Quantification of Urinary 8-iso-Prostaglandin $F_{2\alpha}$ Using Liquid Chromatography–Tandem Mass Spectrometry During Cardiac Valve Surgery

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Oxidative stress is an unavoidable event during many complex surgical procedures. 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-PGF $_{2\alpha}$) is a reliable biomarker for the evaluation of oxidative stress in vivo. The aim of this study is to develop simple and accurate liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods for the detection of urinary 8-iso-PGF $_{2\alpha}$ in samples collected from patients who received a cardiopulmonary bypass (CPB) during cardiac valve surgery. Urine samples of 14 patients with cardiac valve diseases were collected before, during, and after CPB. The level of 8-iso-PGF $_{2\alpha}$ was detected via selected-reaction monitoring triple quadrupole MS/MS and the result was compared with 12 healthy volunteers. The method's

detection limit (3S/N) was 0.25 pg for 8-iso-PGF $_{2\alpha}$, with a linear working range of 0.25–20 ng/ml. For patients with cardiac valve disease, the 8-iso-PGF $_{2\alpha}$ levels before the bypasses were the same as those of healthy individuals ($P > 0.05$) and the 8-iso-PGF $_{2\alpha}$ levels during and after CPB were significantly higher than those before the bypasses ($P < 0.05$). In conclusion, we present a simple and specific protocol for LC-MS/MS quantification of urinary 8-iso-PGF $_{2\alpha}$ collected during CPB. Using this technique, it would be feasible to assess the levels of oxidative stress during cardiac surgery and thereby helpful for the management of oxidative injury. *J. Clin. Lab. Anal.* 24:237–245, 2010. © 2010 Wiley-Liss, Inc.

Key words: oxidative stress; liquid chromatography–tandem mass spectrometry; isoprostanes; cardiopulmonary bypass; cardiac surgery

INTRODUCTION

Enhanced production of oxygen-free radicals can lead to the generation of oxidative stress, which is harmful to human tissue and organs. Reactive oxygen species (ROS) are released abruptly during some surgical procedures, and they are the major causes of ischemia–reperfusion injuries. With different biomarkers and detection methods, increased oxidative stress has been reported during several types of surgeries, including total knee replacement under ischemic tourniquet (1), coronary artery bypass grafting surgery (2), one-lung thoroscopic surgery (3), and laparoscopic abdominal

surgery (4). Oxidative stress can result in hemodynamic instability, arrhythmia, impaired immune system, and impaired cardiac and pulmonary function during or after surgery (5,6). Therefore, accurately monitoring and

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avoiding the occurrence of oxidative stress during surgery is an important clinical issue.

Isoprostanes are a series of prostaglandin-like compounds produced by non-enzymatic peroxidation of arachidonic acid (7). 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-PGF $_{2\alpha}$), one of a large number of isoprostane isomers, is an indicator of lipid peroxidation resulting from free radical generation. It has been recognized as an excellent biomarker for the quantification of oxidative stress, oxidative damage, and antioxidant deficiency in vivo (8–10). Although 8-iso-PGF $_{2\alpha}$ can be detected in various biological fluids or tissues, the urinary assay is the most widely used for clinical studies. The advantages of urine sampling, when compared with collection of other biological fluids, are that collection is easy and non-invasive, samples require less complicated preparation, and there is less potential for artifactual generation (10). All of these features make 8-iso-PGF $_{2\alpha}$ suitable for large clinical trials.

Many analytical methods, including enzyme-linked immunosorbent assay (ELISA) (11,12) and gas chromatography/mass spectrometry (GC/MS) (13,14), have been used to detect 8-iso-PGF $_{2\alpha}$ and its isomers in urine. However, there are several challenges inherent in detecting 8-iso-PGF $_{2\alpha}$. First, 8-iso-PGF $_{2\alpha}$ is one of 64 isoprostane isomers (15), and therefore a highly specific method is needed to distinguish it from related compounds. Second, 8-iso-PGF $_{2\alpha}$ is usually present at very low concentrations in biological samples and thus sensitivity can also be a problem.

The ELISA and radioimmunoassay methods are relatively inexpensive and easy to perform. However, the inconsistent performance, the high average coefficient of variation (>40%) (16), and the cross-reactivity of these approaches with other isoprostanes (17,18) constitute significant disadvantages. Therefore, these methods are not appropriate for clinical practice.

Compared with other immunoassay methods, chromatographic detection by GC/MS is a very sensitive technique for isoprostane analysis. The major drawback of this assay is that it is quite time-consuming and labor intensive, requiring extensive sample preparation steps. In addition, GC/MS requires the derivatization of the analytes, complicating the analysis and potentially leads to loss of target compounds and lower recovery yields (19,20). Furthermore, GC/MS does not allow the isolation of a single isoprostane isomer. All these factors make this technique less than ideal for handling large numbers of clinical samples and for determining 8-iso-PGF $_{2\alpha}$ concentrations.

Liquid chromatography–tandem mass spectrometry (LC-MS/MS) with electrospray ionization is a superior method for highly specific and sensitive quantification of many analytes in the biological laboratory. In recent

years, several reports have been published using LC-MS/MS with solid phase extraction (SPE) to quantify urinary 8-iso-PGF $_{2\alpha}$ in smokers (21), patients with diabetes (22), and patients with Alzheimer's disease (19).

However, it is still unknown whether LC-MS/MS can be used effectively for the analysis of intra-operative urinary 8-iso-PGF $_{2\alpha}$. Cardiopulmonary bypass (CPB) is a standard procedure during cardiac surgery. During cardiac operations, especially in the case of CPB, the exact quantification of any biomarker in the urine is very difficult. First, large amounts of intravenous fluid are injected during surgery, and this makes the urine extremely dilute. Second, the urinary matrix of patients during surgery can become more complex because of the presence of additional fluid with electrolytes that may be transfused, and medications that may be infused during CPB. Finally, the protocol suggested by previous reports requires time-consuming steps for sample preparation and analysis that would be prohibitively cumbersome in large clinical trials. In this study, we seek to detect changes in 8-iso-PGF $_{2\alpha}$ concentrations for patients during and after CPB in cardiac valve surgery.

The aim of this study was to report an improved LC-MS/MS method that is rapid, simple, and accurately quantifies urinary 8-iso-PGF $_{2\alpha}$. To this end, we have developed a simple method for SPE and a rapid procedure for HPLC separation with ESI–tandem MS. This approach could be used to analyze the oxidative stress biomarkers present in urine samples collected from patients receiving cardiac valve surgery with CPB.

MATERIALS AND METHODS

8-iso-PGF $_{2\alpha}$, 8-iso-PGF $_{2\alpha}$ -d $_4$, prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$), and prostaglandin $F_{2\alpha}$ -d $_4$ (PGF $_{2\alpha}$ -d $_4$) were obtained from Cayman Chemical Company (Ann Arbor, MI). All standards were diluted into adequate volumes of ethanol to generate stock solutions, which were then aliquoted into small vials and stored at -20°C . High purity acetonitrile, methanol, and hexane were obtained from J.T. Baker (Phillipsburg, NJ). Ammonium hydroxide (25% NH_4OH) and hydrochloric acid (37% HCl) were obtained from Merck Co. (Darmstadt, Germany). Oasis HLB 96-well extraction plates (30 μm , 10 mg) were obtained from Waters Corporation (Ireland). Extraction was performed on a Waters 96-well plate manifold (Ireland) with a customized insert to adapt shallow 0.5-ml 96-well plates (Agilent Technologies, Palo Alto, CA) for collection.

Clinical Sample Collection

After approval of the ethics committee and with fully informed consent, 12 healthy volunteers and 14 patients scheduled for cardiac valve surgery and CPB were

TABLE 1. Clinical Characteristics of Patients

Number	14
Male/female	6/8
Age (yr)	49.0 ± 15.9
BMI	23.8 ± 4.4
Mean PA (mmHg)	30.7 ± 12.3
EF (%)	56.8 ± 16.4
Surgery	
Mitral valve surgery	8
Aortic valve surgery	6
Cardiopulmonary bypass time (min)	146.9 ± 46.8
Cross-clamp time (min)	101.8 ± 36.6

Data are presented as mean ± standard deviation.

enrolled in this study. Normal human subjects were defined as healthy adults who were not taking any medications, and random spot urine samples were collected from these subjects.

The 14 patients receiving cardiac valve surgery were in ASA II-III physical status, and their ages were between 25–80 years old. We excluded any patients with trauma, infection, low ejection fraction (less than 25%), liver cirrhosis, acute renal failure, or emergency surgery. There was no operative mortality in this study. The clinical characteristics of all patients are shown in Table 1.

In all patients, anesthesia was induced with midazolam 5–10 mg, fentanyl 3–5 µg kg⁻¹, propofol 0.5–1.5 mg kg⁻¹, and cisatracurium 0.2 mg kg⁻¹ for tracheal intubation. After tracheal intubation, mechanical ventilation was controlled with tidal volume of 8–10 ml kg⁻¹, and respiratory rate was adjusted to maintain an end-tidal carbon dioxide value between 30 and 35 mmHg. Anesthesia was maintained with the use of volatile anesthetics and cisatracurium until the end of surgery. Volatile anesthetic concentrations were adjusted to sevoflurane 1–1.2 MAC to maintain adequate anesthetic depth. Patients were monitored via electrocardiography, invasive blood pressure from arterial lines, peripheral oxygen saturation measurements, and continuous cardiac output as measured by a Swan-Guans catheter. All patients receiving cardiac valve surgery were operated on using the standard CPB technique. CPB was established by cannulation of the ascending aorta and both vena cava, with a moderate systemic hypothermia (26°C) and moderate hemodilution (hematocrit 22–25%). Ten milliliters of urine was collected from patients at the following times: baseline before anesthesia, 30 min before CPB, 25 min (median, 25 min; range, 20–40 min) after the start of CPB, 25 min (median, 25 min; range, 20–40 min) after the end of CPB, and 2 hr after CPB. It has been recognized that the detection of urinary 8-iso-PGF_{2α} shows an approximately 20-min time lag relative to its detection in plasma. Therefore, we collected urine

samples 25 min after the start and end of CPB to match the time lag of this biomarker source relative to the CPB event. For some patients, additional urine samples at 4 and 20 hr after CPB were also collected. Baseline urine samples were collected between 6 and 7 a.m. preoperatively, and all urine samples were portioned into 50-ml plastic centrifuge tubes and stored at –80°C before analysis.

Sample Preparation

The sample preparation was performed using a modified protocol published by Yan et al. (21). For each sample, the freshly thawed urine was mixed for a few seconds on a vortex shaker and centrifuged at 12,000 rpm for 15 min to precipitate solids. Urine supernatant (1.8 ml) was then pipetted into an Eppendorf tube and spiked with 18 µl of 8-iso-PGF_{2α}-d₄ (1 µg/ml) and PGF_{2α}-d₄ as the internal standard. For urine samples with creatinine concentrations <20 mg/dl, 3.6 ml of urine supernatant was used. Samples were then acidified to approximately pH 3 by the addition of 40 µl of 1 N HCl. After gentle vortexing, the sample was centrifuged (12,000 rpm for 10 min) to remove suspended material. The supernatant was then loaded into an HLB 96-well extraction plate (10 mg) that had been preconditioned with 1 ml of methanol and equilibrated with 1 ml of water. The plate was sequentially washed with 0.5 ml of 0.1 N HCl, 0.5 ml of 5:95 methanol:water, and 0.1 ml of hexane. Then, analytes and the internal standard were eluted into the sample collection plate with two aliquots of 80 µl of methanol containing 0.5% NH₄OH. After the addition of 20 µl water, the eluent was transferred to autosampler vials. Five microliters of each specimen was injected into the LC-MS/MS system. The measurement of urinary creatinine levels was performed by the Chang Gung Memory Hospital Clinical Pathology Lab using the Jaffe method. The absolute 8-iso-PGF_{2α} levels detected were then normalized by urine creatinine (ng/mg creatinine).

LC-MS/MS Instrumentation and Analysis

The HPLC analysis was carried out using an LC packing system (Ultimate Plus, the Netherlands) equipped with an autosampler (Famos, the Netherlands). The LC method used was a modified version of the procedure reported by Saenger et al. (23). Five microliters of the specimen was injected onto a Phenomenex (Torrance, CA) Gemini C18 (50 × 2.0 mm, 3 µm particle size) analytical column. The sample was delivered at a flow rate of 200 µl/min. Gradient elution was used with two mobile phases: (A) 0.1% ammonium hydroxide in water and (B) 0.1% ammonium hydroxide in acetonitrile. The LC separation was performed with a solvent

gradient program of: 11% A at time 0 and a linear increase to 20% A at 6 min. This composition was held until 7 min, at which time the column was re-equilibrated again, with a total cycle time of 10 min. Typical elution times for 8-iso-PGF_{2α} and 8-iso-PGF_{2α}-d₄ were both approximately 5 min.

The HPLC system was directly interfaced with a triple stage quadrupole mass spectrometer (Finnigan TSQ Quantum Discovery MAX; Thermo Electron Corporation, San Jose, CA) equipped with an ESI source. The electrospray ionization was performed in the negative mode. Instrument control, data acquisition, and data analysis were controlled using Xcalibur software (Thermo Finnigan, San Jose, CA). The position of the ESI probe and the parameters used for MS/MS tuning were optimized for maximum sensitivity by injecting 1 µg/ml of 8-iso-PGF_{2α} and 8-iso-PGF_{2α}-d₄ standard solutions at a microsyringe pump flow rate of 5 µl/min. The instrument was operated in the selected-reaction monitoring (SRM) mode. The interfaced spray voltage was maintained at 3,800 V. Nitrogen was used for both the sheath and the auxiliary gases, which were set to 52 and 8 (arbitrary units), respectively. The capillary temperature was operated at 220°C, and source collision-induced dissociation energy was maintained at 14.0 V. Collision gas (argon) pressure was 0.8 mTorr with a collision energy of 33 eV. The ion pairs with *m/z* 353/193, *m/z* 357/197 were used to monitor 8-iso-PGF_{2α} and 8-iso-PGF_{2α}-d₄, respectively. A seven-point linear calibration curve was established using internal standards. Quantification was performed using integrated peak area ratios of 8-iso-PGF_{2α}/8-iso-PGF_{2α}-d₄.

LC-MS/MS Method Validation

The linearity, limit of detection, precision, accuracy, and recovery were all evaluated to assess the performance characteristics of the assay. The seven-point linear calibration curve was established using serial dilutions of standard stock solutions. Seven standard solutions containing 20, 10, 5, 2.5, 1.25, 0.5, and 0.25 ng/ml of 8-iso-PGF_{2α} standard and 100 ng/ml of internal standard were injected into the LC-MS/MS. Integrated peak area ratios of the standard and the internal standard at every concentration were recorded by the Xcalibur software package, and a regression curve was calculated.

Detection limit for 8-iso-PGF_{2α} was determined using a minimum signal-to-noise ratio (S/N) of three. The limit of quantification was defined as the lowest standard concentration on the calibration curve. To test the assay stability and reproducibility, the precision and accuracy of intra-day and inter-day measurements were assessed. Intra-day precision was assessed by measuring the seven standard solutions mentioned

above four times per day. Inter-day precision used seven standard solutions continuously running for 3 days. The calibration standards were measured at the beginning and the end of the actual sample measurements for quality control. One level of internal standard was injected at the beginning and every 10 samplings to monitor the assay precision. Analytical recovery was determined by adding 8-iso-PGF_{2α} to a urine sample at three concentrations (2.5, 5.0, and 10.0 ng/ml) and analyzing the specimens. There was no ion suppression observed; however, the addition of a deuterated internal standard, adequate preparation steps, and the use of electrospray ionization can compensate for this artifact if it occurs.

Statistical Analysis

Data were analyzed using SPSS ver11.5.0 software. A nonparametric Wilcoxon Signed Rank Test was used to compare the levels of 8-iso-PGF_{2α} measured before and after CPB. Results were considered to be statistically significant when *P* values were smaller than 0.05. Figure analysis was performed with SigmaPlot ver10.0. software.

RESULTS

Under negative electrospray ionization mode, the most abundant molecular ions were *m/z* 353 for 8-iso-PGF_{2α} and *m/z* 357 for 8-iso-PGF_{2α}-d₄, respectively. The molecular structures and product ion spectra of 8-iso-PGF_{2α} and 8-iso-PGF_{2α}-d₄ are shown in Figure 1. The most common daughter ions were *m/z* 193 for 8-iso-PGF_{2α} and *m/z* 197 for 8-iso-PGF_{2α}-d₄, respectively.

Figure 2 shows the LC-MS/MS chromatograms of a standard solution, containing 20 ng/ml of 8-iso-PGF_{2α} with 100 ng/ml internal standards, respectively, detected under SRM detection mode for ion pairs of *m/z* 353/193, and using 357/197 as an internal standard. Retention times of 8-iso-PGF_{2α} and 8-iso-PGF_{2α}-d₄ were 5.82 and 5.79 min, respectively.

The SRM chromatograms of 8-iso-PGF_{2α} and its isomers extracted from urine samples are presented in Figs. 3A and B, which show the results from healthy volunteers and patients sampled during surgery, respectively. The analysis of these chromatograms shows near baseline separation of 8-iso-PGF_{2α} from other compounds, suggesting that other isomers are unlikely to interfere with quantification. It is important to develop a good LC program for isomer separation because similar fragmentation patterns will be observed among the various isomers.

The performance of the LC-MS/MS method was subsequently evaluated. Seven standard solutions, containing 0.25–20 ng/ml of 8-iso-PGF_{2α}, were used to

Fig. 1. Product ion spectra of 8-iso-PGF_{2α} (A) and 8-iso-PGF_{2α}-d₄ (B). Product ions *m/z* 193 and *m/z* 197 were monitored for 8-iso-PGF_{2α} and 8-iso-PGF_{2α}-d₄, respectively. 8-iso-PGF_{2α}, 8-iso-prostaglandin F_{2α}.

Fig. 2. LC-MS/MS chromatogram of a standard solution of 5 ng/ml 8-iso-PGF_{2α} and PGF_{2α} with their internal standards 100 ng/ml. Peak (A) 8-iso-PGF_{2α} (B) PGF_{2α}. LC-MS/MS, liquid chromatography–tandem mass spectrometry; 8-iso-PGF_{2α}, 8-iso-prostaglandin F_{2α}.

evaluate the sensitivity and linearity of the method. The calibration graphs for 8-iso-PGF_{2α} were obtained by plotting the peak area ratio of the standard to that of the internal standard. The assay showed good linear behavior within the tested ranges, with a correlation coefficient of $r^2 > 0.995$. When the S/N was three, the

limit of detection was calculated for 8-iso-PGF_{2α} to be 0.25 pg (5 μ l) injected on the column, corresponding to a sample concentration of 0.05 ng/ml. Inter-assay CVs were 2.51–6.67% and intra-assay CVs were 2.02–10.87%. The accuracy of inter-day and intra-day measurements ranged from 87.9 to 108.1%. Absolute recovery of 8-iso-PGF_{2α} from spiked urine samples ranged from 85 to 103% at all three investigated levels. After injecting 100 ng/ml of the available standards, the carry-over was less than 0.01%.

For the 12 healthy volunteers (five women and seven men, 36.8 ± 10.6 years old), the concentration of urinary 8-iso-PGF_{2α} ranged from 0.187 to 0.761 ng/mg creatinine, and the average level $\pm$ standard deviation was 0.481 ± 0.212 ng/mg creatinine (data not shown). Our results were comparable to previously published reference ranges (16,22,24). Urine samples were collected from 14 patients receiving cardiac surgery combined with CPB at different time points. Figure 4 shows the results for 8-iso-PGF_{2α} detected by using LC-MS/MS in urinary samples from cardiac surgery patients at different steps during the procedure. The urinary 8-iso-PGF_{2α} levels in samples collected before anesthesia ranged from approximately 0.122 to 1.004 ng/mg

Fig. 3. LC-MS/MS chromatograms of 8-iso-PGF_{2α} and its isomers extracted from urine samples spiked with the internal standards (A) healthy volunteer sample (B) patient's samples during surgery. Peak (1) 8-iso-PGF_{2α}; (2) PGF_{2α}; peak (a) 8-iso-PGF_{2α}-d₄; (b) PGF_{2α}-d₄. LC-MS/MS, liquid chromatography–tandem mass spectrometry; 8-iso-PGF_{2α}, 8-iso-prostaglandin F_{2α}.

Fig. 4. The results of 8-iso-PGF_{2α} detected by LC-MS/MS in a urinary sample from cardiac valve surgery patients at different steps of cardiopulmonary bypass. Values are presented as mean ± standard error. (T0 = baseline before anesthesia; T1 = 30 min before CPB; T2 = 25 min after the start of CPB; T3 = 25 min after the end of CPB; T4 = 2 hr after CPB; T5 = 4 hr after CPB; and T6 = 20 hr after CPB, **P* < 0.05). LC-MS/MS, liquid chromatography–tandem mass spectrometry; 8-iso-PGF_{2α}, 8-iso-prostaglandin F_{2α}.

creatinine with an average of 0.405 ± 0.239 ng/mg creatinine (mean ± standard deviation). These results showed no statistical difference from those of healthy volunteers (*P* > 0.05). Therefore, the status of pre-operative oxidative stress in patients with cardiac valve diseases is the same as that of normal individuals. From our results, the levels of 8-iso-PGF_{2α} significantly increased after bypass procedures (*P* < 0.05). At 25 min after the end of CPB, it reached a maximum that was about four times the baseline value, approximately 1.861 ± 0.883 ng/mg creatinine. Then, the concentrations of 8-iso-PGF_{2α} gradually declined and returned to the baseline after the end of CPB.

DISCUSSION

To date, coronary artery and cardiac valve diseases are the two most common indications for cardiac surgery. CPB is a standard procedure during cardiac surgery. It temporarily arrests the heart so that the surgeon can perform the operation with precision. However, during and after the bypass, significant concentrations of oxygen-free radicals can be generated, and substantial oxidative stress frequently occurs. Oxidative stress can lead to hemodynamic instability, arrhythmia, impaired immune function, as well as compromised cardiac and pulmonary functions during and after surgery (5,6). These conditions can endanger patients who already show impaired cardiopulmonary and renal function and symptoms that are commonly seen in candidates for cardiac surgery. Therefore, it is important to minimize the impact of oxidative stress generated during CPB for patients receiving cardiac surgery.

In previous clinical studies for the analysis of oxidative stress during cardiac and laparoscopic surgeries (2,4), plasma malondialdehyde (MDA) was widely used as a biomarker to evaluate free radical insult to tissues, with the thiobarbituric acid-reactive substance (TBARS) being the most commonly chosen in vitro method. However, there are problems associated with the application of this method to the analysis of biological fluids. First, many substances in biological fluids, such as sugars, amino acids, and carbohydrates, can interfere with the measurement of TBARS. Second, MDA is not only a specific product of lipid peroxidation but also formed via thromboxane synthesis in the cyclooxygenase cascade. Furthermore, free MDA produced during lipid peroxidation can react with lysine residues to form a Schiff base adduct, which will not be detected by the TBARS test (25). Finally, the MDA levels measured by TBARS are influenced by the rates of peroxide breakdown and the metabolism of both peroxides and aldehydes. Thus, peroxides and aldehydes

in foods can potentially confound the measurement of MDA and TBARS *in vivo* (26). Therefore, it is necessary to find a more sensitive and specific biomarker that can substitute for plasma MDA in the evaluation of oxidative stress during surgery.

8-iso-PGF_{2α} is a product of the free radical-catalyzed peroxidation of arachidonic acid, which is produced independently of the cyclooxygenase enzyme. It has been widely accepted as the most reliable marker for endogenous oxidative stress production. There are several advantages of urinary 8-iso-PGF_{2α} such as its chemical stability, the fact that it is a specific product of peroxidation, and that 8-iso-PGF_{2α} levels are unaffected by lipid content in diet. In addition, samples are easy to collect using non-invasive techniques, and no significant daily concentration variability is observed in healthy subjects (27). Therefore, it is a more appropriate biomarker for the evaluation of oxidative stress in patients during surgery. Work previously published by Delanty et al. (28) measured 8-iso-PGF_{2α} by using GC/MS in five patients receiving coronary artery bypass grafting. However, for the quantification of 8-iso-PGF_{2α}, the accuracy and precision of GC/MS are not as good as those achieved by LC-MS/MS. Therefore, using the latter technology to develop an increasingly accurate and efficient quantification method for the detection of urinary 8-iso-PGF_{2α} is of great interest. We herein present a rapid and precise LC-MS/MS assay for the quantification of urinary 8-iso-PGF_{2α} that can be applied to samples collected periodically during cardiac surgery, and in particular during procedures such as CPB.

In previous reports, several useful protocols for the quantification of urinary 8-iso-PGF_{2α} by using LC-MS/MS have been presented. However, new challenges were encountered in attempting to adapt the SPE sample preparation methods of other groups, such as the modified SPE procedures reported by Liang et al. (16). Although the results from healthy volunteers and pre-operative patients were satisfactory, the urinary samples collected after CPB presented much interference in the sample matrix, and SPE purification failed to remove this interference. This resulted in signal attenuation and decreased ability to detect the internal standard. Furthermore, larger volumes of eluent were needed to couple SPE with the LC-MS/MS measurements. To achieve detectable concentrations, samples had to be evaporated to dryness and reconstituted. This was a time-consuming step of the procedure, requiring at least 90 min in our experience. By contrast, the multidimensional SPE method introduced by Zhang et al. (29), which incorporates size exclusion, reverse-phase chromatography, and normal phase chromatography, enabled isoprostane isomers to be separated at detectable concentrations while controlling for matrix effects.

However, extensive sample washing and elution steps were involved in this procedure, which resulted in long preparation times and low-recovery rates. In our experience, more than 3 hr was required for the extraction step, and recovery rates were less than 30%. The complexity of this procedure makes it less than ideal for analyzing large numbers of clinical samples. In the case of the method developed by Saenger et al. (23), which benefits from reduced ion suppression by the ESI source, much less sample preparation time was required. However, some samples collected during CPB contained very low concentrations of the biomarker, and matrix effects in these samples masked the detection of the analyte at such low concentrations. Finally, we modified the SPE procedures introduced by Yan et al. (21) by increasing the volume of organic solvent used for analyte elution to 160 μl. This resulted in an increased recovery rate of more than 90%. The SPE step succeeded in efficiently eliminating interference from the matrix while achieving an acceptable recovery rate with less overall processing time than other methods.

Several problems were also encountered with the LC methods. As described above, urine samples collected during surgery were very complex, making the LC mobile phase critical to the performance of the method. Although a variety of mobile phases had been described by previous reports for the detection of 8-iso-PGF_{2α} (16,21,23), none of these produced suitable detection levels in this study. In the method described by Yan et al. (21), the use of buffering with ammonium hydroxide resulted in improved ionization intensities than that with ammonium acetate. However, the pH value of mobile phases was greatly increased up to 11, and the column (Phenomenex Luna C18) was destroyed, with subsequent contamination of the spray cone interface. While applying Saenger's protocol (23), the Phenomenex Gemini C18 column tolerated the high pH values, but the LC program failed to produce complete separation of 8-iso-PGF_{2α} isomers. Finally, we used ammonium hydroxide as the buffer solution in a Phenomenex Gemini C18 column and modified the linear gradient of the LC program with the initial organic solvent concentration increased from 5 to 11% and a linear increase to 20% after 6 min, resulting in complete separation of the 8-iso-PGF_{2α} isomers.

Thus, after modifying the previous methods mentioned above, our protocol was successful in rapid sample extraction and excellent LC separation. These modifications also shortened total run times from 20 to 10 minutes compared with previous reports.

An unexpected finding in this study was that the pre-operative levels of urinary 8-iso-PGF_{2α} in patients with cardiac valve diseases were the same as those of healthy individuals ($P > 0.05$). This finding may suggest that

increased levels of 8-iso-PGF_{2α} are only observed in patients with severe pulmonary hypertension and congestive heart failure (30,31). We also observed that the levels of urinary 8-iso-PGF_{2α} increased up to three times the baseline value after the bypass procedure had been underway for 25 min, reaching maximum values that were approximately four times baseline levels at 25 min after the end of CPB. Therefore, our study clearly demonstrated the great impact of oxidative stress during and after CPB. In addition, we also noted that the highest levels of plasma 8-iso-PGF_{2α} occurred after the end of CPB, and this was also a period in which patients presented the greatest hemodynamic instability during surgery. It has been reported that 8-iso-PGF_{2α} is a potent vasoconstrictor in the lungs and kidneys (31), and it could increase the pulmonary artery pressure after the end of CPB, or even result in impairment of the left ventricular preload, compromising the hemodynamics of the ventricle. It is known that patients receiving CPB experience acute renal injury with an incidence of up to 30%, and it has been suggested that the cause of these complications is related to neutrophil activation, which is a major source of ROS release (32). Therefore, prescribing antioxidants before or during CPB could potentially be helpful for these patients. With our LC-MS/MS protocol, it becomes possible to accurately evaluate the oxidative injury at any time point during operation. This would be useful for comparing and selecting better operative procedures with less oxidative stress and for monitoring the effects of antioxidant therapy with precision in future clinical trials.

In addition to 8-iso-PGF_{2α}, some new biomarkers for the evaluation of oxidative stress had also been discovered recently. Isofurans, a class of oxidation products of arachidonic acid, were newly reported as markers of oxidative injury (33,34). There had been several in vitro and animal studies showing that in settings of high oxygen tension, the levels of isofurans increased dramatically, whereas isoprostanes increased only slightly or did not change (33). Therefore, isofurans could potentially be a more sensitive marker for oxidative injury during surgeries in which oxygen inhalation is usually supplied. It would be worth examination this finding in further human studies.

CONCLUSIONS

The new LC-MS/MS method that we introduce provides a simple, rapid, and specific assay for the quantification of urinary 8-iso-PGF_{2α} in samples with complex urine matrices, such as specimens collected during CPB. This method may be used to precisely assess oxidative status in large clinical studies during complex surgical procedures.

ABBREVIATION

8-iso-PGF _{2α}	= 8-iso-prostaglandin F _{2α}
LC-MS/MS	= liquid chromatography–tandem mass spectrometry
ESI	= electrospray ionization
SRM	= selected-reaction monitoring mode
SPE	= solid phase extraction
CV	= coefficient variation
CPB	= cardiopulmonary bypass

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