Increased Plasma Levels of 15-Deoxy Δ Prostaglandin J₂ Are Associated With Good Outcome in Acute Atherothrombotic Ischemic Stroke

Miguel Blanco, MD, PhD; María Ángeles Moro, PhD; Antonio Dávalos, MD, PhD; Rogelio Leira, MD, PhD; Mar Castellanos, MD, PhD; Joaquín Serena, MD, PhD; José Vivancos, MD, PhD; Manuel Rodríguez-Yáñez, MD; Ignacio Lizasoain, MD, PhD; José Castillo, MD, PhD

Background and Purpose—The 15-deoxy Δ prostaglandin J₂ (15-dPGJ₂) 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-dPGJ₂ 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-dPGJ₂ 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-dPGJ₂ 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-dPGJ₂ 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-dPGJ₂, 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-dPGJ₂ concentration is associated with good early and late neurological outcome and smaller infarct volume. These findings suggest a neuroprotective effect of 15-dPGJ₂ in atherothrombotic ischemic stroke.

Key Words: atherosclerosis • diabetes mellitus • hypertension • inflammation • stroke
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 neurologi-
cal disorders in which inflammation contributes to cell death or damage, such as stroke.\(^12\) To our knowledge, the concent-
ration 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),\(^3\) the influence of blood pressure during the acute phase of ischemia (352 patients),\(^13\) and the phenomenon of ischemic tolerance (521 pa-
tients).\(^14\) These 3 registers were pooled in a single database, because they did not share any patient and fulfilled the following require-
ments: 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 3000–cubic centimeters, with a first episode of hemispheric ischemic stroke of years, with a first episode of hemispheric ischemic stroke of 3000–cubic centimeters, with a first episode of hemispheric ischemic stroke of 3000–cubic centimeters. These 3 registers were pooled in a single database, because they did not share any patient and fulfilled the following require-
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### Results

Baseline characteristics and outcome variables of the 552 selected patients are shown in Table 1. Demographic vari-
bles, 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] mg/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 pa-
tients with vascular risk factors (history of hypertension or
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-dPGJ2 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-dPGJ2 concentrations between treated and not treated patients. There was no correlation between 15-dPGJ2 levels and the baseline stroke severity, leukocyte count, and fibrinogen values, either in all patients or by stroke subtype.

By stroke subtype, 15-dPGJ2 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 values, either in all patients or by stroke subtype.

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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-dPGJ2 concentrations were associated with better stroke prognosis. Plasma 15-dPGJ2 concentrations were significantly greater in patients without END and with better stroke prognosis. Plasma 15-dPGJ2 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-dPGJ2 concentrations did not correlate with acute phase reactants such as leukocyte count and fibrinogen levels. Taking together, these findings suggest that high 15-dPGJ2 levels may result from a PPARγ agonist, 15-dPGJ2 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-dPGJ2 may result from a PPARγ-mediated inhibition of the inflammatory cascade triggered by the ischemic insult. 15-dPGJ2 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-dPGJ2, 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**

<table>
<thead>
<tr>
<th>Stroke Subtype</th>
<th>Early Neurological Deterioration</th>
<th>Neurological Outcome at 3 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No (n=424)</td>
<td>Yes (n=128)</td>
</tr>
<tr>
<td>Atherothrombic</td>
<td>120.5 (99.3–151.2)</td>
<td>77.5 (56.9–121.3)</td>
</tr>
<tr>
<td>Lacunar</td>
<td>58.7 (32.7–86.2)</td>
<td>65.7 (34.9–87.4)</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>11.8 (6.5–31.6)</td>
<td>20.4 (6.5–88.4)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>10.5 (5.5–23.6)</td>
<td>16.4 (10.2–25.1)</td>
</tr>
<tr>
<td>All patients</td>
<td>50.9 (11.2–111.1)</td>
<td>69.5 (12.8–69.5)</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>5 (3.8–7.2)</td>
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Discussion
We hereby show for the first time to our knowledge that patients with acute ischemic stroke have higher levels of 15-dPGJ2 in plasma than normal subjects. Furthermore, the present study demonstrates that increased plasma 15-dPGJ2 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-dPGJ2-related favorable outcome was only found in patients with atherothrombotic infarcts. According to the different plasma levels of 15-dPGJ2 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-dPGJ2 levels over the lowest quartile in atherothrombotic infarcts.

High 15-dPGJ2 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-dPGJ2 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).

**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.
ischemia, and in blood after acute stroke, suggest their participation in ischemic brain damage.\textsuperscript{3–5,24–28} Consequently, their repression by 15-dPGJ\textsubscript{2} might explain, at least part, its neuroprotective effects.

The effects of the different PPAR\textsubscript{\gamma} ligands on inflammation do not always correlate with their ability to activate PPAR\textsubscript{\gamma}. Thus, it has been suggested that some ligands, mainly 15-dPGJ\textsubscript{2}, can also act through PPAR\textsubscript{\gamma}-independent mechanisms, which also involve inhibition of NF-\kappaB signaling\textsuperscript{9,10,20} and some kinases involved in the MAPK cascade and, subsequently, the production of inflammatory mediators in macrophages.\textsuperscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2} series, we believe that this fact does not limit our conclusions, because measurements of 15-dPGJ\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{2} or other PPAR\textsubscript{\gamma} ligands might be investigated in acute stroke trials.

**Acknowledgments**

This work was partly supported by the Spanish Ministry of Health, grant FIS PI030314 (M.A.M.).

**References**

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