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Concurrent quantification of multiple biomarkers indicative of oxidative stress status using liquid chromatography-tandem mass spectrometry

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ABSTRACT

8-Hydroxy-2-deoxyguanosine (8-OHdG), 8-nitroguanine (8-NO_2Gua), 8-iso-prostaglandin $F_{2\alpha}$ (8-Iso$PGF_{2\alpha}$), and N-acetyl-S-(tetrahydro-5-hydroxy-2-pentyl-3-furanyl)-L-cysteine (HNE-MA) are well-studied and representative biomarkers for oxidative DNA damage, inflammation, and lipid peroxidation; all of which have been associated with increases in risks of various diseases and cancers. A rapid and highly sensitive isotope-dilution liquid-chromatography tandem mass spectrometry (LC-MS/MS) method was developed to simultaneously quantify the aforementioned biomarkers in urine. Upon validation, this method shows excellent feasibility, sensitivity (0.008–0.03 ng/mL) and satisfactory recoveries (88.7–95.4%); the calibration curves displayed excellent linearity with coefficients of determination (R^2) greater than 0.998. Additionally, low variations were observed in the relative standard deviation for intra- and inter-day measurements for the four analytes. The relative matrix effects for all four analytes ranged from 2.04 to 3.27%, which signaled that interferences from endogenous levels of the analytes were deemed statistically insignificant. This study successfully developed an analytical method capable to simultaneously quantify urinary 8-OHdG, 8-NO_2Gua, 8-Iso$PGF_{2\alpha}$, and HNE-MA. This analytical protocol can be applied towards conducting epidemiological studies to reveal the mechanisms related to disease development, and thus evaluate the associated risks of diseases.

1. Introduction

Oxidative/nitrative stress occurs when cellular antioxidant defenses are inadequate to completely inactivate the generation of reactive oxygen and nitrogen species (ROS/RNS). Byproducts of normal respiratory metabolism, hydroxyl radical (HO), superoxide anion (O_2), and hypochlorous acid (HClO) are all capable of inducing oxidative stress [1,2]. Nitric oxide (NO), a RNS, is generated specifically via inducible nitric oxide synthase (NOS) in inflammatory and epithelial cells during inflammatory response [3–5]. Excess ROS/RNS can damage cellular constituents, such as carbohydrates, nucleic acids, lipids, and proteins. Such damage can promote the generation of various secondary reactive species. This process can trigger a number of cellular responses, including apoptosis, which often lead to disease development. Oxidative/nitrative stress is increasingly reported to demonstrate strong associations with the onset and progression of cancer, as well as, cardiovascular and neurodegenerative diseases [1–10]. Since the determination of ROS/RNS in tissues or body fluids is extremely difficult due to high reactivity and extremely short half-life, the biomonitoring of the modified DNA, proteins, lipids, and sugars is an important practice to gain a better understanding of the relationship between these biomarkers and onset of disease.

8-OHdG, induced by ROS, is the most representative biomarker for oxidative DNA damage and results from the reaction between HO• and 2'-deoxyguanosine in DNA, followed by the nucleotide excision repair pathway [11]. Many studies have revealed that urinary 8-OHdG is detected in patients with intestinal cancers and

Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; 8-Iso$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; HNE-MA, N-acetyl-S-(tetrahydro-5-hydroxy-2-pentyl-3-furanyl)-L-cysteine; 8-NO_2Gua, 8-nitroguanine; LC-MS/MS, liquid chromatography tandem mass spectrometry; ROS, reactive oxygen species; RNS, reactive nitrogen species; ESI, electrospray ionization; HPLC, high-performance liquid chromatography; ID, isotope-dilution; LOD, limit of detection; LOQ, limit of quantification; SPE, solid-phase extraction; MRM, multiple reaction monitoring.

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Alzheimer's disease; urine concentration of 8-OHdG is positively correlated with age and exposure to various toxic agents [12–16]. 8-NO_2Gua, which causes inflammatory responses, is a potential biomarker for nitrative DNA damage that results from the reaction between highly reactive $ONOO^-$ and the 2'-deoxyguanosine in DNA followed by the depuration of DNA during the inflammatory response [17]. 8-NO_2Gua can also be detected at various cancer sites in animals and humans; such finding indicates the excess generation of RNS may be a risk factor for cancer development [3,18,19]. Due to their potential mutagenicity, 8-OHdG and 8-NO_2Gua have also been recognized as indicators of cancer risk [20,21].

In addition to DNA damage, ROS can attack cell membranes, lead to lipid peroxidation and the eventual destruction of membrane lipids [22]. The determination of reliable biomarkers for lipid peroxidation is difficult because an array of lipids exist in biological systems. Arachidonic acid and linoleic acid, two relatively abundant fatty acids in human cells, are essential for the proper formation and functioning of cell membranes, hormones, the immune system and neuronal transmission [23]. Isoprostanes (F_2-iPs) are metabolites from the peroxidation of arachidonic acid; as shown in Fig. 1, up to 32 pairs of enantiomers, i.e. 32 racemates which are diasteromeric to each other, may be formed [24]. Among these, 8-isoPGF$_{2\alpha}$ has attracted special attention since it can be a potent vasoconstrictor of the lung and kidney [25,26]. Moreover, elevated levels of 8-isoPGF$_{2\alpha}$ was also noted in patients with asthma and interstitial lung diseases [27,28]. Additionally, other studies have shown that 8-isoPGF$_{2\alpha}$ is correlated with the severity of diabetes and atherosclerosis [29,30].

4-hydroxy-2-nonenal (HNE), both cytotoxic and genotoxic, is a hydroxyalkenal produced by lipid peroxidation. HNE is formed by the radical-initiated degradation ofω-6-polyunsaturated fatty acids, such as linoleic and arachidonic acids. Via Michael-type addition during biological detoxification, HNE can form HNE-GSH, a glutathione conjugate. When HNE-GSH is metabolized by the liver and kidney, it becomes HNE-mercapturic acid (HNE-MA), a biomarker detectable in urine [31,32]. HNE-MA's stable presence has been detected in rats after an acute oxidative stress insult, demonstrating that HNE-MA can serve as a biomarker indicative of the levels of lipid peroxidation and be used to evaluate oxidative stress in biological systems [29].

Existing studies have developed analytical methods to quantitate four of the abovementioned analytes separately. Barregard et al. addressed several sources of variability in the quantification of 8-OHdG in human urine [33]; Ishii and associates developed a method to quantitatively assay 8-NO_2Gua concomitant with 8-hydroxydeoxyguanosine [34]. Similarly, individual quantitation methods for 8-isoPGF2α and HNE-MA are also available [35,36]. However, to our knowledge, no study is yet to establish a combine panel for 8-OHdG, 8-NO_2Gua, 8-isoPGF2α and HNE-MA, which can be useful to comprehensively evaluate oxidative/nitrative stress status and lipid peroxidation. Thus, this study aims to develop an LC-MS/MS method to simultaneously analyze 8-OHdG, 8-NO_2Gua, 8-isoPGF2α and HNE-MA with high sensitivity and specificity. To accurately quantify these biomarkers in a complex matrix of urine samples, we incorporated stable isotope-dilution quantification,

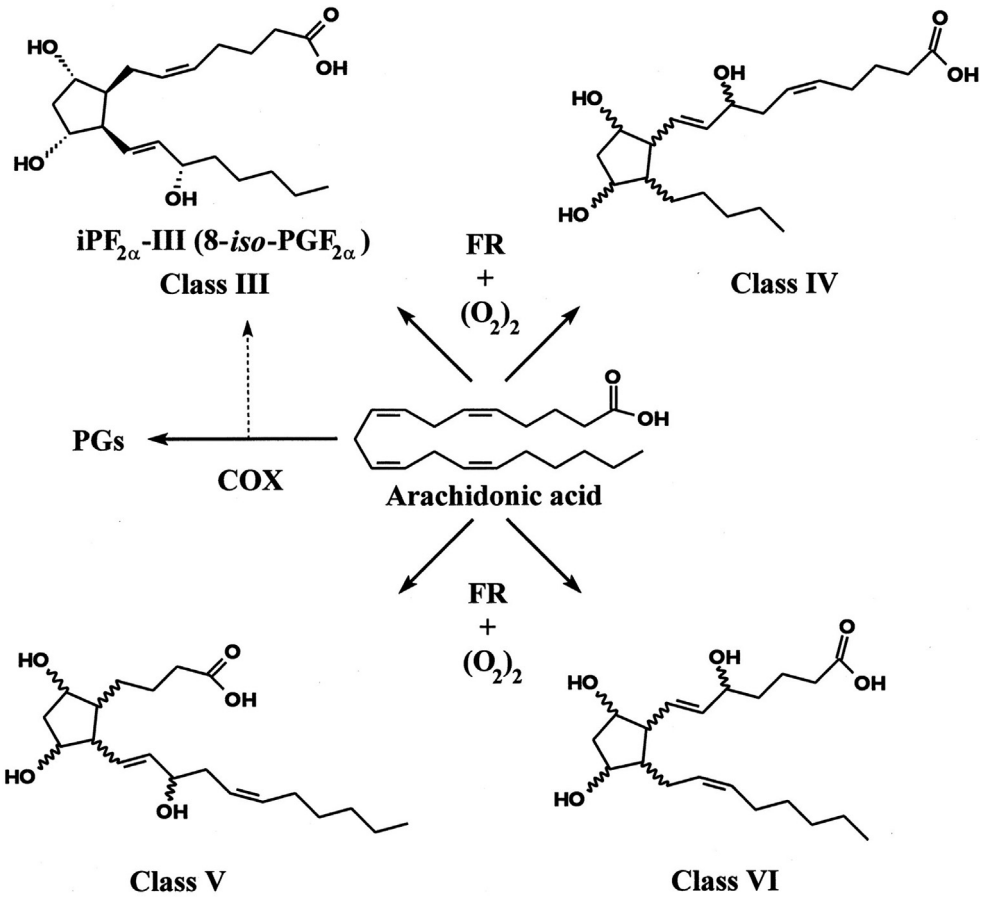

Fig. 1. Structures of the four classes of F2-isoprostanes formed from peroxidation of arachidonic acid. Stereospecific structures of iPF2α-III, 8-isoPGF2α is shown [40]. FR: free radicals.