Increased Plasma Levels of 15-Deoxy Δ Prostaglandin J_2 Are Associated With Good Outcome in Acute Atherothrombotic Ischemic Stroke

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Background and Purpose—The 15-deoxy Δ prostaglandin J_2 (15-dPGJ$_2$) is an anti-inflammatory prostaglandin that has been proposed to be the endogenous ligand of peroxisome proliferator-activated receptor-γ (PPARγ), a nuclear receptor that can exert potent anti-inflammatory actions by repressing inflammatory genes when activated. It has been suggested that 15-dPGJ$_2$ could be beneficial in neurological disorders in which inflammation contributes to cell death such as stroke.

Methods—We investigated the relationship between plasma levels of 15-dPGJ$_2$ and early neurological deterioration (END), infarct volume, and neurologic outcome in 552 patients with an acute stroke admitted within 24 hours after symptoms onset.

Results—Median [quartiles] plasma 15-dPGJ$_2$ levels on admission were significantly higher in patients than in controls (60.5 [11.2 to 109.4] versus 5.0 [3.8 to 7.2] pg/mL; $P<0.0001$). Levels of this prostaglandin were also significantly higher in patients with vascular risk factors (history of hypertension or diabetes) and with atherothrombotic infarcts (113.9 [81.6 to 139.7] pg/mL), than in those with lacunar (58.7 [32.7 to 86.2] pg/mL), cardioembolic (12.1 [6.5 to 39.2] pg/mL), or undetermined origin infarcts (11.4 [5.6 to 24.3] pg/mL) ($P<0.0001$). In the subgroup of patients with atherothrombotic infarcts, the adjusted odds ratio of END and poor outcome for 1 pg/mL increase in 15-dPGJ$_2$ were 0.95 (95% CI, 0.94 to 0.97) and 0.97 (95% CI, 0.96 to 0.98), respectively. In a generalized linear model, by 1 U increase in 15-dPGJ$_2$, there was a reduction of 0.47 mL (95% CI, 0.32 to 0.63) in the mean estimated infarct volume.

Conclusions—Increased plasma 15-dPGJ$_2$ concentration is associated with good early and late neurological outcome and smaller infarct volume. These findings suggest a neuroprotective effect of 15-dPGJ$_2$ in atherothrombotic ischemic stroke. **(*Stroke*. 2005;36:1189-1194.)**

Key Words: atherosclerosis ■ diabetes mellitus ■ hypertension ■ inflammation ■ stroke

One-third of patients with acute ischemic stroke have early neurological deterioration (END), a situation associated with increased mortality and long-term functional disability.[1] Although the underlying mechanisms are not completely understood,[2] high levels of pro-inflammatory and low levels of anti-inflammatory cytokines in the peripheral blood are associated with END, greater extent of cerebral infarct, and poorer clinical outcome in patients with ischemic stroke.[3–6]

In this context, 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (15-dPGJ$_2$) is a natural anti-inflammatory prostaglandin that appears to be the putative endogenous, high-affinity ligand for the peroxisome proliferator-activated receptor (PPAR) subtype PPARγ, which is a ligand-dependent nuclear transcription factor that has been implicated in a broad range of cellular functions, including anti-inflammatory actions.[7,8] 15-dPGJ$_2$ is a natural derivative of PGD_2. PGD_2 is a major product from a COX-catalyzed reaction in a variety of tissues and cells including those of the immune system, such as platelets, T cells, dendritic cells, and macrophages. PGD_2 undergoes dehydration in vivo and in vitro to yield biologically active prostaglandins of the J_2 series, including PGJ_2, $\Delta^{12,14}$-PGJ_2, and 15-dPGJ$_2$. 15-dPGJ$_2$ represses several inflammatory genes such as inducible nitric oxide synthase and tumor

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necrosis factor-α genes through PPARγ-dependent as well as PPARγ-independent mechanisms.[7–10] Regardless of the mechanism, 15-dPGJ$_2$ is present in vivo during the resolution phase of inflammation, suggesting that it may function as a feedback regulator of the inflammatory response.[11]

In the central nervous system, these anti-inflammatory actions of PPARγ agonists could be beneficial in neurological disorders in which inflammation contributes to cell death or damage, such as stroke.[12] To our knowledge, the concentration of 15-dPGJ$_2$ in blood has never been measured. Therefore, in this study we evaluated the relationship between plasma levels of the natural anti-inflammatory ligand 15-dPGJ$_2$ and stroke outcome in patients with acute cerebral ischemia.

Patients and Methods

This is a secondary study of 1122 patients with an acute ischemic stroke included in 3 prospective registers with the aim to investigate a series of molecular markers of ischemia (249 patients),[5] the influence of blood pressure during the acute phase of ischemia (352 patients),[13] and the phenomenon of ischemic tolerance (521 patients).[14] These 3 registers were pooled in a single database, because they did not share any patient and fulfilled the following requirements: they had a large number of common variables including neurological and functional scales, were gathered by the same researchers at the same centers using similar clinical methodology, and stored frozen blood samples for molecular determinations using a common protocol. Inclusion criteria were uniformly predefined in the 3 registers. In summary, they included patients older than 18 years, with a first episode of hemispheric ischemic stroke of $<$24 hours from symptoms onset, and without previous disability (modified Rankin scale score $\leq$1). Common reasons for exclusion were not confirmed cerebral infarction by CT (n=13), randomization in clinical trials (n=80), thrombolytic therapy (n=11), terminal or severe systemic diseases (n=5), and absence of follow-up (n=94). Although the three registers had some distinct exclusion criteria, only treatment with vasoactive drugs (n=15), dementia or psychiatric diseases (n=15), and unstable cardiovascular problems (n=45) were additional prospective exclusion criteria for this pooling analysis. Informed consent was given by the patients or their relatives. For the purpose of this study, we used the following baseline variables: age, sex, history of inflammatory or infectious diseases in the 15 days preceding admission, history of diabetes and hypertension, atrial fibrillation, and time interval from onset to hospital admission.

On admission at the emergency department, systolic and diastolic blood pressure and body temperature were recorded, and blood samples were taken for blood glucose, fibrinogen, and leukocyte count determinations. Suspensions of plasma were centrifuged at 3000*g* for 5 minutes and stored at -80°C for further molecular determinations. Infarct volume was measured in a cranial CT performed between the fourth and seventh day of evolution, in accordance with the formula $0.5 \times a \times b \times c$ (a and b=greatest perpendicular diameters, c=number of sections of 10 mm when the cerebral infarct was apparent).

Only patients who had a systolic blood pressure $\geq$220 mm Hg or a diastolic blood pressure $\geq$120 mm Hg received antihypertensive drugs within the first 48 hours after admission. Treatment with insulin for hyperglycemia (blood glucose $>$160 mg/dL) and with intravenous metamizol or paracetamol for hyperthermia (tympanic temperature $>$37°C) was initiated early after hospitalization. Subcutaneous low-dose heparin as prophylaxis against pulmonary thromboembolism and antiplatelet drugs were prescribed. Anticoagulants were given to patients with a major cardioembolic source but not as a treatment for early neurological deterioration.

Stroke subtype was classified as atherothrombotic, cardioembolic, lacunar, or of undetermined origin after the TOAST criteria.[15] Transcranial and carotid ultrasound studies were performed in all patients, whereas echocardiography and MR angiography or conventional angiography were performed in selected patients. Stroke severity was evaluated by an experienced neurologist using the Canadian Stroke Scale (CSS) assessed on admission, at 48$\pm$6 hours, and at 3 months$\pm$15 days. The CSS ranges from 1.5 (maximum deficit) to 10 (absence of deficit).[16]

From the total of 844 patients who were finally pooled in the database, we selected 552 patients in whom at least 1 aliquot of frozen plasma obtained on admission was available. The 15-dPGJ$_2$ was measured 9 to 68 months after collection of samples with a commercial quantitative enzyme-linked immunosorbent assay kit (Bio Link 2000; Assay Designs, Inc). Normal reference values of plasma 15-dPGJ$_2$ were obtained from 30 healthy subjects (age 69 [63.7 to 72.2] years; males, 53.3%) matched by age and sex, who were not using any type of medication.

Three outcome measures were evaluated: (1) END as a potential sign of enlarging brain injury; (2) infarct volume at day 4 to 7; and (3) poor neurological outcome at 3 months. Following already published criteria,[17] END was diagnosed when the CSS score declined $\geq$1 points within the first 48 hours of hospitalization. Patients in whom the CSS was $<$7 points at 3 months were classified in the poor outcome group. CSS score was equalized to 0 in patients who died during the follow-up.

Statistical Analyses

The results are expressed as percentages for categorical variables and as median (quartiles) for the continuous variables. Proportions were compared using the χ^2 test, and Mann–Whitney or Kruskal–Wallis tests were used to compare 2 groups, or 3 or more groups, respectively. Spearman analysis was used for bivariate correlations between 15-dPGJ$_2$ levels and time from stroke onset to inclusion, and infarct volume.

The influence of plasma 15-dPGJ$_2$ concentrations on admission on END and poor neurological outcome was evaluated by logistic regression analysis. Likewise, to assess the influence of 15-dPGJ$_2$ levels on the volume of the infarct, generalized linear models were used. Models were adjusted for factors that were related to the outcome variables and infarct volume in the bivariate analyses ($P<0.05$). We fitted the models in a customized way by forced regression logistic method. $P<0.05$ were considered to be statistically significant in all tests.

Results

Baseline characteristics and outcome variables of the 552 selected patients are shown in Table 1. Demographic variables, stroke subtype, and outcome were comparable with those from excluded patients. However, history of diabetes was more frequent in the selected group ($P<0.0001$), which showed higher blood glucose levels ($P=0.003$) and lower systolic blood pressure on admission ($P=0.004$) than the group of excluded patients.

Median plasma 15-dPGJ$_2$ concentration was significantly higher in patients than in normal subjects (60.5 [11.2 to 109.4] versus 5 [3.8 to 7.2] pg/mL; $P<0.0001$). In patients, there was no correlation between 15-dPGJ$_2$ levels and the time interval from stroke onset to the time blood samples were taken ($r=0.059$, $P=0.166$). 15-dPGJ$_2$ in plasma samples did not significantly degrade during storage across the study period, because no differences in 15-dPGJ$_2$ levels were seen between registers and no correlation was found between them and the time interval from sampling to laboratory determinations ($r=-0.032$, $P=0.434$).

Plasma 15-dPGJ$_2$ levels were significantly higher in patients with vascular risk factors (history of hypertension or

TABLE 1. Baseline Characteristics and Outcome Variables in Selected and Excluded Patients

	Patients Selected n=552	Patients Excluded n=292	*P*
Age, y	70 (65–76)	71 (66–76)	0.103
Sex (males), %	57.1	52.4	0.110
Time from onset to admission, h	6.2 (4–11.5)	6.7 (4.6–10)	0.242
History of hypertension, %	54.0	49.0	0.094
History of diabetes, %	29.5	18.8	<0.0001
Systolic blood pressure, mm Hg	160 (130.7–181.7)	168.5 (153–181)	0.004
Diastolic blood pressure, mm Hg	90 (72–100)	90 (77.2–100)	0.158
Body temperature, °C	37 (36.5–37.6)	36.9 (36.5–37.6)	0.239
CSS score	5.5 (3.5–7)	5.5 (3.5–6.5)	0.075
Leukocyte count $\times 10^3$/mm	6.1 (4.2–8.2)	6.4 (4.7–7.8)	0.129
Serum glucose, mg/dL	146.5 (123–209)	139 (119–171.2)	0.003
Fibrinogen, mg/dL	405 (347–511.2)	410 (346–463.7)	0.425
Stroke subtype, %			0.334
Atherothrombotic	40.0	39.0	
Cardioembolic	43.1	45.9	
Lacunar	9.8	11.0	
Undetermined	7.1	4.1	
Early neurological deterioration, %	23.2	20.2	0.339
Infarct volume, mL	49.5 (15–103)	57 (16–104.7)	0.433
Poor neurological outcome at 3 mo, %	37.1	43.8	0.640

CSS indicates Canadian Stroke Scale.

diabetes) than in those without, with the latter showing levels similar to the reference normal values (Figure 1). This effect was caused by both higher concentrations in diabetic patients (111.6 [91.8 to 137.2] versus 25.3 [8.3 to 77.5] pg/mL; $P<0.0001$) and in hypertensive patients (96.5 [50.3 to 127.9] versus 10.5 [5.9 to 69] pg/mL; $P<0.0001$) in comparison with patients without such a risk factor. History of atrial fibrillation, and previous inflammatory or infectious diseases, were not associated with greater 15-dPGJ$_2$ concentrations. No patients were in treatment with COX-2 inhibitors. Regarding previous antiplatelet therapy and nonsteroidal anti-inflammatory drugs, we did not find significant differences in 15-dPGJ$_2$ concentrations between treated and not treated patients. There was no correlation between 15-dPGJ$_2$ levels and the baseline stroke severity, leukocyte count, and fibrinogen values, either in all patients or by stroke subtype.

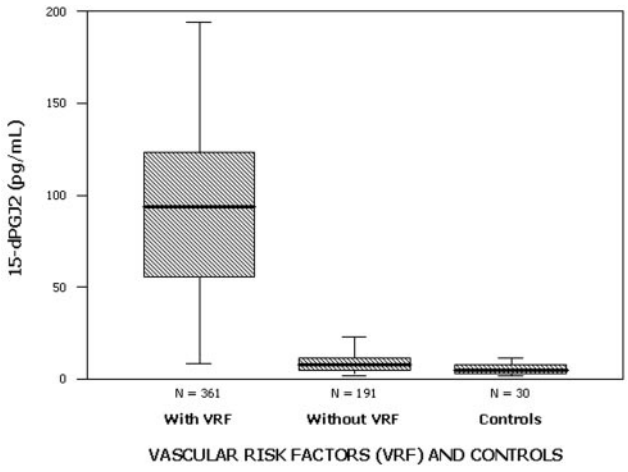

Figure 1. Boxplots showing median values (horizontal line inside the box), quartiles (box boundaries), and the largest and smallest observed values (lines drawn from the end of the box). Plasma 15-dPGJ2 concentrations on admission in patients with vascular risk factors, in patients without vascular risk factors, and in normal subjects.

By stroke subtype, 15-dPGJ$_2$ levels were significantly higher in patients with atherothrombotic infarcts (113.9 [81.6 to 139.7] pg/mL) than in those with lacunar (58.7 [32.7 to 86.2] pg/mL), cardioembolic (12.1 [6.5 to 39.2] pg/mL), or of undetermined origin infarcts (11.4 [5.6 to 24.3] pg/mL) ($P<0.0001$; overall test for trend) (Figure 2).

END was observed in 128 (23.2%) patients and poor neurological outcome at 3 months in 205 (37.1%) patients. By stroke subtype, END and poor outcome were recorded in 30% and 42% of patients with atherothrombotic infarcts, in 3% and 0% of patients with lacunar infarcts, in 21% and 42% of patients with cardioembolic infarcts, and in 18% and 31% of patients with infarcts of undetermined origin. Bivariate analyses showed that a lack of history of hypertension, low systolic and diastolic blood pressure, high body temperature and fibrinogen levels were significantly associated with END, whereas low systolic and diastolic blood pressure, high body temperature, and serum glucose, and low CSS score were associated with poor neurological outcome. These variables

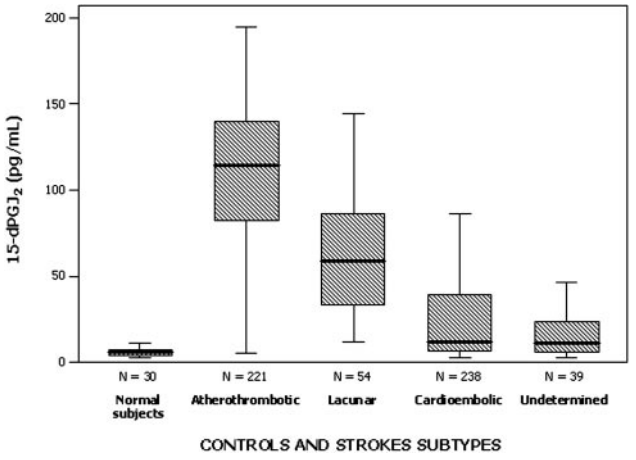

Figure 2. Boxplots showing median values (horizontal line inside the box), quartiles (box boundaries), and the largest and smallest observed values (lines drawn from the end of the box). Plasma 15-dPGJ2 concentrations on admission in normal subjects and by stroke subtype.

in addition to age were also associated with greater infarct volume (data not shown). In the subgroup of patients with atherothrombotic infarcts, but not in the other stroke subtypes, increased 15-dPGJ$_2$ concentrations were associated with better stroke prognosis. Plasma 15-dPGJ$_2$ concentrations were significantly greater in patients without END and with good neurological outcome at 3 months (Table 2). Furthermore, there was a negative correlation between 15-dPGJ$_2$ levels and infarct volume in the atherothrombotic subgroup ($r=-0.221$, $P=0.001$), but not in the other stroke subtypes.

In patients with atherothrombotic infarcts, logistic regression analyses showed that for every unit increase in 15-dPGJ$_2$ there was a 5% (odds ratio [OR], 0.95; 95% CI, 0.94 to 0.97; $P<0.0001$) reduction in the odds of END and a 3% (OR, 0.97; 95% CI, 0.96 to 0.98; $P<0.0001$) reduction in the odds of poor neurological outcome after adjusting for other risk factors. Generalized linear models showed that patients with atherothrombotic infarcts had, for every unit increase in 15-dPGJ2 levels, a 0.47-mL (95% CI, 0.32 to 0.63; $P<0.0001$) decrease in the mean estimated infarct volume.

Discussion

We hereby show for the first time to our knowledge that patients with acute ischemic stroke have higher levels of 15-dPGJ$_2$ in plasma than normal subjects. Furthermore, the present study demonstrates that increased plasma 15-dPGJ$_2$ is associated with better early and late neurological outcome, and with reduced infarct volume, and that this effect is independent on the effect of other important prognostic variables. However, this 15-dPGJ$_2$–related favorable outcome was only found in patients with atherothrombotic infarcts. According to the different plasma levels of 15-dPGJ$_2$ by stroke subtype, we hypothesize that the beneficial effect of this prostaglandin would only occur at concentrations higher than those observed in patients with nonatherothrombotic infarcts. It is notable that <20% of patients with cardioembolic or undetermined infarcts had 15-dPGJ$_2$ levels over the lowest quartile in atherothrombotic infarcts.

High 15-dPGJ$_2$ levels found in patients with atherothrombotic infarct and with vascular risk factors indicate that atherosclerosis, hypertension, and diabetes may be powerful chronic stimuli for the synthesis of this prostaglandin. Interestingly, 15-dPGJ$_2$ concentrations in patients without vascular risk factors were comparable to the normal reference values, despite the fact that they had a similar stroke severity as patients with atherothrombotic infarcts (data not shown). Furthermore, 15-dPGJ$_2$ concentrations did not correlate with acute phase reactants such as leukocyte count and fibrinogen levels. Taking together, these findings suggest that high 15-dPGJ$_2$ levels may be reflecting a previous inflammatory condition underlying vascular diseases, in which COX-2 could be responsible for the 15-dPGJ$_2$ synthesis.[11]

The potential neuroprotective role of 15-dPGJ$_2$ might be attributed to anti-inflammatory mechanisms in the ischemic brain. As a PPARγ agonist, 15-dPGJ$_2$ may exert potent anti-inflammatory actions that modulate the vascular inflammation and the atherosclerotic process.[18,19] Furthermore, because the presence of both PPARγ mRNA and protein have been described in brain,[20,21] the neuroprotective effect of 15-dPGJ$_2$ may result from a PPARγ-mediated inhibition of the inflammatory cascade triggered by the ischemic insult. 15-dPGJ$_2$ may concentrate into the cells of the ischemic tissue because it has nanomolar affinity for active transporters distributed in the whole brain and peripheral blood leukocytes.[22]

PPARγ agonists, including 15-dPGJ$_2$, are known to inhibit the upregulation of the expression of certain genes, such as the iNOS, tumor necrosis factor-α, IL-1, IL-6, and matrix metalloproteinase-9, by antagonizing the activities of transcription factors such as STAT1, NF-κB, and AP-1.[7,8,23] The increase of these molecules in brain tissue after experimental

TABLE 2. Median (Quartiles) Plasma 15-dPGJ2 Concentrations (pg/mL) by Outcome Variables in Each Stroke Subtype

Stroke Subtype	Early Neurological Deterioration			Neurological Outcome at 3 Months		
	No (n=424)	Yes (n=128)	*P*	Good (n=347)	Poor (n=205)	*P*
Atherothrombotic	120.5 (99.3–151.2)	77.5 (56.9–121.3)	<0.0001	121.7 (99.2–151.3)	94.6 (58.6–123.2)	<0.0001
Lacunar	58.7 (32.7–86.2)	65.7 (34.9–87.4)	0.974	56.9 (32.2–86.3)	...	...
Cardioembolic	11.8 (6.5–31.6)	20.4 (6.5–88.4)	0.195	12.3 (7.1–38.1)	11.7 (6.2–80.5)	0.970
Undetermined	10.5 (5.5–23.6)	16.4 (10.2–25.1)	0.578	11.5 (4.1–22.9)	10.8 (6.3–28.7)	0.893
All patients	50.9 (11.2–111.1)	69.5 (12.8–69.5)	0.880	61.7 (12.6–115.7)	54.9 (9.3–99.4)	0.038
Normal subjects	5 (3.8–7.2)					

ischemia, and in blood after acute stroke, suggest their participation in ischemic brain damage.[3–5,24–28] Consequently, their repression by 15-dPGJ$_2$ might explain, at least part, its neuroprotective effects.

The effects of the different PPARγ ligands on inflammation do not always correlate with their ability to activate PPARγ. Thus, it has been suggested that some ligands, mainly 15-dPGJ$_2$, can also act through PPARγ-independent mechanisms, which also involve inhibition of NF-κB signaling,[9,10,29] and some kinases involved in the MAPK cascade and, subsequently, the production of inflammatory mediators in macrophages.[30]

The main strengths of this study are a large sample size, the use of the same assay on stored blood samples collected around the time of the acute event for all subjects, and a prospective 3-month follow-up using CT and accepted neurological scales. However, this study has a number of limitations that must be acknowledged. First, this is a retrospective study, so the results should be cautiously interpreted. The suggested neuroprotective effects of 15-dPGJ$_2$ in atherothrombotic infarctions could be attributed to differences in uncontrolled baseline characteristics such as current therapy before stroke, the severity of the occlusive lesions, and other nonreported vascular risk factors. However, the beneficial effect of 15-dPGJ$_2$ in the present subset of patients is consistent, because it was independent of relevant prognostic variables such as age, initial stroke severity, body temperature, and serum glucose. Second, although some of our results suggest that increased levels of 15-dPGJ$_2$ within the first 24 hours of an acute ischemic stroke are not likely related to the acute phase response or cerebral ischemia, this hypothesis has not been confirmed with 15-dPGJ$_2$ determinations at the end of follow-up. Third, although the specific enzyme-linked immunosorbent assay kit used has cross-reactivity between prostaglandins of the J$_2$ series, we believe that this fact does not limit our conclusions, because measurements of 15-dPGJ$_2$ levels between different groups were performed in similar conditions. Finally, we cannot completely rule out a selection bias because of the exclusion of patients without stored blood samples, because excluded patients showed a lower frequency of history of diabetes. However, we believe our results may be generalized to patients with acute stroke, because we used similar study protocols in the 3 registers and we pooled the data bases to control for potential heterogeneities between them.

In summary, increased plasma 15-dPGJ$_2$ is associated with good early and late neurological outcome and smaller infarct volume in atherothrombotic ischemic stroke. These findings suggest a neuroprotective role of 15-dPGJ$_2$ that is likely to result from the inhibition of the inflammatory cascade triggered by the ischemic insult. To our knowledge, our study is the first evidence reporting values of 15-dPGJ$_2$ in human plasma and may open a fruitful line of investigation regarding the endogenous actions of this prostaglandin in vascular diseases. If these results are replicated by others, the potential neuroprotective actions of 15-dPGJ$_2$ or other PPARγ ligands might be investigated in acute stroke trials.

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