Plasma Levels of Brain-Derived Neurotrophic Factor are Associated with Prognosis in the Acute Phase of Ischemic Stroke

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Context: Brain-derived neurotrophic factor (BDNF) is a neurotrophin involved in neuronal survival, differentiation, and maturation. *Purpose*: To evaluate the levels of BDNF in the acute phase of stroke and their potential association with neurological impairment. *Methods:* Patients in the acute phase of ischemic stroke were evaluated with the following clinical tools: National Institutes of Health Stroke Scale, modified Rankin scale, Gugging Swallowing Screen and Alberta Stroke Program Early CT Score. Blood samples were collected at 3 different moments of hospital stay. BDNF was measured through enzyme-linked immunosorbent assay. *Results:* Patients who were discharged after 10 days had worse clinical outcomes and higher levels of BDNF since admission. There was correlation between BDNF levels and clinical parameters. *Conclusion:* BDNF levels were associated with clinical prognosis in the acute phase of ischemic stroke.
Key Words: Brain-derived neurotrophic factor—biomarker—stroke—ichemic stroke—stroke outcome—prognosis
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Introduction

Blood biomarkers may be useful in the differential diagnosis and/or defining clinical outcome in the acute phase of ischemic stroke.[1,2] Several biomarkers have been associated with the volume of cerebral infarction and the severity of neurological deficits, including S-100B,[3] IL-6,[4] TNF-α, ICAM-1,[5] glutamate, and nitric oxide.[6] However, so far there are no valid biomarkers that can be used in the clinical setting for stroke diagnosis or risk prediction.[1,2,4,5]

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that promotes neuronal differentiation, maturation, and survival in the nervous system.[7-12] BDNF can also exert neuroprotective effects in the presence of adverse conditions such as cerebral ischemia.[13-18] Low levels of BDNF have been described in patients with risk factors for stroke,[19] higher volume of cerebral infarction,[20] and worse neurological deficits.[21-25] Conversely, high levels of BDNF have been reported in stroke patients undergoing neurological rehabilitation.[26,27] Experimental studies have suggested that the first 10 days after stroke are a critical period when several pathophysiological processes happen, including the increase of BDNF levels in the brain.[7] It has not been defined yet whether BDNF levels vary during the acute phase of ischemic stroke.

The purpose of the current study was 2-fold: (1) to investigate changes in the plasma levels of BDNF in the acute phase of ischemic stroke and (2) to evaluate whether BDNF levels are associated with neurological impairment and functional changes.

Methods

Patients

Patients in the acute phase of ischemic stroke were enrolled in this study. The inclusion criteria were patients older than 18 years and diagnosis of cerebral ischemia within 24 hours of ictus. Patients who received thrombolysis, developed hemorrhagic transformation, had transient ischemic attack, diagnosis of other neurological diseases and/or psychiatric disorders, and clinical instability were excluded.

The study was conducted in accordance with the Resolution 466/2012. The project was evaluated and approved by the Research Ethics Committee of the Federal University of Minas Gerais, Project: CAAE—32809514.4.4.0000.5149.

BDNF Measurement

To measure BDNF plasma levels approximately 20 mL of venous blood were collected from the ulnar vein in disposable vacuum tubes made of plastic (BD Vacutainer) with ethylenediaminetetraacetic acid . Samples were immediately placed on ice until their centrifugation at 2500 rpm for 15 minutes. After centrifugation, the supernatant was separated and aliquoted in Eppendorf type conical tube of 500 μL. The samples were then stored in a freezer at −80°C. BDNF plasma levels were measured by enzyme-linked immunosorbent assay according to the instructions of the manufacturer (R&D Systems, Minnesota, MN). Samples were assayed in duplicate. The detection limit of the assay was 5 pg/mL. The inter- and intra-assay coefficients of variation were below 7%.

Clinical Evaluation

Sociodemographic and clinical data were extracted from electronic medical records and/or interview with the patients. The National Institutes of Health Stroke Scale (NIHSS)[28] was used to determine neurological impairment, the modified Rankin scale to assess the degree of disability and dependence in the activities of daily living,[28] and the Gugging Swallowing Screen (GUSS) for the evaluation of swallowing.[29] Regarding neuroimaging, the Alberta Stroke Program Early CT Score (ASPECTS)[30] was applied to quantify and characterize the topography of the stroke.

Procedures

Clinical and molecular evaluations were carried out at 3 different moments: at hospital admission (within 24 hours of ictus), 72 hours of hospitalization and hospital discharge. The ASPECTS was determined by a single neurologist who was blinded to the clinical data through the interpretation of the cranial computed tomography. The NIHSS and modified Rankin scale were applied through interview and clinical examination by 2 trained professionals (a speech therapist and an occupational therapist) with a very good interobserver agreement (kappa = 0.85). The GUSS was applied by a speech therapist.

Statistical Analysis

Categorical variables were expressed as frequencies and percentages. The Statistical Package for the Social Sciences software version 20.0 was used for statistical analysis. A bilateral P value lower than .05 was adopted as the level of significance for all statistical tests.

Fisher's exact test was used for the comparison of categorical variables. All variables were assessed for normality using the Shapiro-Wilk test. Nonparametric tests were preferentially used since most variables failed to show normal distribution. Due to the variation in hospital stay, patients were divided in those who were discharged within 10 days of hospitalization and after than 10 days.[7] For continuous variables, the Mann-Whitney and Friedman tests were applied for intergroup and intragroup comparisons, respectively, when evaluating 3 time points. The Wilcoxon test with Bonferroni correction was applied when comparing the time points in pairs. Spearman's rank test was used to assess correlations between independent variables.

Finally, a linear regression model was fitted using the backward method considering a P value lower than .20 for covariate. The fit of the model was assessed using the adjusted R^2 statistics.

Results

Fifty participants were evaluated at 3 different moments: hospitalization (up to 24 hours of stroke), 72 hours of hospitalization and discharge. The average age of the patients was 65.5 and the average hospital stay was 10.3 days. Half of the patients had history of past stroke with a minimum interval of 6 months between the previous episode and the current ischemic event (Table 1).

Comparison of plasma levels of BDNF at 3 different moments (admission, 72 hours and hospital discharge) showed no significant difference, although the values fluctuated during hospitalization (Fig 1).

There was negative correlation between BDNF levels at 72 hours and clinical parameters. Patients with lower BDNF levels had worse neurological deficits (NIHSS), overall functional performance (Rankin) and swallowing (GUSS; Table 2). In the linear regression model, an adjusted R coefficient of 0.101 was found, indicating that BDNF levels were independently associated with the prognosis of the patients (coefficient B = −147.126;

Table 1. *Sociodemographic and clinical characteristics of patients with acute ischemic stroke*

Sociodemographic and clinical variables	Patients (N = 50)	
	N	Ratio (%)
Sex		
Male	28	56.0
Female	22	44.0
Age (years)		
Mean ± SD		65.5 ± 11.7
Median (min-max)		64.5 (37-93)
Length of stay (days)		
Mean ± SD		10.3 ± 6.8
Median (min-max)		9.0 (4-30)
Previous history of stroke	25	50.0
Signs of previous ischemia at CCT	17	34.0
TOAST		
Small artery occlusion	25	50.0
Undetermined mechanism	10	20.0
Large-artery atherosclerosis	8	16.0
Cardioembolic	6	12.0
Other etiologies	1	2.0

Abbreviations: CCT, cranial computed tomography; max, maximum; min, minimum; N, number of patients; SD, standard deviation; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

P value = .014; confidence interval 95% = −262.940 to 31.312).

Patients who were discharged after 10 days of hospitalization had worse clinical parameters since admission compared to patients discharged earlier. There was also significant difference in BDNF levels at 72 hours and at discharge between these 2 groups, with patients with late discharge exhibiting lower levels of BDNF (Table 3).

Discussion

Our study showed that, despite some fluctuation, the plasma levels of BDNF did not vary significantly in patients with ischemic stroke through their hospital stay.
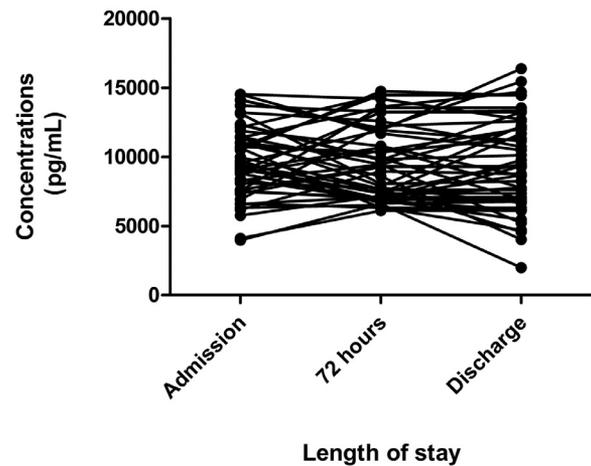
Brain-Derived Neurotrophic Factor

Figure 1. *Comparison of concentration of the brain-derived neurotrophic factor of same patient in the acute phase of ischemic stroke.*
P value > .005. Friedman test.

More importantly, BDNF levels at 72 hours correlated with different clinical outcomes.

This finding is in line with previous studies reporting the association between circulating levels of BDNF on admission and functional outcome at later stages, ie, after 3 months[22,24] and 2 years of stroke.[23] Accordingly, plasma levels of BDNF in the first days after stroke are promising biomarkers of short- and long-term outcomes.

In animal models, an increase in cerebral and circulating levels of BDNF is shown immediately after ischemic events.[11,12] In these models, BDNF seems to limit the ischemic area, being associated with motor recovery.[11,12] Neurons are the predominant source of BDNF in the ischemic brain, while the contribution of non-neuronal cells, such as microglia, astrocytes, and endothelial cells, can occur in the context of significant neuronal death.[11,12] Possibly, when the tissue damage is extensive, affecting

Table 2. *Correlations between BDNF levels and measures of neurological and functional impairment in the acute phase of ischemic stroke*

Variables	Admission		72 hours		Discharge	
	Rho	P value	Rho	P value*	Rho	P value
Age (year)	−0.182	0.206	−0.055	0.704	−0.235	0.100
Previous history of stroke (number)	−0.193	0.180	−0.305	**0.031***	**-0.293**	**0.039***
ASPECTS	0.307	**0.030***	0.196	0.172	0.207	0.149
NIHSS	−0.030	0.804	−0.287	**0.044***	−0.153	0.289
Rankin	−0.093	0.519	−0.270	**0.049***	−0.248	0.082
GUSS	0.194	0.176	−0.288	**0.043***	0.061	0.675

Abbreviations: ASPECTS, Alberta Stroke Program Early CT; GUSS, Gugging Swallowing Screen; NIHSS, National Institutes of Health Stroke Scale; Rho, Spearman's rank correlation coefficient.
P value < .05 was adopted as the level of significance for all statistical tests.
*Spearman's rank test.

Table 3. *Comparisons between ischemic stroke patients who were discharged within 10 days and after 10 days of hospitalization*

	Admission				72 hours				Discharge			
	Discharge ≤ 10 (N = 32)	Discharge > 10 (N = 18)	Z	P value	Discharge ≤ 10 (N = 32)	Discharge > 10 (N = 18)	Z	P value	Discharge ≤ 10 (N = 32)	Discharge > 10 (N = 18)	Z	P value
BDNF												
Mean ± SD	9715.00 ± 2561.15	9736.61 ± 2873.95	−0.200	0.984	10166.43 ± 2728.41	8265.27 ± 2053.30	−2.365	**0.018***	10682.00 ± 3147.47	7470.28 ± 2788.00	−3.173	**0.002***
Median (min-max)	9513 (4101-14131)	9230 (3975-14531)			10082 (6113-14739)	7587 (6325-14200)			10932 (4579-16383)	6867.5 (1998-12697		
ASPECTS												
Mean ± SD	9.2 ± 1.3	8.8 ± 1.4	−0.932	0.351	8.7 ± 1.7	8.4 ± 2.0	−0.613	0.540	8.7 ± 1.7	8.2 ± 2.1	−0.874	0.382
Median (min-max)	10 (5-10)	9 (5-10)			9 (2-10)	9 (2-10)			9 (2-10)	9 (2-10)		
NIHSS												
Mean ± SD	5.6 ± 3.8	12.4 ± 7.4	−3.275	**0.001***	5.6 ± 3.9	11.9 ± 7.5	−3.174	**0.002***	3.7 ± 3.2	9.8 ± 8.1	−3.006	**0.003***
Median (min-max)	6 (0-17)	13.5 (0-30)			5.5 (0-12)	12 (0-30)			3.5 (0-12)	8.5 (0-31)		
Rankin												
Mean ± SD	2.4 ± 1.6	3.5 ± 1.4	−2.777	**0.005***	2.1 ± 1.7	3.5 ± 1.4	−2.883	**0.004***	1.7 ± 1.7	3.0 ± 1.7	−2.684	**0.007***
Median (min-max)	3 (0-4)	4 (0-5)			3 (0-4)	4 (0-5)			1 (0-4)	4 (0-5)		
GUSS												
Mean ± SD	19 ± 2.4	16 ± 6.7	−1.756	0.079	19.7 ± 1.2	17.7 ± 5.1	−2.173	**0.030***	19.9 ± 0.24	19.2 ± 2.1	−.1.720	0.085
Median (min-max)	20 (11-20)	19 (0-20)			19 (14-20)	20 (1-20)			20 (19-20)	20 (11-20)		

Abbreviations: ASPECTS, Alberta Stroke Program Early CT; BDNF, Brain-derived neurotrophic factor; GUSS, Gugging Swallowing Screen; max, maximum; min, minimum; NIHSS, National Institutes of Health Stroke Scale; SD, standard deviation.

P value < .05 was adopted as the level of significance for all statistical tests.

*Mann-Whitney test.

the capacity of BDNF production by neurons, the clinical outcome is worse, delaying, for example, the discharge of patients from the hospital. The fact that the clinical parameters differ since admission between groups with early versus late discharge reinforces this hypothesis.

There was some variability in the days of hospitalization (from 4 to 30) possibly influencing the interpretation of the BDNF results at hospital discharge. Conversely, there was no significant variability in BDNF levels during hospital stay when comparing the 3 assessments. The production of BDNF by multiple sources that could have been affected at different time points might explain this finding. Actually the neurochemical process triggered by cerebral ischemia is very complex, involving local and systemic changes of several molecular pathways, including BDNF. As 72-hour poststroke is an established time point and BDNF levels were associated with clinical outcomes, future studies should focus on the evaluation of BDNF levels at 72 hours after stroke.

It is also interesting to notice that BDNF was associated with the ASPECTS only at baseline and not at a later time point (72 hours) when it correlated with the clinical outcomes. An explanation for this finding is not straightforward. This initial association can be merely a coincidence. Alternatively, BDNF levels could reflect only local changes in the beginning, explaining its correlation with ASPECTS. As BDNF can be produced/released by other tissues, its later correlation with clinical parameters and outcome may reflect not only the development of local, but also other organ/tissue responses. This hypothesis deserves further investigation.

The results of the study should be interpreted in the light of its limitations, including the sample size and the follow-up of the patients only during their hospitalization. Moreover, the frequency and intensity of neurological rehabilitation carried by speech therapist, physiotherapist, and occupational therapist, which may have influenced BDNF levels, were not controlled.[26,27] On the other hand, the strict inclusion and exclusion criteria, and the careful follow-up of the patients during hospitalization can be pointed out as strengths of the study. Future clinical studies enrolling more patients, controlling for other variables (such as rehabilitation), and with longer follow-ups must be performed to confirm the role of BDNF as a prognostic biomarker of ischemic stroke.

Conclusions

Plasma levels of BDNF at 72 hours were associated with neurological impairment and length of hospital stay after stroke.

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