Identification of the Major Urinary Metabolite of the Highly Reactive Cyclopentenone Isoprostane 15-A2t-Isoprostane in Vivo*

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The cyclopentenone isoprostanes (A2/J2-IsoPs) are formed in significant amounts in humans and rodents esterified in tissue phospholipids. Nonetheless, they have not been detected unesterified in the free form, presumably because of their marked reactivity. A2/J2-IsoPs, similar to other electrophilic lipids such as 15-deoxy-Δ12,14-prostaglandin J2 and 4-hydroxynonenal, contain a highly reactive α,β-unsaturated carbonyl, which allows these compounds to react with thiol-containing biomolecules to produce a range of biological effects. We sought to identify and characterize in rats the major urinary metabolite of 15-A2t-IsoP, one of the most abundant A2-IsoPs produced in vivo, in order to develop a specific biomarker that can be used to quantify the in vivo production of these molecules. Following intravenous administration of 15-A2t-IsoP containing small amounts of [3H4]15-A2t-IsoP, 80% of the radioactivity excreted in the urine remained in aqueous solution after extraction with organic solvents, indicating the formation of a polar conjugate(s). Using high pressure liquid chromatography/mass spectrometry, the major urinary metabolite of 15-A2t-IsoP was determined to be the mercapturic acid sulfoxide conjugate in which the carbonyl at C9 was reduced to an alcohol. The structure was confirmed by direct comparison to a synthesized standard and via various chemical derivatizations. In addition, this metabolite was found to be formed in significant quantities in urine from rats exposed to an oxidant stress. The identification of this metabolite combined with the finding that these metabolites are produced in in vivo settings of oxidant stress makes it possible to use this method to quantify, for the first time, the in vivo production of cyclopentenone prostanoids.

Oxidative stress has been implicated in a number of human diseases including atherosclerosis, cancer, neurodegenerative disorders, and even aging (1–4). Free radical damage to tissue biomolecules, such as the peroxidation of lipids, is a central feature of oxidant stress. Previously, we reported that a group of novel prostaglandin (PG)1-like products, termed the isoprostanes (IsoPs), are formed from the free radical-initiated peroxidation of arachidonic acid in vivo in humans (5). These compounds are isomeric to PGs differing only in the stereochemical relationship of the two side chains on the five-membered prostane ring. The side chains of PGs are in the trans-configuration with respect to the prostane ring, whereas the majority of IsoPs contain side chains that are in the thermodynamically less stable cis-configuration. Several different classes of IsoPs have been characterized, including those with F-type prostane rings (E2/IsoPs) (5) and those with E-type and D-type prostane rings (E2/D2-IsoPs) (6). Furthermore, more recently, we have reported that E2/D2-IsoPs rapidly dehydration to yield cyclopentenone IsoPs (A2/J2-IsoPs) (Fig. 1), respectively (7).

Cyclopentenone IsoPs are unique as a class of IsoPs, because they are highly reactive and thus are thought to be responsible for some of the adverse biological effects of oxidant stress. These molecules have been shown to impair normal cell function in a variety of cell types including neurons and macrophages (8). The marked reactivity of these compounds is attributed to the presence of an α,β-unsaturated carbonyl in the prostane ring. This functional group renders cyclopentenone IsoPs susceptible to nucleophilic addition reactions with relevant biomolecules, especially thiol-containing proteins and peptides in vivo. The biological activities of the A2/J2-IsoPs are most probably attributable to either the direct adduction of proteins or the alteration of cellular redox status due to protein adduction (9, 10).

The cyclopentenone IsoPs are probably biologically relevant in vivo settings, because, similar to other classes of IsoPs, they are found in vivo in large amounts and are esterified in lipids in various tissues (7, 11). However, unlike F-ring and D/E-ring IsoPs, the A2/J2-IsoPs have not been detected in plasma or urine unesterified as the free acids, probably due to their marked reactivity. In recent years, research efforts in this laboratory have focused on exploring the metabolic fate of these molecules. We have shown that 15-A2t-IsoP (Fig. 1), one of the abundant cyclopentenone IsoPs formed in vivo (11), readily adds to the cysteine residue of GSH in vitro in the presence of glutathione transferases (12). In addition, when 15-A2t-IsoP is incubated with HepG2 cells, this molecule is readily conjugated with GSH to yield four major metabolites (13). Interestingly, after adduc-

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1 The abbreviations used are: PG, prostaglandin; PGA2, prostaglandin A2; C16Δ7, carbon tetrachloride; ent-, enantioomer; GSH, glutathione; HPLC, high performance liquid chromatography; IsoP, isoprostane; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; rac-, racemic; SRM, selected reaction monitoring; SIM, selected ion monitoring.

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tion with GSH, the biological activity of 15-A2t-IsoP is negated, probably as a result of the loss of the highly reactive α,β-unsaturated carbonyl moiety. Thus, it is likely that addition with GSH is a mechanism by which cells detoxify this and other related reactive cyclopentenone-containing compounds. Herein, we sought to explore the metabolic fate of 15-A2t-IsoP in rodents. Through these studies, we hoped to gain a better understanding of in vivo pathways responsible for the inactivation and excretion of these cytotoxic molecules as well as identify a specific biomarker that could be used to quantify, for the first time, the in vivo production of cyclopentenone prostanoids.

**MATERIALS AND METHODS**

**Chemical Reagents and Supplies**—Chemicals and enzymes were purchased from Sigma unless otherwise stated. [3H]-15-E2t-IsoP (specific activity 250 μCi/μmol) was custom-synthesized by Amersham Biosciences 3 15-E2t-IsoP was purchased from Cayman Chemicals (Ann Arbor, MI). All of the solvents were of HPLC quality and purchased from EM Science (Gibbstown, NJ). C18 Sep-Paks were purchased from Waters Corporation (Milford, MA).

**Preparation of [3H]-15-A2t-IsoP—[3H]-15-A2t-IsoP was prepared by incubating ~10 μCi of [3H]-15-E2t-IsoP and a small amount of unlabeled 15-E2t-IsoP (~50 μg) in ethanolic solution (100 μl) in the presence of 0.1 M HCl (200 μl) for 5 h at 37 °C. Subsequently, the product was extracted in ethyl acetate. The solvent was removed by evaporation under a stream of dry nitrogen. The concentrate was suspended in 50% methylene chloride and ethyl acetate to gain an understanding of the chemical characteristics of the metabolites.

**Synthesis of 15-A2t-IsoP—**The single enantiomer 15-A2t-IsoP was synthesized (14, 15). The purity of the compound was confirmed using HPLC as described above. A single peak representing 15-A2t-IsoP was eluted at 20 min.

**Infusion of 15-A2t-IsoP and [3H]-15-A2t-IsoP into a Rat**—1 ml of 15-A2t-IsoP combined with ~1 μCi of [3H]-15-A2t-IsoP was taken up in 75 μl of ethanol and then suspended in 1 ml of normal sterile saline. The suspension was then infused into the superficial femoral vein of a 300-g Sprague-Dawley male rat, which had been anesthetized by inhalation of isoflurane, over 5 min. After the infusion, the animal was placed in a metabolic cage, and urine was collected in aliquots for 24 h. The radioactivity was counted after the sample adapted to room temperature, and the reaction was allowed to proceed for 30 min. To stop the reaction and purify the product, the reaction was diluted with 10 ml of water, acidified to pH 3 with 1 n HCl. The samples were loaded onto a C18 Sep-Pak that had been preconditioned by rinsing with acetonitrile and 50 mm aqueous ammonium acetate, pH 3.4. The C18 Sep-Pak was rinsed with 10 ml each of ammonium acetate and heptane, and the sample was eluted with 50 μl of ammonium acetate and heptane, and the sample was eluted with 50% ethanol at 80 °C until LC/MS analysis.

**Extraction and HPLC/MS Analysis of 15-A2t-IsoP Metabolites—**Before extraction using a C18 Sep-Pak, aliquots of urine (0.5–2 ml) were diluted to a total volume of 10 ml with distilled deionized water and then acidified to pH 3 with 1 n HCl. The samples were loaded onto a C18 Sep-Pak that had been preconditioned by rinsing with acetonitrile and 50 mm aqueous ammonium acetate, pH 3.4. The C18 Sep-Pak was rinsed with 10 ml each of ammonium acetate and heptane, and the sample was eluted with 50% ethanol. The ethanol was removed by evaporation under a stream of dry nitrogen, and samples were stored in 100 μl of 95% ethanol at −80 °C until LC/MS analysis.

**Extraction and Analysis**—The ThermoFinnigan Surveyor MS Pump 2.0 equipped with a Discovery C18 column (2.1 mm × 50 cm, 5-μm particle size) utilizing a linear gradient (mobile phase A: water/mobile phase B: acetic acid (95/5, v/v); mobile phase B: acetonitrile/methanol/acetic acid (95/5/0.1, v/v/v) at a flow rate of 1 ml/min. Cyclopentenone Isopentenone prostanoids.

**Extraction of 15-A2t-IsoP—**In this figure, the dehydration of 15-E2t-IsoP and 15-D2t-IsoP to 15-A2t-IsoP and 15-J2t-IsoP, respectively, is shown.

**Preparation of [3H]-15-E2t-IsoP—**[3H]-15-E2t-IsoP was synthesized by isomerization of [3H]-PGE2. 3 [3H]-15-E2t-IsoP was synthesized by isomerization of [3H]-PGE2. 4 A. Porta, G. Zanoni, G. Vidari, G. L. Milne, and J. D. Morrow, manuscript in preparation.
stream of dry nitrogen. The purity of the products was confirmed by MS after each reaction, and the yield of each reaction was almost quantitative. The N-acetyl cysteine sulfoxides of 15-A₂r-IsoP and [³H]PGA₂, in which the carbonyl groups at C9 were not reduced, were also synthesized using this method, leaving out the sodium borohydride reduction.

Quantification of Cyclopentenone Isoprostane Metabolites in Urine from Rats Treated with Carbon Tetrachloride (CCL₄) — CCL₄ (~ 2 mg/kg in 2 ml of corn oil) was administered via intragastric injection to a Sprague-Dawley rat. The animal was then placed in a metabolic cage, and urine was collected at 8 and 24 h. After collection, urine was immediately acidified to pH 4 for storage at −80 °C. For analysis, aliquots of urine (2 ml) were diluted to a total volume of 10 ml with deionized water acidified to pH 3 and extracted using a C₁₈ Sep-Pak as described above. An internal standard, the N-acetyl cysteine sulfoxide conjugate of d₇-PGA₂ (5 ng/2 ml urine), was added to the diluted acidified urine before Sep-Pak extraction. As a control, urine was also collected from a Sprague-Dawley rat that had not been treated with CCL₄ and was handled as described herein. The HPLC/MS method used to identify the metabolite of 15-A₂r-IsoP was employed to analyze these samples.

RESULTS

Infusion of 15-A₂r-IsoP — 15-A₂r-IsoP containing a small amount of [³H]15-A₂r-IsoP was infused into a rat, which had been anesthetized with isoflurane, over the course of 5 min. The rat was then placed in a metabolic cage and allowed to recover. Urine was collected in aliquots up to 24 h post-infusion. 18% total radioactivity infused was recovered in the urine during that time period, and 75% radioactivity was excreted in the first 5.5 h. To account for the remainder of the radioactivity infused into the animal, the feces excreted during the 24 h after infusion were collected and the major organs were harvested after the animal was sacrificed. The tissue samples and feces were solubilized as described above, and the radioactivity was measured. The distribution of [³H]15-A₂r-IsoP in the rat is shown in Fig. 2. Approximately 75% of the [³H]15-A₂r-IsoP infused into the rat remained in the tissues, mostly likely bound to proteins, after 24 h. Importantly, the majority of the radioactivity excreted was found in the urine (18%), rather than in the feces (7%).

In addition, to confirm that the percentage of radioactivity excreted in the urine was not altered by the large bolus (1 mg) of 15-A₂r-IsoP, which was infused into the animal, a second rat was infused with [³H]15-A₂r-IsoP and a small amount (10 µg) of unlabeled 15-A₂r-IsoP. Urine was collected for 24 h, and excretion of the [³H]15-A₂r-IsoP was very similar to the first infusion. Approximately 13% radioactivity infused into the animal was excreted in the urine during this second infusion.

To determine the structure of this metabolite, tandem MS experiments were conducted in both the positive and the negative modes. For these experiments, a flow-splitter was employed post-column and approximately 25% of the sample was directed to a fraction collector. Fractions were collected each minute during the course of the run in order to follow the elution of radioactivity. The vast majority of the radioactivity was eluted in one peak in fractions 9 and 10. The most abundant peak in fractions 9 and 10 contained the highest amount of radioactivity (was eluted between 9 and 10 min).

HPLC/MS Analysis of 15-A₂r-IsoP Metabolites — The eluate from the Sep-Pak was analyzed by reversed-phase HPLC/electrospray ionization-MS using the conditions described under “Materials and Methods.” Initially, the mass spectrometer was operated in the full scan mode monitoring m/z 300–1100. Analyses were performed in both the positive mode and the negative mode. For these experiments, a flow-splitter was employed post-column and approximately 25% of the sample was directed to a fraction collector. Fractions were collected each minute during the course of the run in order to follow the elution of radioactivity. The vast majority of the radioactivity was eluted in one peak in fractions 9 and 10. The most abundant peak found in the full-scan spectrum of those fractions was m/z 514 in the negative mode (Fig. 3A) and, correspondingly, m/z 516 in the positive mode (Fig. 3B).

To understand better the chemical characteristics of the 15-A₂r-IsoP urinary metabolites, small aliquots of urine were acidified to pH 3 and extracted with organic solvents. After extraction with either methylene chloride or ethyl acetate, ~70% radioactivity remained in aqueous solution, suggesting the formation of a polar conjugate(s). Therefore, for purification of the conjugate(s) before LC/MS analysis, the urine was extracted using a C₁₈ Sep-Pak as previously described (13). 90% of the radioactivity applied to the Sep-Pak cartridge was eluted in 95% ethanol after washing with 50 mM ammonium acetate, pH 3.4, and heptane.
and loss of water. Fig. 4B shows the CID spectrum obtained from the analysis of precursor ion m/z 516 in the positive mode. The major ions of importance in the spectrum were m/z 498 [M + H - H₂O]⁺, m/z 480 [M + H - 2H₂O]⁺, m/z 462 [M + H - 3H₂O]⁺, m/z 389 (see the structure in Fig. 4D), m/z 351 (m/z 389 with loss of water), m/z 337 (the eicosanoid portion of the molecule in which one double bond is reduced), m/z 319 (m/z 337 with loss of water), m/z 301 (m/z 337 with loss of two molecules of water), m/z 283 (m/z 337 with loss of three molecules of water), and m/z 275 (decarboxylation of m/z 319). On the basis of these mass spectra, the metabolite was determined to be the N-acetyl cysteine conjugate of 15-A₂t-IsoP in which the sulfur on the cysteine is oxidized to the sulfoxide and a double bond, either the carbonyl on the prostane ring or one of the olefins on the sides chains, on the eicosanoid portion of the molecule is reduced (Fig. 4D).

To confirm that the structure was identified correctly, we sought to synthesize a standard of the metabolite (Scheme 1). N-Acetyl cysteine was first conjugated with 15-A₂t-IsoP that had been previously synthesized by Zanoni and co-workers (14, 15)⁴ using rat liver glutathione transferase as a catalyst. The carbonyl at the C9 position of the eicosanoid was subsequently reduced to the alcohol with sodium borohydride. Finally, the cysteinyl sulfur was oxidized to the sulfoxide by treatment with

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hydrogen peroxide. As can be seen in Fig. 5, this synthetic standard (Fig. 5B) co-chromatographs with the urinary metabolite of 15-A2t-IsoP (Fig. 5A). Additionally, the CID spectra in both the positive and negative modes are identical to those observed for the urinary metabolite (data not shown). Although the double bond of the carbonyl group on the prostane ring was reduced when synthesizing the standard of the 15-A2t-IsoP metabolite, the eicosanoid double bond, which was reduced in vivo, had not yet been determined. The double bond of the carbonyl was chosen to be reduced for purposes of the synthesis for two reasons. 1) The chemical reduction was easy to perform and specific to that double bond. 2) We previously observed that this carbonyl is reduced when cyclopentenone IsoPs or PGs are metabolized by cells in culture (13, 18). However, there are two other double bonds on 15-A2t-IsoP, one at the C5 position and one at the C13 position, that could be reduced during in vivo metabolism. To determine which moiety is reduced in vivo, a small portion of the HPLC-purified urinary conjugate was reacted with methoxyamine HCl. It would be predicted that, if the carbonyl group on the prostane ring had been reduced, it would not be capable of forming a methyloxime derivative. On the other hand, if reduction of one of the carbon-carbon double bonds had occurred, the reaction of the conjugate with methoxyamine HCl would yield a methyloxime derivative. Conversion of a carbonyl moiety to a methyloxime derivative shifts the molecular mass 29 Da due to the loss of the carbonyl oxygen and the addition of NOCH$_3$. As can be seen in Fig. 6B, $C_i$ and $C_{ii}$, shows that the results of treatment of this conjugate with methoxyamine HCl as evident, the $m/z$ 514 peak did not shift to $m/z$ 543. As a control for these experiments, a synthesized $N$-acetyl cysteine sulfoxide conjugate of 15-A2t-IsoP in which the carbonyl was not reduced (Fig. 6A) was treated with methoxyamine HCl and its molecular weight shifted from $m/z$ 512 upwards 29 Da to $m/z$ 541 (Fig. 6, $D_i$ and $D_{ii}$) as would be predicted. Additionally, the synthesized standard of the urinary metabolite of 15-A2t-IsoP was incubated with methoxyamine HCl. As can be seen in Fig. 6, $E_i$ and $E_{ii}$, like the urinary conjugate, there is no shift in the $m/z$, thus indicating that a methyloxime derivative could not form. Taken together, these observations support the contention that the major urinary metabolite of 15-A2t-IsoP in rats is the $N$-acetyl cysteine sulfoxide conjugate in which the carbonyl at the C9 position of the eicosanoid is reduced.

**Quantification of Cyclopentenone Isoprostanoid Metabolites Formed in Vivo during Settings of Oxidant Stress**—Previously, we showed that cyclopentenone IsoPs are formed in vivo in rats by treatment with CCl$_4$, an inducer of oxidant stress. $A_2$-J-$\Delta_2$-IsoPs are esterified in liver lipids at levels as high as 5.1 ng/g tissue after treatment with CCl$_4$. Metabolites of the $A_2$-J-$\Delta_2$-IsoPs should also be generated under these conditions. Also, because of the general structure of these compounds, the many different $A_2$-J-$\Delta_2$-IsoP stereoisomers should be metabolized in the same way as 15-A2t-IsoP. To analyze for these metabolites, urine from both a control rat and a rat treated with CCl$_4$ was collected for 24 h, extracted via Sep-Pak as described above, and analyzed by LC/MS. The mass spectrometer was operated in the selected reaction monitoring (SRM) mode in the negative mode monitoring the transition of the metabolite precursor ion $m/z$ 514 to the specific product ion $m/z$ 385. As can be seen from the chromatograms in Fig. 7, very little cyclopentenone IsoP metabolites were observed in the control urine (Fig. 7A), whereas the metabolites were readily detectable in the urine from the CCl$_4$-treated animals (Fig. 7B). As would be expected, a range of peaks representing the metabolites of the many $A_2$- and $J_2$-IsoP stereoisomers was detected in the mass spectrometer. The 15-A2t-IsoP metabolite is represented by the peak at 9.30 min. The $N$-acetyl cysteine sulfoxide of $d_2$-PGA$_2$ was added prior to extraction of the urine to serve as an internal standard so that metabolite formation could be quantified (Fig. 7C). To quantify all of the $A_2$-J-$\Delta_2$-IsoP metabolites, the area under all of the peaks eluting between 9.5 and 12.5 min was calculated. These compounds were found to be present in the urine after treatment with CCl$_4$ at levels of ~6 ng/ml. Metabolite formation in the control urine was below the limit of detection.

**DISCUSSION**

The cyclopentenone ($A_2$/$J_2$) IsoPs are highly reactive products of the free radical-initiated peroxidation of arachidonic acid. These compounds are formed via the dehydration of D$_2$/H$_9$004 cyclopentenone acids as a class because of the high reactivity of cyclopentenone eicosanoids as a class because of the high reactivity of the cycloxygenase-derived stereoisomers of the A$_2$-/$J_2$-IsoPs, which differ only in the stereochemical orientation of the side chains in relation to the prostane ring. The side chains of PGs are primarily in the trans-configuration with respect to the prostane ring, whereas the IsoPs contain side chains are in the thermodynamically less stable cis-configuration. Similar to the cyclopentenone IsoPs, the cyclopentenone PGs contain a highly reactive $\alpha,\beta$-unsaturated carbonyl on the prostane ring and have been shown to possess potent biological activities. The J-series PG, 15-deoxy-$\Delta^{12,14}$-PGJ$_2$, has been postulated to...
be an endogenous ligand for the peroxisome proliferator-activated receptor γ and an agent that modulates cell proliferation and maturation (19). In addition, it inhibits NF-κB-dependent gene transcription via covalent modification of critical cysteine-binding residues in IκB kinase and the DNA-binding domains of NF-κB subunits (20).

In this study, we identified, for the first time, the major urinary metabolite of a cyclopentenone eicosanoid, 15-A2t-IsoP, in the rat. The metabolite was identified as the N-acetyl cysteine (or mercapturic acid) sulfoxide conjugate of 15-A2t-IsoP in which the carbonyl on the prostane ring of the IsoP was reduced to the alcohol (Fig. 4C). N-Acetyl cysteine conjugates are common metabolites of molecules that contain α,β-unsaturated carbonyl moieties, including the aldehydic product of lipid peroxidation 4-hydroxynonenal (21, 22). These conjugates originate from the corresponding GSH conjugate. The conversion to the N-acetyl cysteine conjugate results from a series of enzymatic reactions involving sequentially γ-glutamyltranspeptidases, cysteinyl-glycine dipeptidase, and N-acetyltransferases (23). The sulfur on the N-acetyl cysteine is oxidized to the sulfoxide through enzymatic reactions as well. Such sulfoxidation reactions have been shown to be catalyzed by both cytochrome P450s and flavin monoxygenases in rat liver microsomes (24–26). These reactions have also been shown to occur in human liver microsomes, albeit at a slower rate (27). Reduction of the carbonyl group on the prostane ring most probably occurs after conjugation with GSH. If reduced first, the reactive α,β-unsaturated carbonyl moiety would be destroyed and the molecule would be less likely to conjugate GSH (28). Previously, Atsmon et al. (28) showed that the corresponding carbonyl group of cyclopentenone PGs is reduced in Chinese hamster ovary and hepatoma cells and we showed that the carbonyl in the prostane ring of 15-A2t-IsoP is reduced in the presence of HepG2 cells (13). Most probably, this reduction is catalyzed by various intracellular ketoreductases (29–31).

The addition of 15-A2t-IsoP with GSH and subsequent metabolism probably relates to the biological activity of these molecules. Conjugation of cyclopentenone eicosanoids with GSH has been shown to negate their bioactivity, probably as a result of the loss of the highly reactive unsaturated carbonyl moiety (32). Thus, it is likely that addition of 15-A2t-IsoP with GSH is a mechanism by which cells detoxify this and other related reactive cyclopentenone-containing compounds. Indeed, in this regard, we have shown that 15-A2t-IsoP impairs...
the normal function of macrophages but that the GSH conjugate of the compound has no effect on the cells (8).

The identification of the major urinary metabolite of 15-A2t-ISOp has important implications regarding the total amount of cyclopentenone ISOps in vivo. As noted, A2t/J2-ISOps are readily detected esterified in tissues of animals exposed to an oxidant stress, yet they are undetectable in the free form in the circulation. Thus, direct evidence of the in vivo production of cyclopentenone cyclopentenone ISOps in which the carbonyl at the C9 position on the eicosanoid is reduced to the alcohols. The mass spectrometer was operated in the SRM mode monitoring the precursor-to-product transition of m/z 516–387 in the negative mode.

In summary, we report that the cyclopentenone ISOp 15-A2t-ISOp, which is produced in large amounts in vivo, is rapidly metabolized in rats to the N-acetyl cysteine sulfoxide conjugate in which the carbonyl at the C9 position on the eicosanoid is reduced to the alcohol. These findings provide, for the first time, direct evidence of the in vivo pathways by which these highly reactive molecules are detoxified. In addition, identification of the major urinary metabolite of 15-A2t-ISOp provides a biomarker that could be used to assay the in vivo production of cyclopentenone ISOps. We demonstrated the usefulness of this assay by quantifying the formation of cyclopentenone ISOp metabolites excreted from a rat exposed to an oxidant stress. Because the metabolism of eicosanoids can differ between different animal species, efforts are currently underway in our laboratory to further develop this methodology by determining the major urinary metabolite of 15-A2t-ISOp in primates. Through these studies, we hope to identify a metabolite that could be used as a biomarker for the formation of cyclopentenone eicosanoids in human disease.

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