We did not detect BJP and had no false-positive results (data not shown). Given the occurrence of false positives in cases such as hepatitis where oligoclonal banding is common, interpretation of the result together with other clinical data is important.

Increased BJP indicates a poor prognosis and aggravation of monoclonal gammopathies. The quantification of urinary BJP is important for observing the course and evaluating treatment effects. In patients, the urinary total protein concentration varies. Because low urinary total protein concentrations cannot be determined precisely, the BJP concentration cannot be calculated by multiplying the amount of total protein by the percentage of the BJP band. When we used \( \alpha \) or \( \beta \) solutions as calibrators for semiquantification of BJP, their electrophoretic patterns were smeared. In this study, therefore, we used albumin as a calibrator, for which the electrophoretic pattern was a single sharp band. The calibration curve based on albumin calibrators has been shown to be acceptably linear. More accurate quantification is possible in overflow-type urine samples, which show a high correlation between the values obtained by our semiquantitative method and that obtained by immunonephelometry. However, for electrophoretic images of urine samples containing high amounts of protein, a decrease in the quantification accuracy must also be taken into consideration. In the recent study by Levinson (18), BJP concentrations were expressed as semiquantitative values based on data obtained by UPE and IFE. If the results obtained by our method are also considered to be semiquantitative rather than quantitative, our method may be useful for observing and evaluating the clinical course of monoclonal gammopathies.

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References

Stability of Busulfan in Frozen Plasma and Whole Blood Samples, Poovizhali Balasubramanian,* Alok Srivastava, and Mammen Chandy (Department of Hematology, Christian Medical College Hospital, Vellore, India; *author for correspondence: fax 91-416-232035, e-mail bala@hemato.cmc.ernet.in)

Busulfan is widely used as a component of myeloablate conditioning therapy for bone marrow transplantation (1–5). Busulfan is most often used at a fixed total dose of 16 mg/kg. Wide interindividual variation in the bioavailability of busulfan has been recognized. Pharmacokinetic analyses to achieve target plasma concentrations and dose adjustments are increasingly being used to improve the outcome of bone marrow transplantation (6–8). Studies on busulfan kinetics have demonstrated that busulfan concentrations in stored plasma samples are stable for up to 3 months at −20 °C and that blood samples for busulfan analysis should be centrifuged within 3 h of collection and plasma frozen if not analyzed immediately (9–11). The stability of busulfan in blood and plasma samples stored for longer time periods and at lower temperatures has not been reported. Because pharmacokinetic analysis of busulfan requires collection of multiple samples at frequent intervals, such data will be useful in planning transport and analysis of blood and plasma samples.

This study was undertaken to determine the stability of busulfan in whole blood samples stored up to 24 h at 4 °C and in plasma samples stored up to 2 years at −80 °C. Busulfan analysis was performed on plasma samples by HPLC as described previously (12). Briefly, busulfan in plasma was extracted with toluene and derivatized with 1 mol/L tetrafluorothiophenol under alkaline conditions for 2 h at 70 °C. The tetrafluorothiophenol derivative of busulfan thus formed was reextracted with 1 mol/L...
sodium hydroxide and toluene. The toluene layer was then removed and evaporated to dryness under nitrogen gas at 60 °C. The residue was dissolved with 200 μL of 800 mL/L methanol, vortex-mixed, and filtered through 0.45 μm microspin filters (Millipore India Ltd). A 20-μL aliquot of the sample was injected into an isocratic HPLC system (Bio-Rad) by an automatic sampler. Detection was performed using an ultraviolet detector (Bio-Rad) at 275 nm. A calibration curve was created with each set of samples using known concentrations of busulfan calibrator in acetone. This method did not involve the use of an internal standard. The minimum quantifiable concentration of busulfan was 50 μg/L, with linearity up to 2000 μg/L. The detection limit of the assay, corresponding to a signal-to-noise-ratio of 3, was 25 μg/L. The inter- and intraday CVs were <3% for the calibrators used for the calibration curve (12). Samples analyzed both by this HPLC method and the gas chromatography–mass spectrometry method showed good correlation ($r^2 = 0.95$) (13). The area under the busulfan concentration–time curve (AUC) was calculated using TOPFIT by noncompartmental analysis (14).

For the blood collection and stability studies, the protocol was approved by the Institutional Review Board. Whole blood samples (8–9 mL) were collected 0, 0.5, 1, 1.5, 2, 4, and 6 h after the 13th dose of busulfan from three patient with β-thalassemia before bone marrow transplantation. Each sample was divided into six aliquots. Plasma was separated from one aliquot by centrifugation at 1900g for 10 min at 4 °C immediately after collection and stored at −80 °C until analysis [immediate sample (IS)]. The other five aliquots were processed similarly at 2, 4, 6, 12, and 24 h. Busulfan concentrations in all plasma samples were analyzed within 48 h of sample collection. Linear regression analysis comparing the busulfan concentrations in the IS with those obtained by centrifuging whole blood 2, 4, and 6 h after collection showed a variation <5% ($R^2 = 0.999, 0.997$, and 0.991, respectively; Fig. 1). However, a 7.7–9.8% decrease occurred in busulfan concentrations ($r^2 = 0.91$ and 0.88, respectively) in plasma samples separated after 12 and 24 h compared with the IS.

The AUC calculated from plasma samples centrifuged and separated within 2–6 h of collection showed good agreement with that calculated from the IS with a maximum CV of 4.7%; samples collected after 12 and 24 h showed CVs of 5.4% and 7.2%, respectively. No data regarding the stability of busulfan in whole blood are currently available. Henner et al. (15) determined the stability of busulfan in buffer (50 mmol/L phosphate–0.15 mol/L sodium chloride, pH 7.4) and plasma incubated at 0, 22, and 37 °C for periods up to 22 h. They suggested that samples in buffer were stable for >22 h with <15% decomposition at all three temperatures, but plasma samples were stable at 0 and 22 °C only; plasma samples incubated at 37 °C decomposed with a half-time of ~14 h. It was concluded that busulfan is relatively stable at physiologic temperatures (half-life, 14 h), unlike other alkylating drugs such as mustine (half-life, 20–25 min). Our data show that busulfan is stable in whole blood samples with a <5% reduction in concentration and AUC when stored at 4 °C and a <10% reduction in concentration and AUC up to 24 h.

To examine the stability of busulfan in plasma at −80 °C, blood samples were collected as described above, centrifuged immediately after collection, and stored at

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**Table 1. Stability of busulfan in plasma and whole blood samples.**

<table>
<thead>
<tr>
<th></th>
<th>AUC (mean ± SD)</th>
<th>R²</th>
<th>Decrease, %</th>
<th>R²b</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Stability of busulfan in whole blood at 4 °C</strong></td>
<td>After 2 h</td>
<td>After 4 h</td>
<td>After 6 h</td>
<td>After 12 h</td>
</tr>
<tr>
<td>Immediate</td>
<td>4206 ± 1543</td>
<td>4082 ± 1528</td>
<td>4005 ± 1728</td>
<td>3980 ± 1567</td>
</tr>
<tr>
<td>Decrease, %</td>
<td>2.0</td>
<td>2.9</td>
<td>4.7</td>
<td>5.37</td>
</tr>
<tr>
<td>R²b</td>
<td>0.999</td>
<td>0.997</td>
<td>0.991</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>B. Stability of busulfan in frozen plasma samples at −80 °C</strong></td>
<td>After 3 months</td>
<td>After 6 months</td>
<td>After 1 year</td>
<td>After 2 years</td>
</tr>
<tr>
<td>Immediate</td>
<td>5626 ± 1514</td>
<td>5362 ± 1509</td>
<td>5239 ± 1356</td>
<td>5193 ± 1438</td>
</tr>
<tr>
<td>Decrease, %</td>
<td>1.6</td>
<td>4.7</td>
<td>6.9</td>
<td>7.7</td>
</tr>
<tr>
<td>R²b</td>
<td>0.99</td>
<td>0.98</td>
<td>0.95</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*a AUC values expressed as μg · h/L and represent the mean ± SD AUCs for three patients.  
*b R² values are regression coefficients for busulfan concentrations in “immediate samples” vs those analyzed after various times.
−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 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 Proeicosome Proliferator-activated Receptor γ Ligand, in Urine, Chantal Thévenon, Michel Guichardant, and Michel Lagarde (INSERM U352, Biochimie and Pharmacologie INSA-Lyon, Bldg. 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 dehydration, 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 antinflammatory 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...