Pharmacological Reports
2008, 60, 1014–1018
ISSN 1734-1140

Short communication

Simvastatin inhibits the increase in serum tau protein levels in the acute phase of ischemic stroke

Jacek Kurzepa[1], Joanna Bielewicz[2], Halina Bartosik-Psujek[2], Anna Szczepańska-Szerej[2], Zbigniew Stelmasiak[2]

[1]Department of Biochemistry and Molecular Biology, Medical University of Lublin, Chodźki 1, PL 20-093 Lublin, Poland

[2]Department of Neurology, Medical University of Lublin, Jaczewskiego 9, PL 20-954 Lublin, Poland

Correspondence: Jacek Kurzepa, e-mail: kurzepa@onet.pl

Abstract:
Our goal was to analyze the effects of treatment with a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (simvastatin, 40 mg/day) on serum S100BB and tau protein levels during the acute ischemic stroke (IS). Twenty four patients with IS were divided into two equal groups; treated and untreated with simvastatin. Blood was obtained four times during acute IS. Tau protein was noticed in six patients from treated group and in five patients from untreated group. The serum tau protein levels significantly increased on the 10th day only in patients untreated with simvastatin ($p < 0.05$). Simvastatin did not exert an effect on serum S100BB protein levels.

Key words:
stroke, simvastatin, tau protein, S100BB protein

Abbreviations: BBB – blood-brain barrier , BI – Barthel Index, CSF – cerebrospinal fluid, HMG-CoA – 3-hydroxy-3-methylglutaryl coenzyme A, IS – ischemic stroke, MMP – matrix metalloproteinase, NIHSS – National Institute of Health Stroke Scale

Introduction

The S100BB protein is physiologically present in astroglial cells. During pathological conditions, it is released to the cerebrospinal fluid (CSF) as well as, due to its small size, to the blood [6]. Serum S100BB levels at 48 and 72 h after the ischemic stroke (IS) onset highly correlate with infarct volume of the brain tissue [5]. S100BB exerts an apoptotic effect on neurons and predicts blood-brain barrier (BBB) disruption [6, 7]. Moreover, some authors indicate that the serum S100BB level can function as a noninvasive marker of BBB damage [8]. Other studies have highlighted the role of the S100BB protein in neuroplasticity and its positive correlation with the neurological status of stroke patients at three months after the stroke [17]. In contrast to S100BB, the tau protein, a biological marker of neuron or axonal damage, rises in the serum of approximately 30% of stroke patients. In such cases, the serum tau level also correlates with the se-

verity of stroke and infarct volume [2]. The large tau protein can be secreted across a damaged BBB and released into systemic circulation after the disintegration of neuron cell membranes, which results from either apoptosis or necrosis [2]. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors (statins) are widely applied for the primary or secondary prevention of IS [1]. Experimental data have shown that the pleiotropic effects of statins might be responsible for their role in neuroprotection, in addition to their lipid-lowering properties. Clinical studies with statins have suggested that there could be significant benefits of treating with simvastatin in the acute phase of IS (see [11, 13] for review).

Our goal was to evaluate whether the pleiotropic properties of simvastatin are reflected in either the serum protein concentrations of S100BB and tau or in the clinical outcome of patients in the acute phase of IS.

Materials and Methods

Our study group consisted of 24 patients admitted to the Stroke Unit at the Department of Neurology, Medical University of Lublin. The diagnosis of IS was confirmed by computed tomography scan (CT). Patients were randomly assigned into two groups. In group 1 [n = 12, mean age: 71.7 years (SD: 6.9) range: 60–81 years; male/female: 7/5], simvastatin (Zocor, MSD, Switzerland) at 40 mg/day was administrated *po* within 24 h of the stroke onset. Group 2 [n = 12, mean age: 69.8 years (SD: 8.9) range: 54–83 years; male/female: 5/7] was not treated with simvastatin during the first 10 days after the stroke. Our exclusion criteria were: treatment with statins during the last six months prior to the stroke, inflammatory disorders during the last two weeks and a history of autoimmune diseases and cancer. No persons from the study groups suffered from diabetes – 21 were hypertensive patients. Within the first four days of stroke, antihypertensive drugs were not administrated to the patients according to standard protocol. In cases of increased of blood pressure, 12.5 mg of captopril (Captopril, Polpharma, Poland) *po* was administrated. All patients received, acetylsalcylic acid at 75 mg/day *po* (Acard, WZF Polfa, Poland), 0.9% NaCl 1500–2000 ml *iv* and a potassium supplement in accordance with the actual potassium serum level. It was unnecessary to administer osmotic agents, such as mannitol, which could disrupt BBB [16] and affect the findings of the study. A healthy control group, which approximately matched the experimental group in age and sex, was also included in the study [n = 17, mean age: 64.9 years (SD: 7.1) range: 51–77 years; male/female: 6/11]. Written informed consent was obtained from each patient (or from family members when necessary). The Ethics Committee (Medical University of Lublin) accepted the protocol of the study.

Blood samples were obtained at four time-points: during the first 24 h of stroke (between 6 and 24 h, before treatment with simvastatin) and on the 3rd, 5th and 10th days after the onset of symptoms. At the same time-points, the neurological examinations were performed with the National Institute of Health Stroke Scale (NIHSS). The Barthel Index (BI), which measures a patient's disability, was performed at the above mentioned time-points and at three months after the stroke onset.

Control blood samples were obtained only once.

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to evaluate serum S100BB (CanAg, Fujirebio Diagnostic AB, Sweden) and tau levels (Innotest, Innogenetics NV, Belgium) according to the product instructions. The Innotest tau ELISA kit was originally accredited for CSF, but it has since been applied to measure the tau level in serum samples [2]. The detection limit of the tau protein assay was 60 pg/ml, which corresponds to the lowest standard in the calibration curve. All values below the detection limit were not used in the analysis. The optical density was determined with a microplate reader set to 450 nm. An ANOVA test and Student *t*-test were used for S100BB evaluation. Due to the small number of individuals, non-parametric repeated measures Friedman test and Mann-Whitney test were applied for the statistical analysis of the tau protein. Statistically significant values were considered when $p < 0.05$.

Results

The baseline clinical characteristics and etiology of IS (thrombosis, embolism, unknown etiology) did not significantly differ between groups. There were no significant differences in the neurological status (NIHSS) and patient disability (BI) between both groups at day 1

Tab. 1. The score of National Institute of Health Stroke Scale (NIHSS) and Barthel Index (BI) during acute stroke. BI was additionally scored at three months after the stroke. Median values (1st – 3rd quartiles). No differences were observed between Groups 1 and 2 (treated and untreated with simvastatin) during the evaluated time-points. NA – not applicable; M-W – Mann-Whitney test

	Day 1	Day 3	Day 5	Day 10	Month 3	
NIHSS						M-W test
Group 1	12 [7–18]	11 [3–13]	11 [3–11]	10 [2–11]	NA	$p > 0.05$
Group 2	13 [7–17]	12 [6–15]	12 [4–14]	11 [3.5–13]	NA	
BI						M-W test
Group 1	0 [0–45]	0 [0–15]	10 [0–65]	15 [0–65]	50 [45–85]	$p > 0.05$
Group 2	0 [0–0]	5 [0–21.5]	12.5 [0–47.5]	12.5 [0–58.5]	80 [60–90]	

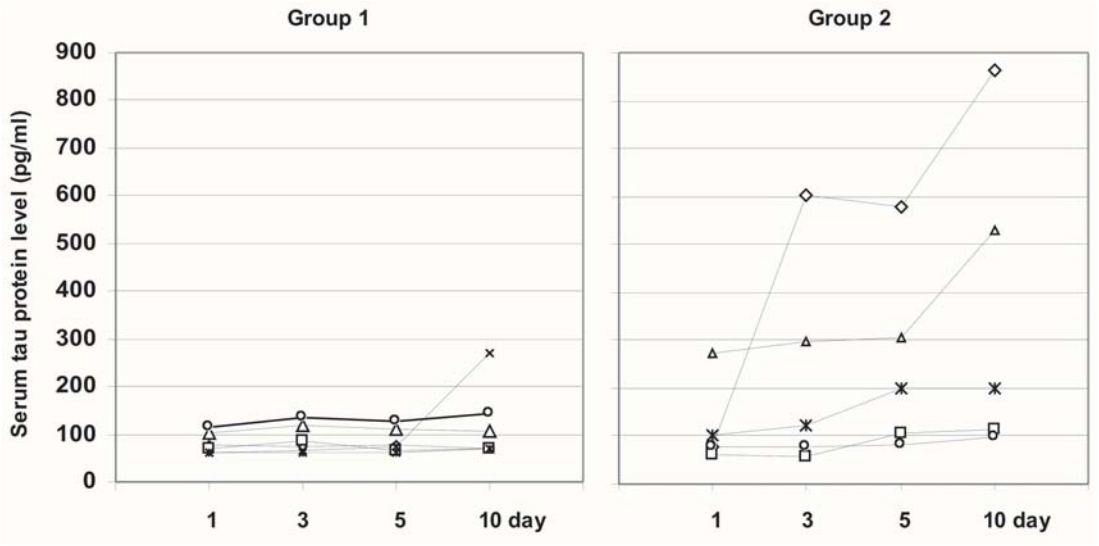

Fig. 1. Temporal profiles of serum tau protein levels in patients treated (Group 1) or untreated (Group 2) with simvastatin in the acute phase of ischemic stroke. Six patients from Group 1 and five from Group 2 had detectable serum tau protein levels. In Group 1 only, serum tau protein levels did not increase during the first 10 days of stroke. Detection limit: 60 pg/ml

of IS (Tab. 1). The gradual improvement of neurological status resulted in decreasing NIHSS; an augmentation of BI scores was observed during the first 10 days after the stroke in all patients. The differences in NIHSS and BI scores between both groups were not statistically significant throughout the evaluated time-points. After three months, three patients from group 1 and one from group 2 had passed away. The median value of BI score was higher in group 2 after three months from the onset of symptoms; however, these differences were insignificant.

The tau protein was found to be above the mentioned detection limit in the sera of six patients (50%) from group 1 and five patients (42%) from group 2. The tau protein was not detected in control sera. A gradual increase in the tau protein level during the study was noticed only in patients from group 2. The median tau protein level increased approximately 2-fold over the 3rd and 5th days and greater than 3-fold by the 10th day in patients not treated with simvastatin ($p < 0.05$, Friedman test). There was no significant increase in the serum tau protein level during those same time-points in group 1 ($p > 0.1$, Friedman test). There were no statistical differences in the measurements of serum tau protein levels in groups 1 and 2 ($p > 0.1$, Mann-Whitney test) (Fig. 1).

The changes in S100BB concentration at the evaluated time-points were also not statistically significant

Tab. 2. Serum S100BB and tau protein levels in acute phase of ischemic stroke. The concentration of tau protein significantly increased at day 10 after the stroke from the level at the stroke onset only in patients that were not treated with simvastatin (* $p < 0.05$, Dunn's *post-hoc* test). SD – standard deviation

	Day 1	Day 3	Day 5	Day 10	Test
S100BB (pg/ml)					ANOVA
Group 1 mean (SD)	47.7 (23.53)	78.05 (59.92)	57.8 (36.1)	42.85 (17.6)	p = 0.07
Group 2 mean (SD)	64.31 (28.43)	83.88 (68.78)	74.08 (40.47)	41.33 (15.12)	p = 0.11
tau (pg/ml)					Friedman
Group 1 median	73.24	80.94	75.81	87.07	p = 0.53
1st quartile	63.42	69.01	68.62	68.92	
3rd quartile	94.89	111.65	102.19	134.85	
Group 2 median	78.11	187.4	203.82	320.44*	p = 0.003
1st quartile	73.69	73.01	98.41	108.32	
3rd quartile	127.53	372.9	372.34	612.29	

(in both groups 1 and 2, $p > 0.1$, ANOVA test). Control serum S100BB levels were significantly lower than the test groups at all time-points. There were also no significant differences between serum S100BB protein levels in groups 1 and 2 measured at the same time-points ($p > 0.1$, Student *t*-test). The exact results are given in Table 2.

Discussion

The benefits of statins for the clinical outcome of stroke patients have been indicated in numerous studies. In addition to the well-known lipid-lowering properties, statins have multiple neuroprotective features, such as the ability to decrease nuclear factor-κB, reactive oxygen species, platelet reactivity, thromboxane A_2, c-reactive protein, matrix metalloproteinases (MMPs) and tumor necrosis factor α and the ability to increase endothelial nitric oxide synthase and tissue-type plasminogen activator [11, 13, 14]. These effects are not associated with the decrease in serum cholesterol level. The acute phase of stroke is too short to respond to the classic mechanism of the statins action [10]. As such, simvastatin has been administrated to patients regardless of their lipid profile. To our knowledge, this study is the first to focus on the effect of an early treatment with simvastatin on serum tau and S100BB protein levels during acute IS. We observed that the S100BB protein level in serum does not depend on simvastatin treatment. This finding suggests that simvastatin probably does not exert an effect on astroglial damage within the ischemic focus. Previous studies have shown that serum S100BB protein levels correlate with the volume of brain infarct [5]. Our results show that simvastatin does not influence serum S100BB protein levels. Therefore, it is highly probable that simvastatin does not influence the volume of ischemic focus either. This conclusion needs to be confirmed with CT and magnetic resonance imaging scans to examine the volume of the brain infarct. In contrast, simvastatin treatment within 24 h of IS onset was found to prevent the augmentation of serum tau protein levels. The neuroprotective properties of statins can exert biochemical and clinical effects. The reduction of neuronal death decreases the release of neuronal damage biomarkers, such as the tau protein. While there have been promising experimental data on the early administration of statins, the clinical benefits of an early treatment with simvastatin are not clear [12]. Our investigation does not confirm the clinical efficacy of an early treatment with statin.

The second potential cause of high tau protein levels in serum could be an intense BBB disruption. Undamaged BBB blocks the release of the tau protein into the blood. The function of the BBB can be assayed by measuring the albumin CSF/serum ratio, but a lumbar puncture is essential for this kind of analysis [15]. Previous studies have indicated that the serum

S100BB level is a noninvasive, sensitive marker of BBB disruption [8]. In our study, the highest levels of S100BB protein were observed on the 3rd day of IS; no differences were observed between study groups. This finding suggests that the BBB damage is most intense on the 3rd day. On the other hand, serum tau protein levels increased only in the group that was not treated with simvastatin until the 10th day of IS. Previously, we showed that the administration of simvastatin (40 mg/day) from the first day of IS prevents from the augmentation of serum MMP-9 activity in the acute phase of IS [9]. Considering that MMP-9 is involved in BBB disruption [4], simvastatin can potentially decrease BBB permeability and effectively decrease the release of tau protein into the blood. Unfortunately, the lack of an effect of simvastatin on serum S100BB levels fails to confirm its role in BBB function and limits the positive effects of simvastatin in neuroprotection.

The fact that the tau protein was not detected in the blood of all stroke patients could also be the result of the different reperfusion times. Spontaneous recanalization restores the blood flow within part of the ischemic focus and may allow the tau protein to be washed out from the damaged neurons or axons [2]. MMP-9 is also involved in the recanalization process [3]; therefore, the above mentioned inhibition of MMP-9 activity by simvastatin [9] can postpone the onset of reperfusion. Doppler sonography should help explain the relationship between reperfusion and the presence of the tau protein in the serum of stroke patients in some cases.

Future investigations in this field ought to be performed with larger groups of individuals. In conclusion, we suggest that simvastatin treatment that is implemented within 24 h of IS onset exerts a neuroprotective effect that is manifested by a decrease in serum tau protein levels in the acute phase of IS.

References:

1. Athyros VG, Kakafika AI, Tziomalos K, Papageorgiou AA, Karagiannis A: Statins for the prevention of first or recurrent stroke. Curr Vasc Pharmacol, 2008, 6, 124–133.
2. Bitsch A, Horn C, Kemmling Y, Seipelt M, Hellenbrand U, Stiefel M, Ciesielczyk B et al.: Serum tau protein level as a marker of axonal damage in acute ischemic stroke. Eur Neurol, 2002, 47, 45–51.
3. Bouzeghrane F, Darsaut T, Salazkin I, Ogoudikpe C, Gevry G, Raymond J: Matrix metalloproteinase-9 may play a role in recanalization and recurrence after therapeutic embolization of aneurysms or arteries. J Vasc Interv Radiol, 2007, 18, 1271–1279.
4. Castellanos M, Leira R, Serena J, Pumar JM, Lizasoain I, Castillo J, Davalos A: Plasma metalloproteinase-9 concentration predicts hemorrhagic transformation in acute ischemic stroke. Stroke, 2003, 34, 40–46.
5. Foerch C, Singer OC, Neumann-Haefelin T, du Mensil de Rochemont R, Steinmeiz H, Sitzer M: Evaluation of serum S100B as a surrogate marker for long-term outcome and infarct volume in acute middle cerebral artery infarction. Arch Neurol, 2005, 62, 1130–1134.
6. Foerch C, Wunderlich MT, Dvorak F, Humpich M, Kahles T, Goertler M, Alvarez-Sabin J et al.: Elevated serum S100B levels indicate a higher risk of hemorrhagic transformation after thrombolytic therapy in acute stroke. Stroke, 2007, 38, 2491–2495.
7. Hu J, Ferreira A, Van Eldik LJ: S100β induces neuronal cell death through nitric oxide release from astrocytes. J Neurochem, 1997, 69, 2294–2301.
8. Kanner AA, Marchi N, Fazio V, Mayberg MR, Koltz MT, Siomin V, Stevens GH et al.: Serum S100β: a noninvasive marker of blood-brain barrier function and brain lesions. Cancer, 2003, 97, 2806–2813.
9. Kurzepa J, Szczepańska-Szerej A, Stryjecka-Zimmer M, Małecka-Massalska T, Stelmasiak Z: Simvastatin could prevent increase of the serum MMP-9/TIMP-1 ratio in acute ischaemic stroke. Folia Biol (Praha), 2006, 52, 181–183.
10. Laws PE, Spark JI, Cowled PA, Fitridge RA: The role of statins in vascular disease. Eur J Vasc Endovasc Surg, 2004, 27, 6–16.
11. Montaner J: Treatment with statins in the acute phase of ischemic stroke. Expert Rev Neurother, 2005, 5, 211–221.
12. Montaner J, Chacon P, Krupiski J, Rubio F, Millan M, Molina CA, Hereu P et al.: Simvastatin in the acute phase of ischemic stroke: a safety and efficacy pilot trial. Eur J Neurol, 2008, 15, 82–90.
13. Stępień K, Tomaszewski M, Czuczwar SJ: Neuroprotective properties of statins. Pharmacol Rep, 2005, 57, 561–569.
14. Szczepańska-Szerej A, Kurzepa J, Wojczal J, Stelmasiak Z: Simvastatin-induced prevention of the increase of TNF-α level in the acute phase of ischemic stroke. Pharmacol Rep, 2007, 59, 94–97.
15. Wallin A, Blennow K, Rosengren L: Cerebrospinal fluid markers of pathogenetic processes in vascular dementia, with special reference to the subcortical subtype. Alzheimer Dis Assoc Disord, 1999, 13, Suppl 3, S102–105.
16. Wang M, Etu J, Joshi S: Enhanced disruption of the blood brain barrier by intracarotid mannitol injection during transient cerebral hypoperfusion in rabbits. J Neurosurg Anesthesiol, 2007, 19, 249–256.
17. Węglewski A, Ryglewicz D, Mular A, Juryńczyk J: Changes of protein S100β serum concentration during ischemic and hemorrhagic stroke in relation to the volume of stroke lesion (Polish). Neurol Neurochir Pol, 2005, 39, 310–317.

Received:
April 13, 2008; in revised form: November 24, 2008.