Acute ischemic stroke severity, progression and outcome relates to changes in dipeptidyl peptidase IV and fibroblast activation protein activity

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Abstract:	Background Dipeptidyl peptidase IV (DPPIV) inhibition may be a promising therapeutic strategy for acute stroke treatment, given its potential to prolong the biological half-life of neuroprotective substrates. A related protease, fibroblast activation protein (FAP), was recently shown to inactivate the same substrates. Therefore, it should also be investigated as a potential target in stroke. Aims To investigate whether stroke severity and outcome correlates with DPPIV and FAP activities and their kinetics shortly after acute ischemic stroke. Methods

	DPPIV and FAP activities were analyzed in the serum of 50 hyperacute stroke patients at admission, 1 day, 3 days and 7 days after stroke onset and in 50 age-matched healthy controls. This was done as part of the Middelheim's Interdisciplinary Stroke Study. Results DPPIV activity tended to increase shortly after stroke compared to the control population. DPPIV and FAP activities steadily decreased in the first week after stroke onset. Higher infarct volumes (≥ 5 ml) and a more severe stroke (NIHSS > 7) at admission were correlated with a stronger decrease in the activities of both enzymes. Moreover, these patients more often developed a progressive stroke, were more often institutionalized and had a higher degree of disability after three months (modified Rankin scale ≥ 4). Conclusions Patients with a stronger increase in DPPIV activity at admission and decrease in the activity of both DPPIV and FAP during the first week after stroke onset had a more severe stroke and worse short-term and long-term outcomes.

***Reviewer #1*: The authors provide a revised version of the manuscript entiteled "Acute ischemic stroke severity, progression and outcome relates to changes in dipeptidyl peptidase IV and fibroblast activation protein activity".**

The authors provide an itemized, point-by-point response to the comments of the reviewers. The manuscript is revised in response to the comments and suggestions made by reviewer #1 and #2. The comments made by the reviewers were considered and adressed in an adequate manner.

As requested by both reviewers the authors perfomed a multifactorial analysis for encymes studied in this paper. They performed a multivariate analysis by backward logistic regression analysis to evaluate whether dipeptidyl peptidase IV (DPPIV) and fibroblast activation protein (FAP) activities were independently associated with infarct volume, stroke progression, the need for institutionalization at discharge, or long-term functional outcome (mRS score at 3 months). The multivariate analysis revealed DPPIV and FAP activity as independent predictors for short-term outcome after stroke. The method section and results section oft the manuscript was adapted and the results were discussed in the discussion section.

The statement in the conclusion section of the abstract, that patients with a stronger increase in DPPIV activity at admission and decrease in the activity of both DPPIV and FAP during the first week after stroke onset had a worse long-term outcome is not supported by the data and the part regarding long-term outcome shoud be omitted.

We would like to thank reviewer 1 for another carefull reading of our manuscript. As reviewer 1 suggested we have omitted the statement in the abstract (results and conclusion) regarding the long-term outcome of the stroke patients.

ABSTRACT

Results

DPPIV activity tended to increase shortly after stroke compared to the control population. DPPIV and FAP activities steadily decreased in the first week after stroke onset. Higher infarct volumes (≥ 5 ml) and a more severe stroke (NIHSS > 7) at admission were correlated with a stronger decrease in the activities of both enzymes. Moreover, these patients more often developed a progressive stroke, were more often institutionalized.

Conclusions

Patients with a stronger increase in DPPIV activity at admission and decrease in the activity of both DPPIV and FAP during the first week after stroke onset had a more severe stroke and worse short-term outcomes.

As suggested by *reviewer #2*, the authors additionally discussed the potentialy sources of the studied encymes in the blood. By giving more attention to this point, the authors provide more background that helps to better understand the aim of their study.

We are pleased that our revision met the expectations of reviewer 2.Once again we would like to thank reviewer 2 for his suggestions.

Acute ischemic stroke severity, progression and outcome relates to changes in dipeptidyl peptidase IV and fibroblast activation protein activity

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Abstract

Background

Dipeptidyl peptidase IV (DPPIV) inhibition may be a promising therapeutic strategy for acute stroke treatment, given its potential to prolong the biological half-life of neuroprotective substrates. A related protease, fibroblast activation protein (FAP), was recently shown to inactivate the same substrates. Therefore, it should also be investigated as a potential target in stroke.

Aims

To investigate whether stroke severity and outcome correlates with DPPIV and FAP activities and their kinetics shortly after acute ischemic stroke.

Methods

DPPIV and FAP activities were analyzed in the serum of 50 hyperacute stroke patients at admission, 1 day, 3 days and 7 days after stroke onset and in 50 age-matched healthy controls. This was done as part of the Middelheim's Interdisciplinary Stroke Study.

Results

DPPIV activity tended to increase shortly after stroke compared to the control population. DPPIV and FAP activities steadily decreased in the first week after stroke onset. Higher infarct volumes ($\geq$ 5 ml) and a more severe stroke (NIHSS > 7) at admission were correlated with a stronger decrease in the activities of both enzymes. Moreover, these patients more often developed a progressive stroke, were more often institutionalized.

Conclusions

Patients with a stronger increase in DPPIV activity at admission and decrease in the activity of both DPPIV and FAP during the first week after stroke onset had a more severe stroke and worse short-term outcomes.

Keywords

Acute ischemic stroke, Dipeptidyl peptidase IV (DPP IV), Fibroblast activation protein (FAP), Stroke evolution, Stroke outcome, Stroke severity

Introduction

In recent years, evidence which points to a role of Dipeptidyl Peptidase IV (DPPIV) in stroke has been growing. Darsalia *et al.* showed that the DPPIV inhibitor linagliptin counteracts stroke in the normal and diabetic mouse brain [1]. This is supported by a meta-analysis that found a trend towards a lower stroke incidence in patients with type 2 diabetes using DPPIV inhibitors [2]. The protective effect is not only attributable to an improved glycemic control alone but also to the prolonged half-life of neuroprotective DPPIV substrates [1, 3, 4].

Several DPPIV substrates have been shown to be implicated in stroke. For example, stromal derived cell factor 1, neuropeptide Y and B-type natriuretic peptide are correlated with stroke outcome and are proposed as biomarkers [5, 6]. Interestingly, Fibroblast Activation Protein (FAP) a DPPIV-related serine protease and member of the same prolyl oligopeptidase family, is able to inactivate several of these substrates [7]. This is in agreement with Röhnert *et al.*, who suggested that DPPIV and proteases with similar substrate specificity are potential therapeutic targets in cerebral ischemia [8]. For more information on DPPIV and its substrates in ischemia we refer to the review by Matheeussen et al [9].

In this study we aimed to investigate if stroke severity, progression and outcome were correlated with serum DPPIV and FAP activities and their kinetics shortly after stroke onset.

Materials and Methods

Study population

This study is part of the Middelheim's Interdisciplinary Stroke Study (ZNA Middelheim Hospital, Antwerp; 2005-2008). Patients with ischemic stroke underwent neuroimaging and were clinically, biochemically, neuropsychologically and electrophysiologically evaluated. The subtype of the ischemic stroke was classified as described by the TOAST criteria (Trial of Org 10172 in Acute Stroke Treatment) [10]. The results of other biochemical analyses have been reported elsewhere [11–15]. Serum samples from 50 patients, who did not receive thrombolytic treatment, were selected for analysis of DPPIV and FAP activities. Leftover samples from outpatients with C-reactive protein

levels and glomerular filtration rates within normal ranges were used to create an age-matched control group of 50 samples. Patient and stroke characteristics can be found in table 1 and table 2, respectively. This study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committees of ZNA Antwerp and the University of Antwerp.

Evaluation of stroke severity, evolution and outcome

Trained stroke physicians determined neurological impairment according to the National Institutes of Health Stroke Scale (NIHSS) at admission, day 1, day 3 and day 7. Progressing stroke was evaluated as described by the European Progressing Stroke Study criteria [16]. A brain MRI scan was performed to confirm the diagnosis of acute ischemic stroke. The infarct volume was assessed by two independent neurologists, as previously described [11, 15]. After three months, stroke outcome was assessed with the modified Rankin Scale, severe disability was defined as an mRS score ≥ 4 [15]. The mRS was obtained by trained and certified stroke physicians, in the majority of cases during face-to-face follow-up visits at the out-patient clinic. Assessment of the mRS via phone was performed if face-to-face was not possible. The individual distribution of the DPPIV and FAP activities for the different parameters of stroke severity, evolution and outcome can be found in the supplemental data.

Blood collection and DPPIV and FAP activity measurement

Venous blood samples were collected at admission, 1 day, 3 days and 7 days after stroke onset, in serum tubes. After a centrifugation at 2000 $\times g$ for 15 min at 4°C, serum was frozen in liquid nitrogen and stored at −80 °C.

The DPPIV activity was measured using Gly-Pro-pNA as described by Matheeussen et al [17]. FAP activity was measured using Z-Gly-Pro-AMC. Standards and samples were diluted in a 0.1 M Tris buffer (pH 8.0) containing 300 mM NaF, 1 mM NaN2, 1 mM EDTA and 50 mM salicylic acid. Substrate was added and incubated for 2 h at 37 °C. Reactions were stopped with 1.5 M acetic acid and fluorescence was measured (λ_{ex} = 370 nm, λ_{em} = 440 nm). In these assay conditions all measured activity is attributable to FAP.

Statistical analysis

All statistical tests were performed using the *Statistical Package for the Social Sciences* (SPSS) version 15. A one-way ANOVA or t-test for independent samples was used to compare enzyme activities between groups, while a t-test for paired samples was used for time-points within a group. Bonferroni post hoc analysis or correction was applied for multiple pairwise comparisons to prevent inflation of the type I error rate. Correlations between stroke severity, outcome or evolution and changes in enzyme activity over 7 days after stroke onset ($\Delta DPPIV_{day7}$ and ΔFAP_{day7}) were assessed with bivariate analysis. For continuous data such as infarct volume a Pearson correlation was used after dichotomization, while ordinal data, for example stroke progression, was assessed with a Spearman rank correlation coefficient. Thresholds were prospectively selected as defined by Kehoe *et al.*[15]. Multivariate analysis was performed by backward logistic regression analysis (using inclusion criterion of $P<0.010$) to evaluate whether DPPIV and FAP activities were independently associated with infarct volume, stroke progression, the need for institutionalization at discharge, or long-term functional outcome (mRS score at 3 months). For this, a model including patient characteristics (age, gender), the interval between stroke onset and time of blood sampling, vascular risk factors (arterial hypertension, atrial fibrillation, heart failure, familial stroke, previous stroke, previous myocardial infarction, peripheral artery disease, diabetes mellitus, dyslipidemia, smoking, and alcohol abuse), and the use of antidiabetic medication was constructed. Differences were considered significant at $p < 0.05$. All results are shown with the standard error of the mean.

Results

DPPIV and FAP activity in serum

DPPIV and FAP activities from the patient samples are shown in Fig. 1. Compared to the controls, DPPIV showed a trend towards an increase immediately after stroke onset and decreased afterwards. FAP activities decreased significantly in the first week after admission. The lowest activities in patients were found at day 7.

At admission, DPPIV and FAP activities were significantly correlated. This correlation was also seen for the kinetics of both enzymes over 7 days. On average, activities decreased by 11% for DPPIV and 19% for FAP. Enzyme activities of both enzymes at admission progressed independently from age, gender or interval between stroke onset and time of blood sampling (table 3).

Relation between patient characteristics and enzyme activity

No relation was observed between the enzyme activities or their kinetics and risk factors associated with stroke (arterial hypertension, atrial fibrillation, heart failure, familial stroke, previous stroke, previous myocardial infarction, diabetes mellitus, dyslipidemia, smoking or alcohol abuse), with the exception of DPPIV kinetics and dyslipidemia. Peripheral artery disease was correlated with DPPIV activities at admission (table 3).

Both systolic and diastolic blood pressures, at presentation, were correlated with DPPIV and FAP activities at the same time and with their kinetics after 7 days. The same was true for HDL levels at admission. Glycemia at admission and after 7 days was correlated with the change in DPPIV activity over the same period. Inflammatory parameters were also correlated with the enzyme activities. FAP admission levels inversely related with CRP levels at admission and its kinetics with CRP levels after 7 days. All the data is summarized in table 3.

DPPIV and FAP activity in relation to stroke severity

DPPIV activities at admission were significantly higher in patients with higher infarct volumes ($\geq$ 5 ml) and showed a trend towards a more pronounced decrease of DPPIV and FAP during the first week after stroke onset (Fig 2A and 2C). Logistic regression analysis confirmed DPPIV activity at admission as an independent predictor for infarct volume (Exp(β) (95% CI): 1.134 (1.001-1.285), P=0.048).

Patients were divided in two groups based on their NIHSS score. Mild stroke was defined as NIHSS $\leq$ 7 and more severe stroke as NIHSS > 7. Patients with a more severe stroke at admission showed a more pronounced decrease in DPPIV activity during the first week after stroke (Fig 2B, 2D and table 3).

DPPIV and FAP activity in relation to short-term stroke outcome and evolution

Patients who developed progressive stroke, according to the EPSS criteria, had a more pronounced decrease in DPPIV and FAP activity than those with a more favorable outcome. In addition, changes in NIHSS during the first week were related with DPPIV levels at admission and the kinetics of both enzymes over 7 days (Fig 3A and 3C). Patients who were institutionalized after discharge had higher DPPIV and FAP activities at admission and a more pronounced decrease towards day 7 (Fig 3B and 3D). The decrease in DPPIV and FAP activities were confirmed to be independent predictors of stroke progression (Exp(β) (95% CI): 1.326 (1.008-1.745), P=0.044 and Exp(β) (95% CI): 1.746(1.144-1074.156), P=0.042) by logistic regression analysis. The same model showed that DPPIV and FAP activity at admission were predictive for institutionalization at discharge (Exp(β) (95% CI): 1.212 (1.039-1.412), P=0.014 and Exp(β) (95% CI): 1.746(1.144-1074.156), P=0.042).

DPPIV and FAP activity in relation to long-term stroke outcome

Long-term outcome was assessed with the mRS score after 3 months. Patients with a severe disability (mRS $\geq$ 4) had a more pronounced decrease in DPPIV and FAP activities during the first week after stroke onset (Fig 4). However, after logistic regression the decrease in DPPIV and FAP activities, during the first week, was shown not to be an independent predictor of long term stroke outcome.

Result validation

In order to validate the results, hematocrit was chosen as an internal standard. As erythrocytes have a long half-life, this can be considered as a stable parameter. No significant difference was observed in change in hematocrit after 7 days between most of the investigated subgroups. In the groups with a change in hematocrit, the DPPIV or FAP activities showed the reverse trend (i.e. inclined where hematocrit declined), excluding that differences between groups were influenced by factors such as changes in plasma volume.

Discussion

In recent years, there has been a growing interest in dipeptidyl peptidases in stroke. As most research focuses on the effects of inhibition or on the role of physiological substrates of DPPIV [1, 8, 18], there exists a knowledge-gap on DPPIV activity levels in stroke. While the involvement of DPPIV activity and related proteases has been reported in cerebral ischemia induced inflammation in rats [8], we are the first to report on the kinetics of DPPIV and FAP activity in patient serum in hyperacute stroke and the 7 days following stroke onset. While DPPIV activities only tended to increase shortly after stroke and decrease thereafter, FAP activities decreased significantly during the first week after stroke onset. Both enzymes reached their lowest levels at day 7.

The increase in soluble DPPIV shortly after stroke onset is in agreement with Röhrborn *et al.* who showed *in vitro* that DPPIV is shed during hypoxia [19]. After admission this hypoxic state is relieved and the activity lowers to baseline. Since we did not observe a significant increase in FAP activity, we have no indication for a similar mechanism for FAP. Interestingly, DPPIV shedding is also increased by inflammation [20]. Consequently, small inflammatory molecules generated intra-cerebrally might cross the blood brain barrier (BBB) and induce DPPIV activity systemically. Alternatively, intracerebral inflammation is known to increase the BBB permeability. As a consequence, cerebral DPPIV/FAP might also leak into the bloodstream [21].

This hypothesis might help explain the beneficial effect of a pretreatment with DPPIV inhibitors in an animal model [1]. This is most likely a class effect as both linagliptin and alogliptin show a beneficial effect [1, 18]. As inhibitors prevent the sudden rise in DPPIV, the biological half-life of neuroprotective substrates such as glucagon-like peptide 1 (GLP-1) is extended [3]. Similar to DPPIV inhibitors, DPPIV and FAP are unable to cross the BBB. In contrast, several DPPIV and FAP substrates are able to cross the BBB [22, 23]. The higher enzyme activities result in biological inactive substrates or even antagonists that might cross the BBB and abolish the neuroprotective effect of their intact counterparts [24]. Nevertheless, the effect on hyperglycemia should not be forgotten, as hyperglycemia is associated with worse outcomes in stroke [25].

The fact that we did not find a correlation between cardiovascular risk factors and enzyme activities, especially of DPPIV, is surprising. A recent meta-analysis found a reduction in all-cause mortality and major cardiovascular events during DPPIV inhibitor treatment [2]. This might be explained by the fact that the meta-analysis only included patients with diabetes mellitus type 2.

We showed that both the systolic as the diastolic blood pressure increased with higher DPPIV and FAP activities. The role of DPPIV in blood pressure is controversial. While the majority of authors state that DPPIV inhibitors can lower blood pressure, others claim a rise [9, 26]. However, the truth probably lies in between as Jackson *et al.* showed that the effect of DPPIV inhibitors is context dependent [26]. We are the first to show a correlation of FAP levels with blood pressure. Nonetheless, the mechanism behind this remains unclear as its expression is tightly regulated [27]. An explanation might be sought in the overlap with DPPIV concerning its cardiovascular-active substrates.

The correlation between glycemia and DPPIV kinetics is not very surprising, as its best known substrate, GLP-1, is involved in glucose homeostasis [9]. The correlation of inflammation with FAP activity is equally unsurprising as it was shown earlier that its expression is induced during inflammation through TNFα [28].

To conclude, this study describes the decrease in DPPIV and FAP activities during the first week after stroke onset and its correlation with stroke severity, progression and outcome. These novel insights help us to better understand the biological significance of both enzymes in stroke and warrant further investigation of the use of DPPIV inhibitors in stroke in humans.

Compliance with Ethical Standards

This work was supported by the University of Antwerp Research Fund (Grant FFB3551), the Institute Born-Bunge, the Antwerp Biobank, the Interuniversity Attraction Poles (IAP) programme of the Belgian Science Policy Office and the Flemish Government Methusalem excellence program, Belgium.

The authors have no conflict of interest to declare. This study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committees of ZNA Antwerp and the University of Antwerp. For the stroke patients of ZNA Middelheim hospital, written informed consent was obtained from each participating subject or proxy in case of reduced consciousness. The (leftover) control samples from the UZA hospital were number coded and were obtained according to the FDA"Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable".

Limitations

The first limitation of this study is that the age-matched control population consisted of 'apparently healthy' outpatients who came to the hospital for a check-up (C-reactive protein and glomerular filtration rate were within the normal range) A second limitation is the fact that it is impossible to exclude the effect of all confounding factors.

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References

1. Darsalia V, Ortsäter H, Olverling A, Darlöf E, Wolbert P, Nyström T, Klein T, Sjöholm Å, Patrone C (2013) The DPP-4 inhibitor linagliptin counteracts stroke in the normal and diabetic mouse brain: a comparison with glimepiride. Diabetes 62:1289–96

2. Monami M, Ahrén B, Dicembrini I, Mannucci E (2013) Dipeptidyl peptidase-4 inhibitors and cardiovascular risk: a meta-analysis of randomized clinical trials. Diabetes Obes Metab 15:112–20

3. Shannon RP (2013) DPP-4 inhibition and neuroprotection: do mechanisms matter? Diabetes 62:1029–31

4. Robin AM, Zhang ZG, Wang L, Zhang RL, Katakowski M, Zhang L, Wang Y, Zhang C, Chopp M (2006) Stromal cell-derived factor 1alpha mediates neural progenitor cell motility after focal cerebral ischemia. J Cereb Blood Flow Metab 26:125–34

5. Paczkowska E, Kucia M, Koziarska D, et al (2009) Clinical evidence that very small embryonic-like stem cells are mobilized into peripheral blood in patients after stroke. Stroke 40:1237–44

6. Rost NS, Biffi A, Cloonan L, Chorba J, Kelly P, Greer D, Ellinor P, Furie KL (2012) Brain natriuretic peptide predicts functional outcome in ischemic stroke. Stroke 43:441–5

7. Keane FM, Nadvi NA, Yao T-W, Gorrell MD (2011) Neuropeptide Y, B-type natriuretic

peptide, substance P and peptide YY are novel substrates of fibroblast activation protein-α. FEBS J 278:1316–32

8. Röhnert P, Schmidt W, Emmerlich P, et al (2012) Dipeptidyl peptidase IV, aminopeptidase N and DPIV/APN-like proteases in cerebral ischemia. J Neuroinflammation 9:44

9. Matheeussen V, Jungraithmayr W, De Meester I (2012) Dipeptidyl peptidase 4 as a therapeutic target in ischemia/reperfusion injury. Pharmacol Ther 136:267–82

10. 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

11. 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

12. Brouns R, Sheorajpanday R, Kunnen J, De Surgeloose D, De Deyn PP (2009) Clinical, biochemical and neuroimaging parameters after thrombolytic therapy predict long-term stroke outcome. Eur Neurol 62:9–15

13. 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

14. 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 34:1642–9

15. Kehoe K, Brouns R, Verkerk R, Engelborghs S, De Deyn PP, Hendriks D, De Meester I (2015) Prolyl carboxypeptidase activity decline correlates with severity and short-term outcome in acute ischemic stroke. Neurochem Res 40:81–8

16. Birschel P, Ellul J, Barer D (2004) Progressing stroke: towards an internationally agreed definition. Cerebrovasc Dis 17:242–52

17. Matheeussen V, Lambeir A-M, Jungraithmayr W, Gomez N, Mc Entee K, Van der Veken P, Scharpé S, De Meester I (2012) Method comparison of dipeptidyl peptidase IV activity assays and their application in biological samples containing reversible inhibitors. Clin Chim Acta 413:456–62

18. Yang D, Nakajo Y, Iihara K, Kataoka H, Yanamoto H (2013) Alogliptin, a dipeptidylpeptidase-4 inhibitor, for patients with diabetes mellitus type 2, induces tolerance to focal cerebral ischemia in non-diabetic, normal mice. Brain Res 1517:104–13

19. Röhrborn D, Eckel J, Sell H (2014) Shedding of dipeptidyl peptidase 4 is mediated by metalloproteases and up-regulated by hypoxia in human adipocytes and smooth muscle cells. FEBS Lett 588:3870–7

20. Lamers D, Famulla S, Wronkowitz N, et al (2011) Dipeptidyl peptidase 4 is a novel adipokine potentially linking obesity to the metabolic syndrome. Diabetes 60:1917–25

21. Borlongan C V, Glover LE, Sanberg PR, Hess DC (2012) Permeating the blood brain barrier and abrogating the inflammation in stroke: implications for stroke therapy. Curr Pharm Des 18:3670–6

22. Nonaka N, Shioda S, Niehoff ML, Banks WA (2003) Characterization of blood-brain barrier permeability to PYY3-36 in the mouse. J Pharmacol Exp Ther 306:948–53

23. Kastin AJ, Akerstrom V (1999) Nonsaturable entry of neuropeptide Y into brain. Am J Physiol 276:E479–82

24. Casrouge A, Decalf J, Ahloulay M, et al (2011) Evidence for an antagonist form of the chemokine CXCL10 in patients chronically infected with HCV. J Clin Invest 121:308–17

25. Olsen TS (2009) Blood glucose in acute stroke. Expert Rev Neurother 9:409–19

26. Jackson EK, Mi Z, Tofovic SP, Gillespie DG (2015) Effect of dipeptidyl peptidase 4 inhibition on arterial blood pressure is context dependent. Hypertension 65:238–49

27. Jacob M, Chang L, Puré E (2012) Fibroblast activation protein in remodeling tissues. Curr Mol Med 12:1220–43

28. Brokopp CE, Schoenauer R, Richards P, et al (2011) Fibroblast activation protein is induced by inflammation and degrades type I collagen in thin-cap fibroatheromata. Eur Heart J 32:2713–22

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Figure 1: Enzyme activities of the total study population and controls. (A) DPPIV activity at admission showed a trend towards higher levels compared to controls and tended to decrease afterwards. (B) FAP activity decreased steadily after stroke and reached statistical significance 7 days after stroke. (* $p < 0.05$)

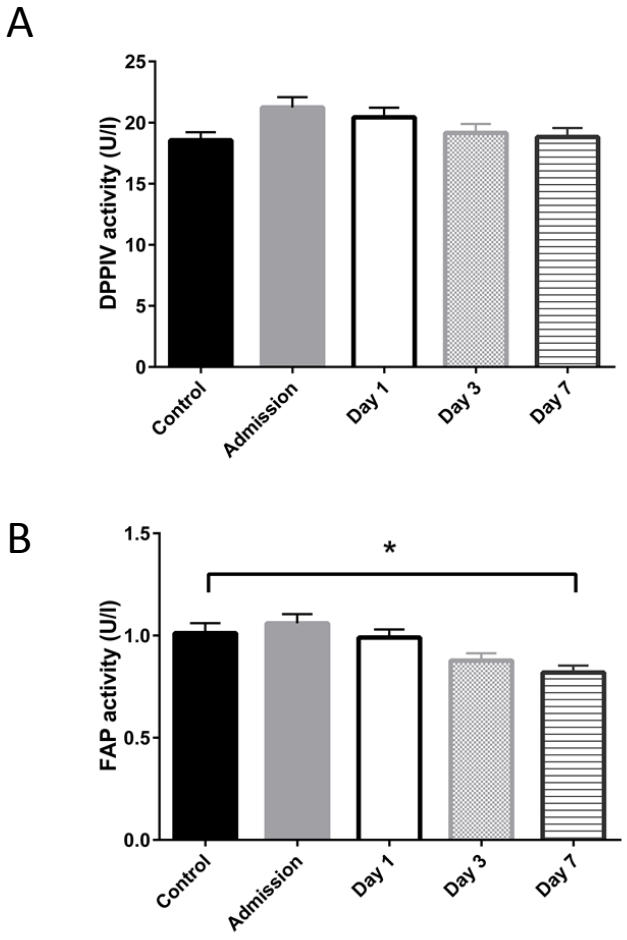

Figure 2: Enzyme activity kinetics and stroke severity. (A) Patients with large infarct volumes (≥ 5 ml) have significantly higher DPPIV activities at admission. (B) Its activity did not differ at any time-point between patients with high or low NIHSS scores (≤ 7). (C) FAP activity did not differ significantly between patients with large or small infarct volumes (D) or high or low NIHSS scores. (* $p < 0.05$)

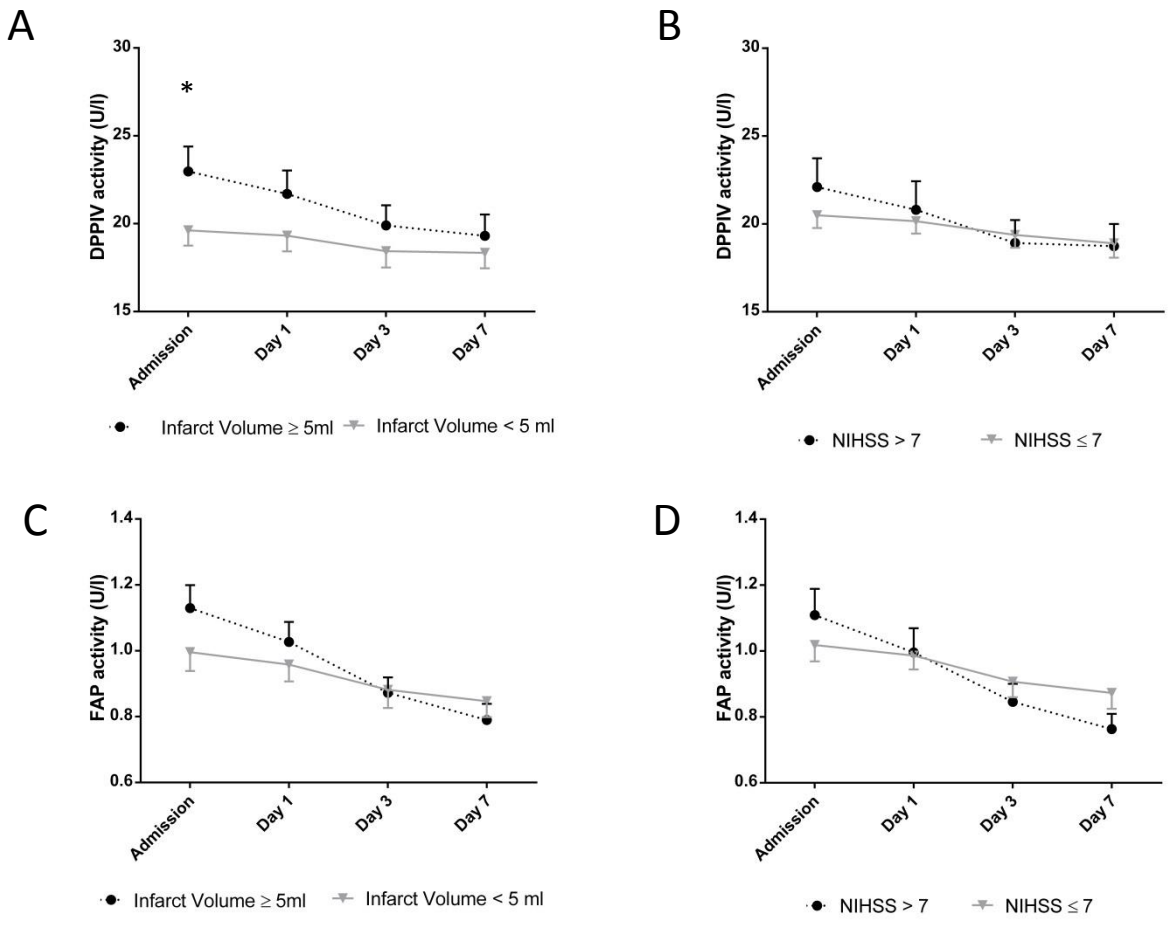

Figure 3: Enzyme activity kinetics and short-term outcome. (A) DPPIV activities were higher in patients with progressive stroke after 1 and 3 days . (B) Patients who were institutionalized after discharge had significantly higher DPPIV activities at admission. (C) FAP activities tended to be higher at admission, 1 day and 3 days after stroke in patients who developed a progressive stroke. (D) FAP activities were significantly higher at admission in patients who had to be institutionalized after discharge . (* $p < 0.05$)

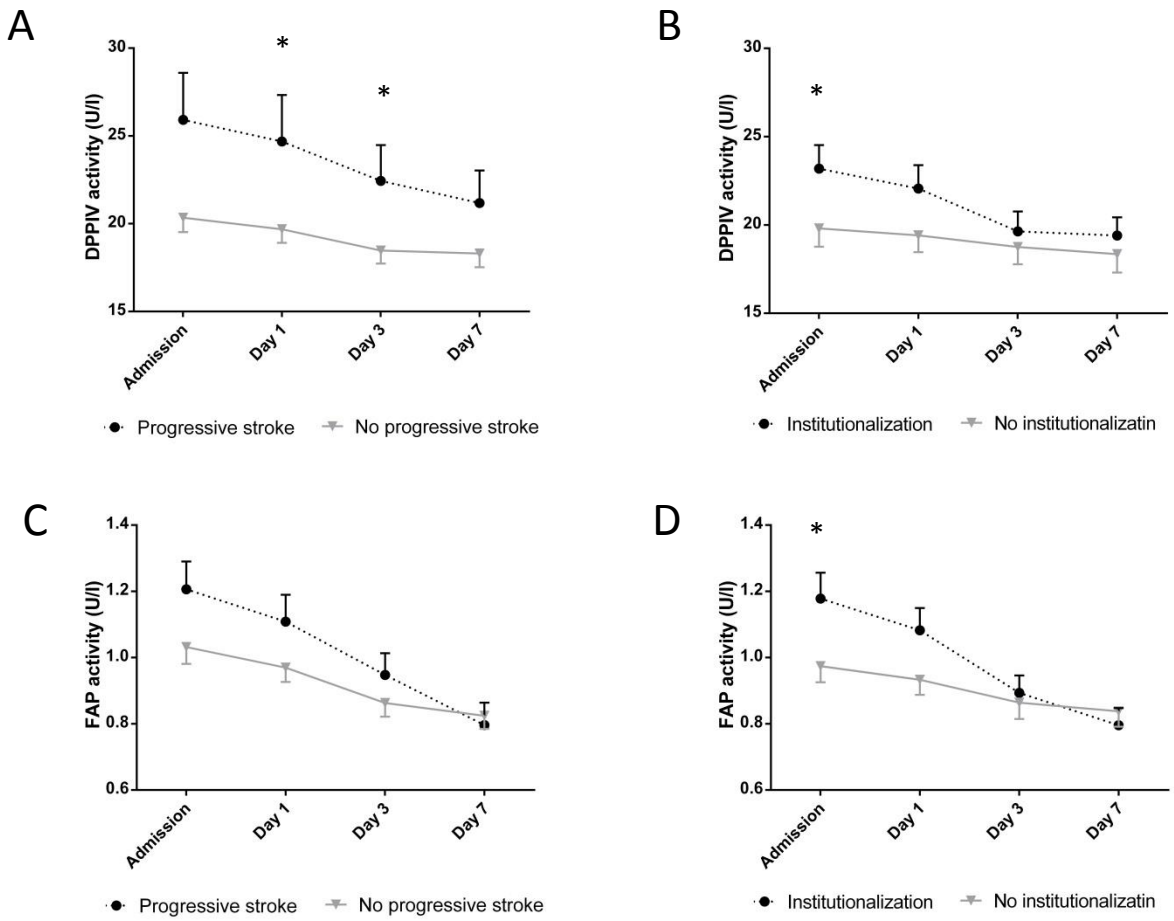

Figure 4: Enzyme activity kinetics and long-term outcome. No difference could be found in DPPIV (A) or FAP activity (B) between patients with a severe disability and no or mild disability at any of the time-points. (* $p < 0.05$)

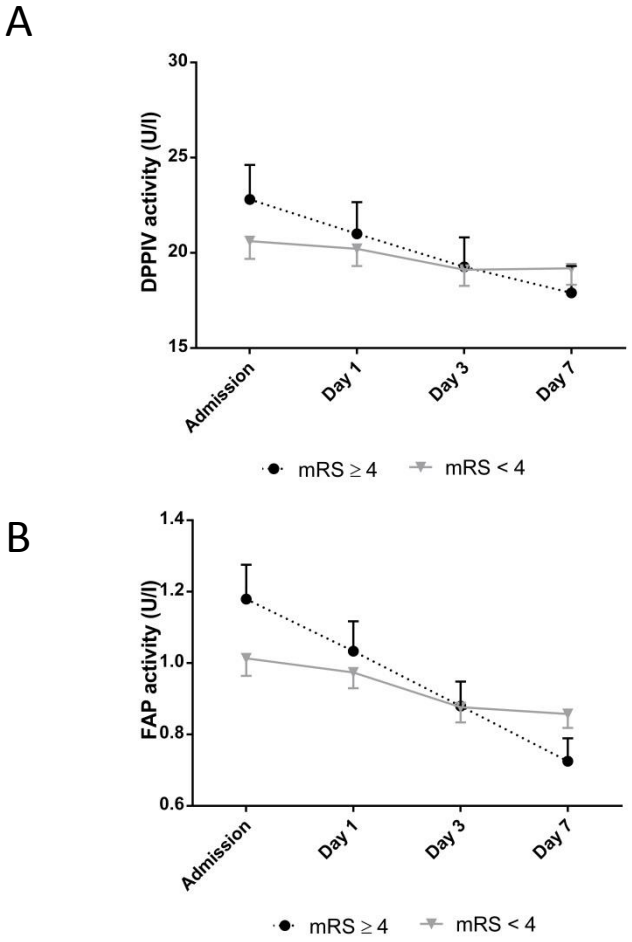

Table 1: Characteristics of the entire population.

		Patients	Controls
Age	(years)	75 ± 7	73 ± 5
Gender	(male)	26 (52%)	31 (62%)
Caucasian	(%)	100%	100%
Time to blood sampling after onset stroke symptoms	(h)	3 ± 2	

Table 2: Stroke characteristics of the patients.

Stroke characteristic		Value
Stroke severity		
	NIHSS at admission	9 ± 9
	Infarct volume	4.3 ml [IQR; 0.8-40.]
Stroke etiology (TOAST criteria)		
	Atherothrombotic	16%
	Cardioembolic	53%
	Lacunar	12%
	Specific	2%
	Undetermined	16%
Subacute stroke evolution (after 7 days)		
	Normalized gain in NIHSS	46% ± 60%
	Progressive stroke (EPSS criteria)	16%
Short-term stroke outcome		
	Institutionalization	43%
	mRS score after 7 days	3 ± 2
	Mortality after 7 days	2%
Long-term stroke outcome		
	mRS score after 3 months	3 ± 2
	Mortality after 3 months	12%

EPSS European Progression Stroke Study Criteria

mRS modified Rankin Scale

NIHSS National Institutes of Health Stroke Scale

TOAST Trial of Org 10172 in Acute Stroke Treatment

Table 3: The correlation between the parameters analysed and DPPIV and FAP activities at admission and their kinetics.

		DPPIV activity at admission			$\Delta DPP4_{day7}$			FAP activity at admission			ΔFAP_{day7}		
		Pearson correlation	T-value	P-value	Pearson correlation	T-value	P-value	Pearson correlation	T-value	P-value	Pearson correlation	T-value	P-value
	Age	-0.022		0.88	0.102		0.506	0.065		0.654	0.164		0.28
	Gender		1.791	0.08		1.545	0.13		0.567	0.574		0.962	0.342
	Interval stroke onset and blood sampling	0.17		0.239	n.a.		n.a.	0.088		0.542	n.a.		n.a.
Cardiovascular													
	Arterial hypertension		0.344	0.733		-0.062	0.951		0.108	0.915		-0.35	0.728
	Atrial fibrillation		-0.227	0.822		-0.842	0.404		-0.098	0.922		-0.14	0.89
	Heart failure		0.592	0.557		-0.111	0.913		1.456	0.152		1.328	0.191
	Peripheral artery disease		2.447	**0.018***		1.213	0.232		2.29	**0.026***		1.939	0.059
	Familial stroke		0.02	0.984		0.397	0.693		-0.758	0.452		0.05	0.96
	Previous stroke		-0.031	0.975		0.157	0.876		-1.72	0.092		-1.08	0.286
	Previous myocardial infarction		-0.17	0.866		-1.221	0.229		0.86	0.394		0.2	0.843
	Diabetes mellitus		0.222	0.825		-1.025	0.311		0.598	0.552		-0.376	0.709
	Dyslipidemia		-0.859	0.395		-2.571	**0.014***		-0.659	0.513		-1.543	0.13
	Previous smoking		0.73	0.469		0.401	0.691		0.364	0.718		0.487	0.629
	Current smoking		1.268	0.211		1.574	0.123		1.416	0.163		0.992	0.393
	Alcohol abuse		0.47	0.64		0.303	0.764		-0.383	0.703		-0.027	0.978
Biochemistry													
	$Glycemia_{admission}$	0.211		0.142	0.322		**0.031***	0.003		0.983	0.162		0.287
	$Glycemia_{day7}$	0.126		0.409	0.346		**0.020***	-0.121		0.427	0.081		0.595
	Total triglycerides	0.031		0.835	0.035		0.828	-0.109		0.465	-0.116		0.464
	Total cholesterol	0.154		0.3	-0.101		0.526	0.264		0.072	0.137		0.386
	High-density lipoprotein	0.459		**0.001***	0.348		**0.026***	0.413		**0.004***	0.468		0.088
	Low-density lipoprotein	-0.003		0.986	-0.273		0.084	0.12		0.427	-0.36		0.821
	$CRP_{admission}$	-0.207		0.149	-0.035		0.819	-0.342		**0.015***	-0.132		0.387
	CRP_{day7}	0.007		0.963	0.331		0.026	0.043		0.78	0.466		**0.001***
	$Sedimentation\ rate_{admission}$	-0.158		0.31	0.006		0.972	-0.258		0.095	-0.117		0.483
	$Sedimentation\ rate_{day7}$	0.007		0.965	0.293		0.056	-0.272		0.077	0.042		0.79
Stroke													
	Infarct volume	0.323		**0.022***	0.382		**0.010***	0.162		0.262	0.299		**0.046***
	$NIHSS\ score_{admission}$	-0.073		0.617	0.307		**0.040***	-0.09		0.534	0.249		0.099
	$\Delta NIHSS\ score_{admission\text{-}day7}$	-0.285		**0.045***	-0.089		0.561	-0.353		**0.012***	-0.248		0.101
	Progressive stroke		-1.959	0.081		-2.339	**0.024***		-1.425	0.161		-2.316	**0.025***
	Institutionalization		-2.038	**0.047***		-3.444	**0.001***		-2.311	**0.025***		-4.19	**0.000***
	$mRS\ score_{day7}$	0.166		0.249	0.55		**0.000***	0.091		0.529	0.528		**0.000***
	$mRS\ score_{month3}$	0.03		0.839	0.495		**0.001***	0.028		0.849	0.474		**0.001***