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Determination of dihydroxynaphthalenes in human urine by gas chromatography–mass spectrometry

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Abstract

A gas chromatography–mass spectrometry (GC–MS) method was developed for measuring 1,2-dihydroxynaphthalene (1,2-DHN) and 1,4-dihydroxynaphthalene (1,4-DHN) in urine. The method involves enzymatic digestion of urinary conjugates to release the DHNs which were then analyzed as trimethylsilyl derivatives by GC–MS. For 1,2-DHN and 1,4-DHN, respectively, the assay limits of detection were 0.21 and 0.15 μg/l, the assay limits of quantitation were 0.69 and 0.44 μg/l, and the coefficients of variation were 14.7 and 10.9%. This method was successfully applied to determine urinary levels of 1,2-DHN and 1,4-DHN in coke workers (14 top workers and 13 side-bottom workers) and 21 matching control workers from the steel industry of northern China. The geometric mean (GM) levels of 1,2-DHN were approximately 100 and 30 times higher than those of 1,4-DHN in exposed and control subjects, respectively. The GM levels 1,2-DHN and 1,4-DHN were significantly higher for coke workers (1,2-DHN: top workers – 552 μg/l, side-bottom workers – 260 μg/l; 1,4-DHN: top workers – 3.42 μg/l, side-bottom workers – 3.56 μg/l) than for controls (1,2-DHN: 38.8 μg/l; 1,4-DHN: 1.21 μg/l) ($p \leq 0.0031$). In each exposure category, levels of the DHNs were marginally greater in smokers than in nonsmokers ($p = 0.0646$). Strong correlations were observed among 1,2-DHN and 1,4-DHN and previously measured urinary levels of naphthalene, 1-hydroxynaphthalene, and 2-hydroxynaphthalene in these subjects ($r_s \geq 0.623$; $p < 0.0001$). Also, levels of 1,2-DHN were significantly correlated with those of serum albumin adducts of l,2-naphthoquinone ($r_s = 0.492$, $p = 0.0004$). These results indicate that 1,2- and 1,4-DHN are good biomarkers for assessment of naphthalene exposure in coke workers. Since the DHNs are precursors of the naphthoquinones, which have been implicated as toxic products of naphthalene metabolism, measurements of urinary DHNs may have toxicological significance.

Keywords: 1,2-Dihydroxynaphthalene; 1,4-Dihydroxynaphthalene; Gas chromatography–mass spectrometry; Urine; Coke workers; Biomarker; Naphthalene

1. Introduction

Naphthalene is the most abundant member of the class of compounds known as polycyclic aromatic hydrocarbons (PAHs), which are produced primarily from incomplete combustion of hydrocarbon fuels [1,2]. Humans exposed to high levels of PAH mixtures have been shown to be at increased risk of cancer of the lung, skin, and bladder [3]. Although PAHs with the greatest carcinogenic potency tend to have four or more aromatic rings, naphthalene (with two rings) has produced respiratory-tract tumors in rats and mice of both sexes [4–7]. The demonstrated carcinogenicity of naphthalene in two mammalian species, coupled with the abundance of naphthalene in indoor and outdoor air (it is the only PAH to exist almost exclusively in the gas phase), recently motivated the International Agency for Research on Cancer (IARC) [8] and the U.S. Environmental Protection Agency (EPA) [9–11] to reclassify naphthalene as a possible human carcinogen.

Abbreviations: CYP, cytochrome P450; DHN, dihydroxynaphthalene; EI, electron impact; GC–MS, gas chromatography–mass spectrometry; GM, geometric mean; GSD, geometric standard deviation; LOD, limit of detection; PAH, polycyclic aromatic hydrocarbon; SIM, selected ion monitoring; TMS, trimethylsilyl

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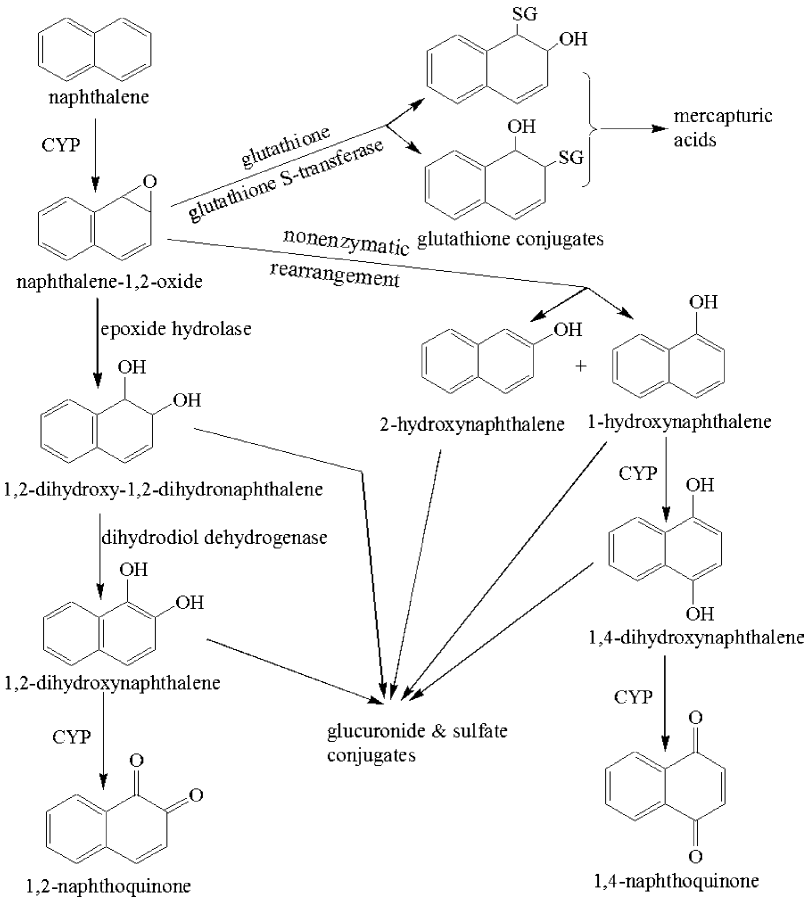

Fig. 1. Proposed metabolic pathways for naphthalene in human.

The mammalian metabolism of naphthalene is complicated [1,12]. As shown in Fig. 1, naphthalene is metabolized by various CYP enzymes (CYP 1A1, 1A2, 2A1, 2E1, 2F1, 2F2, CYP3A5, and CYP3A7) to naphthalene-1,2-oxide [7,13–15]. All other metabolites are thought to arise from naphthalene-1,2-oxide via the following pathways: spontaneous rearrangement to 1-hydroxynaphthalene and 2-hydroxynaphthalene, conjugation with glutathione and further rearrangement to the mercapturic acid, and conversion to 1,2-dihydroxy-1,2-dihydronaphthalene via epoxide hydrolase. Further CYP oxidation of 1-hydroxynaphthalene leads to 1,4-dihydroxynaphthalene (1,4-DHN) while reduction of 1,2-dihydroxy-1,2-dihydronaphthalene via dihydrodiol dehydrogenase leads to 1,2-DHN. The DHNs can be oxidized, either enzymatically or nonenzymatically, to 1,2- and 1,4-naphthoquinone, both of which are capable of binding with macromolecules [16–22].

In considering the mode of naphthalene's toxic action, attention has focused primarily upon the naphthoquinones and their precursors, the DHNs [16–22]. We have shown previously that levels of urinary naphthalene, urinary 1- and 2-hydroxynaphthalene, and albumin adducts of 1,2-naphthoquinone were all significantly increased in coke workers compared to control workers in the steel industry of northern China [2,16,23]. However, since analytical methods were not available to measure the DHNs, we could not complete our analysis of important urinary naphthalene metabolites in those samples. In the current study, we report a simple GC–MS method to quantify 1,2- and 1,4-DHN in urine and then we apply the assay to urine from the same 28 coke workers and 22 control workers, previously investigated. To our knowledge, this is the first report of measurements of urinary 1,2- and 1,4-DHN in humans.

2. Experimental

2.1. Chemicals and reagents

[$^2H_8$]Naphthalene (+98% isotopic purity) was obtained from Aldrich Chemical (Milwaukee, WI). 1,4- and 1,2-DHN were obtained from Tokyo Kasei Kogyo Co. (Toshima, Kita-Ku, Tokyo). β-Glucuronidase/aryl sulfatase (type H-2 from Helix pomatia; β-glucuronidase activity 98,000 units/ml and sulfatase activity 2400 units/ml) was purchased from Sigma Chemical Co. (St Louis, MO). Tri-Sil®-TBT reagent was obtained from Pierce Chemical (Rockford, IL). Magnesium sulfate, sodium dithionite, sodium acetate, anhydrous sodium sulfate, diethyl ether, acetonitrile, ethyl acetate, methanol and hexane were obtained from Mallinckrodt Baker, Inc. (Phillipsburg, NJ). Concentrated sulfuric acid, sodium chloride, ceric ammonium nitrate and L-ascorbic acid were purchased from Fisher Scientific Co. (Norcross, GA).