−80 °C in five different aliquots. The busulfan concentration in one aliquot was measured within 48 h of storage (IS). The other four aliquots were analyzed after 3 months, 6 months, 1 year, and 2 years of storage at −80 °C, respectively. The AUC was calculated as mentioned earlier. Good correlation was found between busulfan concentrations obtained for the IS and for the other aliquots after storage with a maximum decrease of 7.8% at 2 years (Table 1B). The decrease in AUC values was 4.7%, 6.9%, and 7.3% at 6, 12, and 24 months, respectively. These data (Table 1B). The decrease in AUC values was 4.7%, 6.9%, and 7.3% at 6, 12, and 24 months, respectively. These data suggest that plasma samples collected for busulfan assay can be stored at −80 °C for up to 6 months with a <5% reduction in concentration and AUC and a <10% reduction in these values for up to 2 years. Henner et al (13) reported that plasma samples supplemented with 0.5–20 µmol/L busulfan and stored at −20 °C for 0, 16, and 57 days showed values identical to that of the plasma sample analyzed immediately after the addition of busulfan (+5%). Our data show that busulfan concentrations in plasma samples stored at −80 °C are stable for up to 2 years.

In conclusion, we have shown that busulfan concentrations are stable in whole blood for 24 h at 4 °C and in plasma for 2 years at −80 °C. There is a <5% variation in concentration and AUC if plasma is isolated within 6 h of blood collection and analyzed within 6 months of storage. These data will be very useful for evaluation of busulfan kinetics in situations where sample analysis cannot be undertaken immediately.

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References

Gas Chromatographic-Mass Spectrometric Measurement of 15-Deoxy-Δ12,14-prostaglandin J2, the Peroxisome Proliferator-activated Receptor γ Ligand, in Urine, Chantal Thévenon, Michel Guichardant, and Michel Lagarde *(INSERM U352, Biochimie and Pharmacologie INSERM Lyon, Blvd. Louis Pasteur, 20 Avenue A. Einstein, 69621 Villeurbanne, France; * author for correspondence: fax 33-4-72-43-85-24, e-mail michel.lagarde@insa-lyon.fr)

Prostaglandin D2 (PGD2) together with its positional isomer PGE2 is a direct metabolite of PGH2 arising from the dioxygenation of arachidonic acid (1). More than 15 years ago, PGJ2, the 9-deoxy derivative of PGD2 via dehydrogenation, was identified and described as a mitogen (2). Later, the Δ12 isomer of PGJ2 was found in human urine (3).

A new dehydration product of PGJ2, 15-deoxy-Δ12,14-PGJ2 (15dPGJ2), has been described as a specific ligand of peroxisome proliferator-activated receptor γ, which is associated with adipocyte differentiation (4, 5). Considerable interest has arisen in cyclopentenone derivatives of prostaglandins (6), particularly 15dPGJ2, which has been described as an active compound in cancer (7, 8) and in cell apoptosis (9, 10), in addition to adipogenesis (11). 15dPGJ2 has recently been reported to have antiinflammatory activity (12). Indeed, it prevents cytokine- and endotoxin-stimulated activation of peripheral and resident tissue macrophages and cytokine-induced inducible nitric oxide synthase expression in B cells by inhibition of transcriptional activation and induction of the heat-shock response.

Despite the growing interest in this prostaglandin metabolite, no reliable assay has been reported, especially by gas chromatography–mass spectrometry (GC-MS), a reference method for prostanooids (13). The lack of such an assay presumably reflects the difficulty in derivatizing this peculiar prostaglandin, which has three conjugated double bonds with the ketone group. We report here a
simple and sensitive measurement method for 15dPGJ2 as well as its detection in human urine.

All prostaglandins were obtained from Cayman Chemical (SPI Bio); all other chemicals and reagents were analytical grade and were purchased from Sigma-Fluka-Aldrich Chemical Co. Analytical-grade organic solvents and silica thin-layer chromatography plates were from Merck.

Derivatizations were performed as described previously (14, 15). 15dPGJ2 and its 3,3,4,4-deuterated form (d4-15dPGJ2, used as internal standard) were dissolved in 30 µL of acetonitrile, after which 10 µL of 350 g/L 2,3,4,5,6-pentafluorobenzyl bromide in acetonitrile and 10 µL of N,N-diisopropylamine were added; the solution was then heated to 40 °C for 20 min. The resulting molecules were the pentafluorobenzyl (PFB) esters.

D12-PGJ2 was derivatized to PFB esters as discussed above, and its 15-hydroxyl group was converted to silyl ether by overnight treatment at room temperature with 50 µL of N,O-bis-(trimethylsilyl)-trifluoroacetamide to obtain a trimethylsilyl (TMS) ether. The resulting compound was the PFB,TMS-D12-PGJ2 derivative.

PGJ2 and PGE1 were also used for comparing sensitivities. These two derivatizations were preceded by overnight treatment at room temperature with 100 µL of methoxylamine hydrochloride in anhydrous pyridine (5 g/L) to make a methoxime (MOX) in place of the oxo group. The resulting derivatives were MOX,PFB,TMS-prostaglandins.

Urine samples were collected from donors who gave informed consent. Urine samples (5 mL) were subjected to lipid extraction three times with 50 mL of ethyl acetate. Appropriate amounts of the internal standard d4-15dPGJ2 were added before lipid extraction when needed. In some experiments, urine samples were acidified to pH 3 with 3 mol/L HCl for 30 min before lipid extraction. The dried lipid extracts were purified by thin-layer chromatography with chloroform-methanol-acetic acid (90:5:2 by volume) as mobile phase to separate 15dPGJ2 from its parent prostaglandins (2). In this system the Rf was 0.41 for PGD2, 0.66 for PGJ2/D12-PGJ2, and 0.79 for 15dPGJ2. The 15dPGJ2 spot was extracted three times from the silica gel with ethyl acetate, and the resulting extracts were dried and derivatized into PFB-15dPGJ2 as described above.

To measure recovery of 15dPGJ2 added to urine, increasing amounts (0–250 ng) of 15dPGJ2 were added to each 5-mL urine sample containing 100 ng of d4-15dPGJ2 as internal standard. 15dPGJ2 was extracted and derivatized as described previously.

GC-MS was carried out on a Agilent Technologies gas chromatograph (Model 6890) interfaced with a quadrupole mass spectrometer (Model 5973). The gas chromatograph was equipped with a HP-1MS fused-silica capillary column [30 m × 0.25 mm (i.d.); 0.25 µm film thickness; Agilent Technologies] that was held at 57 °C. The following oven temperature program was used: 2 min at 57 °C, then increased to 180 °C at a rate of 20 °C/min, followed by an increase to 280 °C at a rate of 4 °C/min. The interface, injector, and ion source were kept at 280, 280, and 130 °C, respectively. Electron energy was set at 70 eV. Helium (N55; 1 mL/min) and methane were used as the carrier and reagent gases, respectively. The electron multiplier voltage was usually set at 1400 V. Mass spectra were acquired from m/z 50 to m/z 600 in electron impact mode and from m/z 100 to m/z 600 in negative ion chemical ionization (NICI) mode.

The classical derivatization of the carbonyl group as methoxime reported for PGE1 (16) was also effective for PGJ2. Their NICI mass spectra (results not shown) indicated a specific fragmentation at [M-181]− leading to main fragments at m/z 526 and 422, respectively. In contrast, methoxime formation was not observed for D12-PGJ2 or for 15dPGJ2 because of the high conjugation...
of their carbonyl groups and the double bonds located in the cyclopentenone and the $\omega$ chain. However, these prostaglandins could easily be measured by MS without the oximation step. For example, the 15dPGJ$_2$ spectrum acquired using the NICI mode (Fig. 1A) showed a unique fragment at $m/z$ 315, which corresponded to the loss of the PFB ester group. Interestingly, the detection limit was not affected by the absence of the oxime derivatization as indicated (Table 1). Different prostaglandins treated or not with methoxylamine hydrochloride were analyzed using the NICI mode. The signals of the selected single ion monitoring corresponding to the [M-181]$^+$ fragment of the derivatized prostaglandins PGE$_1$, PGJ$_2$, $\Delta^{12}$-PGJ$_2$, and 15dPGJ$_2$ were recorded. The detection limits for PGE$_1$ and PGJ$_2$ (Table 1) were similar with or without the carbonyl derivatization and were $\sim$0.10 and 1 pg, respectively. The detection limits of both $\Delta^{12}$-PGJ$_2$ and 15dPGJ$_2$ without oxime derivatization (Table 1) were very close (1 pg). In consequence, further analyses were performed without the oximation step.

PFB-15dPGJ$_2$ was analyzed first by electron impact GC-MS. Its mass spectrum (Fig. 1B) shows two important characteristic ions: one at $m/z$ 496, which represents the molecular ion [M]$^+$, and the other at $m/z$ 425, which corresponds to the loss of the N-terminal alkyl chain (C$_{6}$H$_{11}$) [M-71]$^+$. The presence of the carbonyl group leads to an abundant ion at $m/z$ 181, which corresponds to the PFB group used for the PFB ester derivatization. The different amounts of 15dPGJ$_2$ added to urine were measured as described above by NICI. The response (area of 15dPGJ$_2$/area of d$_4$-15dPGJ2) was linearly related to added 15dPGJ$_2$ in the interval 0–250 ng ($r = 0.99$). The intercept of the correlation linear curve with the y axis allowed us to calculate a concentration of 1.2 $\mu$g/L for 15dPGJ$_2$ in urine. It is noteworthy that the prostaglandin extraction step needs to be performed without acidification. Indeed, prior experiments revealed that acidic extraction (pH 3) of urine provoked a 20-fold increase in 15dPGJ$_2$ (15.57 ± 5.64 $\mu$g/L with acidification vs 0.68 ± 0.19 $\mu$g/L without acidification; $n = 3$).

The excretion of 15dPGJ$_2$ was significantly ($P < 0.05$) higher in women (13.2 ± 3.5 $\mu$g/mol of creatinine, mean ± SD; $n = 4$) than in men (5.7 ± 1.4 $\mu$g/mol of creatinine; $n = 3$).

This is the first report of quantification of 15dPGJ$_2$ excretion. The difference observed between males and females needs to be further investigated.

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### Table 1. Detection limits for the different derivatized standard prostaglandins.

<table>
<thead>
<tr>
<th>Derivatized prostaglandin</th>
<th>Detection limit, fg</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFB-15dPGJ$_2$</td>
<td>880</td>
</tr>
<tr>
<td>PFB, TMS-$\Delta^{12}$-PGJ$_2$</td>
<td>340</td>
</tr>
<tr>
<td>PFB, TMS-PGJ$_2$</td>
<td>900</td>
</tr>
<tr>
<td>MOX, PFB, TMS-PGJ$_2$</td>
<td>1020</td>
</tr>
<tr>
<td>PFB, TMS-PGE$_1$</td>
<td>85</td>
</tr>
<tr>
<td>MOX, PFB, TMS-PGE$_1$</td>
<td>90</td>
</tr>
</tbody>
</table>

*a The analysis was performed using the NICI mode with selected-ion monitoring of the respective [M-181]$^+$ ions. The detection limit was defined as a signal-to-noise ratio of 10 in samples prepared from standard prostaglandins.

References