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Metabolism of 8-iso-prostaglandin $F_{2\alpha}$

S. Basu*

Department of Geriatrics, Faculty of Medicine, Uppsala University, S-751 25 Uppsala, Sweden

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Abstract Tritium labelled ($\bar{x}$ =1.1 MBq/17.7 μg/kg) and unlabelled 8-iso-$PGF_{2\alpha}$ (43 μg/kg) were administered intravenously to female rabbits and frequent blood and continuous urinary samples were collected up to 4 h. The total radioactivity was lost rapidly from the circulation. About 80% of the total radioactivity was found in urine within 4 h. The plasma half-life of 8-iso-$PGF_{2\alpha}$ is found to be 1 min at the distribution phase. The terminal elimination phase half-life was about 4 min. At 1.5 min after administration 64%, 19% and 13% of the plasma radioactivity represented 8-iso-$PGF_{2\alpha}$, 15-keto-8-iso-$PGF_{2\alpha}$ and β-oxidised products, respectively. The values for 20-min plasma were 5%, 2% and 88%. The radiochromatograms from 10 min–4 h urinary samples were dominated by more polar β-oxidised products. α-Tetranor-15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$ was identified as a major urinary metabolite.Thus, 8-iso-$PGF_{2\alpha}$ metabolises in the rabbit mainly to several degraded polar metabolites through dehydrogenation at C-15, reduction of Δ^{13}-double bond and β-oxidation, and excretes efficiently into the urine.

Key words: Metabolism; Isoprostane; 8-iso-Prostaglandin $F_{2\alpha}$; Eicosanoid; Pharmacokinetics; Lipid peroxidation

1. Introduction

Free radicals are believed to be involved in the aging process as well as in a number of diseases including atherosclerosis, diabetes, cancer, Alzheimer's disease, etc. [1–5]. Non-enzymatic lipid peroxidation through free radical catalysis of unsaturated fatty acid oxidation is presumed to be implicated in several diseases involving oxidative injury. Isoprostanes, a series of newly discovered prostaglandin like compounds, are biosynthesised in vivo through free radical oxidation of arachidonic acid, circulate in peripheral blood and are excreted in urine in man and animals [6–9]. 8-Iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) which is a major isoprostane and exhibits potent biological activity is elevated in several diseases that are shown to be associated with various forms of oxidant injury. These include smoking, diabetes, liver cirrhosis, vascular reperfusion and excess alcohol intake [10–14]. Occurrence of F-ring isoprostane metabolites in human urine has previously been reported [15]. However, a systematic metabolism study of 8-iso-$PGF_{2\alpha}$ is lacking. In this study, the in vivo metabolism of 8-iso-$PGF_{2\alpha}$ in the rabbit is investigated.

*Fax: (46) (18) 177976.
E-mail: Samar.Basu@geriatrik.uu.se

Abbreviations: GC-MS, gas chromatography-mass spectrometry; RIA, radioimmunoassay; TMS, trimethylsilyl; BSTFA, bis(trimethylsilyl)trifluroacetamide; HPLC, high pressure liquid chromatography; RP, reversed-phase; SP, straight-phase; i.v., intravenous; PG, prostaglandin; 15-PGDH, 15-hydroxy prostaglandin dehydrogenase

2. Materials and methods

2.1. Materials

Unlabelled 8-iso-$PGF_{2\alpha}$, other related isoprostanes and prostaglandins were purchased from Cayman Chemicals, Ann Arbor, MI, USA. Tris-HCl, Tris-base, EDTA-disodium salt and bovine-γ-globulin were purchased from Sigma Chemicals (St. Louis, MO, USA). Instagel scintillation cocktail was obtained from Packard Instruments Co. (Meriden, CT, USA). Polyethylene glycol (MW 4000) was purchased from Merck (Germany). Tris-HCl buffer 0.05 M, pH 7.8, was used in the radioimmunoassay (RIA). Bovine γ-globulin 0.5% was prepared in the RIA buffer. Glucose solution was obtained from Pharmacia&Upjohn, Uppsala, Sweden. Unlabelled 8-iso-$PGF_{2\alpha}$ standards, tritium labelled tracer and working antibody solution were prepared in the RIA buffer. The tritium labelled 8-iso-$PGF_{2\alpha}$ (specific activity: 608 GBq/mmol) was synthesized and purified as described previously [16,17]. The tritium labelled 8-iso-$PGF_{2\alpha}$ was further diluted with unlabelled 8-iso-$PGF_{2\alpha}$ to obtain a final concentration of the substance which was subsequently administered to the experimental animals.

2.2. Animal experiment

Two rabbits (New Zealand White rabbits, female, Lidköping Kanin Farm, Lidköping, Sweden) of body weight 3.7 and 4.5 kg were used in this study after acclimatisation to the laboratory conditions. The animals were anaesthesized by a bolus dose of 3 ml Ketalar and Rompun (3:1) and further anaesthesia (0.5–1 ml) was given to the animals at about every half-hour until the end of the experiment. Blood pressure, respiration, electrolytes and body temperature were checked continuously during the experiments. One ml of saline was given to the animals through the vena femoralis every half hour. The animals were killed at the end of the experiment by the administration of an excess dose of Mebumal Vet (Apoteksbolaget, Sweden).

2.3. Experiment 1

Tritium labelled 8-iso-$PGF_{2\alpha}$ (1.24 MBq/22.2 μg/kg) in glucose solution was administered intravenously (vena femoralis) to a rabbit. Blood samples were collected in heparinised tubes from femoral artery through a catheter at different intervals (blood: 1.5, 5, 20, 30, 60, 120 and 240 min; urine: 0–30, 30–60, 60–90, 90–120, 120–180 and 180–240 min). Plasma was separated and stored frozen at −70°C until analysis. Similarly, urinary samples were collected continuously in portions in glass vials at different intervals from urinary bladder after catheterization. The samples were stored frozen at −70°C until analysis.

2.4. Experiment 2

Unlabelled 8-iso-$PGF_{2\alpha}$ (43 μg/kg) in glucose solution was administered intravenously (vena femoralis) to a rabbit. Blood samples were collected as in experiment 1 at 0, 1.5, 5, 10, 15, 20, 30, 45, 60, 90, 120 and 150 min after the administration of the substance. Urinary samples were collected similarly as in experiment 1 at different intervals (0–10, 10–20, 20–30, 30–60, 60–90, 90–120 and 120–150 min). Samples were treated as before and stored frozen at −70°C until analysis.

2.5. Experiment 3

After the end of the second experiment tritium labelled 8-iso-$PGF_{2\alpha}$ (0.9 MBq/13.2 μg/kg) in glucose solution was administered intravenously (vena femoralis) to the same rabbit. Blood samples were collected as in experiment 1 at 1.5, 5, 10, 15, 20, 30, 45, 60 and 90 min. Urinary samples were collected at different intervals of 0–10, 10–20, 20–30, 30–60 and 60–90 min. Samples were treated as before and stored frozen at −70°C until analysis.

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2.6. Purification, separation and identification

Total radioactivity was counted in all plasma and urinary samples before chromatography. To extract 8-iso-$PGF_{2\alpha}$ and its metabolites, the plasma and urinary samples were purified by using SEP-PAK C_{18} column containing octadecylsilyl silica gel (Waters Associates, USA) according to Basu and Kindahl with minor modifications [18]. In brief, radiolabelled plasma and urinary samples were acidified to pH 3.5 by 0.1 M HCl. The samples were passed through the SEP-PAK C_{18} columns and the columns were eluted with 5 ml of 20% ethanol and 10 ml of petroleum ether. 8-iso-$PGF_{2\alpha}$ and its metabolites were eluted with 5 ml of methyl formate. Ten ml of 80% ethanol was used to elute the rest material from the column. Radioactivity was counted in all fractions. The major portion of the radioactivity was found in the methyl formate fractions which were evaporated to dryness under N_2 and dissolved in ethanol. The samples were analysed by RP-HPLC (Merck-Hitachi L-6000, Germany) using 5 μM Nucleosil C_{18} column (Machery and Nagel, Germany) and the products were detected by an on-line radioactivity detector (Flow-one A-200, Radiomatic Inc., USA). Separation of 8-iso-$PGF_{2\alpha}$ and its metabolites was performed by using a solvent system consisting of 35% acetonitrile and 65% water containing 0.1% acetic acid (v/v) as previously described for prostaglandins [19]. Flow rate was constant at 1 ml/min. Fractions from the major metabolite were collected after RP-HPLC separation, evaporated under N_2 and further purified by an SP-phase HPLC. This separation was performed by using a Nucleosil 50-5 column with a solvent system of 15% isopropanol and 85% hexane with 0.1% acetic acid (v/v). Flow rate was constant at 1.5 ml/min. Three separate peaks were obtained from the major peak collected from RP-HPLC (A, Fig. 2, right panel). Both RP- and SP-HPLC fractions from these peaks were subjected to GC-MS identification after derivatisation.

2.7. Derivatisation and GC-MS

Urinary samples from HPLC purified peaks were evaporated to dryness under N_2 and trimethylsilyl ethers were prepared by treatment with BSTFA and pyridine [20]. Methyl esters were prepared with diazomethane [20]. A capillary GC (Varian 3100) with a non-polar column (30 m, DB-5, J&W Scientific, film, 0.25 μm; diameter, 0.25 mm, carrier gas He, 15 psi) and an ion trap mass spectrometer (ITS40, Finnigan MAT) were used [20]. After splitless injections of samples in heptane, the GC was programmed from 120 to 200°C with 40°C/min to 260°C with 28°C/min and then to 285°C with 3°C/min.

2.8. Radioimmunoassay of 8-iso-$PGF_{2\alpha}$

The plasma and urinary samples from the pharmacokinetic study with unlabelled 8-iso-$PGF_{2\alpha}$ were analysed for 8-iso-$PGF_{2\alpha}$ by a newly developed radioimmunoassay at our laboratory as described elsewhere [16]. In brief, unextracted plasma and urinary samples were used in the assay. The cross-reactivity of the 8-iso-$PGF_{2\alpha}$ antibody with 15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$, 8-iso-$PGF_{2\beta}$, $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$, TXB_2, 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$ and 8-iso-$PGF_{3\alpha}$, was, respectively, 1.7, 9.8, 1.1, 0.01, 0.01, 0.1, 0.03, 1.8 and 0.6%. The detection limit of the assay was about 23 pmol/l.

3. Results

3.1. Pharmacokinetics of 8-iso-$PGF_{2\alpha}$

The total radioactivity in the plasma samples collected after intravenous administration of 3.14 MBq ^{3}H-labelled 8-iso-$PGF_{2\alpha}$ to a rabbit is shown in Fig. 1 (upper panel). Similarly, the concentration of radioactivity corresponding to 8-iso-$PGF_{2\alpha}$ levels as calculated in the radiochromatograms after separation of the substance from the total radioactivity profile is shown in Fig. 1 (upper panel). The highest level of radioactivity and 8-iso-$PGF_{2\alpha}$ was found at 1.5 min after the intravenous administration of the radiolabelled 8-iso-$PGF_{2\alpha}$. The total excretion of radioactivity in urine was about 80% during 4 h after the administration of the tritium labelled 8-iso-$PGF_{2\alpha}$.

The appearance and disappearance of 8-iso-$PGF_{2\alpha}$ in the plasma and urine after intravenous administration of 159 μg

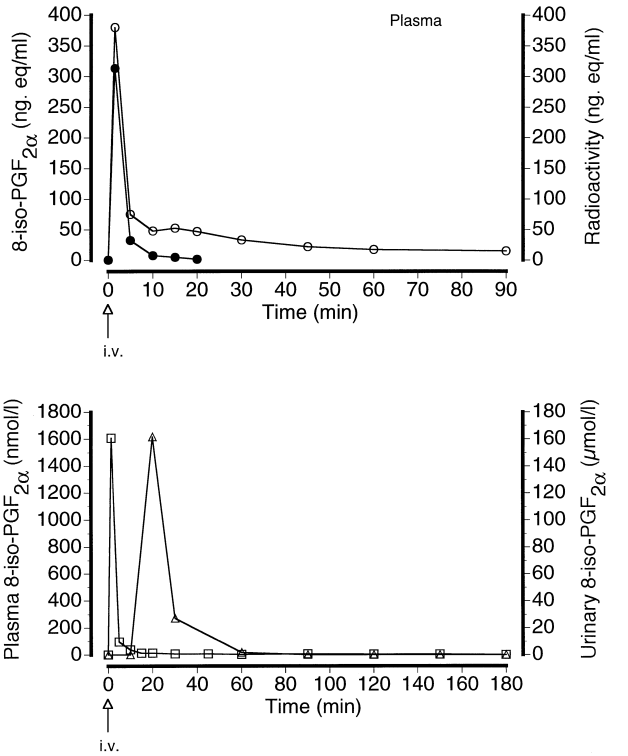

Fig. 1. The kinetics of total radioactivity (open circle) and [^{3}H]8-iso-$PGF_{2\alpha}$ (solid circle) in plasma following i.v. administration of 0.9 MBq/13.2 μg/kg (corresponding to 49 μg) of [^{3}H]8-iso-$PGF_{2\alpha}$ to a rabbit (upper panel). The kinetics are shown of 8-iso-$PGF_{2\alpha}$ in plasma (open square) and urine (open triangle) following i.v. administration of 43 μg/kg (corresponding to 159 μg) of unlabelled 8-iso-$PGF_{2\alpha}$ to a rabbit (lower panel).

of unlabelled 8-iso-$PGF_{2\alpha}$ to a rabbit as analysed by the RIA is shown in Fig. 1 (lower panel). Following 8-iso-$PGF_{2\alpha}$ administration, the increase in 8-iso-$PGF_{2\alpha}$ in the circulation was very rapid. Maximum concentration (1606 nmol/l) in the peripheral plasma was reached at 1.5 min after the administration of the substance. The level then decreased rapidly. Three hours after application the 8-iso-$PGF_{2\alpha}$ level decreased to 0.1 nmol/l (lower panel, Fig. 1). The urinary concentration of 8-iso-$PGF_{2\alpha}$ reached its height (161 μmol/l) at 20 min following the administration of 8-iso-$PGF_{2\alpha}$. Three hours after the application the 8-iso-$PGF_{2\alpha}$ levels decreased to 62 nmol/l (lower panel, Fig. 1). The plasma distribution phase half-life (α-phase) of 8-iso-$PGF_{2\alpha}$ is found to be about 1 min. The terminal elimination phase half-life (β-phase) is shown to be about 4 min.

3.2. Chromatographic profiles of [^{3}H]8-iso-$PGF_{2\alpha}$

Representative chromatographic profiles (RP-HPLC) obtained in plasma samples at various times (1.5, 5 and 20 min) after the administration of [^{3}H]8-iso-$PGF_{2\alpha}$ to a rabbit is shown in Fig. 2 (left panel). Peak 1 chromatographed as 8-iso-$PGF_{2\alpha}$, peak 2 as 15-keto-8-iso-$PGF_{2\alpha}$ and peaks with retention times between 1 and 5 min represented degraded shorter polar metabolites of 8-iso-$PGF_{2\alpha}$. About 83, 43 and 2% of the total radioactivity represented unconverted [^{3}H]8-iso-$PGF_{2\alpha}$ at 1.5, 5 and 20 min, respectively, after the administration of [^{3}H]8-iso-$PGF_{2\alpha}$.

Similarly, representative chromatographic profiles obtained

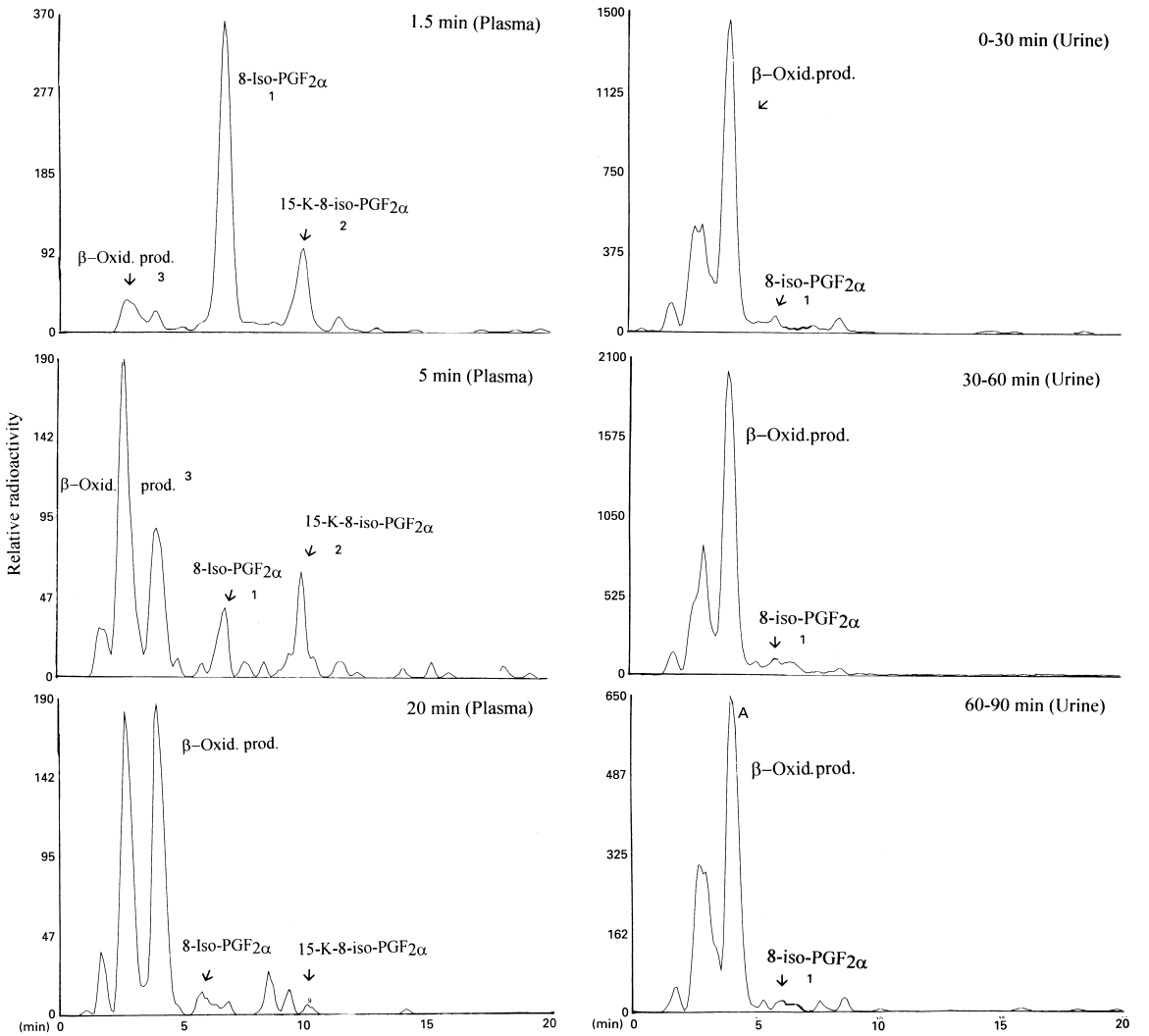

Fig. 2. Representative RP-HPLC separation of plasma (left panel) and urine (right panel) at different times after i.v. administration of 1.24 MBq/22.2 μg/kg (corresponding to 100 μg) of [^{3}H]8-iso-PGF$_{2\alpha}$ to a rabbit. Known peaks are designated in the chromatogram.

in urinary samples at various times (0–30, 30–60 and 60–90 min) after the administration of [^{3}H]8-iso-PGF$_{2\alpha}$ to a rabbit are shown in Fig. 2 (right panel). Peak 1 chromatographed as 8-iso-PGF$_{2\alpha}$ and peaks with retention times between 1 and 5 min represented degraded polar metabolites of 8-iso-PGF$_{2\alpha}$. About 2 and 7% of the total radioactivity represented unconverted [^{3}H]8-iso-PGF$_{2\alpha}$ at 0–30 min and 10–20 min (data not shown), respectively, after the administration of [^{3}H]8-iso-PGF$_{2\alpha}$.

3.3. Identification of β-oxidation metabolites of 8-iso-PGF$_{2\alpha}$

The RP-HPLC fractions from the urinary major peak (A, Fig. 2, right panel) separated by SP-HPLC into three major peaks. All these peaks (peak A from RP-HPLC and three peaks from SP-HPLC) were analysed by GC-MS as methyl ester TMS ether derivatives. Except peak A (see below) from RP-HPLC none of the peaks contained enough quantity of material for final structural determination. Thus, no further final identification of the other peaks was possible. However, the mass spectra from these peaks showed that these peaks contained most probably several β-oxidised products.

3.4. α-Tetranor-15-keto-13,14-dihydro-8-iso-PGF$_{2\alpha}$

The mass spectrum and derivatised molecular structure of this metabolite (RP-HPLC, peak A, Fig. 2, $C_{23}H_{46}O_5Si_2$) are shown in Fig. 3. The mass spectrum showed signals at *m/z* 458 (M^+) which is in accordance with the expected molecular ion of this metabolite. Strong signals were obtained at *m/z* 443 ($M^+ -15$), 368 ($M^+ -90$), 353 ($M^+ -90+15$) indicating cleavages of CH_3-, $(CH_3)_3SiO$- and $(CH_3)_3SiO$-CH_3-groups, respectively. α and β cleavages indicating a keto group at C-11 were observed at *m/z* 387 ($M^+ -90+71$), 254 ($M^+ -90+b+1$) and 181 ($M^+ -2\times89+d$). Other prominent ions were observed at *m/z* 281 ($M^+ -90+a$) and 241 ($M^+ -90+c$) due to the loss of the entire ω-side chain containing a keto group at C-15 and a reduced double bond at C-13,14 position. Other strong signals were seen at *m/z* 278 ($M^+ -2\times90$), 266 ($M^+ -2\times90+15$), 247 ($M^+ -2\times90+31$), 217 and 191. The final structure of α-tetranor-15-keto-13,14-dihydro-8-iso-PGF$_{2\alpha}$, a major urinary metabolite of 8-iso-PGF$_{2\alpha}$, is shown in Fig. 4.

4. Discussion

This in vivo metabolism study of 8-iso-PGF$_{2\alpha}$ in the rabbit

demonstrates that this compound eliminates from the circulation very rapidly after a single intravenous administration of unlabelled or tritium labelled 8-iso-$PGF_{2\alpha}$ as described for other cyclooxygenase catalysed prostaglandins [21,22]. Results from the radio-HPLC and RIA after the administration of both unlabelled and radiolabelled substances correlated well with each other. After intravenous administration, a brief distribution phase with a half-life (α-phase) of about 1 min was observed in the plasma. The terminal elimination phase half-life (β-phase) was found to be about 4 min. Only 1.5 and 5 min after administration 36% and 92% of tritium labelled 8-iso-$PGF_{2\alpha}$ was found to be as degraded metabolites of the parent compound, respectively. It was previously shown that most of the primary prostaglandins administered intravenously were degraded within a few minutes [21–23].

It was also observed that most of the radioactivity excreted via urine (about 80% during 4 h) mainly consists of several degraded polar metabolites and a low percentage of the parent compound. A certain amount of unmetabolised 8-iso-$PGF_{2\alpha}$ were also found in the urine when it was quantified by a specific radioimmunoassay as also seen in the radiochromatography of the labelled urinary samples. The excretion of unmetabolised $PGF_{2\alpha}$ or its major metabolite 15-keto-13,14-dihydro-$PGF_{2\alpha}$ had previously been described [24–26].

The RP-HPLC separation of 8-iso-$PGF_{2\alpha}$ and its metabolites in plasma shows that already 5 min after administration 8-iso-$PGF_{2\alpha}$ had degraded to several metabolites. Twenty minutes after administration most of the parent compound was found to be metabolised to several other polar compounds. Similar polar compounds were found in the urine collected between 10–20 min. However, urine collected between 0–10 min contained only 8-iso-$PGF_{2\alpha}$ (data not shown). After 20 min most of the products found in the urine consist of several β-oxidised products as identified by different chromatographic systems and also by GC-MS. One of the major urinary metabolites was identified as α-tetranor-15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$. Several other polar metabolites of 8-iso-$PGF_{2\alpha}$ were also seen in the GC-MS analysis of the urinary samples. However, no final structural determination of these metabolites was possible due to the limited amounts of the unlabelled purified compounds for further GC-MS analysis. These unidentified polar metabolites are most probably various forms of β-oxidised products as seen for $PGF_{2\alpha}$ [22].

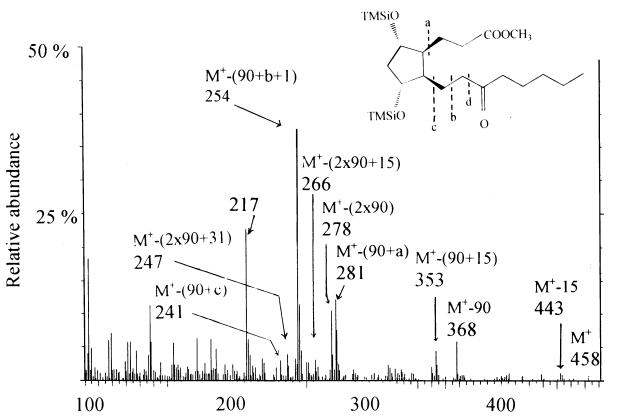

Fig. 3. Mass-spectrum of the methyl ester and TMS derivative of a major urinary metabolite, α-tetranor-15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$ (peak A, Fig. 2).

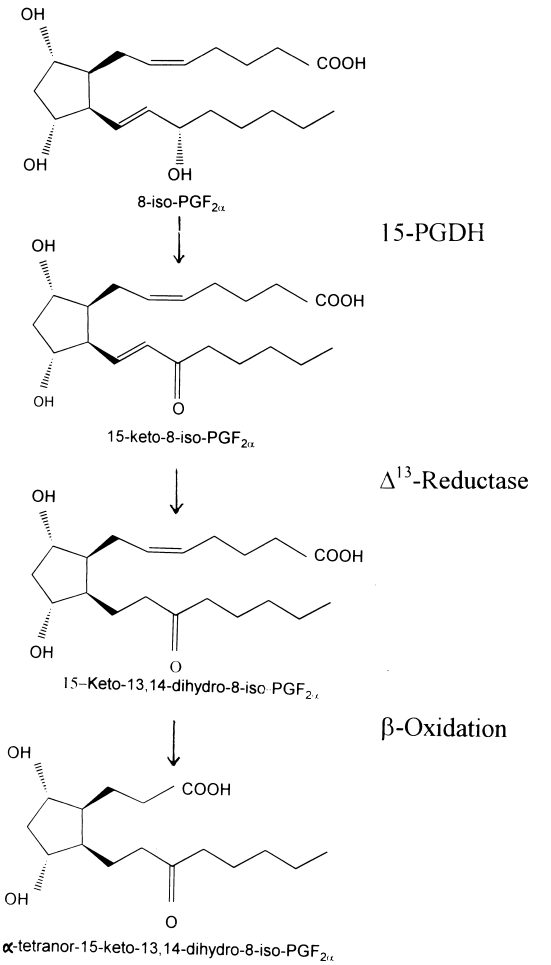

Fig. 4. Tentative metabolic pathway of 8-iso-$PGF_{2\alpha}$ in the rabbit.

The metabolism of 8-iso-$PGF_{2\alpha}$ to α-tetranor-15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$ and other β-oxidised products occurs in several steps in the rabbit which is very similar to $PGF_{2\alpha}$ metabolism in this species [21–23]. A tentative metabolic pathway of 8-iso-$PGF_{2\alpha}$ in the rabbit is shown in Fig. 4. In vitro studies with tissue enzymes (unpublished observations, Basu) and in vivo experiments (this study) in plasma show that oxidation of the 15-hydroxy group at C-15 by 15-PGDH was the first step of 8-iso-$PGF_{2\alpha}$ metabolism. A reduction of C-13,14 double bond by Δ^{13}-reductase and formation of 15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$ was shown to occur in the second step of metabolism as previously demonstrated for $PGF_{2\alpha}$ metabolism in various species [21–23]. Both β-oxidation and ω-oxidation are very common steps of prostaglandin metabolism. This study also clearly shows that 15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$ rapidly metabolises through β-oxidation mainly to α-tetranor-15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$ and several other β-oxidised metabolites. β-Oxidised metabolites of 8-iso-$PGF_{2\alpha}$ was also identified in human urine [15].

In conclusion, this study shows that 8-iso-$PGF_{2\alpha}$ when administered intravenously is metabolised mainly through dehydrogenation at C-15, reduction of C-13,14 double bond and β-oxidation of the α-chain to several polar compounds and eliminates rapidly from the circulation in a similar manner as the other primary prostaglandins. One of the major urinary

metabolites of 8-iso-$PGF_{2\alpha}$ in the rabbit is identified as α-tetranor-15-keto-13,14-dihydro-8-iso-$PGF_{2\alpha}$. The plasma elimination half-life was found to be short and a high recovery of the radioactivity as determined by polar β-oxidised compounds was observed in the urine. Unmetabolised 8-iso-$PGF_{2\alpha}$ was also found in the urine to some extent.

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