Brief Communications

Brain-derived neurotrophic factor in peripheral blood mononuclear cells and stroke outcome

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Impact statement

There are a great number of arguments suggesting that BDNF could be involved in stroke recovery dependent of neuroplasticity. Methods that can enhance BDNF levels in the ischemic brain could therefore have great clinical value. Peripheral blood mononuclear cells (PBMC) that contain BDNF and infiltrate early and sustainably the ischemic brain might be used as a cellular vector to deliver BDNF to the ischemic brain and consequently promote recovery. This work is important in this field to show if this BDNF derived from BDNF could exert a positive action on stroke recovery. Our main results showed that a high BDNF level at day 3 after hospital admission was associated with a 12.4 fold increase in favorable outcome after adjusting for still recognized prognostic markers. The new information in this field is this finding identifies PBMC as an attractive cellular vector to deliver BDNF to the ischemic brain.

Abstract

Stroke outcome is dependent on brain-derived neurotrophic factor (BDNF)-dependent neuroplasticity. As peripheral blood mononuclear cells (PBMC) contain BDNF, diapedesis of these cells might be followed by BDNF delivery to the ischemic brain. To test this hypothesis, we investigated the association between BDNF levels in PBMC and functional outcome in patients with ischemic stroke. BDNF was measured in PBMC that were isolated from ischemic stroke patients ($n = 40$) just before (day 0) and after (days 1 and 3) fibrinolysis. Three months after stroke, patients were stratified using the modified Rankin Scale (mRS) according to the unfavorable (mRS scores 3–6) and favorable (mRS scores 0–2) functional outcome. We used univariate and multivariate logistic regressions to assess the relationship between BDNF levels in PBMC and functional outcome. BDNF levels in PBMC decreased from day 0 to day 3 in patients with unfavorable outcome, while they remained stable in patients with favorable outcome. Patients with favorable outcome exhibited at day 3 higher PBMC-BDNF levels than patients with unfavorable outcome and the levels were associated with good outcome (odd ratio: 12.0; 95% confidence interval, 1.4–106.2, $P = 0.023$). PBMC-BDNF levels remained a predictor of stroke outcome after adjusting from cardiovascular risk, interval between admission and fibrinolysis, stroke severity from hospital admission to discharge, lymphocytes count, neutrophils/lymphocytes ratio at admission. Favorable functional outcome in ischemic stroke patients that benefited from fibrinolysis was predicted by a high BDNF level in PBMC, suggesting that PBMC might serve as a cellular vector to deliver BDNF to the ischemic brain.

Keywords: Brain-derived neurotrophic factor, inflammation, ischemia, outcome, peripheral blood mononuclear cell, stroke

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Introduction

Ischemic stroke was consistently reported to result in the elevation of brain-derived neurotrophic factor (BDNF), a crucial actor in synaptic remodeling, in ischemic regions.[1,2] Moreover, local administration of BDNF ameliorates the functional motor recovery in ischemic stroke models.[3] Assuming that circulating BDNF levels mirror brain levels, measurement of serum BDNF levels in the early phase of stroke was logically expected to be useful for outcome prediction in patients. However, conflicting data were obtained. While certain studies reported an association between low serum BDNF levels in the acute stroke period and poor short term (seven days to three months) outcome,[4–6] other did not.[7] In the blood, BDNF is not only present in platelets from which it is secreted in response to coagulation process but also in lymphocytes and

monocytes.[8] As these peripheral blood mononuclear cells (PBMCs) infiltrate the ischemic brain early and sustainably,[9] they represent a potential source of BDNF for the ischemic tissue and could consequently favorably influence stroke outcome. Therefore, the present study investigated the hypothesis that BDNF present in PBMC may influence outcome in ischemic stroke patients. As the infiltration of ischemic tissue by PBMC requires the return of blood flow to the ischemic regions, the study was conducted in patients treated with alteplase (tissue-plasminogen activator, t-PA) within the 4.5 h following stroke onset.

Methods

Study patients

A cross-sectional observational study was realized at Hospital of Dijon in 2017 as part of PARADISE study (Prognosis after Revascularization therApy in the Dijon Ischemic Stroke Evaluation study). The PARADISE study received a favorable opinion from the Committee for the Protection of People (N° CPP EST I: 2015/32), the French equivalent of institutional review board. Patients were admitted in neurovascular intensive care unit for ischemic stroke. All received t-PA within the 4.5 h following stroke onset. Patients or a close relative gave an informed consent.

Baseline information

At hospital admission, demographic and anthropometric data (age, sex, body mass index), and conventional cardiovascular risk factors such as hypertension (defined as antihypertensive drug intake or systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg at exit), diabetes (defined as anti-diabetic drug intake or HbA1c ≥6.5%), tobacco consumption (current or ex-smoker), hypercholesterolemia (defined as statins or fibrates intake, fasting low-density lipoprotein cholesterol >160 mg/dL, or total cholesterol >240 mg/dL) were listed. The European Society of Cardiology (ESC) scale measured from age, gender, systolic blood pressure calculated at admission, total cholesterol and smoking status was used to assess the cardiovascular health of each patient. Stroke severity was assessed at admission (D0, before fibrinolysis), at day 1 after admission (D1), and at hospital discharge using NIHSS (National Institute of Health Stroke Scale). TOAST (Trial of ORG 10172 in Acute Stroke Treatment) criteria were used to classify the stroke etiology. Non-inclusion criteria were hemorrhagic stroke, patients benefiting from thrombectomy, transient ischemic attack, severe infection and human immunodeficiency virus (defined by questioning the patient himself or a close relative), prior psychiatric diseases (defined as use of antipsychotic medication), Alzheimer disease (defined by use of donepezil, galantamine, rivastigmine, or memantine) and depression (defined as use of antidepressant medication) as these diseases influenced serum BDNF levels.

Preparation of samples and analysis of BDNF

Venous blood was collected in two tubes (an EDTA tube and a gel and clot activator tube for PBMC and serum preparation, respectively) at hospital admission before fibrinolysis (D0) and one (D1) and three days (D3) after fibrinolysis for the determination of complete blood count (D0) and measurement of BDNF levels in serum and PBMC (D0, D1, and D3). PBMC that were isolated from blood using Ficoll-Hypaque (Eurobio, Courtaboeuf, France) gradient method, were then washed twice with phosphate buffer saline to avoid contamination by platelets before to be lysed in 400 µL of RIPA buffer (Hepes 50 mM, NaCl 150 mM, EDTA 5 mM, NP40 1%, a cocktail of protease inhibitors 1%). After centrifugation, levels of proteins and BDNF (expressed as ng/mg of protein) in supernatants were determined using the Lowry method and an ELISA kit (Biosensis, BEK-2211-2P, Thebarton, Australia). Serum BDNF levels (expressed as ng/mL) were measured with the same ELISA kit. The sensitivity of the kit is 2 pg/mL BDNF in assay diluents.

Study outcome

Three months after hospital admission, a telephone interview was given to collect the modified Rankin Scale (mRS). Clinical outcome was considered as favorable when the mRS was included between 0 and 2, whereas it was considered as unfavorable when the mRS was included between 3 and 6.

Statistical review

For analysis of continuous variables, results were expressed as medians (interquartile ranges (IQR)), whereas for categorical variables, results were expressed as percentages. A χ^2 test with the application of the Yates correction was realized to compare categorical variables, while a nonparametric Mann–Whitney U test was used to compare continuous variables. Differences between values at D0, D1 and D3 were assessed using repeated measures ANOVA test followed by Bonferroni's correction. The influence of BDNF levels in PBMC on NIHSS and mRS was performed using univariate and multivariate logistic regression analysis. Results were expressed as adjusted odds ratios (OR) (95% confidence interval (95% CI)). The determination of the optimal BDNF cutoff value maximizing the sum of sensitivity and sensibility was obtained using a receiver operating characteristic (ROC) analysis, in which results were reported as area under the curve (AUC). The Sigmaplot®11.0 software was used to perform all statistical analysis and statistical significance was defined as $P < 0.05$.

Results

Characteristics of patients

Patient's characteristics ($n = 40$) are summarized in Table 1. In the totality of patients, 62.5% had a favorable outcome with a median mRS of 1. In other patients including dead patients ($n = 3$ between hospital discharge and month 3),

Table 1. Characteristics of patients stratified by stroke outcome.

	Favorable outcome mRS 0–2 ($n = 25$)	Unfavorable outcome mRS 3–6 ($n = 15$)	*P*
Demographic characteristics			
Age, years	76.0 (65.8–83.5)	81.0 (70.0–88.0)	NS[a]
Sex (% of males)	48.0	53.3	NS[b]
BMI, kg/m^2	26.0 (23.1–27.9)	25.8 (23.1–27.7)	NS[a]
Cardiovascular risk factors			
ESC score (%)	11.6 (3.9–17.6)	16.4 (12.1–37.9)	0.005[a]
Active smoker (%)	44.0	40.0	NS[b]
Diabetes (%)	12.0	6.7	NS[b]
Hypertension (%)	64.0	60.0	NS[b]
Hypercholesterolemia (%)	28.0	33.3	NS[b]
Interval between admission and fibrinolysis, median [IQR], min	160.0 (127.5–187.5)	195.0 (160.0–213.8)	0.014[a]
Stroke etiology (TOAST classification)			
Large-artery atherosclerosis (%)	8.0	6.7	NS[b]
Cardioembolism (%)	20.0	33.3	NS[b]
Stroke of other determined etiology (%)	4.0	6.7	NS[b]
Stroke of undetermined etiology (%)	68.0	46.6	NS[b]
Multiple causes possible (%)	0.0	6.7	NS[b]
Stroke severity			
NIHSS at day 0	7.0 (4.0–9.0)	8.0 (5.0–12.0)	0.007[a]
NIHSS at day 1	2.0 (0.8–4.3)	6.5 (3.0–12.0)	$<$0.001[a]
NIHSS at discharge	1.0 (0.0–2.0)	2.0 (1.0–5.8)	$<$0.001[a]
Hematological parameters at D0			
Leucocytes, G/L	9.2 (8.0–10.3)	8.9 (6.6–11.1)	NS[a]
Lymphocytes, G/L	1.7 (1.3–2.5)	1.3 (1.1–1.5)	0.013[a]
Monocytes, G/L	0.6 (0.5–0.8)	0.8 (0.5–1.0)	NS[a]
Neutrophils, G/L	6.0 (5.1–7.7)	6.5 (4.4–9.2)	NS[a]
Neutrophils /lymphocytes ratio	3.5 (2.3–5.5)	5.4 (2.6–7.8)	0.008[a]
Platelets, G/L	246.0 (214.5–300.0)	232.0 (198.0–268.0)	NS[a]
BDNF in serum (ng/mL)			
Day 0	30.4 (27.8–34.0)	31.3 (24.6–34.8)	NS[a]
Day 1	29.6 (26.4–32.6)	28.9 (25.0–32.4)	NS[a]
Day 3	29.3 (25.4–32.4)	28.3 (21.3–33.3)	NS[a]
BDNF in PBMC (ng/mg of protein)			
Day 0	7.5 (2.6–12.5)	9.0 (4.3–17.4)	NS[a]
Day 1	5.7 (2.8–8.1)	5.9 (1.2–8.1)	NS[a]
Day 3	6.3 (3.7–7.2)	4.4 (2.1–6.0)	0.034[a]

Note: Values are expressed as median and interquartile range (IQR) or percentage. Parameters were obtained just before (day 0) and after (day 1, day 3) fibrinolysis of stroke patients.

[a]Differences between the two groups were analyzed using the non-parametric Mann–Whitney-U test ($P < 0.05$). NS: not significant.

[b]Differences between the two groups were assessed using χ^2 test to which the Yates correction was applied ($P < 0.05$). NS: not significant.

n: number of patients; mRS: modified rankin scale; BMI: body mass index; ESC: European society of cardiology; TOAST: trial of ORG 1072 in acute stroke treatment; NIHSS: national institutes of health stroke scale; BDNF: brain-derived neurotrophic factor.

the median mRS reached 3. Statistical analysis showed that there was no difference between patients with favorable and unfavorable outcome concerning demographics and stroke etiology. By contrast, ESC score, the delay between admission and fibrinolysis and stroke severity from hospital admission to discharge was lower in patients with favorable outcome than in patients with unfavorable outcome. Concerning hematological parameters, patients with favorable outcome exhibited lower lymphocytes count and higher neutrophils/lymphocytes ratio as compared to patients with unfavorable outcome. Importantly, platelet count did not differ between the groups.

Association between BDNF either in serum or in PBMC and stroke outcome

Consistent with a lack of difference in platelet count between patients with favorable and unfavorable outcome, there is no difference concerning serum BDNF levels between these two groups of patients from D0 to D3 (Table 1). By contrast, BDNF levels in PBMC were more important in patients with favorable than in patients with unfavorable outcome at least at D3. Indeed, at earlier times (D0 and D1), no difference was observed between groups (Table 1). Importantly, while BDNF levels in PBMC decreased from D0 to D3 in patients with unfavorable outcome (-64%, $P = 0.013$), the levels did not show significant changes in patients with favorable outcome. We then more precisely explored the relationship between BDNF levels in PBMC at D3 and stroke outcome. Among patients with PBMC-BDNF levels within the third tertile, 92% had a favorable outcome, while this percentage dropped to 53.4% and 46.1% when BDNF levels in PBMC were within the first and second tertile, respectively (Figure 1 (a)). Univariate logistic regression analysis showed that PBMC–BDNF levels were strongly associated with good

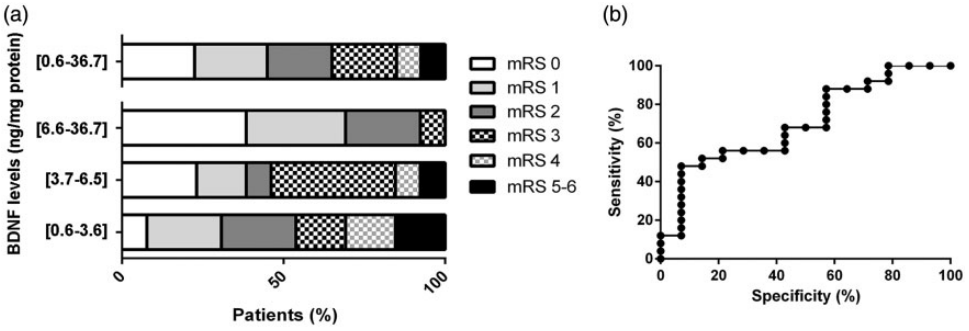

Figure 1. (a) Distribution of modified Rankin scale (mRS) scores at month 3 after stroke onset according to the global or tertiles of BDNF (brain-derived neurotrophic factor) levels in PBMC (peripheral blood mononuclear cells) at day 3 after stroke onset. (b) Receiver operating characteristics (ROC) curve was utilized to determine the optimal cutoff value of PBMC-BDNF levels to predict a good outcome.

outcome (OR = 12.0, 95% CI, 1.4–106.2, $P = 0.023$). Multivariate logistic regression indicated that PBMC-BDNF levels remained a predictor of stroke outcome (OR = 12.4, 95% CI, 1.4–112.2, $P = 0.046$) after adjusting for other predictors (ESC score, interval between admission and fibrinolysis, NIHSS from hospital admission to discharge, lymphocytes and neutrophils/lymphocytes ratio at admission). Finally, based on ROC curve (Figure 1(b)) and AUC (AUC = 0.703, 95% CI: 0.533–0.873, $P = 0.038$), the optimal cutoff value of PBMC-BDNF levels to predict a good outcome was 6.66 ng/mg protein with a sensitivity and specificity at 48.0% and 92.9%, respectively.

Discussion

Our study highlights the BDNF levels in PBMC within the acute stage of stroke decreased in patients with unfavorable, whereas they did not change in patients with favorable outcome as assessed from mRS at month 3. It also reveals that high PBMC-BDNF levels at day 3 after stroke onset are associated with favorable outcome after adjusting for variables that have been associated with mRS including cardiovascular risk factors, delay of fibrinolysis, NIHSS from hospital admission to discharge, lymphocytes count and neutrophils to lymphocytes ratio. It also confirms our previous study[7] showing that mRS at month 3 is not influenced by serum BDNF levels irrespective from time sampling (from admission to day 3 after admission).

The role of PBMC on the ischemic brain is complex and not well understood. These cells exert both adverse and beneficial effects, which might depend on time window and cell subsets.[10] While cytokines secreted by infiltrated PBMC (interleukin 6, tumor necrosis factor α) have been largely involved in their adverse effect, the mediators underlying their beneficial effect in stroke remain largely speculative. The present study supports the idea that infiltration of the ischemic brain by PBMC might contribute to functional recovery and that this effect likely relates to their ability to produce and deliver BDNF to the ischemic brain. Consistently, BDNF levels in PBMC > 6.66 ng/mg of proteins at day 3 after hospital admission were associated with a 12.4 fold increase in favorable outcome. These data fit well with the association observed between impaired cognition and reduced BDNF production by PBMC in patients with multiple sclerosis.[11] They also resonate with a recent study that reported a better outcome at month 6 after stroke onset in patients with high percentage of CD4(+) BDNF(+) Treg cells 24 h after stroke onset than in those with lower percentage.[12] In the present study, BDNF levels in PBMC decreased from admission to day 3 after stroke onset in patients with unfavorable outcome, but remained stable in patients with favorable outcome, suggesting that recovery after stroke might be dependent on still unidentified circulating factors able to inhibit BDNF synthesis by PBMC.

Some limitations of the present study in addition to that linked small number of included patients should be considered. First, all stroke patients enrolled in the present study benefited from fibrinolysis, which is expected to restore blood flow to the ischemic region, which is an obligatory step for extravasation of white blood cells. However, success of reperfusion was not controlled. Second, BDNF gene polymorphism was not evaluated in the present study, while the Val66Met polymorphism was combined with poor motor rehabilitation in stroke patients.[13] However, BDNF secretion by immune cells was reported to be independent on the Val66Met polymorphism.[14]

Authors' contributions: All authors were involved in drafting the article, and all authors approved the final version to be published. Mr. Pedard had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Pedard, Marie, Bejot and Vergely conducted study conception and design, Pedard, Breniere and Pernet acquired data and Pedard and Marie analyzed and interpreted data.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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