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Short communication

Simvastatin-induced prevention of the increase in TNF-α level in the acute phase of ischemic stroke

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Abstract:
Like other proinflammatory cytokines, TNF-α may play an important role in the development of central nervous system injury following ischemic stroke. The aim of this study was to evaluate the influence of early treatment with simvastatin, an HMG-CoA reductase inhibitor, on serum TNF-α level in acute ischemic stroke (AIS). Patients with AIS (n = 36) were randomly assigned to the two groups: Group I (n = 18) treated with simvastatin 40 mg/day within 24 h after the onset of stroke and Group II (n = 18) not treated with the statin. Blood samples were obtained on days 1, 3 and 7 after stroke onset. Serum TNF-α level was significantly elevated on day 3 after the stroke onset in comparison to day 1 only in the simvastatin-treated group (increase in median values by 16.2% [p = 0.028] and 6.1% within 3 days in Group II and I, respectively). These findings indicate that simvastatin given within 24 h after the onset of stroke could prevent the increase in serum TNF-α level within 3 days.

Key words:
TNF-α, simvastatin, ischemic stroke

Abbreviations: AIS – acute ischemic stroke, HMG-CoA – 3-hydroxy-3-methylglutaryl coenzyme A, NF-κB – nuclear factor kappa-B, TNF-α – tumor necrosis factor alfa

Introduction

Although HMG-CoA reductase inhibitors (statins) are being used for the secondary prevention of ischemic stroke, recent experimental data have shown new pleiotropic effects of these drugs independent of their lipid-lowering properties that might be responsible for their role in neuroprotection. There are some additional evidences that treatment with statins is not only associated with a reduced incidence of strokes but with reduced stroke severity, as well [3, 6, 11]. In a small pilot trial with simvastatin in acute ischemic stroke (AIS), Montaner et al. showed more cases of complete functional recovery after 3 months of treatment with simvastatin (40 mg/day) in comparison with the non-treated group ($p < 0.05$). The significant improvement was observed on day 3 of treatment [10].

Animal studies confirmed the observations obtained in clinical trials and provided additional data

Tab. 1. Characteristics of the study groups

		No.	Age (years)	Males/females
Group I	Simvastatin (+)	n = 18	73.4 (SD 6.4; range 60–81)	8/10
Group II	Simvastatin (–)	n = 18	74.3 (SD 5.9; range 57–79)	7/11
Control		n = 15	73.2 (SD 5.3; range 57–85)	6/9

on the putative mechanisms of action underlying this beneficial effect [1, 4, 12]. Benefits of statin treatment can be explained by activation of endothelial nitric oxide synthase (eNOS), reducing activity of TNF-α and interleukin-1β (IL-1β), decreasing the level of reactive oxygen species and inhibition of matrix metalloproteinases (MMPs) (see [13] for review). The inflammatory response is a critical component of the complex pathological response to stroke. TNF-α, a proinflammatory cytokine, is strongly involved in the pathogenesis of ischemic stroke. As intraventricular injection of TNF-α enlarges infarct volume in the animal model [2], thus inhibition of TNF-α induced by statins could be promising in treatment of strokes.

This study investigated the effect of simvastatin 40 mg/day administered during AIS on serum TNF-α level.

Material and Methods

The study group consisted of 36 patients in the acute phase of ischemic stroke consecutively admitted to the Stroke Unit in the Neurological Department of the Medical University of Lublin. The diagnosis of AIS was established clinically and by CT-scan. Patients were randomly assigned to two groups: **Group I** was treated with 40 mg/day of simvastatin (Zocor, MSD) *po* within 24 h of stroke onset and **Group II** was not treated with simvastatin. Seven days after stroke, simvastatin (40 mg/day) was also added to the treatment in group II. Treatment with simvastatin was continued in both groups during the entire hospitalization period. A healthy control group which approximately matched the experimental group in age and sex was also included in the study. The general descriptions of the patients and controls are given in Table 1.

Exclusion criteria were: treatment with statins during the last 6 months before the stroke, inflammatory disorders during the last 2 weeks as well as the history of autoimmunological diseases and cancer.

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 (KE-0254/204/2004).

Blood samples were obtained three times after stroke: during the first 24 h from the stroke (before treatment with statin) and on day 3 and 7 after the stroke onset. For the control group, blood samples were taken only once. A commercially available high-sensitivity ELISA kit was used to evaluate the serum TNF-α level (R&D System HSTA00C) according to the product instructions. The optical density was determined by using a microplate reader, set to 450 nm (correction 540 nm). All measurements were performed in duplicate.

A Friedman test was performed to compare serum TNF-α level on day 1, 3 and 7 and a Wilcoxon test was used to compare TNF-α values on day 1 and 3 in both study groups. Additionally, a Man-Whitney test was used to compare TNF-α values on day 1 in stroke patients with control values and to compare between both groups on day 1, 3 and 7. Statistical analyses were performed with the use of GraphPad InStat v. 3.06. Results were presented in the figures as the median, and as 1st and 3rd quartile. Values were considered statistically significant when $p < 0.05$.

Results and Discussion

Serum concentration of TNF-α was not significantly different between controls and all stroke patients during the first 24 h after stroke onset. The increase in TNF-α level by 0.31 pg (16.2%) was observed on day 3 after the stroke onset only in those patients not treated with simvastatin ($p = 0.028$). The increase in TNF-α level (by 0.12 pg – 6.2%) was also observed

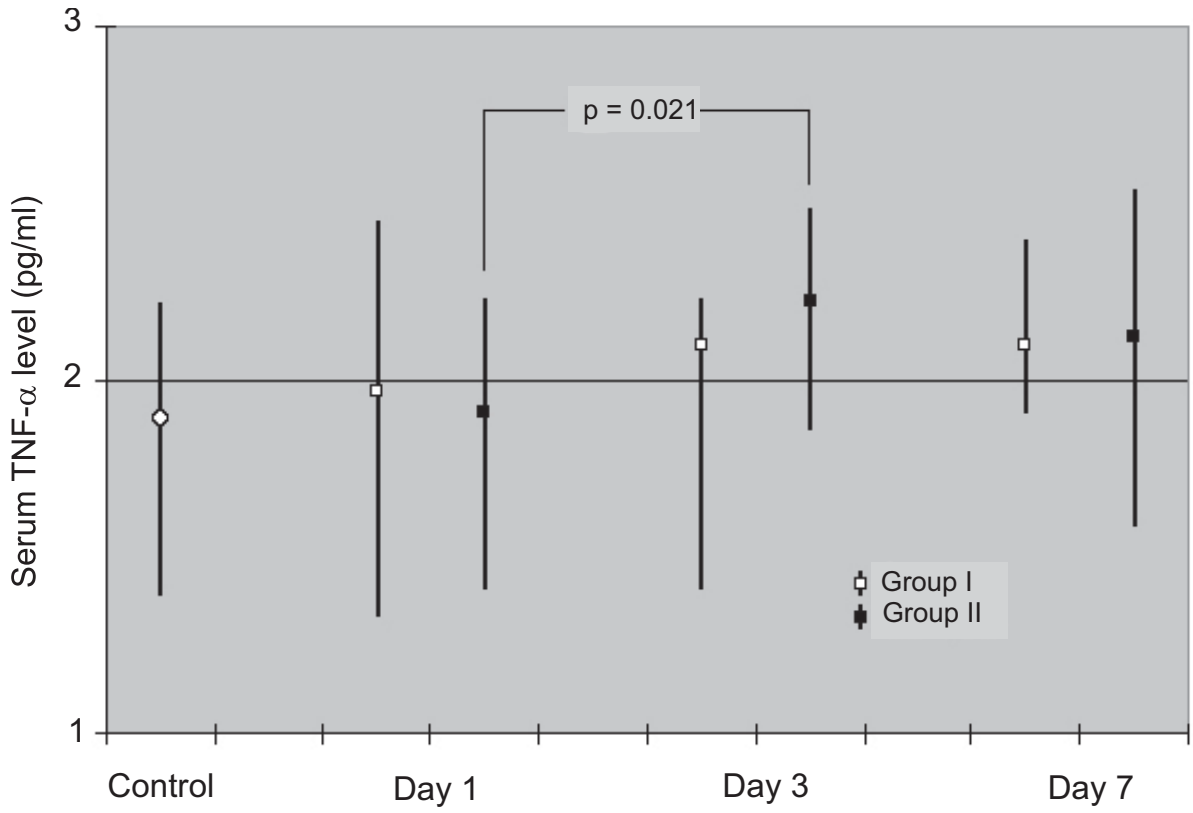

Fig. 1. Serum TNF-α concentration (pg/ml) in acute phase of ischemic stroke. Significant increase in TNF-α level on day 3 after stroke onset was observed in patients from Group II (Wilcoxon test, p = 0.021). Data are presented as the 1st quartile, median value and the 3rd quartile

in the group of patients treated with simvastatin but it was not statistically significant (p > 0.05). The difference between both study groups on day 1, 3 and 7 was not statistically significant probably due to the small number of patients. All results are shown in Table 2.

The classic mechanism of statin action depends on the decreasing of low-density lipoprotein serum level. This effect appears approximately 2 weeks after treatment onset. Beyond lipid-lowering properties, statins are known as anti-inflammatory drugs mainly through the inactivation of NF-κB, a key activator of cytokine transcription and through the resulting decrease in the expression of numerous proinflammatory cytokines including TNF-α [9]. On the basis of our observations, we noticed for the first time the possibility that early treatment with simvastatin could inhibit serum TNF-α in the acute phase of ischemic stroke. In both study groups, TNF-α levels did not differ from control group within the first 24 hours. These values increased on day 3 in the patients not treated with simvastatin. Intiso et al. observed an increase in serum TNF-α level on the first days after ischemic stroke

Tab. 2. Serum concentration of TNF-α at three time-points (pg/ml)

	1 day	3 day	7 day	Friedman test
Group I	1.97 (1.33–2.44)	2.09 (1.41–2.21)	2.09 (1.91–2.38)	p = 0.532
Group II	1.91 (1.46–2.21)	2.22 (1.86–2.48)	2.11 (1.57–2.53)	p = 0.061
Control		1.89 (1.39–2.21)		

Group I was treated with simvastatin from the first day of stroke. Group II was not treated with simvastatin. Data are presented as the median (quartiles)

onset of approximately 70% on day 7 *vs.* day 1 or *vs.* the control [7]. Patients with polygenic hypercholesterolemia treated with simvastatin (20 mg/day) could show a decrease in monocyte expression of proinflammatory cytokines as well as of TNF-α after eight weeks of therapy [5]. Our results suggest that this effect could be observed as early as on day 3 of treatment. In this study, we did not focus on the influence of simvastatin on serum cholesterol level because the observation period (7 days) was too short to show the classic mechanism of statin action [9]. For this reason, we administrated simvastatin (40 mg/day) regardless of lipid profile.

Our previous studies showed that early treatment with simvastatin could decrease the augmented serum MMP-9/TIMP-1 ratio within the 7 days after ischemic stroke onset which probably occurred *via* the same mechanism as TNF-α inhibition [8].

In conclusion, we suggest that simvastatin included in the therapy within 24 hours after ischemic stroke onset could inhibit the increase in serum TNF-α level in the acute phase of ischemic stroke. The anti-inflammatory effects of statins my have clinical impact on this vascular disease. Further studies are needed.

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