Serum microtubule associated protein tau and myelin basic protein as the potential markers of brain ischaemia-reperfusion injury in patients undergoing carotid endarterectomy

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Abstract

Introduction. *In the prevention of ischaemic stroke the recommended surgical procedure is carotid endarterectomy (CEA). However, surgical treatment of atherosclerotic stenosis may cause neurological complications. The aim of the study was to investigate consequential brain ischaemia-reperfusion injury by measuring the cerebral specific markers, the microtubule associated protein tau (MAPt) and myelin basic protein (MBP) in the serum of patients that underwent CEA.*

Material and methods. *This study involved 25 participants who underwent CEA due to internal carotid artery stenosis. Blood samples were taken from each patient at three different intervals; within 24 hours prior to surgery, 12 hours after the surgery, and 48 hours after the surgery. Serum MAPt and MBP levels were measured by a commercially available enzyme-linked immunosorbent assay (ELISA).*

Results. *The study showed that serum MAPt and MBP levels were statistically significantly decreased 12 hours after CEA compared to the level before the surgery (p < 0.05), but MAPt and MBP levels were normalized 48 hours after CEA. There was statistically significant correlation in serum MAPt levels with the velocity of blood flow in the internal carotid artery 12 and 48 hours after CEA (p < 0.05).*

Conclusions. *Data from our study showed that CEA affects serum neuromarkers levels, such as MAPt and MBP, in patients with significant internal carotid artery stenosis. MAPt and MBP levels showed characteristic time curve in patients who underwent CEA and did not experience any neurological deficit in perioperative period. Possible alterations of this time curve may potentially be an index of a neurological event occurrence.*

Key words: carotid endarterectomy, microtubule associated protein tau, myelin basic protein, neuromarkers, serum

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Introduction

Stroke, of which most cases are ischaemic, is a major source of morbidity in patients. Various therapeutic strategies may improve the prognosis, reduce mortality of patients and costs of their hospitalization [1].

Carotid endarterectomy (CEA) is widely accepted surgical treatment of the chronic brain ischaemia caused by haemodynamically significant stenosis of internal carotid artery [2]. However, surgical treatment of atherosclerotic stenosis may cause neurological complications during the perioperative period. These complications include micro and macro embolism resulting in ischaemic brain injury. Moreover, acute ischaemia and reperfusion by clamping and declamping of the internal carotid artery during CEA may produce oxygen-derived free radicals resulting in impairment of cerebrovascular autoregulation, postischaemic hyperperfusion, and brain oedema [3–6].

The microtubule associated protein tau (MAPt) gene encodes the soluble tau protein. This protein is found in abundant quantities in the central nervous system, and subsequently functions to assemble and stabilize microtubules in order to maintain the cytoskeletal structure [7]. As a result of alternative splicing of the MAPt transcripts, six specific isoforms of the tau proteins are generated. They range in size from 352 to 441 amino acid residues in length and are expressed in the human brain [8]. Data from the literature showed that tau protein may be a marker of brain damage in different neurological diseases, such as stroke, traumatic brain injury, Creutzfeldt-Jakob disease, Parkinson disease, and amyotrophic lateral sclerosis [9–13].

Myelin basic protein (MBP), a constituent of the myelin sheath, is essential for normal myelination and conduction of axonal signal. This protein mediates adhesion between cytoplasmic surfaces of individual myelin layers [14]. MBP is located within the serous surface of myelin sheath, where it gets integrated with the myelin lipids. MBP was proved to stabilize the structural properties of myelin and thus influencing its function in central nervous system. MBP is nervous tissue specific, and this protein may reflect the severity of myelin and central nervous system damage [15]. MBP was described as marker of brain injury in neurological conditions, such as cerebral ischaemia, traumatic brain injury, and multiple sclerosis [16–19].

Therefore, MAPt and MBP could also be neuro-chemical markers of brain ischaemia-reperfusion injury in patients undergoing CEA.

The aim of the present investigation was to assess serum MAPt and MBP levels and their dynamics in time in patients who underwent CEA.

Material and methods

The study involved patients hospitalized in the Department of Vascular Surgery and Angiology, Medical University of Lublin, Poland, undergoing CEA due to stenosis of the internal carotid artery.

Patients were qualified for surgical treatment according to the guidelines of the European Society of Vascular Surgery based on Doppler studies performed using a Toshiba Aplio 500 ultrasound [20]. Patients with high grade stenotic carotid arteries were identified and measured using the guidelines set forth by NASCET (North American Symptomatic Carotid Endarterectomy Trial) [21].

The study group consisted of 25 participants (15 male, 10 female) aged from 54 to 88 years with an average of 69 years. Patients with the occlusion of the internal carotid artery were not qualified for surgery and research. The degree of the internal carotid artery stenosis ranged from 60 to 90%. The average clamping time of internal carotid artery during CEA was 8.5 minutes. Conventional CEA was performed under local anaesthesia. Shunt was not used. No complications associated with CEA were observed. The average velocity of blood flow in the internal carotid artery before surgery was 208.4 cm/s, and after the surgery — 89.5 cm/s. In the postoperative period neurological state was monitored by a neurologist. Neurological state after CEA, in all patients participating in the study, did not differ from the neurological state before surgery. Past medical histories of the subjects included symptomatic patients; 8 patients with previous ischaemic stroke, 7 patients with transient ischaemic attack, and 10 patients with asymptomatic internal carotid artery stenosis.

Blood samples were taken from each patient from the antecubital vein at the following time points: within 24 hours prior to surgery (measurement 1), 12 hours after surgery (measurement 2), and 48 hours after surgery (measurement 3). Samples of serum were collected and centrifuged immediately, then stored at −80 Celsius degrees to the time of analysis.

Table 1. Serum MAPt and MBP levels, and a comparative analysis

Measurement	MAPt level [pg/mL]	MBP level [ng/mL]
Before surgery — 1	2.97 (0.31–36.82)	0.21 (0.03–0.50)
12 hours after surgery — 2	2.09 (0.57–25.64)	0.14 (0.02–0.38)
48 hours after surgery — 3	2.83 (0.44–28.16)	0.23 (0.03–0.37)
Comparison		
1–2	p = 0.022*	p = 0.001*
2–3	p = 0.017*	p = 0.024*
1–3	p = 0.716	p = 0.39

Data are expressed as median and range; Wilcoxon test; p* — statistically significant

Serum MAPt and MBP levels were measured by a commercially available enzyme-linked immunosorbent assay (Enzyme-linked Immunosorbent Assay Kit for Microtubule Associated Protein Tau (MAPt) and Enzyme-linked Immunosorbent Assay Kit for Myelin Basic Protein (MBP); Cloud Clone Corp./USCN, Houston, TX, USA).

Both commercially available enzyme-linked immunosorbent assay (ELISA) systems were performed according to the instructions of the manufacturer.

Statistical analysis was performed using STATISTICA, version 12 software (StatSoft, Inc., Poland). The data were not normally distributed and therefore the nonparametric Wilcoxon, Mann-Whitney and Friedman tests were used to measure the differences between variables. The association between variables was tested using Spearman's rank correlation coefficient. The MAPt levels were measured in pg/mL, and the MBP levels are expressed in ng/ml (median and range). The level of statistical significance was $p < 0.05$.

The study was approved by the Ethics Committee of Medical University in Lublin (KE-0254/218/2014) and was conducted according to Declaration of Helsinki given by World Medical Association. All participants signed an informed consent before entering the study.

Results

The study showed that serum MAPt level was statistically significantly decreased 12 hours after CEA compared to level before surgery ($p < 0.05$), and MAPt level was normalized 48 hours after CEA. The serum MAPt levels and a comparative analysis are presented in Table 1 and in Figure 1.
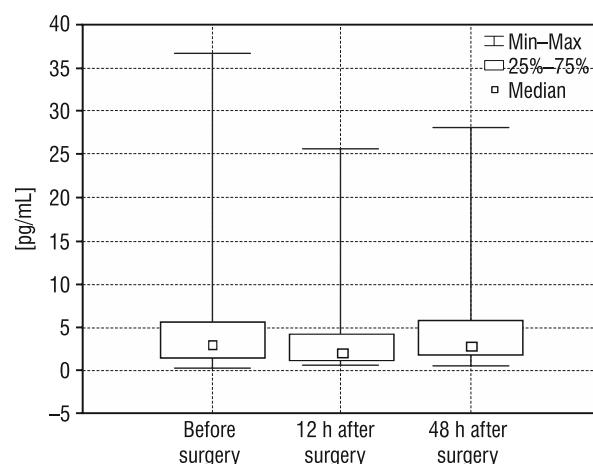
**Figure 1.** Serum microtubule associated protein tau in patients

Analysis of variance showed that the sampling time does not significantly affect MAPt levels in the serum ($p = 0.08$).

There was no statistical significant difference in serum MAPt levels between symptomatic and asymptomatic patients ($p > 0.05$).

Additionally, there was no statistically significant correlation between serum MAPt level and age of patients ($p = 0.88$).

There was no statistically significant correlation in serum MAPt level 12 and 48 hours after surgery with the clamping time ($r = -0.07$, $p = 0.72$ and $r = -0.36$, $p = 0.07$; respectively).

There was also no statistically significant correlation in the serum MAPt levels with the velocity of blood flow in the internal carotid artery before CEA ($r = 0.09$, $p = 0.65$). However, 12 and 48 hours after surgery the correlation was statistically significant ($r = 0.58$, $p = 0.002$ and $r = 0.53$, $p = 0.006$; respectively).
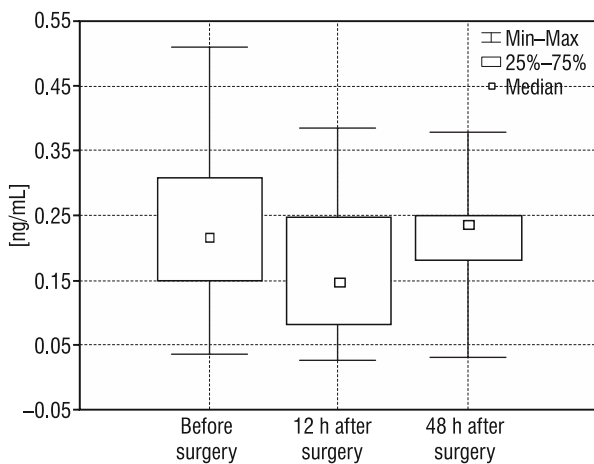
Figure 2. Serum myelin basic protein in patients

The study showed that serum MBP level was statistically significantly decreased 12 hours after CEA compared with level before surgery (p < 0.05), and MBP level was normalized 48 hours after CEA. The serum MBP levels and a comparative analysis are presented in Table 1 and in Figure 2.

The analysis of variance showed that there were statistically significant differences in serum MBP levels between all three recorded measurements (p = 0.0038).

There was no statistical significant difference in serum MBP levels between symptomatic and asymptomatic patients (p > 0.05).

There was no statistically significant correlation between serum MBP level and age of patients (p = 0.07).

There was no statistically significant correlation in serum MBP level 12 and 48 hours after the surgery with the clamping time (r = –0.11, p = 0.59 and r = –0.09, p = 0.64; respectively).

There was also no significant correlation in the serum MBP levels with the velocity of blood flow in the internal carotid artery before CEA (r = 0.14, p = 0.47) and after surgery (r = 0.04, p = 0.83 and r = 0.25, p = 0.22; respectively).

There were no statistically significant correlations between serum MAPt and MBP levels in all three measurements (p = 0.13, p = 0.67, and p = 0.77; respectively).

Discussion

CEA may cause ischaemic-hyperperfusion brain injury. According to Komoribayashi et al. [22] hyperper-

fusion syndrome after CEA is directly related to preoperative and additive, intraoperative ischaemia evoked by clamping of common carotid artery during surgery.

Transient brain ischaemia — also occurring during CEA — may induce hyperphosphorylation of MAPt. The results of the study conducted by Song et al. [23] confirmed that tau protein is dephosphorylated during brain ischaemia. However, after reperfusion, this protein is hyperphosphorylated. Thus, the theory above may play an important role in the evolution of brain damage after brain ischaemia. Zheng et al. [24] hypothesize that the alterations in phosphorylation of tau are important for metabolism and microtubule dynamics, and contribute to the pathophysiology of brain ischaemia and/or reperfusion. Wen et al. [25] suggested that tau hyperphosphorylation can cause neurological injury in the mechanism related to transient brain ischaemia, and may play important role in the pathophysiology of neurodegeneration after ischaemic stroke.

The effects of cerebral ischaemia and reperfusion on phosphorylation of MAPt were measured in a canine model of cardiac arrest. The study conducted by Mailliot et al. [26] reports a complete dephosphorylation of tau proteins during ischaemia. The authors concluded, that monitoring of the recovery of tau phosphorylation, which is a sequential and differential process after ischaemia-reperfusion syndrome, may be an important marker in the assessment of the neuronal integrity after brain ischaemia.

According to Bitsch et al. [27], serum tau protein was detectable within 5 days after ischaemic stroke in 7/20 patients and in 2/10 patients with transient ischaemic attack. Tau protein was detectable within the 6h after occurrence of symptoms, reaching the peak level after 3–5 days and correlated with infarct volume as well as the grade of disability evaluated 3 months after the event. The authors concluded that serum tau protein may be a marker of axonal injury. Wunderlich et al. [28] observed that in patients with ischaemic stroke, serum tau protein levels were increased and correlated with severity of neurological state of patients, infarct volume, and with the functional outcome after 3 months.

Experimental investigation conducted on rats, demonstrated that myelin sheath is generally very vulnerable to ischaemia. The MBP mRNA levels decreased 2 days after ischaemia, and continued to decrease, what was observed at each measurement at four points in

time. The MBP levels significantly decreased at the 2nd and 4th day, but at day 7 recovery was observed, reaching the control levels at day 14 [29]. This finding is with agreement of Chen et al. study, who found that the consequences of the ischaemia on cerebral white matter are time sensitive, however there was no typical scenario over the time. Chida et al. [30] investigated the alterations in oligodendrocytes and myelin following chronic cerebral ischaemia in rats and observed that the amount of MBP was decreased significantly. Cai et al. [31] demonstrated a rat model of chronic cerebral hypoperfusion. On the 30th day after ischaemia, white matter oligodendrocytes presented more severe loss and myelin disruption than in control group. The study conducted by other authors showed that hypoxia/ /ischaemia induces serious hypomyelination [32]. Fan et al. [33] observed after bilateral carotid artery occlusion and hypoxic insult resulted in brain injury with impaired myelination and decreased MBP immunostaining in the rat brain. The authors concluded that the degree of brain injury is dependent on the hypoxic/ischaemic condition, including the exposure time to hypoxia.

Ischaemic injury to the brain, leads to cellular activation and disintegration with subsequent release of cell-type-specific proteins into the cerebrospinal fluid (CSF), including MBP, Brouns et al. [34]. The authors observed that CSF MBP level was significantly higher in patients with subcortical infarcts compared with those with cortical infarcts, and concluded that this protein may also be a marker for infarct location. Hjalmarsson et al. [35] revealed significantly higher levels of protein tau and MBP in CSF of patients with ischaemic stroke which correlated with clinical severity of the stroke.

There are no studies in the literature concerning serum MAPt and MBP levels after CEA. Our study showed that serum MAPt and MBP levels were significantly decreased 12 hours after CEA compared to level before surgery, and that these serum biomarkers normalized 48 hours after CEA. Similar results were observed in study conducted by Rasmussen et al. [36]. The authors measured serum levels of another marker of brain injury, neuron-specific enolase (NSE), in patients before CEA and postoperatively at 12, 24, 36, and 48 hours. Postoperatively, the serum NSE level decreased significantly after uncomplicated CEA. Brightwell et al. [37] hypothesize that a significant internal carotid artery stenosis may cause an increased background level of

NSE release. After CEA brain perfusion gets normalised and levels of NSE subsequently fall with time.

It is difficult to say why MAPt and MBP levels were higher before CEA in our study. Perhaps this is a result of brain damage caused by chronic ischaemia due to a significant stenosis of the internal carotid artery. It can be hypothesized that a significant decrease in the levels of serum MAPt and MBP in the early period after CEA may be associated with improved blood supply to the brain while normalization of these markers may be result of ischaemic-hyperperfusion brain damage at a later date after surgery.

The results of the study conducted by Céspedes et al. [38] suggest that in ischaemic rat models, the immunoreactivity of the hyperphosphorylated tau protein marker (AT-8) steadily increased until 72 hours after ischaemia. This finding suggests that the progression of excitotoxicity and resultant alteration of enzymes was involved in phosphorylation of cytoskeletal proteins. Progressing brain damage caused by ischaemia-hyperperfusion associated with the CEA procedure, could therefore explain the observation in our study of the increased serum MAPt and MBP levels between 12 and 48 hours after surgery.

In our study, we observed similar dynamics of MAPt and MBP changes in serum of patients. Seiberlich et al. [39] showed that tau knockdown may affect overproduction and leads to a decreased expression of MBP. The authors concluded that disturbances in the metabolism of tau protein may cause abnormal oligodendrocyte differentiation, neuron-glia adhesion and the early processes of myelination. Similar dynamics of serum MAPt and MBP changes observed during our study may reflect this process.

Data from our study showed that there was statistically significant correlation in serum MAPt levels with the velocity of blood flow in the internal carotid artery 12 and 48 hours after CEA. An increase in serum MAPt levels in association with higher blood flow may be caused by cerebral hyperperfusion. In our opinion, after CEA, when stenosis is already absent, higher velocity of blood flow probably correlates with blood flow higher value.

The study revealed the lack of difference in serum MAPt and MBP levels between symptomatic and asymptomatic patients. Such a division of the patients have been due to the patients' medical histories. However,

on the day of the surgery and blood sampling, all patients were asymptomatic, which explains the result.

Our study evaluated the effect of endarterectomy on the levels of MAPt and MBP in serum, the level before the surgery was a control. The surgery was effective because the levels of the tested parameters declined significantly immediately after endarterectomy. In our opinion, the recovery levels of these markers observed 48 hours after surgery, does not reflect the lack of effectiveness of the surgery, but it could be the expression of neurological complications associated with this surgical procedure, such as brain ischaemia/hyperperfusion syndrome, as discussed in the manuscript. As a result of ischaemia//hyperperfusion syndrome-associated endarterectomy damage may occur to the blood-brain barrier moving MAPt and MBP in the blood which increases their levels. Various concentrations of MAPt and MBP in serum of individual patients after surgery may indicate varying degrees of ischaemia/hyperperfusion syndrome, and the various levels of these markers before surgery can be an exponent of the degree of carotid stenosis and severity of chronic brain ischaemia.

Conclusions

Data from our study showed that CEA affects serum neuromarkers levels, such as MAPt and MBP, in patients with significant internal carotid artery stenosis. MAPt and MBP levels showed characteristic time curve in patients who underwent CEA and did not experience any neurological deficit in perioperative period. Possible alterations of this time curve may potentially be an index of a neurological event occurrence.

Acknowledgment

Conflict of interest

None declared.

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