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Measuring urinary *N*-acetyl-*S*-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine (IPMA3) as a potential biomarker of isoprene exposure

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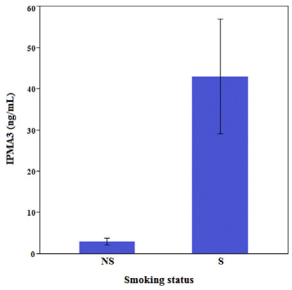
HIGHLIGHTS

- Identified a novel urinary biomarker (IPMA3) for isoprene exposure.
- Suggested a metabolic pathway for IPMA3 formation.
- Measured IPMA3 using a modified UPLC/ESI-MSMS method.
- Found elevated IPMA3 levels in smokers compared with non-smokers.
- Recommend using IPMA3 for isoprene exposure because of selectivity and abundance.

GRAPHICAL ABSTRACT



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ABSTRACT

Isoprene, the 2-methyl analog of 1,3-butadiene, is identified as a possible human carcinogen by the International Agency for Research on Cancer (IARC). Isoprene is ubiquitous in the environment with numerous natural and anthropogenic sources. Tobacco smoke is the main exogenous source of isoprene exposure in indoor environments. Among smoke constituents, isoprene is thought to contribute significantly to cancer risk; however, no selective urinary biomarkers of isoprene exposure have been identified for humans. In this manuscript, we measured the minor isoprene metabolite IPMA1 (mixture of *N*-acetyl-*S*-(1-[hydroxymethyl]-2-methyl-2-propen-1-yl)-L-cysteine and *N*-acetyl-*S*-(2-hydroxy-3-methyl-3-buten-1-yl)-L-cysteine), and we identified IPMA3 (*N*-acetyl-*S*-(4-hydroxy-2-methyl-2-buten-1-yl)-L-cysteine) as a major isoprene metabolite and novel isoprene exposure biomarker for humans. Urinary isoprene metabolites were measured using ultra high performance liquid chromatography coupled with electrospray ionization triple quad tandem mass spectrometry (UPLC/ESI-MSMS). The detection rates of IPMA1 and IPMA3 are <20% and 82%, respectively. The selectivity and abundance of IPMA3 make it a useful urinary biomarker of isoprene exposure. The limit of detection of IPMA3 in urine was 0.5 ng mL^{-1}. IPMA3 was stable under different storage temperatures and following ten freeze-thaw cycles. The average recovery of urine spiked with IPMA3 at three different levels was 99%. IPMA3 was measured in urine samples received from 75 anonymous subjects; the median (25th percentile, 75th percentile) IPMA3 level in smokers was 36.2 (18.2, 56.8) ng mL^{-1} and non-smokers 2.31 (2.31, 4.38) ng mL^{-1}. Application of this method to large population studies will help to characterize isoprene exposure and assess potential health impact.

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1. Introduction

Isoprene is a possible (Group 2B) human carcinogen [1] that is the 2-methyl analog of 1,3-butadiene, a known human carcinogen [2]. The industrial use of isoprene is principally in the manufacture of synthetic rubber [3]. Isoprene is ubiquitous in the environment with a number of natural sources (e.g. foods and endogenous formation) [4,5] and anthropogenic sources (e.g. ethylene production by cracking naphtha, wood pulping, petroleum product burning, wood and other biomass burning, and tobacco smoking) [1,3]. The primary source of isoprene in indoor air is environmental tobacco smoke [3]. In a theoretical cancer hazard index of cigarettes sold in China, isoprene contributed 25% to the calculated incremental lifetime cancer risk [6]. The yield of isoprene in tobacco smoke ranges from 0.39 to 617 μg per cigarette [7] and a level of 657 μg m^{-3} was detected in secondhand smoke [8]. The US Food and Drug Administration includes isoprene on their list of harmful and potentially harmful constituents in tobacco smoke [9]. Isoprene is also formed as a byproduct of cholesterol biosynthesis making it the major endogenous hydrocarbon in human breath [10]. In addition to exhalation, isoprene is detected in blood as well, in the range of 15–70 nmol L^{-1} [11]. The endogenous production rate of isoprene was calculated to be 0.15 pmol kg^{-1} per hour and the quantity exhaled per day per individual was estimated at 2–4 mg per 24 h [4,10]. A recent study investigating levels of volatile organic compounds (VOCs) in exhaled breath at different stages of fibrosis in liver reported that isoprene in exhaled breath was a potential biomarker for advanced fibrosis in patients with chronic liver disease [12]. While isoprene appears to be ubiquitous in exhaled human breath, breath isoprene has not been shown to increase in people with increased isoprene exposure [13].

Isoprene has been studied extensively to characterize metabolism and mutagenicity [14]; biotransformation of isoprene and isoprene mono-epoxides by human cytochrome P450 enzymes [15]; stereo-chemical and kinetic comparisons of mono- and di-epoxide formation in vitro, in rats, mice, and humans [16–18]; and conjugation of isoprene mono-epoxides with glutathione by glutathione *S*-transferases of rats and humans [19]. Despite the extensive past work on isoprene, no methods have been published for biomonitoring of isoprene exposure in humans for large scale studies. In this manuscript, we identify and quantify urinary mercapturic acids consistent with previously suggested glutathione conjugation [19]. Among the predicted isoprene metabolites, we characterize the most abundant isomer in human urine, and describe an effective method for measuring isoprene exposure by modifying an existing multi-analyte method for urinary VOC metabolites [20].

2. Experimental

2.1. Materials and method

We custom synthesized a mixture of *N*-acetyl-*S*-(1-[hydroxymethyl]-2-methyl-2-propen-1-yl)-L-cysteine and *N*-acetyl-*S*-(2-hydroxy-3-methyl-3-buten-1-yl)-L-cysteine (IPMA1, chemical purity 95%), *N*-acetyl-*S*-(2-hydroxy-2-methyl-3-buten-1-yl)-L-cysteine (IPMA2a, chemical purity 90%), *N*-acetyl-*S*-(4-hydroxy-2-methyl-2-*trans*-buten-1-yl)-L-cysteine (IPMA3, chemical purity 97%) and the labeled internal standards, a mixture of *N*-acetyl-*S*-(2-hydroxy-3-methyl-3-buten-1-yl)-L-cysteine_$^2H_3$ plus *N*-acetyl-*S*-(1-[hydroxymethyl]-2-methyl-2-propen-1-yl)-L-cysteine_$^2H_3$ (IPMA1_$^2H_3$, chemical purity 96%, isotopic purity 98%), *N*-acetyl-*S*-(2-hydroxy-2-methyl-3-buten-1-yl)-L-cysteine_$^2H_3$ (IPMA2_$^2H_3$, chemical purity 90%, isotopic purity 98%), *N*-acetyl-*S*-(4-hydroxy-2-methyl-2-*trans*-buten-1-yl)-L-cysteine_$^2H_3$ (IPMA3_$^2H_3$, chemical purity 97%, isotopic purity 98%) (Toronto Research Chemicals, Canada) for this study. TRC provided a certificate of analysis for each compound synthesized. The certificate gives the name of the analyte, the structure, molecular formula, molecular weight, appearance, solubility, long-term storage conditions, methods used to determine the identity of the compound, purity (chemical and isotopic), TLC conditions, elemental analysis data, QC test date and the retest date. To determine the structure of isoprene metabolites TRC used ^{1}H NMR (DMSO_$^2H_6$) and mass spectrometer (MS). To determine chemical purity, ^{1}H NMR and TLC or HPLC were used and for isotopic purity MS was used. The ^{1}H NMR and MS spectra of IPMA1 and IPMA3 (labeled and unlabeled) received from TRC are attached to the Supplemental Information.

The isoprene metabolites stock standards were prepared individually by dissolving a known amount (~10 mg) of the neat standard in 10 mL of solvent. The solvent used for IPMA1 was a mixture of methanol and DMSO (1:1). Methanol was used as the solvent to prepare IPMA1_$^2H_3$, IPMA2a and IPMA2a_$^2H_3$ standards. A mixture of methanol and water (1:1) was used to prepare stock standards of IPMA3 and IPMA3_$^2H_3$.

2.2. Instruments

An ultra-high performance liquid chromatography system (Waters Inc., Milford, MA) coupled with electrospray tandem mass spectrometry (Sciex API 5500 Triple Quad, Applied Biosystems, Foster City, CA) was employed to quantitatively measure urinary VOC metabolites [20]. An Acquity UPLC® HSS T3 1.8 μm × 2.1 mm × 150 mm (Waters Inc., Milford, MA) column with 15 mM ammonium acetate pH 6.8 (Solvent A) and acetonitrile (Solvent B) as mobile phases with gradient were used for chromatographic separation of analytes. The eluent from the column was ionized using an electrospray interface to generate and transmit negative ions into the mass spectrometer. Comparison of relative response factors (ratio of native analyte to stable isotope labeled internal standard) with known standard concentrations yielded individual analyte concentrations for unknowns. The Analyst software (version 1.5.1, Applied Biosystems, Foster City, CA) was used to operate both the Acquity UPLC and the AB Sciex API5500. For mass accuracy we tuned the Sciex 5500 at unit resolution for a calibration <0.1 mass shift. The mass spectrometer was operated under scheduled multiple reaction monitoring (SMRM) mode for negative ions, the ion source temperature was kept at 650 °C, and the electrospray ion voltage at −4000 v. The mass spectrometric parameters were optimized for each analyte and labeled analog separately. The MRM transitions used for quantification, confirmation and internal standard for IPMA1 and IPMA3 were given in Table 1.

2.3. Limit of detection (LOD)

The limit of detection was determined using Taylor's method [21] by running freshly prepared calibrators daily for five consecutive days, and calculating $3S_0$ (3 times the standard deviation at zero concentration) as LOD. To confirm adequate signal to noise at the LOD, we spiked different low concentrations of analyte standards (IPMA1, IPMA3) into separate aliquots of non-smoker urine from a mixed pool, and assayed. This matrix spike confirmed adequate signal to noise to quantify target analytes in urine matrix at the LOD.

2.4. Calibration

A set of 9 calibrators was analyzed with each set of unknown samples. A weighted, 1/x (where x is the standard concentration),