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F_2-isoprostane induced prostaglandin formation in the rabbit

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Abstract

F_2-isoprostanes, non-enzymatic free radical mediated products of arachidonic acid, have shown to form during various oxidant stress status and have potent biological effects. This study investigates to what extent 8-iso-$PGF_{2\alpha}$ (a major F_2-isoprostane), a bioactive product of lipid peroxidation can modify endogenous prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) formation since prostaglandins are inflammatory as well as potent vasoregulatory substances that modulate diverse important physiological functions, and also form during acute and chronic inflammation. An immediate appearance and disappearance of 8-iso-$PGF_{2\alpha}$ was seen in both plasma and urine within a short interval after i.v. administration of 43 μg/kg of 8-iso-$PGF_{2\alpha}$ to the rabbits. A successive but differential formation of $PGF_{2\alpha}$ resulted in a rapid and pulsatile increase of plasma 15-keto-dihydro-$PGF_{2\alpha}$, a major metabolite of primary $PGF_{2\alpha}$. Later, this compound was excreted efficiently as intact compound into the urine during the 3 h of experiment. A 8-fold increase of $PGF_{2\alpha}$ metabolite in plasma at 10 min and 12-fold increase in the urine at 30–60 after the i.v. administration of 8-iso-$PGF_{2\alpha}$ was observed which continued throughout the 3 h of experiment. This observation suggests that pharmacologically administered or endogenously produced 8-iso-$PGF_{2\alpha}$ during oxidant stress induces prostaglandin formation presumbly through the classical cyclooxygenase-catalysed arachidonic acid oxidation which might be inflammatory itself to the cells and exerts further vasoconstrictive effects.

Keywords: *Isoprostanes, prostaglandins, cyclooxygenases, free radicals, inflammation, oxidative stress*

Abbreviations: *PG, prostaglandin; 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; 15-keto-dihydro-$PGF_{2\alpha}$, 15-keto-13,14-dihydro-prostaglandin $F_{2\alpha}$; BSA, bovine serum albumin; TXB_2, Thromboxane B_2; COX, cyclooxygenase*

Introduction

Isoprostanes are novel prostaglandin isomers biosynthesized *in vivo* by free-radical catalysed peroxidation of arachidonic acid [1,2]. This reaction mainly occurs independent of cyclooxygenases (COX) in the body. 8-Iso-$PGF_{2\alpha}$, one of the major isoprostanes, exibits potent biological activity and is increased in peripheral plasma and urine in several syndromes that are considered to be associated with oxidant injury, and also after daily intake of some polyunsaturated fatty acids [2–11].

It was earlier shown that oxidative injury mediated non-enzymatic formation of 8-iso-$PGF_{2\alpha}$ induces COX catalysed formation of $PGF_{2\alpha}$ in experimental hepatotoxicity by CCl_4 in rats [12]. The formation of 8-iso-$PGF_{2\alpha}$ preceded the biosynthesis and release of primary $PGF_{2\alpha}$. Conversely, it was also shown that rapid formation of enzymatically catalysed $PGF_{2\alpha}$ and subsequent formation and release of 8-iso-$PGF_{2\alpha}$ through non-enzymatic free radical catalysed oxidation of arachidonic acid occur in plasma and urine during experimental septic shock [5]. These studies reveal that the formation of these two closely related but distinct compounds (8-iso-$PGF_{2\alpha}$ by non-enzymatically and $PGF_{2\alpha}$ by enzymatically catalysed peroxidation of arachidonic acid which are indicators of oxidative injury and inflammatory response, respectively, Figure 1) are

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closely associated in different pathophysiological situations although their mechanism of biosynthesis, magnitude of formation, biological half-lives and kinetics of release and excretion profile are very different [1,2,5,12,13]. It was previously shown that 8-iso-$PGF_{2\alpha}$ is a vasoconstrictor compound, and the biological action of this compound is shown to be mediated partly by interaction with the vascular TXA_2 and PGH_2 receptors [14]. F_2-isoprostanes are biologically potent compounds which are found to be increased in various human diseases that are characterised by oxidative stress exposer [2,4,7–11]. The possible role of endogenously formed 8-iso-$PGF_{2\alpha}$ during oxidant stress or exogenously administered 8-iso-$PGF_{2\alpha}$ on arachidonic acid metabolism is largely unknown. This is of potential biological importance since primary prostaglandins, the products of arachidonic acid metabolism are known to control various important signaling pathways that have important role in balancing several physiological (vascular, renal, luteolysis of the corpus luteum, ovulation, induction of labor and parturition etc.) and pathological status (inflammation, vasoconstriction in cardiovascular and other diseases) of the mammalian body. This study is the first report on the unique biological effect of a major F_2-isoprostane (8-iso-$PGF_{2\alpha}$) on the endogenous formation of $PGF_{2\alpha}$ in body fluids in rabbits.

Materials and methods

Materials

Unlabelled 8-iso-$PGF_{2\alpha}$, other related isoprostanes and prostaglandins were purchased from Cayman Chemicals, Ann Arbor, MI, USA. Glucose solution was obtained from Pharmacia, Uppsala, Sweden. The tritium labelled 8-iso-$PGF_{2\alpha}$ (specific activity: 608 GBq $mmol^{-1}$) was synthesized and purified as described previously [15]. The tritium labelled 15-keto-dihydro-$PGF_{2\alpha}$ (specific activity: 6.77 TBq $mmol^{-1}$) was obtained from Amersham (Buckinghamshire, UK). Antibodies against 8-iso-$PGF_{2\alpha}$ and 15-keto-dihydro-$PGF_{2\alpha}$ (15-K-DH-$PGF_{2\alpha}$) were raised at our laboratory and well characterized [15,16].

Animal experiment and sample collection

Four rabbits (New Zealand white rabbits, female, Lidköping Kanin Farm, Lidköping, Sweden) of body weight 3.9–4.5 kg (mean = 4.2 kg) were used in this study after acclimatisation to the laboratory conditions. The animals were anaesthesized by a bolus dose of 3 ml Ketalar^R and Rompun^R (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 millilitre of saline was given intravenously to the animals through the vena femoralis every half hour. The animals were sacrificed at the end of the experiment by the administration of an excess dose of Mebumal Vet^R (Apoteksbolaget, Sweden).

Unlabelled 8-iso-$PGF_{2\alpha}$ (43 μg/kg) in glucose solution was administered intravenously (vena femoralis) to the rabbits. Blood samples were collected in heparinised tubes at 0, 1.5, 5, 10, 15, 20, 30, 45, 60, 90, 120, 150 and 180 min after the administration of the substance. Urinary samples were collected continuously

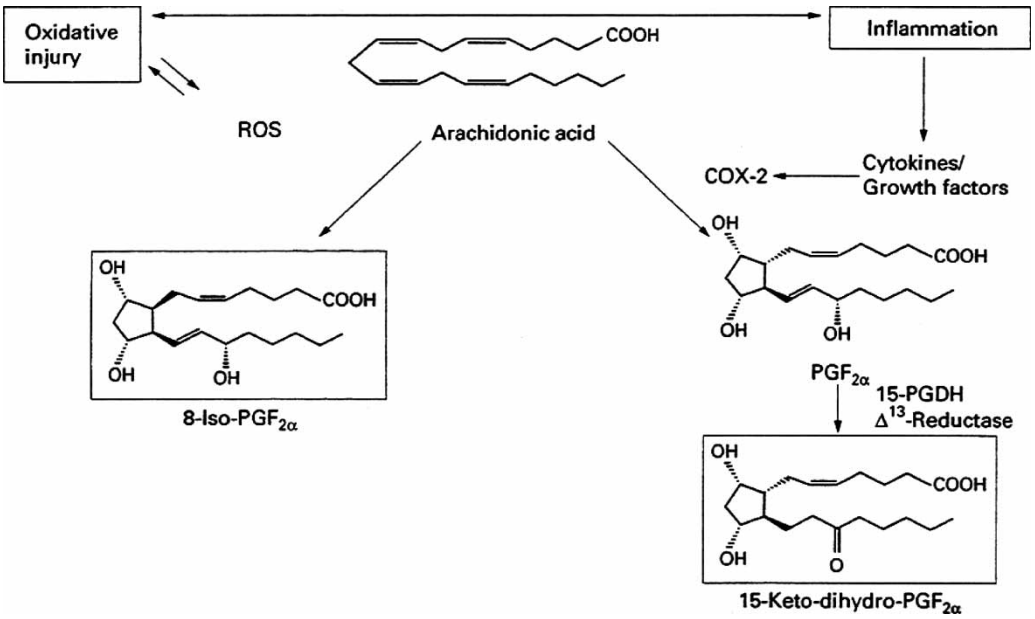

Figure 1. Schematic diagram of the relation between inflammation and oxidative injury, and endogenous formation of 8-iso-$PGF_{2\alpha}$ through a free radical pathway, and $PGF_{2\alpha}$ by cyclooxygenase catalysed oxidation of arachidonic acid. ROS = Reactive oxygen species; COX = Cyclooxygenase; 15-PGDH = 15-hydroxyprostaglandin dehydrogenase [11].

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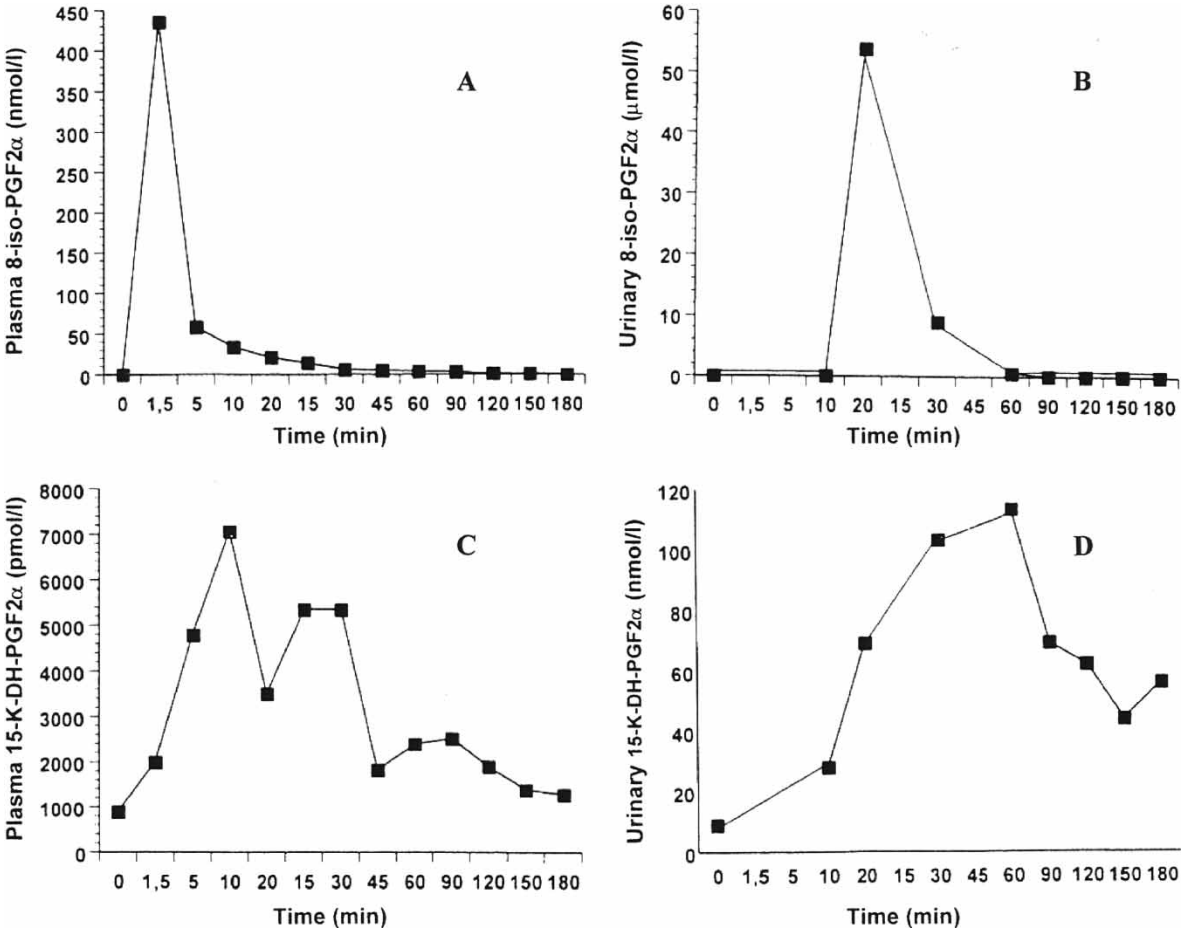

Figure 2. (A) Time course of the elimination of intravenously administered 8-iso-$PGF_{2\alpha}$ in plasma. The kinetics of plasma levels of 8-iso-$PGF_{2\alpha}$ following i.v. administration of unlabelled 8-iso-$PGF_{2\alpha}$ (43 μg/kg) to the rabbits ($n = 4$). Time zero is the time of aministion of 8-iso-$PGF_{2\alpha}$; (B) Time course of the elimination of intravenously administered 8-iso-$PGF_{2\alpha}$ in urine. The kinetics of urinary levels of 8-iso-$PGF_{2\alpha}$ following i.v. administration of unlabelled 8-iso-$PGF_{2\alpha}$ (43 μg/kg) to the rabbits ($n = 4$); (C) Time course of the formation of $PGF_{2\alpha}$ in plasma. The kinetics of plasma levels of 15-keto-dihydro-$PGF_{2\alpha}$ as a result of primary $PGF_{2\alpha}$ formation following i.v. administration of unlabelled 8-iso-$PGF_{2\alpha}$ (43 μg/kg) to the rabbits ($n = 4$) and (D) Time course of the formation of $PGF_{2\alpha}$ in urine. The kinetics of urinary levels of 15-keto-dihydro-$PGF_{2\alpha}$ as a result of primary $PGF_{2\alpha}$ formation following i.v. administration of unlabelled 8-iso-$PGF_{2\alpha}$ (43 μg/kg) to the rabbits ($n = 4$).

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in portions in glass vials at different intervals (0–10, 10–20, 20–30, 30–60, 60–90, 90–120, 120–150 and 150–180 min) from urinary bladder after adequate catheterization. Plasma was separated and stored frozen without any addition of antioxidants for about a month at −70°C until analysis. Similarly, urinary samples were stored frozen at −70°C until analysis. The ethical permission was obtained from the Animal Ethical Committee, Uppsala University, Uppsala, Sweden.

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

The plasma and urinary samples from this study were analysed for free 8-iso-$PGF_{2\alpha}$ without any extraction by a radioimmunoassay developed and characterized by Basu [15]. In brief, an antibody was raised in rabbits by immunization with 8-iso-$PGF_{2\alpha}$ coupled to BSA at the carboxylic acid by 1,1′-carbonyldiimmidazole method. The cross-reactivity of the antibody with 8-iso-15-keto-13,14-dihydro-$PGF_{2\alpha}$, 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 1.7, 9.8, 1.1, 0.01, 0.01, 0.1, 0.03, 1.8 and 0.6%, respectively. The detection limit of the assay was 23 pmol/l.

Radioimmunoassay of 15-keto-dihydro-$PGF_{2\alpha}$

The plasma and urinary samples from this study were also analysed for 15-keto-13,14-dihydro-$PGF_{2\alpha}$ (15-keto-dihydro-$PGF_{2\alpha}$), a major metabolite of $PGF_{2\alpha}$ by a radioimmunoassay developed and characterized by Basu [16]. In brief, an antibody was raised in rabbits by immunization with 15-keto-dihydro-$PGF_{2\alpha}$ coupled to BSA at the carboxylic acid by 1,1′-carbonyldiimmidazole method. The cross-reactivity of the antibody with $PGF_{2\alpha}$, 15-keto-$PGF_{2\alpha}$, PGE_2, 15-keto-13,14-dihydro-PGE_2, 8-iso-15-keto-13,14-dihydro-$PGF_{2\alpha}$ 11β-$PGF_{2\alpha}$, 9β-$PGF_{2\alpha}$, TXB_2 and 8-iso-$PGF_{3\alpha}$ was 0.02, 0.43, <0.001, 0.5, 1.7, <0.001, <0.001, <0.001, 0.01%,

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respectively. The detection limit of the assay was 45 pmol/l.

Results and discussion

Intravenous administration of 43 μg/kg of unlabelled 8-iso-$PGF_{2\alpha}$ to rabbits resulted in a rapid increase of 8-iso-$PGF_{2\alpha}$ levels in the circulation (Figure 2A). The peripheral plasma concentration of 8-iso-$PGF_{2\alpha}$ reached its peak (436 nmol/l) at 1.5–2 min after the administration of the substance. An instant and steady disappearance of the compound from the circulation and its successive appearance in the urine were observed with time. The plasma concentration of 8-iso-$PGF_{2\alpha}$ decreased to 21 nmol/l within 15 min and to 0.7 nmol/l (close to the basal level) within 3 h after the i.v. administration of 8-iso-$PGF_{2\alpha}$. Thus, this compound eliminates rapidly from the circulation like other primary prostaglandins [16–18]. Urinary concentration of 8-iso-$PGF_{2\alpha}$ did not increase until 10 min (Figure 2B). At 20 min after administration of 8-iso-$PGF_{2\alpha}$ the urinary concentration of this compound increased to 53.8 μmol/l from its basal level and it decreased to 0.03 μmol/l within 60 min. Thus, a rapid and efficient excretion of 8-iso-$PGF_{2\alpha}$ from circulation into the urine were observed. It was earlier shown that about 80% of the total intravenously administered 8-iso-$PGF_{2\alpha}$ were recovered in the urine during 4 h after the administration of the tritium labelled 8-iso-$PGF_{2\alpha}$ to the rabbits [19]. The rest of the isoprostanes presumbly excrete during the following hours mainly through the urine. It has recently shown that a part of this isoprostane may undergo conjugation with glucuronic acid in human urine [20]. However, such glucuronidation have not been shown in the rabbit yet.

In this study, we mainly emphasized the biochemical action of intravenously applied 8-iso-$PGF_{2\alpha}$ on the COX catalysed arachidonic acid metabolism since we have first observed that oxidative injury induced 8-iso-$PGF_{2\alpha}$ formation leads to a subsequent but distinct formation of primary bioactive $PGF_{2\alpha}$, as reflected by increased plasma 15-keto-dihydro-$PGF_{2\alpha}$ levels [12]. Primary $PGF_{2\alpha}$ is synthesized and released through COX-2 catalysed oxidation of arachidonic acid. It is well known that arachidonic acid metabolism through the COX pathway, is a physiologically important metabolic pathway in the mammals [17,18]. Prostaglandins, the major products of arachidonic acid metabolism through COXs, are potent bioactive compounds. These compounds not only regulate various important physiological functions (e.g. vascular, renal, luteal function, induction of labour and parturition) [21–25] but also control many pathophysiological consequences of the body in various species, e.g acute and chronic inflammation, fever induction and oxidative injury [5–13]. Prostaglandins are also well known potent mediators of various signaling pathways that controls diverse crucial biochemical events of the body [26].

In this study, we have found that intravenous administration of unlabelled 8-iso-$PGF_{2\alpha}$ (43 μg/kg) to rabbits resulted in a rapid increase of endogenous $PGF_{2\alpha}$ (presumably through the activation of COXs) as reflected by the increase of both plasma (Figure 2C) and urinary levels of 15-keto-dihydro-$PGF_{2\alpha}$ (Figure 2D). The plasma levels of 15-keto-dihydro-$PGF_{2\alpha}$ increased from 894 to 7070 pmol/l (8-fold) within 10 min after administration of the compound (Figure 2C). When comparing the formed prostaglandin levels (at 10 min) with 8-iso-$PGF_{2\alpha}$ levels (1.5 min) at their heights after administration of 8-iso-$PGF_{2\alpha}$ in the circulation it is seen that about 2% of the prostaglandin metabolite has been endogenously formed in this experimental situation. A pulsatile pattern of $PGF_{2\alpha}$ secretion that was observed during the whole experimental period (3 h) could be compared with the physiologically induced endogenous $PGF_{2\alpha}$ secretion pattern during luteolysis of the corpus luteum at the end of the oestrous cycle in the bovine (by increasing 10 times from the basal levels in the plasma) [21,22]. An about 12-fold increase of 15-keto-dihydro-$PGF_{2\alpha}$ levels (9–113 nmol/l) from the basal level was observed in urine at 60 min after administration of 8-iso-$PGF_{2\alpha}$ (Figure 2D). At 3 h, the levels of urinary 15-keto-dihydro-$PGF_{2\alpha}$ were still several fold above the basal level. This corroborates with our previous studies on the elimination profile of the prostaglandin metabolite from the circulation into the urine [22]. Together, these indicate that 8-iso-$PGF_{2\alpha}$ induced $PGF_{2\alpha}$ formation might be a direct effect of the availability of the former in the biological system that might have further physiological or pathophysiological consequences, such as an acute inflammatory response or contribution to the progression of chronic inflammation. This situation may arise both after an endogenous surge of F_2-isoprostanes during acute or chronic oxidative stress/inflammation-related diseases or after a pharmacological or toxicological application of this compound to the mammalian body.

In addition, although 15-keto-dihydro-$PGF_{2\alpha}$ is a major plasma metabolite of primary $PGF_{2\alpha}$, the increasing levels of 15-keto-dihydro-$PGF_{2\alpha}$ in the urine following a pulsatile appearance of this metabolite in plasma clearly demonstate an efficient excretion of the intact compound into the urine. This notion verifies our earlier studies where 15-keto-dihydro-$PGF_{2\alpha}$ metabolite was found to increase not only in the plasma but also in the urine after an endogenous surge of primary $PGF_{2\alpha}$ [22,27].

In conclusion, this study reveals for the first time that intravenously administered 8-iso-$PGF_{2\alpha}$ affects arachidonic acid metabolism presumbly driven by the COXs, and leads to the formation of endogenous bioactive $PGF_{2\alpha}$. COX-catalysed $PGF_{2\alpha}$ formation

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may possibly be a primary, and an important biological consequence, following either F_2-isoprostane administration or its endogenous formation during oxidant stress in various diseases due to the potent role of primary prostaglandins to modulate various physiological and pathophysiological status in the body by affecting through vasoconstrictive, smooth muscle stimulating and inflammatory mechanisms. Thus, it is tempting to speculate that there is a link between oxidative stress induced isoprostane formation and subsequent inflammatory response due to prostaglandin $F_{2\alpha}$ biosynthesis through COX system and its release into the circulation. This observation might have a significant biological role in the pathogenesis of free radical related diseases and initiation of inflammation.

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