Journal of
Diabetes Research & Clinical Metabolism
ISSN 2050-0866

HOAJ
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Original Open Access

Blood biomarkers of insulin resistance in acute stroke patients treated with intravenous thrombolysis: Temporal profile and prognostic value

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Abstract

Background: Metabolic syndrome and insulin resistance may hamper the beneficial effect of intravenous thrombolysis in acute ischemic stroke. We investigated the temporal profile and prognostic value of 11 circulating biomarkers of insulin resistance in acute ischemic stroke patients treated with intravenous thrombolysis.

Methods: We performed a prospective study in acute ischemic stroke patients with a middle cerebral artery (MCA) occlusion who received intravenous tissue plasminogen activator (tPA). Measurement of C-Peptide, Ghrelin, Gastric-inhibitory-polypeptide (GIP), Glucagon-like-peptide-1 (GLP-1), Glucagon, Insulin, Leptin, Resistin, Visfatin, Interleukin-6 (IL-6) and Tumor-necrosis-factor-alpha (TNF-α) was performed in three time-points: before tPA-bolus, right after tPA-infusion and at 24h. Long term clinical outcome, early neurological recovery, MCA-recanalization, infarct volume and hemorrhagic transformation were outcome variables.

Results: Fifty-two patients were included (61%women, mean-age75). Three months after stroke onset, 24 (47%) patients were functionally independent. In a multivariate adjusted regression model, GIP level at 1h≥109.51 pg/ml (OR 13.78 [1.38-137.31], p=0.02) and GIP level at 24h≥82.19 pg/ml (OR 28.46 [1.09-739.82], p=0.04) emerged as independent predictors of good outcome. Baseline GIP and 0-1 h increment in IL-6 and TNF-α were associated with early neurological course. Ghrelin level at 1h and 0-24h increment in Leptin level were independently associated with a larger infarct volume.

Conclusions: Blood concentration of several molecules reflecting a more pronounced insulin resistance status was associated with a worse outcome in acute ischemic stroke patients treated with intravenous thrombolysis. Noteworthy, a higher GIP level at 1h and 24 h after tPA treatment predicted good long-term outcome.

Keywords: Insulin resistance, acute stroke, cytokines

Introduction

Stroke is a major cause of death and disability worldwide. Acute reperfusion therapies are aimed to reduce the amount of brain infarcted tissue, thus improving stroke outcome. Among reperfusion therapies, intravenous thrombolysis still represents the only treatment that has proven to be safe and effective in randomized clinical trials. These trials have shown that intravenous tissue plasminogen activator (tPA) improves functional outcome in ischemic stroke and that the benefits outweigh the risk for patients who receive treatment within 4.5 hours of symptoms onset. Results from the NINDS trial showed that intravenous alteplase improves functional outcome at three months, if given within 3 hours of symptom onset [1]. The ECASS 3 clinical trial found that intravenous alteplase is beneficial when given from 3 to 4.5 hours after stroke onset [2]. However, the beneficial effect of stroke thrombolysis can be hampered by several local and systemic factors, among which the metabolic syndrome has gained attention recently [3,4].

Our group previously reported that insulin resistance, the proposed key pathophysiological mechanism underlying metabolic syndrome [5], is associated with a worse response to intravenous thrombolysis in acute ischemic stroke [6]. In that study, insulin resistance was measured by means of the HOMA index calculated during admission. This approach has the risk of a confounding effect caused by the deleterious role of fasting glycemia included in the HOMA equation [7]. Therefore, we decided to explore the role of other glycemia-independent markers of insulin resistance in acute ischemic stroke, thus ruling out the potential bias caused by the association between 24 hours-glycemia and stroke outcome [8].

Insulin resistance is associated with an increased release and action of several adipose-derived cytokines, the so-called adipokines. These adipokines have been involved in the impairment of insulin sensitivity and exert important actions

doi: 10.7243/2050-0866-2-2

on vessel function and metabolic regulation, thanks to their autocrine and paracrine function [9]. These molecules affect platelet function, coagulation and fibrinolysis, and their increased release may contribute to a prothrombotic and systemic inflammatory state[10]. Whereas a significant number of studies have examined the association of adipokines with incident coronary heart disease [11,12], only a limited number of studies have so far investigated the role of adipokines in ischemic stroke [13]. Most of these studies have evaluated the association between a single adipokine and the risk of having a ischemic stroke in the future [14,15]. However, the role of insulin-resistance-related biomarkers in the response to acute ischemic stroke therapies remains largely unexplored.

Considering our previous research, we designed a prospective study aimed to describe the temporal profile of several insulin-resistance-related molecules during the acute phase of ischemic stroke and to investigate their effect on patients' outcome after intravenous tPA therapy.

Methods

Patient selection

We prospectively studied acute nonlacunar middle cerebral artery (MCA) ischemic stroke patients admitted to our Stroke Unit from September 2009 to July 2011, who had a documented MCA occlusion on prebolus transcranial color coded Duplex (TCCD) examination and fulfilled criteria to receive intravenous thrombolysis according to our institutional protocol. Besides thrombolysis criteria, specific criteria to enter this study were (1) absence of conditions that could affect biomarker levels such as active neoplasm or prior inflammatory diseases, (2) possibility to obtain and process blood samples prior to tPA bolus and at 1 and 24 hours after thrombolysis was started, (3) patient's functional independence prior to stroke, as defined by a modified Rankin scale (mRs) score of 0 or 1, and (4) informed consent obtained from patient or relatives.

During the study period, 52 ischemic stroke patients with an acute TCCD-documented MCA occlusion and treated with intravenous tPA fulfilled all selection criteria and were included in the study. The study protocol was approved by the local ethics committee.

Clinical assessment

All included patients underwent medical history, physical examination, routine blood biochemistry and blood count, electrocardiogram (ECG), chest X ray, urgent cervical Duplex ultrasound, transcranial Duplex examinations, and noncontrast brain computed tomography (CT), upon admission to our Stroke Unit. Intravenous thrombolysis was administered in a 0.9 mg/kg tPA dose as described in Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST) [16]. Prebolus systolic and diastolic blood pressure (BP) values, temperature, and glycemia were determined on admission. Twenty-four hours glycemia were determined in fasting blood sample. Neurologic examinations were performed on admission and periodically during the next 24 h after the initiation of the thrombolytic infusion. Stroke severity was determined with the National Institutes of Health Stroke Scale (NIHSS) [17]. We defined "early neurological improvement" as a reduction in the total NIHSS score by ≥4 points or the complete resolution of the neurologic deficit at 24 hours, based on the methods used in previous studies [1].

During admission, history of vascular risk factors and vascular disease was obtained. Cerebral CT scans were carried out before tPA bolus and repeated after 24 h, or earlier when neurologic deterioration occurred. Early ischemic changes in each patient's CT were evaluated according to the Alberta Stroke Program Early CT Score (ASPECTS) [18]. This is a 10-point quantitative topographic CT scan score developed to assess early ischemic changes on pretreatment CT studies in patients with acute ischemic stroke of the anterior circulation. A normal CT scan receives ASPECTS of 10 points, whereas a score of 0 indicates massive involvement throughout the MCA territory. On follow-up CT we measured infarct volume by using the formula for irregular volumes and we also assessed the presence of symptomatic hemorrhagic transformation (SHT) during admission, according to the SITS-MOST definition [19].

Stroke subtypes were classified using modified Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [20], in agreement with the results of the additional diagnostic procedures performed (echocardiography, ECG–Holter monitor, special coagulation test, and immunologic study).

Clinical long-term outcome was assessed 90 days after symptoms onset by means of the mRS[21]. A mRS score ≤2 determined on day 90 after stroke onset was considered indicative of good long-term functional outcome.

Laboratory methods

Blood samples were drawn right before tPA-bolus, right after tPA infusion and 24h after admission to our Stroke Unit. Blood was also obtained from 10 healthy volunteers without history of stroke or other vascular diseases, who served as laboratory controls to obtain reference values. Plasma was collected by centrifugation at 3.000 rpm for 15 min at 4ºC, aliquoted and stored blind-coded at -80º C until analysis. Biomarker levels in plasma were measured in patients and controls by using the multiplex Bio-Rad assay (Hercules, CA, USA), "Bio-Plex HU Diabetes 12", at the Biomedic Investigation Unit (IBC), HCUV, Valladolid, Spain. This system allows for quantitative measurement of 12 different mediators (C-Peptide, Ghrelin, Gastric inhibitory polypeptide (GIP), Glucagon-like peptide-1 (GLP-1), Glucagon, Insulin, Leptin, Resistin, Visfatin, Interleukin-6 (IL-6), Tumor necrosis factor-alpha (TNF-α) and Plasminogen activator inhibitor-1 (PAI-1)). Concentrations were given in pg/ml for all molecules. Determinations were done by duplicate. Mean coefficients of variation were < 10% in all replicated samples. Results related to PAI-1 are not shown as they are reserved for another publication using different methodology.

Transcranial ultrasound assessment of resistance to clot lysis

All extracranial ultrasound imaging and transcranial Duplex were performed with a Toshiba Aplio XG echograph (Toshiba Medical Systems Europe, Zoetermeer, the Netherlands). TCCD examinations were performed in our Stroke Unit, right before tPA infusion to check the existence of MCA occlusion. If the patient had an inappropriate acoustic window, a single bolus of echocontrast agent (Sonovue) was administered. A follow-up TCCD study was performed by the same neurosonologist 2h after intravenous tPA bolus to monitor arterial status. MCA occlusions were defined according to the Thrombolysis in Brain Ischemia (TIBI) grading system [22], which establishes grade 0 (absent), 1 (minimal), 2 (blunted), or 3 (dampened) as indicative of arterial occlusion. Early arterial recanalization was diagnosed when the TIBI pattern reached a 4 or 5 grade in the 2 hours control TCCD examination.

Statistical analysis

Statistical analyses were performed with the SPSS statistical package (version18.0; SPSS Inc, Chicago III). Statistical significance for intergroup differences was assessed by the χ^2 test for categorical variables and the Student t test and Mann-Whitney U test for continuous variables. All continuous variables except NIHSS score, ASPECTS score, leukocyte and platelet were normally distributed. Non parametric tests were used for molecule analysis. Protein concentrations required a log transformation to satisfy the linearity assumption. Mediators' levels were first compared between patients and controls using Mann-Whitney U-test. Variation of protein concentration during the first 24 hours was assessed by means of Friedman and Wilcoxon tests. Bonferroni correction was used for multiple comparisons and a probability value <0.016 was considered statistically significant.

Long term clinical outcome, early neurological recovery, early MCA recanalization, infarct volume and hemorrhagic transformation were considered outcome variables. The relationship between mediators levels and stroke outcome was analyzed in several steps. First, to evaluate the relationship between every biomarker level (including their variation in concentration from baseline to the 24 hour time-point) and outcome variables, crude logistic regression models were applied. Molecules showing a $p \leq 0.2$ on the respective crude logistic regression analysis were included in a multivariate regression model, and adjustment was done by all baseline variables showing a $p \leq 0.1$ on the respective bivariate analyses. Results of the regression analyses are expressed as odds ratios (ORs) and their corresponding confidence intervals (CI). Second, receivers operating characteristic (ROC) curves were calculated for molecules showing a $p \leq 0.1$ on the multivariate adjusted logistic regression models, in order to determine their predictive value under the curve as well as to obtain the cut-point that better discriminated between favorable and unfavorable stroke outcome. For all tests, a probability value <0.05 was considered statistically significant.

Results

Descriptive analysis and temporal profile of insulin resistance related- biomarkers

We studied 52 consecutive acute ischemic stroke patients with a documented MCA occlusion treated with intravenous tPA. Of them 32(61.53%) were female, mean age was 75.38 ± 8.89 years and median NIHSS was 15 (9-21). **Table 1** summarizes the distribution of demographic characteristics, baseline clinical variables and molecules level.

Table 1: Distribution of demographic characteristics, baseline clinical variables and cytokine levels. Results are mean±SD, No. (%), or median (interquartile range), as appropriate. SBP: Systolic Blood pressure. DBP: Diastolic blood pressure. ASPECTS: Alberta Stroke Program Early CT Score.

VARIABLE	
Sex, female	32 (61.5%)
Age, y	75.38±8.89
Smoking	11 (21.2%)
Hypertension	32 (61.5%)
Diabetes mellitus	6 (11.5%)
Hypercholesterolemia	15 (28.8%)
Cardioembolic etiology	24 (46.2%)
Baseline NIHSS score	15 (9-21)
Pre-bolus glycemia, mg/dL	119.07±27.86
24h fasting glycemia,mg/dL	107.25±33.61
Leukocyte, x103/µl	8.15 (6.43-9.28)
Platelet, x103/µl	193 (165-229)
Admission SBP, mm Hg	153.42±17.16
Admission DBP, mm Hg	79.98±10.67
ASPECTS	9 (8-10)
C_Peptide_0h, pg/ml	1427.10 (1161.46 - 2032.30)
C_Peptide_1h, pg/ml	1925.43 (1471.33 - 2818.98)
C_Peptide_24h, pg/ml	1649.31 (1209.86 - 2173.72)
Ghrelin_0h, pg/ml	62.50 (38.77 - 135.74)
Ghrelin_1h, pg/ml	93.99 (51.40 - 156.83)
Ghrelin_24h, pg/ml	116.66 (84.62 - 150.83)
GIP_0h, pg/ml	46.54 (25.44 - 118.08)
GIP_1h, pg/ml	109.51 (63.51 - 194.32)
GIP_24h, pg/ml	78.10 (58.67 - 133.88)
GLP-1_0h, pg/ml	725.04 (545.93 - 1002.32)
GLP-1_1h, pg/ml	920.71 (459.09 - 1550.04)
GLP-1_24h, pg/ml	1279.23 (1007.16 - 1892.57)
Glucagon_0h, pg/ml	757.57 (624.91 - 855.62)
Glucagon_1h, pg/ml	919.35 (805.26 - 1105.59)
Glucagon_24h, pg/ml	891.56 (805.57 - 990.46)
Insulin_0h, pg/ml	1791.20 (1252.83 - 2731.15)
Insulin_1h, pg/ml	3154.96 (1559.37 - 5279.02)
Insulin_24h, pg/ml	2945.71 (2303.15 - 4827.09)
Leptin_0h, pg/ml	2317.50 (1463.35 - 4120.33)
Leptin_1h, pg/ml	2560.31 (1370.97 - 3990.47)
Leptin_24h, pg/ml	1933.93 (1307.49 - 5403.32)
Resistin_0h, pg/ml	2766.56 (1929.42 - 5640.59)
Resistin_1h, pg/ml	3619.16 (2090.16 - 12247.91)
Resistin_24h, pg/ml	1816.83 (1411.92 - 3251.75)
Visfatin_0h, pg/ml	35.68 (35.68 - 1918.54)
Visfatin_1h, pg/ml	1886.38 (35.68 - 7853.48)
Visfatin_24h, pg/ml	943.55 (12.40 - 1727.51)
IL6_0h, pg/ml	20.17 (14.86 - 31.44)
IL6_1h, pg/ml	28.46 (19.71 - 39.57)
IL6_24h, pg/ml	32.31 (22.63 - 48.08)
TNFa_0h, pg/ml	36.13 (26.27 - 54.19)
TNFa_1h, pg/ml	37.20 (22.83 - 66.67)
TNFa_24h, pg/ml	46.43 (33.71 - 59.33)

doi: 10.7243/2050-0866-2-2

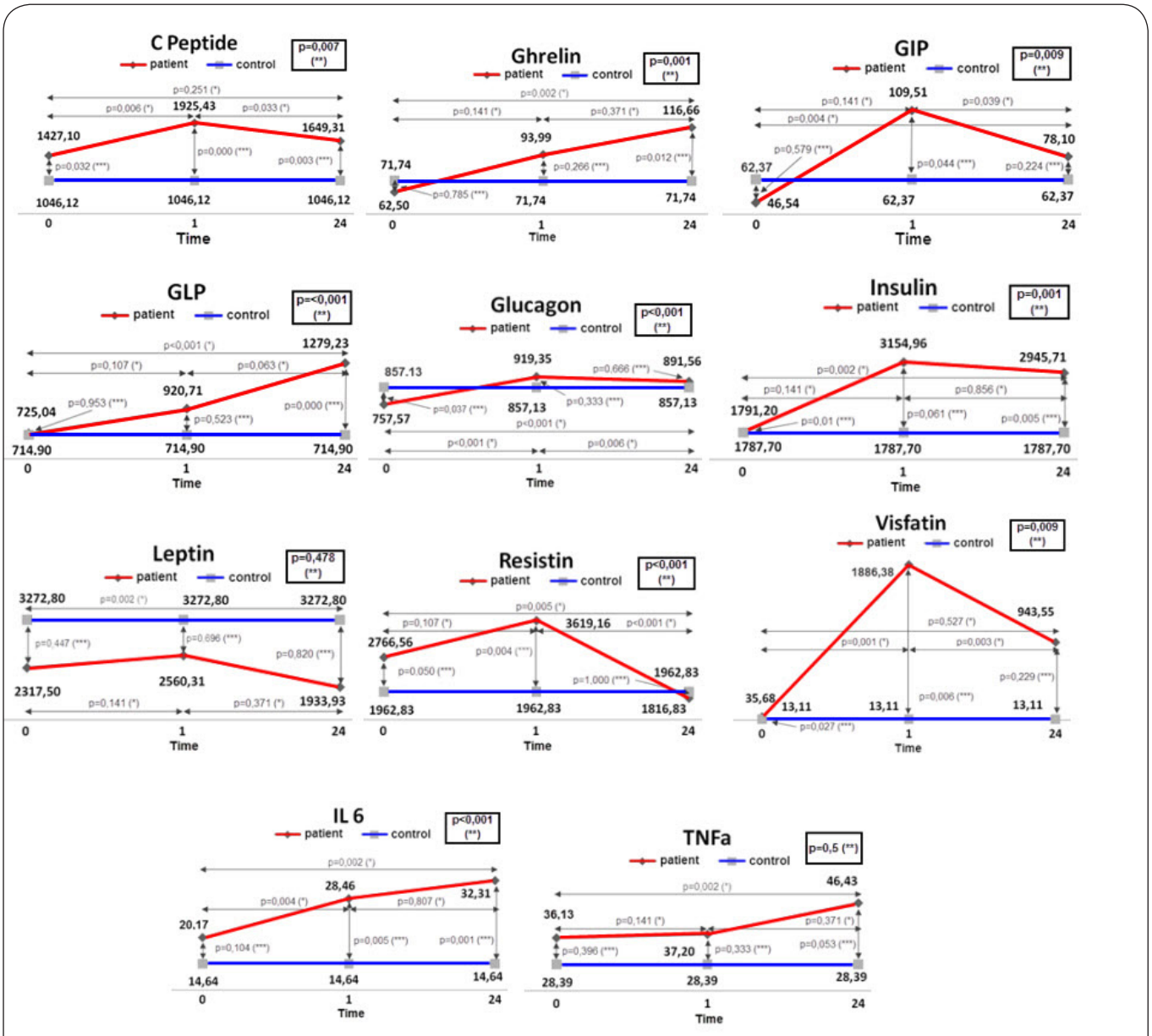

Figure 1. Biomarkers temporal profile. The RED lines show the change in cytokine concentration during the first 24 hours in stroke patients, whereas BLUE lines represent control values.
* p value for the comparison of each time-point concentration with the remaining time-points, assessed by Wilcoxon test in the patients group.
** global p value for the global variation of mediator concentration during the first 24h assessed by Friedman test in the patients group.
*** p value for the comparison of mediator levels between patients and controls.

Studied biomarkers level was higher in stroke patients than in healthy controls for most molecules and in most time-points, with the exception of Leptin. Regarding biomarker variation during the first 24 hours, most molecules experienced a progressive increment, some of them (C-Peptide , GIP, Glucagon, Insulin, Resistin and Visfatin) picking at the first hour and then decreasing until the 24 hour timepoint. The time profile of the studied biomarkers is shown on **Figure 1**.

No significant correlations were found between the baseline level of any molecule and admission's glycemia. Regarding 24-hours glycemia, significant correlations were found for GIP level at 24 hours (r=-0.42, p=0.006) (**Figure 2a**), C-Peptide at 24 hours (r=-0.35, p=0.02), 24- hours Ghrelin (r=-0.34, p=0.03), 24-hours GLP1 (r=-0.43, p=0.006), 1-hour Resistin (r=0.37, p=0.01) and Glucagon increment during the first 24h (r=0.36, p=0.02).

doi: 10.7243/2050-0866-2-2

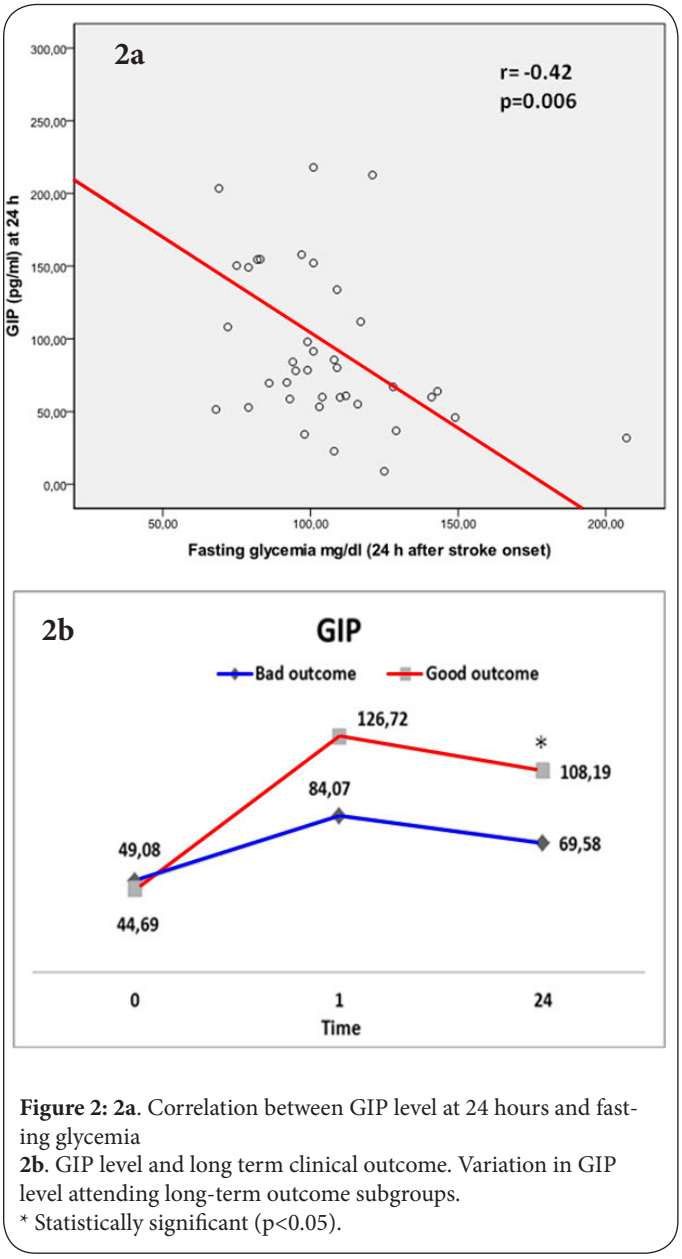

Figure 2: 2a. Correlation between GIP level at 24 hours and fasting glycemia
2b. GIP level and long term clinical outcome. Variation in GIP level attending long-term outcome subgroups.
* Statistically significant (p<0.05).

Table 2: Results of the bivariate analysis of baseline clinical variables associated with poor long-term clinical outcome.
Results are mean±SD, No. (%), or median (interquartile range), as appropriate. SBP: Systolic Blood pressure. DBP: Diastolic blood pressure. ASPECTS: Alberta Stroke Program Early CT Score

VARIABLE	Good outcome at 3 months (n=24)	Poor outcome at 3 months (n=27)	P Value
Sex, female	13 (54.16%)	19 (70.37%)	0.23
Age, y	76.54±8.56	74.22±9.33	0.36
Smoking	5 (20.83%)	6 (22.22%)	0.90
Alcohol	1 (4.16%)	3 (11.11%)	0.24
Hypertension	15 (62.5%)	17 (62.96%)	0.26
Diabetes mellitus	1 (4.16%)	5 (18.51%)	0.11
Hypercholesterolemia	7 (29.16%)	8 (29.62%)	0.97
TOAST category	-	-	0.12
CE	8 (33.33%)	16 (59.25%)	-
ATH	5 (20.83%)	5 (18.51%)	-
IND	11 (45.83%)	5 (18.51%)	-
Others	0 (0%)	1 (3.70%)	-
Baseline NIHSS score	10 (6-14)	19 (15-22)	<0.001
Basal glycemia, mg/dL	111.83±24.28	125.18±30.18	0.091
24h fasting glycemia,mg/dL	94.50±23.01	120.22±38.88	0.50
Leukocyte, x103/µl	7870 (6430-8680)	8730 (6290-9800)	0.32
Platelets, x103/µl	197000 (166500-219500)	193000 (158000-230000)	0.96
Admission SBP, mm Hg	150.58±16.6	155.37±17.68	0.32
Admission DBP, mm Hg	76.58±11.14	83.25±9.48	0.02
ASPECTS	10 (8-10)	8 (7-9)	0.002

Association between molecule concentration and endpoint variables

Long term clinical outcome

Three months after stroke onset, 24 (47%) patients had a good outcome (mRS score ≤ 2). Bivariate analysis, shown on **Table 2**, identified higher baseline NIHSS score (P<0.001), lower baseline NCCT ASPECTS (p=0.002) and higher admission DBP (p=0.02) as significantly associated with a long-term poor clinical outcome. The concentration of the following biomarkers, analyzed as continuous variable, was significantly associated with long-term clinical outcome in the bivariate analyses: 24h-GIP, 1h-Resistin, 1h-Visfatin, GIP, Insulin and Leptin variation during the first 24h, and Visfatin increment during the first hour. These associations did not resist multivariate adjustment for those variables with p<0.1 on bivariate analysis (See **Supplement table 1**). Among molecules showing a p ≤0.1 on the multivariate adjusted logistic regression model, only 1h-GIP, 24h-GIP and 1h-Visfatin exhibited area under ROC (AUROC) values above 0.6. A level of 109.5 pg/ml for 1h-GIP and 82.19 pg/ml for 24h-GIP emerged as the best cutoff values ((sensitivity 71%; specificity 70%) (sensitivity 61%; specificity 77%) respectively). GIP level at 1 and 24 hour higher than the selected cutoff values emerged as independent predictors of good outcome (OR 13.78 [95% CI 1.38-137.31], p=0.02 and OR 28.46 [95% CI 1.09-739.82], p=0.04 respectively), being these associations independent of admission and 24 hours glycemia. **Figure 2b** shows the variation in GIP level attending long-term outcome subgroups.

Early neurological improvement

Twenty four hours after stroke onset, 21 (40.38%) patients experienced early clinical improvement. The multiple logistic regression analysis adjusted for those variables with p<0.1 on bivariate analysis (platelets p=0.04 and admission diastolic

doi: 10.7243/2050-0866-2-2

BP(DBP) p=0.06), identified admission GIP (OR 4.47 [95% CI 1.08-18.51], p=0.03), IL6 (0h-1h) (OR 0.06 [95% CI 0.005-0.73], p=0.02), and TNF-α (0h-1h) (OR 0.13 [95% CI 0.02-0.91], p=0.04), as significantly associated with neurological improvement. Among molecules showing a p ≤0.1 on the multivariate adjusted logistic regression model, only baseline GIP and 24h-GIP exhibited AUROC values above 0.6. A level of 84.91 pg/ml for 24h-GIP emerged as the best cutoff value (sensitivity 55%; specificity 72%). GIP level at 24 hour higher than the selected cutoff value was an independent predictor of early neurological recovery (OR 5.76 [95% CI 1.27-26.16], p=0.02).

Recanalization by TCCD

Control TCCD to assess MCA status at 2 hours was performed in 41/52 patients. Early MCA recanalization was observed in 15 (36%). No significant relationship was found between molecules concentration and the probability of MCA recanalization.

Infarct volume

Median infarct volume measured on follow up CT was 28.8cc (4.43cc-79cc). Linear regression analysis showed that baseline NIHSS (p<0.001), ASPECTS (p<0,001), 1h-Ghrelin (p=0.10), 1h-GLP1 (p=0.06), 1h-IL-6 (p=0.10) and Leptin increment from 0-24h (p=0.006) were associated with infarct volume. A multiple linear regression analysis adjusted for those variables with p<0.1 on bivariate analysis, identified 1h-Ghrelin (B 46.25 [95% CI 2.34-90.16], p=0.03), and Leptin increment (0h-24h) (OR 58.27 [95% CI 6.32-110.22], p=0.02), as independently associated with a larger infarct volume.

Symptomatic hemorrhagic transformation

Two patients (3.8%) patients had a SHT. No significant relationship was found between molecule concentration and the probability of SHT.

Discussion

Among the 11 studied insulin resistance related-biomarkers, we found relevant associations between the plasma level of GIP, leptin, ghrelin, TNF-α and IL-6 and the oucome variables assessed in consecutive MCA ischemic stroke patients treated with i.v. tPA. Of note, a lower level of GIP measured at 1 and 24 hours after tPA bolus predicted a poorer long-term outcome. These results are in line with previous investigations suggesting that insulin resistance may play a deleterious role in acute ischemic stroke, mediated in part by hampering the therapeutic effect of intravenous thrombolysis.

Regarding the time profile of these molecules during the acute phase of ischemic stroke, we were not able to find previous studies in the literature with which to compare our results. When designing the study, we expected to find at least one molecule not suffering significant changes during the acute phase, which could then serve as an indirect indicator of the patients' insulin resistance status prior to stroke. However, most of the studied biomarkers showed an increase in their plasma level during the first 24 hours after admission and could be therefore influenced by stroke-induced acute phase reaction. Interestingly, the associations with stroke outcome were not found for any of the biomarkers' pre-treatment level, but for the change in the molecule concentration observed during the first 24 hours after treatment onset. The magnitude of this acute change in the biomarkers concentration in response to cerebral ischemia showed a wide interindividual variability across the study sample. We suggest that the observed differences in the molecules' time profile might be influenced by pre-stroke insulin resistence status, although this hypothesis needs to be further clarified.

A higher level of GIP at 1 and 24 hours after tPA bolus was associated with a better long-term outcome after thrombolytic therapy. Moreover, a higher level of GIP at admission and at 24 hours was associated with early neurological recovery. GIP is widely recognized as a physiological incretin hormone, which potently stimulates insulin release depending on glucose level. Its secretion is regulated mainly by the presence of nutrients in the gastrointestinal tract [**23**], although the mechanisms regulating GIP release in the context of a stress reaction, such as the one triggered by ischemic stroke, are not well known. In animal models, sustained chemical knockout of GIP receptor signaling results in mild impairment of insulin secretion and glucose homeostasis [**24**]. Consistent with this view, human studies have reported markedly reduced levels of GIP in type 2 diabetic patients[**25**]. However, little is known about the role of this molecule in critically ill patients, and its potential contribution to the development of acute stress hyperglycemia [**26**]. In our series, we found that those patients with a less pronounced increase in GIP level during the first 24 hours, showed a higher glycemia at 24 hours. Hyperglycemia is known to be a strong determinant of poor outcome in stroke patients in general [**27**], and also in ischemic stroke patients treated with intravenous thrombolysis [**28**]. In this context, not only admission's glycemia predicts stroke outcome, but hyperglycemia observed at any time within the first 48 hours may have a deleterious effect on stroke patients (5, 6). In order to explain the independent association found between GIP and outcome, we hypothesize that GIP expression in the setting of acute ischemic stroke might be a part of an insulin-sensitizing response aimed to reduce glucose level, thus counteracting the adverse effect of acute hyperglucemia on stroke outcome. More research is needed to know whether GIP is an active mediator or only an innocent bystander in acute ischemic stroke. If GIP turns to be actively involved in processes determining acute stroke outcome, whether its therapeutic modulation could bring any clinical effect would also deserve further study.

Both leptin and ghrelin were associated with final infarct volume. Leptin is an important adipose derived hormone which regulates energetic intake and expenditure. The potential deleterious role of leptin in acute ischemic stroke, as suggested

by our finding, is also supported by previous experimental studies. Leptin was shown to promote the expression of plasminogen activator inhibitor- 1 (PAI-1) in an in vitro culture of human vascular endothelial cells [29]. Moreover, leptin administration promoted arterial thrombosis in vivo studies in mice [30]. These prothrombotic effects may be inhibited after leptin neutralizing monoclonal antibody administration, as shown in murine models of arterial injury and pulmonary embolism [31]. In line with this experimental data, clinical studies in human found an association between higher leptin levels and endothelial dysfunction [32]. However, these studies, together with our results, are in disagreement with recent investigations performed in a mice model of transient focal cerebral ischemia showing that peripherally administered leptin decreased infarct volume by reducing oxidative stress and neuronal apoptosis [33]. Regarding ghrelin, our finding seems to be in conflict with what has been reported in the literature. Animal models of permanent focal cerebral ischemia in rats showed that ghrelin treatment results in decreased infarct size and neurological deficit through suppression of inflammation, nNOS activity, and apoptosis [34]. In both animal [35] and human [36] studies of cardiac disease, ghrelin improved left ventricular structure and function, exercise capacity and muscle wasting, suggesting that ghrelin could be a new therapeutic approach for the treatment of cardiac heart failure. The role of leptin and ghrelin in human cerebral ischemia needs to be further clarified.

A higher increase in IL-6 and TNF-α level during the first hour after thrombolytic treatment was associated with a worse early neurological course. Both IL-6 and TNF-α are known to be elevated in patients with insulin resistance [9], reflecting the existence of a proinflammatory state. This observation is consistent with previous studies showing an association between these and other inflammatory biomarkers determined during the first 24 hours after stroke onset and the probability of early neurological deterioration [37]. This proinflammatory state associated with insulin resistance [6], among other mechanisms, could contribute to worsen the response to intravenous thrombolysis and stroke outcome in general.

This study has some limitations. First, the final sample was small sized, although highly selected, and therefore our results need to be confirmed in a larger validation sample. Moreover, in the hyperacute stroke setting, the condition of insulin resistance is unknown for the majority of the patients. A larger sample would be also needed to separately evaluate the role of these and other biomarkers in stroke patients with a known history of insulin resistance, such as type 2 diabetics. Second, ultrasound protocol could not be fully accomplished in a significant number of patients, which precluded the selection of early arterial recanalization as a primary outcome variable. Third, other biomarkers associated with metabolic syndrome and insulin resistance were not tested, such as adipokines, coagulation and fibrinolysis factors. Among adipokines, our biomarker panel did not include relevant molecules such as adiponectin. In agreement with our main hypothesis, linking insulin resistance with a poorer stroke outcome, both preclinical studies in rat [38] and mice [39] models of focal cerebral ischemia demonstrated a strikingly positive effect of adiponectin on cerebral flow and infarct size. Moreover, 5-year survival rate after first ever ischemic stroke was found to be higher in patients who had a lower adiponectin level determined within the first 24 hours after stroke onset [40]. Fourth, although arterial recanalization was assessed by TCCD, evaluation of brain reperfusion with neuroimaging would have been desirable.

Conclusions

In conclusion, our findings support the hypothesis that insulin resistance plays a deleterious role in acute ischemic stroke. A higher level of GIP at 1 and 24 hours after tPA bolus, probably reflecting a more intense insulin-sensitizing response, was associated with a better long-term outcome after thrombolytic therapy. Further studies are needed to elucidate the role of GIP and other insulin-resistance related biomarkers as new therapeutic targets in acute ischemic stroke. After this results, our future research plan may include, among other lines, to evaluate the role of GIP in an animal model of focal cerebral ischemia, and to study the role of insulin resistance in acute stroke patients treated with endovascular reperfusion therapies.

Additional file

Supplement Table

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

A.I.C. contributed to the study design, patient selection, clinical work, neurosonology, data collection, analysis and interpretation, and writing of the manuscript. E.C. and P.G.-B. contributed to patient selection, clinical work, neurosonology, and data collection. J.R. contributed to blood sampling and laboratory work. J.F.B. contributed to laboratory work and analysis. M.F.M. contributed to analysis. R.F.-H. contributed comments to the manuscript. J.F.A. contributed to the study design, patient selection, clinical work, neurosonology, data collection, analysis and interpretation, and writing of the manuscript.

Acknowledgement and funding

The authors thank Rosa Alcaide and Edita Sánchez on behalf of the nursing staff of the Stroke Unit, without whom completion of this study would have never been possible. A.I.C. was the recipient of a research grant from Instituto de Salud Carlos III (Río Hortega program), Ministery of Science, Spain (2008-2011), which was cofounded between Instituto de Salud Carlos III and Hospital Clínico Universitario, Salud Castilla y León (SACYL); Deparment of Health, Castilla y León (SACYL); Gerencia Regional de Salud, Castilla y León (SACYL).

Publication history
Received: 19-Nov-2012 Revised: 29-Dec-2012
Accepted: 3-Jan-2013 Published: 18-Jan-2013

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Citation:
Calleja A I, Cortijo E, García-Bermejo P, Reyes J R N, Bermejo J F, Muñoz M F, Fernández-Herranz R and Arenillas J F: **Blood biomarkers of insulin resistance in acute stroke patients treated with intravenous thrombolysis: Temporal profile and prognostic value**. *Journal of Diabetes Research and Clinical Metabolism* 2013, **2**:2.
http://dx.doi.org/10.7243/2050-0866-2-2