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Urinary 8-epi-$PGF_{2\alpha}$ and its endogenous β-oxidation products (2,3-dinor and 2,3-dinor-5,6-dihydro) as biomarkers of total body oxidative stress

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Abstract

Although measurements of plasma F_2-isoprostanes are established markers of oxidative stress, their quantification only reflects acute non-enzymatic lipid peroxidation. In this study, a new approach is described for the rapid isolation and measurement of urinary 8-epi-$PGF_{2\alpha}$ and its endogenous β-oxidation metabolites (2,3-dinor-8-epi-$PGF_{2\alpha}$ and 2,3-dinor-5,6-dihydro-$PGF_{2\alpha}$) for use as index of total body oxidative stress. Isoprostanes were partitioned with ethyl acetate and subsequently purified by chromatography on an aminopropyl (NH_2) and silica (Si) cartridge. Final analysis of F_2-isoprostanes as trimethylsilyl-ester/pentafluorobenzyl ester derivatives was carried out by stable isotope dilution mass spectrometry. Overall recovery of F_2-isoprostanes was $80 \pm 4\%$. Inter- and intra-assay coefficients of variation were 5% and 7%, respectively. In a group of healthy humans, the mean excretion rates expressed as nmol/mmol creatinine for 2,3-dinor-8-epi-$PGF_{2\alpha}$, 2,3-dinor-5,6-dihydro-8-epi-$PGF_{2\alpha}$, and 8-epi-$PGF_{2\alpha}$ were 5.43 ± 1.93, 2.16 ± 0.71, and 0.36 ± 0.16, respectively. Correlations were obtained between 8-epi-$PGF_{2\alpha}$ and 2,3-dinor-8-epi-$PGF_{2\alpha}$ or 2,3-dinor-5,6-dihydro-8-epi-$PGF_{2\alpha}$ ($r = 0.998$ and $r = 0.937$, respectively). A strong relationship was also seen between 2,3-dinor-8-epi-PGF_2 and 2,3-dinor-5,6-dihydro-8-epi-$PGF_{2\alpha}$ ($r = 0.949$). The new technique allows for high sample throughput and avoids the need for HPLC and/or other expensive equipment required for the initial sample preparation. Simultaneous analysis of urinary 8-epi-$PGF_{2\alpha}$ and its metabolites should provide unique tool in clinical trials exploring the role of oxidant injury in human disease.

Keywords: Oxidative stress; Lipid peroxidation; F_2-isoprostanes; 8-epi-$PGF_{2\alpha}$; Prostaglandins

F_2-isoprostanes are a series of prostaglandin (PG)-like compounds produced by non-enzymatic peroxidation of arachidonic acid via a mechanism independent of the cyclooxygenase pathway [1,2]. In total, 64 F_2-isoprostane isomers can be formed and of these, 8-epi-$PGF_{2\alpha}$ has received the most attention because of its cytotoxic biological properties [3,4]. Circulating F_2-isoprostanes are mainly found esterified to phospholipids and are released by the action of phospholipase A_2 [5]. The plasma concentrations of F_2-isoprostanes have been shown to be higher in a number of clinical conditions associated with oxidative stress [5–13]. The usefulness of measurements of 8-epi-$PGF_{2\alpha}$ concentrations in plasma as index of oxidative stress suffers from a number of disadvantages. For example, it is rapidly cleared circulation and thus measurements reflect acute oxidative stress rather than chronic stress [1]. In addition, artefactual generation of 8-epi-$PGF_{2\alpha}$ can occur with improper sample handling and/or prolonged storage leading to spurious results [1,2].

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The quantification of urinary of 8-epi-$PGF_{2\alpha}$ has been proposed as being superior to the measurement of circulating 8-epi-$PGF_{2\alpha}$ since concentrations in urine provide a more precise index of chronic non-enzymatic lipid peroxidation [14]. In addition, the measurement of urinary 8-epi-$PGF_{2\alpha}$ metabolites has been put forward as a more appropriate index of systemic non-enzymatic lipid peroxidation than the parent compound 8-epi-$PGF_{2\alpha}$ [15–17].

Roberts and co-workers [18,19] employed chromatography on octadecyl silica (C_{18}) cartridge followed by two thin layer chromatography (TLC) steps for the isolation of 2,3-dinor-5,6-dihydro-8-epi-$PGF_{2\alpha}$ prior to final analysis by gas chromatography–mass spectrometry (GC–MS). Schwedhelm et al. [20] used a combined C_{18} cartridge and high performance liquid chromatography (HPLC) procedure for the simultaneous isolation of 8-epi-$PGF_{2\alpha}$ and 2,3-dinor-5,6-dihydro-8-epi-$PGF_{2\alpha}$ before final analysis by GC-tandem MS. Liang et al. [21] quantified 8-epi-$PGF_{2\alpha}$ and 2,3-dinor-8-epi-$PGF_{2\alpha}$ by HPLC-tandem MS following initial purification on C_{18} cartridge. However, to date no method has been described for the simultaneous isolation of urinary 8-epi-$PGF_{2\alpha}$ and its metabolites nor any information on the relationship between the levels of 8-epi-$PGF_{2\alpha}$ and its metabolites (2,3-dinor and 2,3-dinor-5,6-dihydro) in human.

The objectives of the present study were to develop a rapid method for the simultaneous isolation of urinary 8-epi-$PGF_{2\alpha}$ and its metabolites, and to explore the relationship between the levels of urinary 8-epi-$PGF_{2\alpha}$ and its metabolites (2,3-dinor and 2,3-dinor-5,6-dihydro) in normal subjects.

Materials and methods

Authentic 9α,11α-, 9α,11β-PGF_2, 9β,11α-PGF_2, 9α,11α-8-epi-PGF_2, 9α,11α-15*R*-8-epi-PGF_2, 9α,11α-15*R*-trihydroxy-2,3-dinor-8-epi-prosta-13*E*-en-1-oic acid (2,3-dinor-8-epi-PGF_2), 3,3′,4,4′-tetradeuterated 9α,11α-PGF_2 (PGF_2-d_4), and 3,3′,4,4′-tetradeuterated 9α,11α-15*S*-8-epi PGF_2 (8-epi-PGF_2-d_4) were obtained from SPI Bio (Massy Cedex, France). *N*,*O*-bis(Trimethylsilyl)trifluoroacetamide (BSTFA), pentafluorobenzyl-bromide (PFB-Br), diisopropylethylamine (DIPEA), and butylated hydroxytoluene (BHT) were purchased from VWR International (Poole, Dorset, UK). Aminopropyl (NH_2) and silica (Si) cartridges (500 mg) were from Waters (Milford, MA, USA). 9α,11α-15*R*-Trihydroxy-^{5,6}Δ-dihydro-2,3-dinor-8-epi-prosta-13*E*-en-1-oic acid (2,3-dinor-5,6-dihydro-8-epi-PGF_2) was a gift from Dr. Thierry Durand (Department of Pharmacy, University of Montpellier, Montpellier, France). All other chemicals were of analytical grade from VWR International (Poole, Dorset).

Subjects and sample collection

Healthy volunteers ($n = 13$; 7 males and 6 females; Age: 34.75 ± 3.36 years) were recruited from the staff of the German Diabetic Research Institute, Düsseldorf, Germany. None of the subjects were obese and all had plasma lipids within the normal range (Body mass index: 22.98 ± 0.94 kg/m^2; triglycerides: 0.85 ± 0.11 mmol/L; total cholesterol: 5.16 ± 0.25 mmol/L; LDL-cholesterol: 3.22 ± 0.21 mmol/L; and HDL-cholesterol: 1.54 ± 0.10 mmol/L). None had any sign of kidney disease and all subjects had normal liver function. In addition, none of the subjects were smokers nor on any special diets or taking antioxidants. This study was approved by the Ethical Committee at Diabetic Research Institute, Düsseldorf.

Urine samples were collected over a 12 h fasting period overnight and aliquots (10 ml) were stored at −85 °C until analysed.

Isoprostane analysis

Total lipid extraction. Urine samples (2 ml) were transferred to glass vials and acidified by addition of HCl (50 μl; 4 M). 8-epi-$PGF_{2\alpha}$-d_4 (2.5 ng in 100 μl ethanol) was added as internal standard and the sample was allowed to equilibrate for 10 min. Ethyl acetate (10 ml) was then added and samples were vortex-mixed followed by centrifugation at 2500*g* for 5 min at room temperature. The organic (upper) phase was aspirated and taken as the total lipid extract [22].

Aminopropyl chromatography and pentafluorobenzyl ester derivatisation. NH_2 cartridges were pre-conditioned with hexane (5 ml) and total lipid extracts were applied. The cartridges were washed with ethyl acetate (10 ml) and isoprostanes were eluted with 5 ml ethyl acetate/methanol/acetic acid (10/85/5, v/v/v). The final extracts were dried under a stream of N_2 at 45 °C. Pentafluorobenzyl (PFB)-Br (40 μl, 10% in acetonitrile, v/v) was added followed by DIPEA (20 μl; 10% in acetonitrile, v/v) and kept at 45 °C for 30 min. The samples were again dried under N_2 at 45 °C and the residues were re-dissolved in 2 ml hexane/ethyl acetate (20/80, v/v) [23,24].

Si chromatography and trimethylsilyl (TMS) ether derivatisation. Samples from PFB-derivatization step were applied to Si cartridges pre-washed with hexane (5 ml). The cartridges were washed with 3 ml hexane/ethyl acetate (20/80, v/v) and isoprostanes were eluted by washing the cartridge with 5 ml ethyl acetate/methanol (95/5, v/v). The eluates were dried under N_2 at 45 °C. BSFTA (50 μl) followed by DIPEA (5 μl; 10% in acetonitrile) was added, and the samples werer heated for 30 min at 45 °C, dried under N_2, and finally re-suspended in *iso*-octane (40 μl).

Gas chromatography–mass spectrometry

Analyses were carried out on a Hewlett–Packard 5890 GC linked to a VG70SEQ MS using the negative ion chemical ionisation (NICI) with ammonia as reagent gas. Separation was carried out on an SPB-1701 column (30 m × 0.25 mm ID; 0.25 μm film thickness, SUPLECO Dorset, UK). Samples (2 μl) were injected into a temperature programmed Gerstel injector (initial temperature 175 °C; initial time: 2 min; rate: 30 °C/min; final temperature: 270 °C; and final time: 30 min). Quantitative analysis of 8-epi-$PGF_{2\alpha}$ and its metabolites as PFB-ester/TMS ether derivatives was performed using selected ion monitoring (SIM) of the carboxylate anion $[M\text{-}PBF]^-$ at *m*/*z* 541, 543, 569, and 573 for 2,3-dinor-5,6-dihydro-8-epi-$PGF_{2\alpha}$, 2,3-dinor-8-epi-$PGF_{2\alpha}$, 8-epi-$PGF_{2\alpha}$, and 8-epi-$PGF_{2\alpha}$-d_4, respectively.

Results

Gas chromatographic–mass spectrometric separation

Fig. 1 shows selected ion monitoring (SIM) of the carboxylate anion $[M\text{-}PFB]^-$ chromatograms of a mixture of authentic PGF_2-like compounds as PFB-ester/TMS ether derivatives. The upper spectrum monitored at *m*/*z* 541 shows the elution of 2,3-dinor-5,6-dihydro-8-epi-$PGF_{2\alpha}$. The second chart shows (*m*/*z* 543) the