Prolyl carboxypeptidase activity decline correlates with severity and short-term outcome in acute ischemic stroke

Prolyl Carboxypeptidase Activity Decline Correlates with Severity and Short-Term Outcome in Acute Ischemic Stroke

Kaat Kehoe [1]

Raf Brouns [2]

Robert Verkerk [1]

Sebastiaan Engelborghs [3,4]

Peter Paul De Deyn [3,4]

Dirk Hendriks [1]

Ingrid De Meester [1,*]

Phone +32 (0)3 265 27 41
Email ingrid.demeester@uantwerpen.be

[1] Laboratory of Medical Biochemistry, Department of Pharmaceutical Sciences, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium

[2] Department of Neurology, Universitair Ziekenhuis Brussel, Center for Neurosciences, Vrije Universiteit Brussel (VUB), Brussels, Belgium

[3] Department of Neurology and Memory Clinic, Hospital Network Antwerp (ZNA) Middelheim and Hoge Beuken, Antwerp, Belgium

[4] Laboratory of Neurochemistry and Behavior, Department of Biomedical Sciences, Institute Born-Bunge, University of Antwerp, Antwerp, Belgium

Abstract

Prolyl carboxypeptidase (PRCP) is an enzyme associated with cerebrovascular risk factors such as hypertension, diabetes mellitus, obesity and hyperlipidemia. We aim to evaluate the relation between serum PRCP activity and severity, evolution and outcome of acute ischemic stroke. We used a specific RP-HPLC activity assay to measure PRCP activity in serum of 50 stroke patients at admission, and at 24 h, 72 h and 7 days after stroke onset to assess correlations with stroke severity based on the National Institutes of Health Stroke scale score (NIHSS), infarct volume on brain MRI scan, stroke outcome based on the modified Rankin scale (mRS) and mortality at 3 months after stroke. The average PRCP activity in serum decreased significantly the first 24 h after stroke onset and returned to baseline values at day 7. High NIHSS scores and infarct volumes at admission were related with a more pronounced decrease of PRCP in the first 24 h after stroke ($\Delta PRCP_{24}$, r = 0.31, $P < 0.05$; r = 0.30, $P < 0.05$). In addition, patients who displayed a more pronounced decrease in PRCP levels during the first 24 h after stroke were more likely to be institutionalized upon discharge (n = 21) ($\Delta PRCP_{24}$ ± SD, 0.05 ± 0.10 U/L vs. 0.17 ± 0.14 U/L, P = 0.001). The decrease in PRCP levels in the first 24 h after stroke onset is associated with stroke severity and an unfavourable short-term stroke outcome.

Keywords

Prolyl carboxypeptidase
Acute ischemic stroke
Enzyme activity
Angiotensin
Angiotensinase C

Abbreviations

proCPU	Procarboxypeptidase U
PRCP	Prolyl carboxypeptidase
Ang-(1-8)	Angiotensin-(1-8)
Ang-(1-7)	Angiotensin-(1-7)
TOAST	Trial of Org 10172 in Acute Stroke Treatment

CRP	C-reactive protein
NIHSS	National Institutes of Health Stroke Scale
EPSS	European Progressing Stroke Study
mRS	Modified Rankin scale

Electronic supplementary material

The online version of this article (doi:10.1007/s11064-014-1468-y) contains supplementary material, which is available to authorized users.

Introduction

Globally, ischemic stroke is one of the most common causes of death and acquired disability in adults [1]. It is caused by an interruption of the blood flow to the brain, leading to a rapid loss of function [2]. Many neurochemical processes underlying acute ischemic stroke remain to be elucidated and still hold the potential to further improve diagnostics and therapeutics for acute cerebrovascular accidents [3–5]. In this light, carboxypeptidases are interesting candidates as it recently has become clear that the plasma concentration of procarboxypeptidase U (proCPU), a zymogen involved in the etiology of thrombosis, is related to thrombotic tendency and that these proCPU plasma levels are associated with stroke characteristics in patients [6].

Prolyl carboxypeptidase (PRCP) is another carboxypeptidase that may play a role in acute ischemic stroke. The enzyme preferentially cleaves off C-terminal amino acids when Proline or Alanine are in the penultimate position [7]. Initially, PRCP was named angiotensinase C because of its ability to metabolize angiotensin-(1-8) (Ang-(1-8)) to angiotensin-(1-7) (Ang-(1-7)), thereby causing a shift from vasoconstriction of blood vessels to vasodilation [8, 9]. The enzyme is also capable of inactivating the vasopressor effects of angiotensin 3, again leading to a decrease in blood pressure. In addition, PRCP is involved in the activation of prekallikrein, resulting in the subsequent release of bradykinin from high-molecular-weight kininogen. Moreover, the anorexigenic effects of neuropeptide α-melanocyte-stimulating hormone 1–13 are suppressed by its cleavage at the C-terminus by PRCP [10–14]. Based on these functions, PRCP has been implicated in the pathophysiology of various diseases among which

hypertension, inflammation, obesity and diabetes mellitus, also known as important cerebrovascular risk factors [15–22]. Given its expression on endothelial cells [23], on blood platelets (unpublished data) and its presence in serum [24], it seems plausible that PRCP may be part of the ischemic cascade following stroke. Furthermore, radioactive in situ hybridization and X-gal staining approaches were performed to determine the distribution of PRCP in the mouse brain. This study demonstrates the broad expression of the PRCP gene in the brain [25]. Buga et al. [26] provided the first indication for its role in cerebral ischemia by identifying PRCP as one of 161 dysregulated genes in a genome-wide analysis of gene expression in the ipsilateral cortex of aged rats after stroke. Additionally, it was shown that PRCP promotes vascular health since PRCP deficiency in mice was associated with decreased endothelial cell growth, wound repair and impaired angiogenesis with vascular injury [27]. In this study, we measured PRCP activity in serum of 50 stroke patients at admission (on average 2.7 h after stroke onset), and at 24 h, 72 h and 7 days after stroke onset, and we evaluated its relation with key stroke characteristics.

Materials and Methods

Study Design and Population

The Middelheim's interdisciplinary stroke study evaluates ischemic stroke patients at ZNA Middelheim Hospital (Antwerp) based on their biochemical, neuroimaging, electrophysiological, neuropsychological and clinical parameters. During this study, many other biochemical parameters were analysed but these results have been published elsewhere [28–31]. Patient samples were collected over a 3-year period (2005–2008). Analysis of PRCP activity in serum was performed in 50 ischemic stroke patients, who were not treated with thrombolytics. The Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria were used to classify stroke etiology [32]. The study was approved by the Ethics Committees of the University of Antwerp and ZNA Antwerp and was conducted according to the ethical guidelines and principles of the revised Declaration of Helsinki (1998). Table 1 shows patient and stroke characteristics of the study group.

Table 1

Patient and stroke characteristics of a group of 50 patients with acute ischemic stroke[a]

Characteristic	Value
Patient characteristics	
Age (years)	75.3 ± 7.4
Gender	Male: 26 (52 %)
	Female: 24 (48 %)
Caucasian race	50 (100 %)
Time to blood sampling at admission (h)	2.7 ± 1.7
Stroke characteristics	
NIHSS at admission	9 ± 9
Stroke progression[b]	8 (16 %)
Infarct volume (mL)	4.3 (IQR: 0.8–40.1)
mRS score at month 3	3 ± 2
Stroke etiology[c]	
Atherotrombotic	8 (16 %)
Cardioembolic	27 (53 %)
Lacunar	6 (12 %)
Specific	1 (2.0 %)
Undetermined	8 (16 %)
NIHSS National Institutes of Health Stroke Scale, *mRS* modified Rankin scale	
[a]Data that follow a normal distribution are given as mean ± SD, non-Gaussian data (infarct volume) are given by median and interquartile range (IQR)	
[b]Stroke progression defined by the European Progressing Stroke Study criteria	
[c]Stroke etiology classified by the TOAST criteria	

Sample Preparation

Serum samples were prepared by drawing whole blood into collection tubes containing no anti-coagulant at admission (mean ± SD, 2.7 ± 1.7 h after onset of stroke symptoms), at 24 h, 72 h and 7 days after stroke onset. Thereafter, a

centrifugation step at 2,000×*g* for 15 min at 4 °C was performed, serum was frozen in liquid nitrogen and then stored at −80 °C before assay.

Measurement Methods

The activity of PRCP was determined by measuring the hydrolysis of substrate *N*-benzyloxycarbonyl-Pro-Phe. The substrate solution was prepared by dissolving 10 mM *N*-benzyloxycarbonyl-Pro-Phe in 0.10 M acetate buffer containing 2 mM EDTA at pH 5.0, the pH optimum of PRCP. Hydrolysis was initiated by incubating 10 μL sample and 75 μL substrate at 37 °C for 2 h. The released *N*-benzyloxycarbonyl-Pro was measured by a RP-HPLC technique as previously described [24]. The PRCP activity assay was done blinded to the case identity. PRCP activity is expressed in units per liter where one unit defines the amount of enzyme that hydrolyses 1 μmole of substrate per minute. C-reactive protein (CRP) was analysed in serum on a Vitros 5.1 FS analyser (Ortho Clinical Diagnostics, Beerse, Belgium) using the dry slide technology. Imprecision ranged from 7.3 % at 28 mg/L to 3 % at 70 mg/L. CRP values <10 mg/L are within the reference interval. Glucose concentration in serum was determined using the Vitros Glu slide method on a Vitros 5600 analyser (Ortho Clinical Diagnostics, Beerse, Belgium). The within lab precision was 1.3 % at 75 mg/dL and 1.3 % at 284 mg/dL.

Evaluation of Stroke Severity, Evolution and Outcome

Trained neurologists used the National Institutes of Health Stroke Scale (NIHSS) score to evaluate the neurological impairment of patients at admission, and at 24 h, 72 h and 7 days after stroke. The European Progressing Stroke Study (EPSS) criteria were used to detect significant changes in the patients' neurological status within the first 72 h of symptom onset. These criteria are based on standardized neurological observations of language function, consciousness, conjugate gaze, and motor function. Stroke progression is defined as a neurological worsening or death within 72 h after onset [33]. As defined by the EPSS criteria, eight patients developed progressing stroke. For all patients the diagnosis of acute ischemic stroke was confirmed on MRI of the brain. As previously described, the infarct volume was determined by two independent neurologists [29, 30]. The modified Rankin scale (mRS) was used to assess stroke outcome at 3 months after stroke onset. On the basis of prior

publications, poor outcome was defined as mRS score >3 [34].

Statistical Analyses

SPSS version 15.0 (SPSS Inc, Chicago, IL, USA) was used to perform all statistical tests. A Kolmogorov–Smirnov test confirmed that the PRCP activity in serum followed a normal distribution. The *t* test for independent samples and *t* test for paired samples was used to compare the PRCP activity between two groups and between various time points within groups. We employed the Bonferroni correction for multiple comparisons between the different time points. Bivariate correlations were used to assess the relation between change in PRCP levels from admission to 24 h after stroke onset ($\Delta PRCP_{24}$) and parameters for stroke severity, evolution and outcome. Pearson correlation coefficient was determined for continuous data such as infarct volume and the NIHSS score at admission, while Spearman rank correlation coefficient was applied for ordinal data (progressing stroke; mRS score at month 3). This statistical approach was similar to the strategy reported by Brouns et al. in a related patient population [6].

Results

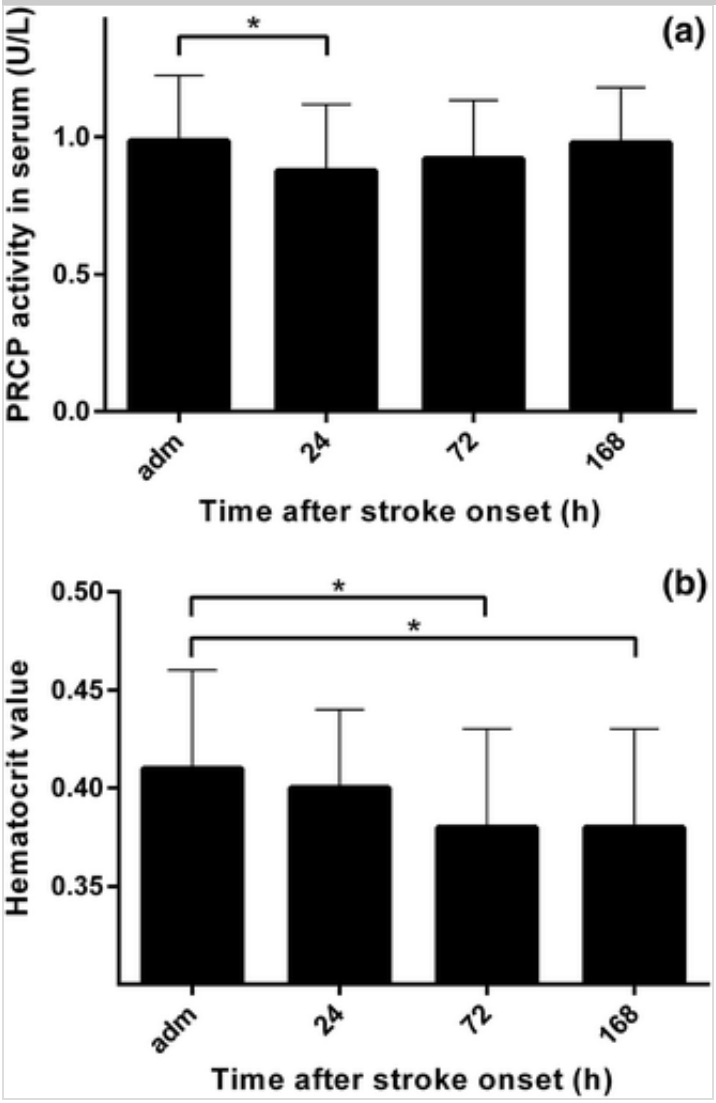
PRCP Activity Levels in Serum

The mean PRCP activity in serum was 0.99 ± 0.24 U/L (mean ± SD) on admission, 0.88 ± 0.24 U/L at 24 h, 0.92 ± 0.21 U/L at 72 h and 0.98 ± 0.20 U/L at day 7, as shown in Fig. 1a. PRCP activity decreases significantly after admission, reaches its nadir at 24 h after stroke onset ($P < 0.001$) and gradually returns to baseline levels towards day 7. The change in PRCP levels from admission to 24 h after stroke onset was 0.10 ± 0.13 U/L ($\Delta PRCP_{24}$ ± SD). To make sure that these differences are not due to changes in plasma volume, we compared the hematocrit values at each time point. The long half-life of red blood cells makes this parameter suitable for the validation of the results. A one-way ANOVA was performed to compare the mean of the hematocrit values at the 4 time points. As shown in Fig. 1b, we observed a significant difference from admission to 72 h (mean ± SD, 0.41 ± 0.05 at admission vs. 0.38 ± 0.05 at 72 h, $P = 0.032$) and from admission to day 7 (0.38 ± 0.05 at day 7, $P = 0.025$). However, there was no significant difference between the hematocrit values at admission and at 24 h (mean ± SD, 0.41 ± 0.05 at admission vs. 0.40 ± 0.04 at

24 h, $P = 0.073$) meaning that the changes in serum PRCP activity at these time points are not linked to alterations in plasma volume. The PRCP activity at admission was not related to the interval between stroke onset and blood sampling ($P = 0.535$).

Fig. 1

a The activity profile of PRCP (mean ± SD) is illustrated for each time point. PRCP activity in serum decreases significantly the first 24 h post stroke and thereafter returns to baseline towards day 7. *Asterisk* (*) shows significant differences between various time points ($P < 0.001$)**. b** The hematocrit value (mean ± SEM) is illustrated for each time point. There is no significant difference between the hematocrit value at admission and at 24 h. However, we did observe a significant decrease from admission to 72 h and to day 7. *Asterisk* (*) shows significant differences between various time points ($P < 0.05$)



PRCP Activity and Cerebrovascular Risk Factors

There was no relation between PRCP activity at admission or its kinetics in the first week after stroke and patient age, gender or cardiovascular risk factors and diseases (heart failure, arterial hypertension, atrial fibrillation, previous myocardial infarction, smoking, alcohol abuse, diabetes mellitus, family history of stroke, previous stroke, obesity and dyslipidemia). We related heart rate, systolic and diastolic blood pressure at admission to PRCP activity at admission and its kinetics. Only the systolic blood pressure showed a positive correlation with the kinetics of PRCP. Glycemia and CRP levels at admission also correlated with PRCP activity at admission. These correlations are presented in graphs in the supplementary material. In this study population, PRCP did not correlate with the body mass index or serum levels of triglycerides, total cholesterol, high-density lipoprotein or low-density lipoprotein. The statistical data are summarized in Table 2.

Table 2

Correlations between PRCP's admission levels or kinetics and the parameters analyzed

Parameters	PRCP activity at admission			$\Delta PRCP_{24}$		
	Pearson correlation	*T* value	*P*	Pearson correlation	*T* value	*P*
Interval between onset and bloodsampling	0.090		0.535	0.074		0.621
Age	−0.025		0.863	0.101		0.498
Gender		0.845	0.402		1.531	0.134
Arterial hypertension		−1.763	0.084		−0.604	0.549
Current smoker		0.217	0.842		0.713	0.480
Previous smoker		0.395	0.695		0.959	0.343
Diabetes mellitus		−0.756	0.453		−0.602	0.550

Alcohol abuse		0.864	0.392		0.030	0.976
Atrial fibrillation		0.906	0.369		1.066	0.292
Previous myocardial infarction		−0.473	0.638		−1.015	0.316
Heart failure		0.827	0.413		0.907	0.373
Previous stroke		−1.004	0.321		−0.445	0.658
Familial stroke		0.317	0.753		0.314	0.755
Dyslipidemia		−1.012	0.317		−0.124	0.902
Systolic blood pressure$_{adm}$	0.180		0.215	0.400		0.006*
Diastolic blood pressure$_{adm}$	0.017		0.909	0.270		0.070
Heart rate$_{adm}$	−0.103		0.486	0.067		0.664
CRP$_{adm}$	0.451		0.001*	−0.117		0.433
CRP$_{24}$	0.149		0.308	−0.016		0.916
CRP$_{72}$	−0.141		0.338	−0.134		0.380
CRP$_{day7}$	−0.198		0.193	0.127		0.423
Glycemia$_{adm}$	0.541		<0.001*	0.228		0.123
Glycemia$_{24}$	0.204		0.155	−0.062		0.681
Glycemia$_{72}$	0.211		0.141	0.118		0.428
Glycemia$_{day7}$	0.304		0.042	0.051		0.747
Body mass index	0.066		0.650	0.019		0.899
Triglycerides	0.077		0.605	0.121		0.429
Total cholesterol	−0.064		0.671	0.160		0.294
High-density lipoprotein	0.041		0.787	0.260		0.088

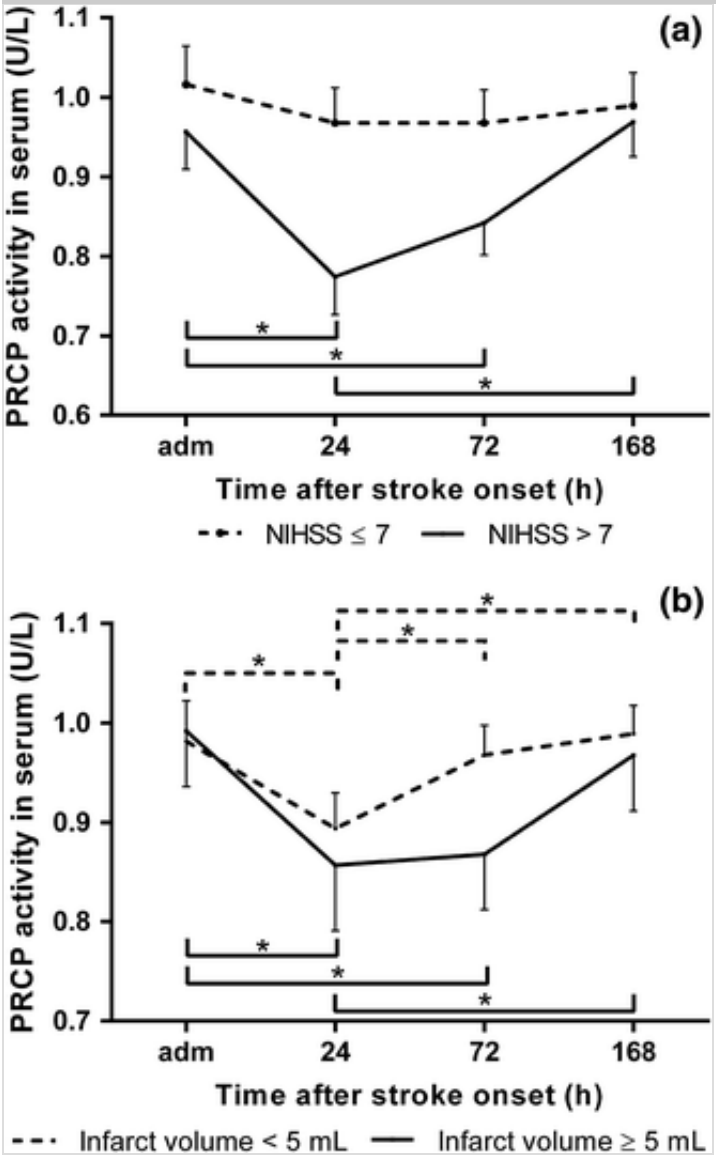
Low-density lipoprotein	−0.089		0.555	0.030		0.845
Significant correlations ($P < 0.01$) are indicated by an asterisk (*)						

PRCP Levels in Relation to Stroke Severity

The NIHSS score at admission was used to differentiate between patients with mild stroke (NIHSS ≤ 7, n = 25) and moderate to severe stroke (NIHSS > 7, n = 25) [35]. In patients with moderate to severe stroke, PRCP levels decreased significantly from admission to 24 h (mean ± SD, 0.96 ± 0.24 U/L at admission to 0.77 ± 0.23 U/L at 24 h, $P < 0.001$). Although the PRCP levels at 72 h were still significantly lower than the levels at admission (mean ± SD, 0.96 ± 0.24 U/L at admission to 0.84 ± 0.20 U/L at 72 h, $P < 0.05$), they were increasing to reach baseline levels at day 7 (mean ± SD, 0.97 ± 0.21 U/L). On the contrary, patients with mild stroke showed non-significant changes in PRCP levels (mean ± SD, 1.02 ± 0.24 U/L at admission, 0.97 ± 0.22 U/L at 24 h, 1.00 ± 0.20 U/L at 72 h and 0.99 ± 0.19 U/L at day 7). In this study group, higher NIHSS scores at admission were related with a more pronounced decrease of PRCP in the first 24 h after stroke (r = 0.31 $P < 0.05$). The parameter infarct volume was used to classify patients into two subgroups: those with small infarctions (<5 mL, n = 27) and those with moderate to large infarctions (≥5 mL, n = 23). In the first subgroup the PRCP levels showed a significant decrease from admission to 24 h (mean ± SD, 0.98 ± 0.21 U/L at admission vs. 0.89 ± 0.18 U/L at 24 h, $P < 0.001$), which significantly returned to initial levels towards 72 h and day 7 (mean ± SD, 0.97 ± 0.15 U/L at 72 h, $P < 0.05$, 0.99 ± 0.14 U/L at day 7, $P < 0.05$). Patients with infarct volumes ≥5 mL showed a more pronounced decrease of PRCP levels (mean ± SD, 0.99 ± 0.27 U/L at admission, 0.86 ± 0.30 U/L at 24 h, 0.87 ± 0.26 U/L at 72 h, 0.97 ± 0.26 U/L at day 7). The change in PRCP levels from admission to 72 h is greater in patients with moderate to large infarctions compared to patients with small infarctions ($\Delta PRCP_{72}$ ± SD, 0.13 ± 0.16 U/L vs. 0.02 ± 0.19). In the total study population, higher infarct volumes were related with a more pronounced decrease of PRCP in the first 24 h after stroke ($\Delta PRCP_{24}$ ± SD, $r = 0.30$, $P < 0.05$). Figure 2a, b display the relation between the PRCP profile and the severity of the stroke.

Fig. 2

The kinetics of serum PRCP activity (mean ± SEM) correlate with initial stroke severity. **a** NIHSS score dichotomized at 7, **b** infarct volume dichotomized at 5 mL



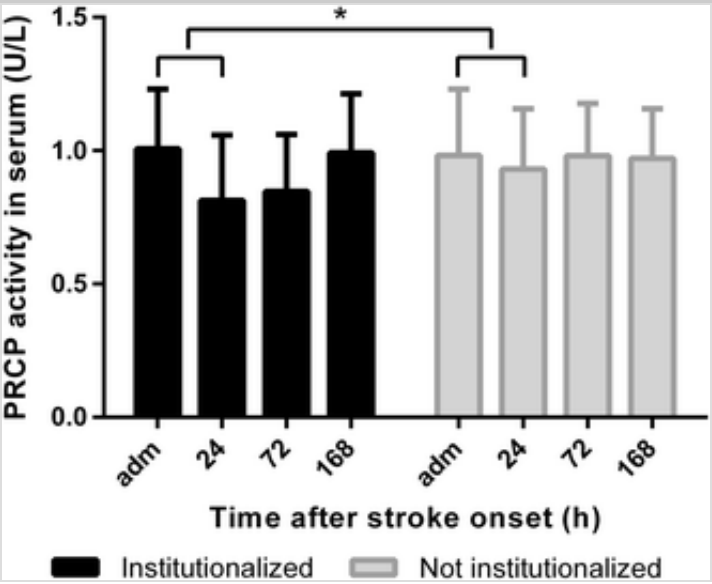
PRCP Levels in Relation to Subacute Stroke Evolution

Based on the EPSS criteria, eight patients (16 %) developed progressive stroke in the first 72 h after stroke onset. There were no significant differences in PRCP activity at admission (mean ± SD, 0.99 ± 0.24 U/L vs. 0.97 ± 0.21 U/L, $P = 0.82$) or its profile between patients with and those without progressive stroke ($\Delta PRCP_{24}$ ± SD, 0.06 ± 0.09 U/L vs. 0.11 ± 0.13 U/L, $P = 0.61$) (supplementary material). Changes in the NIHSS scores between the 4 time points were related to PRCP levels in de first 24 h but no significant correlation

was found ($P > 0.05$). As shown in Fig. 3, patients who displayed a more pronounced decrease in PRCP levels during the first 24 h after stroke were more likely to be institutionalized upon discharge (n = 21, 4 %) ($\Delta PRCP_{24} \pm SD$, 0.05 ± 0.10 U/L vs. 0.17 ± 0.14 U/L, $P = 0.001$).

Fig. 3

Bar graphs demonstrating the mean PRCP activity in serum and SD for each time point. Patients with poor short-term outcome have a more pronounced decrease in PRCP levels in the first 24 h after stroke and are more likely to stay institutionalized. *Asterisk* (*) shows significant differences between various time points ($P < 0.001$)



PRCP Levels in Relation to Long-Term Stroke Outcome

Based on the mRS score at month 3, patients were subdivided into two groups; those with favourable long-term outcome (mRS 0–3, n = 36) and those with unfavourable outcome (mRS 4–6, n = 14). We did not find a significant difference between these groups in PRCP activity at admission (mean ± SD, 1.00 ± 0.23 U/L vs. 0.95 ± 0.26 U/L, $P > 0.05$) (supplementary material). The mRS scores at month 3 did not correlate with $\Delta PRCP_{24}$ ($r = 0.20$, $P = 0.170$). At month 3, six patients were deceased but no significant difference in PRCP activity at admission (mean ± SD, 0.98 ± 0.21 U/L for survivors vs. 1.01 ± 0.37 U/L for deceased, $P = 0.790$) or $\Delta PRCP_{24}$ (mean ± SD, 0.10 ± 0.12 U/L for survivors vs. 0.10 ± 0.18 U/L for deceased, $P > 0.05$) with stroke survivors were

observed. These results demonstrate that PRCP's profile is not related to long-term stroke outcome.

Influence of Pre-stroke Medication on PRCP Levels

For evaluation of a possible influence of pre-stroke medication on PRCP levels, we categorized the medication in the clinically relevant pharmacological groups: antiaggregants, antihypertensive drugs and antidiabetics. An independent *t* test revealed no significant differences in the PRCP activity at admission or the change in PRCP levels from admission to 24 h after stroke onset between patients who received antihypertensive or antidiabetic drugs before admission and those who did not. The influence of antiaggregants on the kinetics of PRCP reached borderline significance ($P = 0.049$). The results are summarized in Table 1 of the supplementary material.

Discussion

This is the first study reporting on kinetics of PRCP activity in serum of patients with acute ischemic stroke. So far, the involvement of PRCP in the etiology and outcome of acute ischemic stroke has never been explored. PRCP is a carboxypeptidase involved in the processing of Ang-(1-8) to Ang-(1-7). Since the renin-angiotensin system has been linked to the development and progression of cerebrovascular disease, further study on PRCP activity in patients with acute ischemic stroke will help shed light on the biological role of PRCP in stroke.

In this study, we observed a decrease of PRCP activity in serum during the first 24 h after stroke onset with subsequent return to baseline towards day 7. Studying the vital parameters at admission (heart rate, systolic and diastolic blood pressure), only the systolic blood pressure correlated with the decrease of PRCP activity from admission to 24 h after stroke. This decrease might be reflected in less Ang-(1-7) formation by PRCP and thus in a physiological decline of the vasodilatory actions of Ang-(1-7), which may cause a subsequent increase in the systolic blood pressure. Searching the literature, we found one study that recently reported on an upregulation of the PRCP, Ace, Calcrl, Ece1, P2rx4 gene at both 3 and 14 days post-stroke in rats. Since these genes are involved in blood pressure control, the authors hypothesize that these results

might indicate a dysregulation of post-stroke blood pressure [26]. High blood pressure is present in 70–80 % of patients with acute ischemic stroke and is independently associated with a poor functional outcome [36]. The present study is the first to assess the PRCP enzyme activity in serum of acute ischemic stroke patients, therefore confirmation of these results is necessary. At this moment, we can only speculate on the possible mechanisms underlying the activity profile of PRCP. We hypothesize that PRCP plays a role in the cleavage of circulating Ang-(1-8) to Ang-(1-7), causing a shift from vasoconstriction and prothrombotic activity towards vasodilatation and antithrombotic actions [37]. Importantly, intracerebroventricular infusion of Ang-(1-7) in a rat model of cerebral ischemia has been shown to reduce cerebral infarct size and neurological deficits. Activation of this cerebroprotective Ang-(1-7)/Mas axis by PRCP might be beneficial during ischemic stroke [38, 39]. Among all the general biochemical parameters we have investigated, we could only observe a correlation between PRCP activity at admission and admission glycemia. This result confirms an earlier report on a strong association of the plasma concentrations of PRCP with metabolic syndrome and diabetes mellitus [20]. PRCP admission activity in serum was related to admission CRP concentration, a parameter reflecting the degree of inflammation in the human body. The role of PRCP in the inflammatory process may be related to its ability to convert prekallikrein to kallikrein. This leads to a subsequent formation of bradykinin, which is an important mediator of vasodilation, inflammation and fibrinolysis.

To investigate the relation between PRCP's levels and stroke severity, we divided the patients into groups based on their NIHSS score and infarct volume. The decrease in PRCP activity in the first 24 h post stroke was more pronounced in patients with more severe stroke and larger infarctions on neuroimaging. These patients were also more likely to be institutionalized upon discharge. Besides stroke severity, we also evaluated the relation to stroke evolution and the long-term stroke outcome. A relation with the evolution in the subacute phase after stroke was absent and PRCP activity was not predictive for long-term functional outcome or for mortality. In 2012, Xu et al. [20] developed a sensitive immunoassay for PRCP and observed a strong association of the plasma concentrations of PRCP with signs of obesity, diabetes mellitus and cardiovascular dysfunction. However, in this study population we have not found a correlation with important cerebrovascular risk factors (e.g. arterial

hypertension, heart failure) and PRCP's levels at admission or kinetics.

An alternative explanation for the differences in PRCP's serum activity at the different time points could be endothelial dysfunction or the presence of confounding factors. It is well documented that PRCP is located on the membrane of endothelial cells [23]. Since endothelial dysfunction is a fundamental step in the development of atherosclerosis and has been observed in stroke patients, we believe that this dysfunction might result in an increased production and release of membrane-bound PRCP into the circulation [20, 40]. We considered a possible effect of the time between stroke onset and blood sampling and the measurement of PRCP activity at admission, but no relation was found. Since there was no relation between PRCP activity and changes in plasma volume, it is unlikely that the observed alterations in PRCP activity are caused by nonspecific mechanisms.

In the present study, an age-matched control population was not available. Previously, we measured PRCP activity in serum of 32 young, healthy individuals. The average PRCP activity in serum of this healthy population was found to be 0.72 ± 0.16 U/L, respectively. This is lower than the average PRCP activity we measured in the present study population. Differences could be due to the variation in the average age of these populations and/or the presence of multiple pathologies in the diseased population or are a result of the stroke itself. Another limitation of this study could be the presence of unknown confounding factors. The strengths of this pilot study include (1) confirmation of acute ischemic stroke by MRI in all patients, (2) very early blood sampling after stroke onset (on average 2.7 h), and (3) all methods used in this study were optimized and validated.

The present study reports on the association of PRCP's activity decline in the first 24 h after stroke onset, with stroke severity and an unfavourable short-term stroke outcome, with the purpose to further elucidate PRCP's physiological role during cerebrovascular events.

Acknowledgments

The authors gratefully thank Nicole Lamoen for excellent technical assistance, Lesley Baerts and Jill Luyckx for logistic support with blood samples. This

work was supported by the Fund for Scientific Research Flanders (Belgium, FWO–Vlaanderen) and the University of Antwerp Research Fund. Kaat Kehoe is a research assistant for FWO–Vlaanderen (Grant 11E4613N). This study was also supported by: the Institute Born-Bunge; the agreement between the Institute Born-Bunge and the University of Antwerp; Interuniversity Poles of Attraction (IAP Network P7/16) of the Belgian Federal Science Policy Office; the Flemish Government initiated Methusalem excellence grant, Belgium; and the Medical Research Foundation Antwerp.

Conflict of interest

The authors declare that they have no conflict of interest.

Electronic supplementary material

Below is the link to the electronic supplementary material.

Supplementary material 1 (DOCX 387 kb)

References

1. Donnan GA, Fisher M, Macleod M, Davis SM (2008) Stroke Lancet 371:1612–1623

2. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Turner MB et al (2013) Heart disease and stroke statistics–2013 update: a report from the American Heart Association. Circulation 127:e6–e245

3. Brouns R, De Deyn PP (2009) The complexity of neurobiological processes in acute ischemic stroke. Clin Neurol Neurosurg 111:483–495

4. Dirnagl U, Iadecola C, Moskowitz MA (1999) Pathobiology of ischaemic stroke: an integrated view. Trends Neurosci 22:391–397

5. Sugawara T, Fujimura M, Noshita N, Kim GW, Saito A, Hayashi T, Narasimhan P, Maier CM, Chan PH (2004) Neuronal death/survival signaling pathways in cerebral ischemia. NeuroRx 1:17–25

6. Brouns R, Heylen E, Willemse JL, Sheorajpanday R, De Surgeloose D, Verkerk R, De Deyn PP, Hendriks DF (2010) The decrease in procarboxypeptidase U (TAFI) concentration in acute ischemic stroke correlates with stroke severity, evolution and outcome. J Thromb Haemost 8:75–80

7. O'Donoghue AJ, Eroy-reveles AA, Knudsen GM, Ingram J, Zhou M, Statnekov JB, Greninger AL, Hostetter DR, Qu G, Maltby DA, Anderson MO, DeRIsi JL, Mckerrow JH, Burlingame AL, Craik CS (2013) Global identification of peptidase specificity by multiplex substrate profiling. Nat Methods 9:1095–1100

8. Mallela J, Yang J, Shariat-Madar Z (2009) Prolylcarboxypeptidase: a cardioprotective enzyme. Int J Biochem Cell Biol 41:477–481

9. Skidgel RA, Erdös EG (1998) Cellular carboxypeptidases. Immunol Rev 161:129–141

10. Shariat-Madar Z, Mahdi F, Schmaier AH (2004) Recombinant prolylcarboxypeptidase activates plasma prekallikrein. Blood 103:4554–4561

11. Chajkowski SM, Mallela J, Watson DE, Wang J, McCurdy CR, Rimoldi JM, Shariat-Madar Z (2011) Highly selective hydrolysis of kinins by recombinant prolylcarboxypeptidase. Biochem Biophys Res Commun 405:338–343

12. Shariat-Madar Z, Rahimy E, Mahdi F, Schmaier AH (2005) Overexpression of prolylcarboxypeptidase enhances plasma prekallikrein activation on Chinese hamster ovary cells. Am J Physiol Heart Circ Physiol 289:H2697–H2703

13. Wallingford N, Perroud B, Gao Q, Coppola A, Gyengesi E, Liu ZW, Goa XB, Diament A, Haus KA, Shariat-Madar Z, Mahdi F, Wardlaw SL, Schmaier AH, Warden CH, Diano S (2009) Prolylcarboxypeptidase regulates food intake by inactivating α-MSH in rodents. J Clin Invest 119:2291–2303

14. Palmiter RD (2009) Reduced levels of neurotransmitter-degrading enzyme PRCP promote obesity. J Clin Invest 119:2130–2133

15. Adams GN, LaRusch GA, Stavrou E, Zhou Y, Nieman MT, Jacobs GH, Cui Y, Lu Y, Jain MK, Mahdi F, Shariat-Madar Z, Okada Y, D'Alecy LG, Schmaier AH (2011) Murine prolylcarboxypeptidase depletion induces vascular dysfunction with hypertension and faster arterial thrombosis. Blood 117:3929–3937

16. Wang L, Feng Y, Zhang Y, Zhou H, Jiang S, Niu T, Wei LJ, Xu X, Xu X, Wang X (2006) Prolylcarboxypeptidase gene, chronic hypertension, and risk of preeclampsia. Am J Obstet Gynecol 195:162–171

17. Ngo ML, Mahdi F, Kolte D, Shariat-Madar Z (2009) Upregulation of prolylcarboxypeptidase (PRCP) in lipopolysaccharide (LPS) treated endothelium promotes inflammation. J Inflamm 6:3

18. Zhu L, Carretero OA, Liao TD, Harding P, Li H, Sumners C, Yang XP (2010) Role of prolylcarboxypeptidase in angiotensin II type 2 receptor-mediated bradykinin release in mouse coronary artery endothelial cells. Hypertension 56:384–390

19. Zhang Y, Hong XM, Xing HX, Li JP, Huo Y, Xu XP (2009) E112D polymorphism in the prolylcarboxypeptidase gene is associated with blood pressure response to benzapril in Chinese hypertensive patients. Chin Med J 122:2461–2465

20. Xu S, Lind L, Zhao L, Lindahl B, Venge P (2012) Plasma prolylcarboxypeptidase (angiotensinase C) is increased in obesity and diabetes mellitus and related to cardiovascular dysfunction. Clin Chem 58:1110–1115

21. Rabey FM, Gadepalli RSVS, Diano S, Cheng Q, Tabrizian T, Gailani D, Rimoldi JM, Shariat-Madar Z (2012) Influence of a novel inhibitor (UM8190) of prolylcarboxypeptidase (PRCP) on appetite and thrombosis. Curr Med Chem 19:4194–4206

22. Renna R, Pilato F, Profice P, Marca GD, Broccolini A, Morosetti R, Frisullo G, Rossi E, De Stefano V, Di Lazzaro V (2014) Risk factor and etiology analysis of ischemic stroke in young adult patients. J Stroke Cerebrovasc Dis 23:1–7

23. Shariat-Madar Z, Mahdi F, Schmaier AH (2002) Identification and characterization of prolylcarboxypeptidase as an endothelial cell prekallikrein activator. J Biol Chem 277:17962–17969

24. Kehoe K, Verkerk R, Sim Y, Waumans Y, Van der Veken P, Lambeir AM, De Meester I (2013) Validation of a specific prolylcarboxypeptidase activity assay and its suitability for plasma and serum measurements. Anal Biochem 443:232–239

25. Jeong JK, Diano S (2014) Prolyl carboxypeptidase mRNA expression in the mouse brain. Brain Res 1542:85–92

26. Buga AM, Scholz CJ, Kumar S, Herndon JG, Alexandru D, Cojocaru GR, Dandekar T, Popa-Wagner A (2012) Identification of new therapeutic targets by genome-wide analysis of gene expression in the ipsilateral cortex of aged rats after stroke. PLoS One 7:e50985

27. Adams GN, Stavrou EX, Fang C, Merkulova A, Alaiti MA, Nakajima K, Morooka T, Merkulov S, LaRusch GA, Simon DI, Jain MK, Schmaier AH (2013) Prolylcarboxypeptidase promotes angiogenesis and vascular repair. Blood 122:1522–1531

28. Willemse JL, Brouns R, Heylen E, De Deyn PP, Hendriks DF (2008) Carboxypeptidase U (TAFIa) activity is induced in vivo in ischemic stroke patients receiving thrombolytic therapy. J Thromb Haemost 6:200–202

29. Brouns R, Heylen E, Sheorajpanday R, Willemse JL, Kunnen J, De Surgeloose D, Hendriks DF, De Deyn PP (2009) Carboxypeptidase U (TAFIa) decreases the efficacy of thrombolytic therapy in ischemic stroke patients. Clin Neurol Neurosurg 111:165–170

30. Brouns R, Sheorajpanday R, Wauters A, De Surgeloose D, Mariën P, De

Deyn PP (2008) Evaluation of lactate as a marker of metabolic stress and cause of secondary damage in acute ischemic stroke or TIA. Clin Chim Acta 397:27–31

31. Brouns R, Marescau B, Possemiers I, Sheorajpanday R, De Deyn PP (2009) Dimethylarginine levels in cerebrospinal fluid of hyperacute ischemic stroke patients are associated with stroke severity. Neurochem Res 34:1642–1649

32. Adams HP, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh EE (1993) Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 24:35–41

33. Birschel P, Ellul J, Barer D (2004) Progressing stroke: towards an internationally agreed definition. Cerebrovasc Dis 17:242–252

34. Uyttenboogaart M, Luijckx GJ, Vroomen PCAJ, Stewart RE, De Keyser J (2007) Measuring disability in stroke: relationship between the modified Rankin scale and the Barthel index. J Neurol 254:1113–1117

35. Selim M, Savitz S, Linfante I, Caplan L, Schlaug G (2005) Effect of pre-stroke use of ACE inhibitors on ischemic stroke severity. BMC Neurol 5:10

36. Manabe Y, Kono S, Tanaka T, Narai H, Omori N (2009) High blood pressure in acute ischemic stroke and clinical outcome. Neurol Int 1:e1

37. Lu J, Jiang T, Wu L, Gao L, Wang Y, Zhou F, Zhang S, Zhang Y (2013) The expression of angiotensin-converting enzyme 2-angiotensin-(1-7)-Mas receptor axis are upregulated after acute cerebral ischemic stroke in rats. Neuropeptides 47:289–295

38. Gironacci MM, Carbajosa NAL, Goldstein J, Cerrato BD (2013) Neuromodulatory role of angiotensin-(1-7) in the central nervous system. Clin Sci (Lond) 125:57–65

39. Mecca AP, Regenhardt RW, O'Connor TE, Joseph JP, Raizada MK, Katovich MJ, Sumners C (2011) Cerebroprotection by angiotensin-1-7 in endothelin-1-induced ischaemic stroke. Exp Physiol 96:1084–1096

40. Blum A, Vaispapir V, Keinan-Boker L, Soboh S, Yehuda H, Tamir S (2012) Endothelial dysfunction and procoagulant activity in acute ischemic stroke. J Vasc Interv Neurol 5:33–39