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Measurement of urinary excretion of 5-hydroxymethyluracil in human by GC/NICI/MS: Correlation with cigarette smoking, urinary TBARS and etheno DNA adduct

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This article is dedicated to Professor Iwao Ojima for his 60th birthday

Abstract

5-Hydroxymethyluracil (5-HMU) is derived from radiation in addition to endogenous oxidative DNA damage and it is one of the most abundant DNA adducts. Human 5-HMU-DNA-glycosylase has been shown to repair this lesion. Whether urinary levels of 5-HMU is a valid biomarker for oxidative DNA damage in vivo has been investigated. However, controversial results on its relation to cigarette smoking were reported. To facilitate analysis of urinary 5-HMU in epidemiological studies, a highly sensitive and specific assay based on stable isotope dilution gas chromatography/negative ion chemical ionization/mass spectrometry was developed. The limit of detection for N^1,N^3-bis(pentafluorobenzyl)-HMU is 10 fg (20 amol) ($S/N = 4$) injected on column and the limit of quantification in urine was 0.7 nM of 5-HMU. Using as little as 10 μL of human urine samples, levels of urinary 5-HMU in 21 healthy volunteers were accurately quantified. No correlation was observed between urinary 5-HMU levels and cigarette smoking. However, there was a statistically significant association between urinary levels of 5-HMU and thiobarbituric acid-reactive substances ($r = 0.71$, $p = 0.0003$). In addition, urinary 5-HMU levels also correlated with urinary levels of 1,N^6-ethenoadenine ($r = 0.54$, $p = 0.01$). These findings suggest that this assay should be valuable in assessing the role of urinary 5-HMU as a biomarker of oxidative DNA damage and repair.
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Keywords: Cigarette smoking; 1,N^6-Ethenoadenine; 5-Hydroxymethyluracil; Mass spectrometry; Oxidative DNA damage; TBARS; Urine

Abbreviations: PFB_2-5-HMU; N^1,N^3-bis(pentafluorobenzyl)-5-HMU; GC/NICI/MS, gas chromatography negative ion chemical ionization mass spectrometry; 5-HMU, 5-hydroxymethyluracil; LC/ESI/MS/MS, liquid chromatography/electrospray ionization tandem mass spectrometry; PFB, pentafluorobenzyl; SIM, selective ion monitoring; SPE, solid-phase extraction; TBARS, thobarbituric acid-reactive substances

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1. Introduction

The production of reactive oxygen species within the cells causes damage of biomolecules and it plays an important role in carcinogenesis and aging. An important type of oxidative DNA damage is hydroxylation of DNA bases, leading to formation of 8-oxo-7,8-dihydroguanine; 5-hydroxymethyluracil (5-HMU); 5-hydroxyuracil; 8-oxo-7,8-dihydroadenine, etc. Hydroxylated DNA adducts have been considered as biomarkers of oxidative DNA damage (Shigenaga and Ames, 1991). Cigarette smoking produces various reactive oxygen species and results in elevation of oxidative stress and lung cancer formation (Pryor and Stone, 1993; Hecht, 1999).

Although the biological consequences of 5-HMU in human are not fully understood, specific glycosylase activities have been identified which remove 5-HMU from DNA (Rusmintratip and Sowers, 2000; Baker et al., 2002). Two possible sources account for formation of 5-HMU: via hydroxylation of thymine, forming the HmU:A mismatched base pair, or hydroxylation followed by deamination of 5-methylcytosine, resulting in HmU:G base pair formation. Although the occurrence of the mismatched HmU:A base pair greatly exceeds that of HmU:G, the latter is removed much more efficiently than the former by human 5-HMU glycosylase activities (Rusmintratip and Sowers, 2000; Baker et al., 2002). Thus, the conversion of 5-methylcytosine to 5-HMU is a potential pathway for the generation of 5-methylcytosine to T-transition mutations often found in human tumors.

Increased 5-HMU formation in tissue DNA has been connected with human diseases, and 5-HMU is considered a biomarker of oxidative DNA damage and of breast cancer (Djuric et al., 1991, 1996; Frenkel et al., 1993). Bianchini and co-workers reported that urinary excretion of 5-HMU is higher than that of other oxidized nucleobases, including 8-oxo-7,8-dihydroguanine, and it is significantly higher in smokers than in non-smokers (Bianchini et al., 1998). However, Pourcelot and co-workers showed that urinary excretion of HMU was similar in the smokers and non-smokers groups (Pourcelot et al., 1999). In this study, a highly sensitive and specific assay based on stable isotope dilution gas chromatography/negative ion chemical ionization/mass spectrometry (GC/NICI/MS) was developed to facilitate the analysis of urinary 5-HMU in epidemiological studies and to examine its correlation with the index of lipid peroxidation and lipid peroxidation-induced DNA adducts in the urine.

2. Materials and methods

2.1. Subjects

The subjects were aged between 20 and 68 years. The population consists of 9 smokers and 12 non-smokers, including 3 female non-smokers. The range of number of cigarettes smoked per day was between 5 and 25 and that of years of smoking was between 3 and 50 years.

2.2. Materials

5-Hydroxymethyluracil, pentafluorobenzyl bromide (PFB-Br), diisopropylethylamine, anhydrous methanol, and anhydrous phosphorous pentoxide were obtained from Aldrich Chemical Co. (Milwaukee, WI). $[^{13}C_2,D_2]$5-Hydroxymethyluracil (4,5-$^{13}C_2$, 98%; 5,5′-D_2, 98%) was purchased from Cambridge Isotope Laboratories, Andover, MA. Bond Elut C18–OH and Si solid-phase extraction (SPE) columns (500 mg, 3 mL) were from Varian (Harbor City, CA).

2.3. Verification of the structure of PFB_2-5-HMU

To a 4 mL vial containing dried 5-HMU (1.0 mg, 7.8 μmol) was added a solution of diisopropylethylamine (28 μL, 186 mmol) and PFB-Br (20 μL, 132 mmol) in 0.2 mL anhydrous methanol and the reaction mixture was stirred at 42 °C for 1 h. The reaction mixture was evaporated under vacuum and purified by two Si SPE columns to afford 3.0 mg PFB_2-5-HMU (76% yield) as a white solid. ^{1}H NMR ($CDCl_3$) δ 7.30 (C6–H, 1H), 5.28 (N3–CH_2 of PFB, 2H), 5.20 (N1–CH_2 of PFB, 2H), 4.40 (C5–CH_2OH, 2H). NICI/MS (assignment and relative abundance in parentheses) m/z 321 ($[M\text{–PFB}]^-$, 100%), 305 ($[M\text{–PFB-O}]^-$, 25%).

2.4. Assay procedures

Urine samples collected over a 24 h period was stored as 1.0 mL aliquots in 1.5 mL Eppendorff tubes

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