-1 ratio on the 1st and 7th day of ischaemic stroke in Group I (treated with simvastatin) and Group II (non-treated with statin). ** $P < 0.01$, paired t-test.

24 h of stroke does not affect the total MMP-9 serum level in patients with AIS. On the 7th day of stroke the serum MMP-9 level increased about 25–27% ($P < 0.05$) in both groups and it seems that simvastatin did not have any influence on this process. Simvastatin also did not affect the serum TIMP-1 level measured on the 7th day of stroke. In both study groups the increase of the TIMP-1 level was not statistically significant. Considering serum concentrations of both MMP-9 and TIMP-1 we evaluated MMP-9/TIMP-1 ratio, which could be an indicator of MMP-9 activity *in vivo*. Despite that MMP-9/TIMP-1 ratio increases on the 7th day compared to 24 h of stroke in both groups, only in Group II the difference was statistically relevant ($P < 0.01$) (13.9% and 19.6% increase on the 7th day in Group I and Group II, respectively). The coincidental increase of serum MMP-9 together with TIMP-1 concentration was more often found in the statin-treated group, so that the MMP-9/TIMP-1 ratio was not elevated to such extent as in the non-statin group.

The known mechanism of statin influence on MMP-9 activity depends mainly on the inhibition of activity of nuclear factor-κB (NF-κB), which is the key to expression of many proinflammatory cytokines and MMP-9 (Laws et al., 2004). TIMP-1 together with MMP-9 are ranked among inducible proteins for which expression of NF-κB plays a critical role. This study did not show any influence of early treatment with simvastatin on the serum MMP-9 and TIMP-1 levels in AIS, except for the expression of MMP-9, which was evaluated only at the protein level. However, further studies with larger study groups evaluating the role of statins on the MMP-9/TIMP-1 ratio in ischaemic stroke are needed.

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