Toxicology Letters 205 (2011) 273–278

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Metabolism of N^2-(4-hydroxyphenyl)guanine, a DNA adduct formed from *p*-benzoquinone, in rat

Igor Linhart[a,*], Petr Mikeš[b], Antonín Králík[a], Jaroslav Mráz[c], Emil Frantík[c]

[a] *Department of Organic Chemistry, Faculty of Chemical Technology, Institute of Chemical Technology, Prague, Technická 1905, CZ-166 28 Prague, Czech Republic*
[b] *Apigenex, Ltd., Poděbradská 56, CZ-180 66 Prague, Czech Republic*
[c] *National Institute of Public Health, Šrobárova 48, CZ-100 42 Prague, Czech Republic*

ARTICLE INFO

Article history:
Received 28 April 2011
Received in revised form 10 June 2011
Accepted 14 June 2011
Available online 28 June 2011

Keywords:
Benzene
p-Benzoquinone
DNA adducts
Biotransformation
Molecular dosimetry

ABSTRACT

Among numerous adducts formed by reaction of DNA with *p*-benzoquinone (*p*-BQ), an electrophilic metabolite of benzene, only N^2-(4-hydroxyphenyl)guanine (N2HPG) has been confirmed *in vivo*. If excreted in urine N2HPG would be a candidate non-invasive biomarker of the DNA damage caused by benzene. To test this hypothesis, biotransformation of N2HPG was studied in rats. Unchanged N2HPG in urine amounted to 8.0 ± 2.2% and 9.1 ± 1.7% (mean ± SE) at the dose of 2 mg/kg excreted within 1 and 2 days after *ip* dosage, respectively. After acidic hydrolysis of the urine a slight but consistent increase in urinary N2HPG to 9.5 ± 3.2% and 11 ± 2.6% of dose was found within 1 and 2 days, respectively, indicating formation of hydrolysable conjugates. An oxidised metabolite was detected by LC–ESI-MS and identified by comparison with authentic standard as N^2-(4-hydroxyphenyl)-8-oxoguanine (N2HPOG). Its excretion amounted to 2.7 ± 0.2% of dose and increased to 12.0 ± 2.7% of dose when N2HPOG was released from its conjugates by acidic hydrolysis. Glucuronides and sulphates of both N2HPG and N2HPOG were confirmed in urine by LC-ESI-MS and by enzymatic treatment with glucuronidase/sulphatase. These results indicate an extensive metabolism of N2HPG *in vivo*, which must be taken into account when considering N2HPG as a possible biomarker of exposure to benzene.

1. Introduction

Benzene is a well-known human and animal carcinogen produced on mass scale and released into the environment mainly from automobile exhausts (IARC, 1987; Whysner et al., 2004). Although many studies have been focused on reactive metabolites of benzene (for reviews see Kalf, 1987; Snyder et al., 1993; Monks et al., 2010) as well as on the DNA adducts formed, the role of these reactive metabolites in the process of carcinogenesis is poorly understood (Whysner et al., 2004). Benzoquinones (BQ) are formed as electrophilic metabolites of benzene by oxidation of hydroquinone (HQ) and catechol (CAT) catalysed by myeloperoxidases in the bone marrow (Eastmond et al., 1986). Numerous adducts have been found by reactions of *o*- and *p*-benzoquinones (*o*- and *p*-BQ) with the DNA (Pongracz et al., 1990; Pongracz and Bodell, 1991; Jowa et al., 1990; Gaskell et al., 2002; Levay et al., 1991; Chenna et al., 1995; Bodell et al., 1996; Norpoth et al., 1996; Cavalieri et al., 2002; Zahid et al., 2010). To our knowledge neither the condensed benzetheno adducts derived from *p*-BQ, nor the depurinating adducts derived from *o*-BQ have ever been detected *in vivo*.

In our previous work we described metabolism of two benzetheno adducts in rat. We found that these adducts are extensively metabolised so that only a small portion of the administered dose was excreted unchanged in urine (Linhart et al., 2011). Their efficient metabolism may be the reason why they could not be found in urine or tissues of animals exposed to benzene. In continuation of our previous study we now focused our attention on the fate of another adduct derived from *p*-BQ, namely, N^2-(4-hydroxyphenyl)guanine (N2HPG). Nucleotide form of N2HPG has been detected by ^{32}P-postlabeling in the bone marrow and leukocytes of mice treated with high doses of benzene (Bodell et al., 1996) as well as in HL-60 cells treated with HQ and *p*-BQ *in vitro* (Levay et al., 1991; Pongracz and Bodell, 1996). Hitherto identified urinary DNA adducts are almost exclusively nucleobase derivatives (Shuker and Farmer, 1992; Farmer et al., 2005) with a significant exception of 8-oxo-2′-deoxyguanosine (Wu et al., 2004). Although DNA adducts at the N^2 position of guanine do not belong to easily depurinating ones, their nucleoside and nucleotide forms are likely to undergo hydrolytical cleavage *in vivo* releasing eventually the corresponding nucleobase adduct (Linhart et al., 2011). Therefore, N2HPG itself rather than its nucleoside or nucleotide precursors can be expected in urine after its corresponding DNA

* Corresponding author. Tel.: +420 220 444 165; fax: +420 220 444 288.
E-mail address: linharti@vscht.cz (I. Linhart).

0378-4274/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2011.06.016

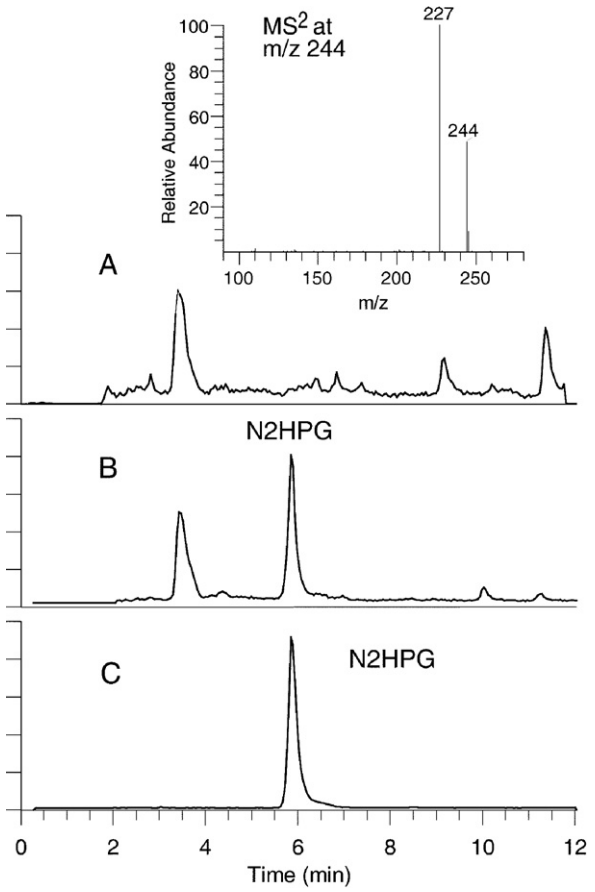

Fig. 1. Analysis of N2HPG in urine by LC-ESI-MS (positive ion chromatograms). MS^2, full scan taken at m/z 244 $(M+H)^+$ for N2HPG was set for traces (A), control rat urine, (B) urine of rat dosed with 7.5 mg/kg N2HPG, and (C) N2HPG standard. MS^2 spectrum of N2HPG at m/z 244 is shown in the insert.

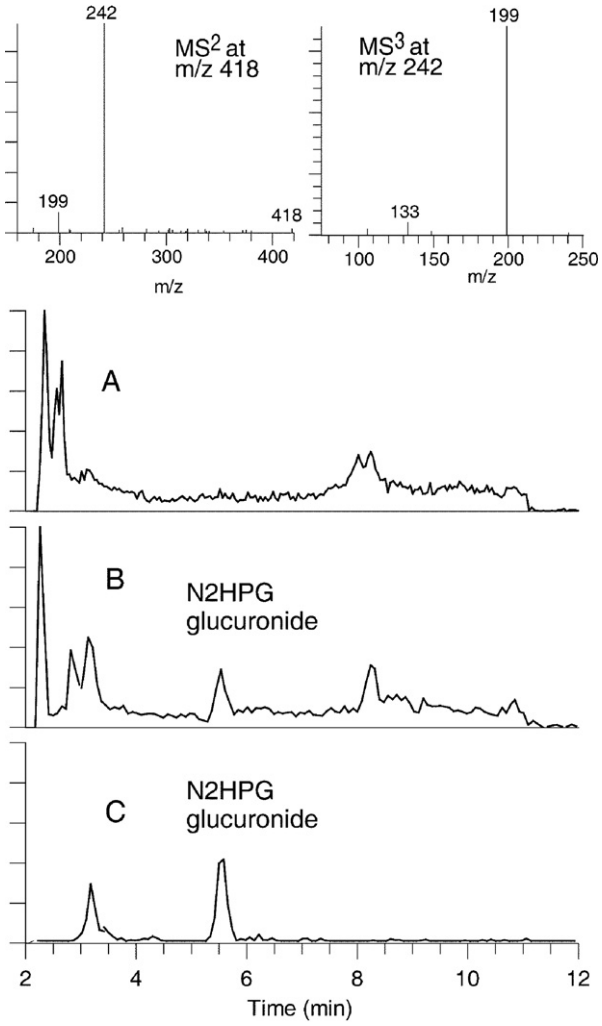

Fig. 2. Analysis of N2HPG metabolites in urine by LC-ESI-MS (negative ion chromatograms). MS^2, full scan taken at m/z 418 $(M-H)^-$ for N2HPG glucuronide was set for traces (A), control rat urine, (B) urine of rat dosed with 7.5 mg/kg N2HPG and (C) urine of rat dosed with 7.5 mg/kg N2HPG. MS^2 and MS^3 spectra of N2HPG glucuronide are shown in the inserts.

adducts are released from the DNA irrespective of the specific repair mechanisms. For known environmental carcinogens such as benzo[*a*]pyrene urinary nucleobase adducts have been found in human urine indicating not only exposure to but also DNA damage caused by these chemicals (Bhattacharya et al., 2003).

The aim of this study is to investigate biotransformation of N2HPG in rats *in vivo* in relation to its possible use in molecular dosimetry of benzene.

2. Materials and methods

2.1. General

Acetonitrile for LC/MS Chromasolv was from Riedel de Haën, formic acid, puriss. p.a. from Fluka. Re-distilled water was used for LC/MS and solid phase extraction. β-Glucuronidase type H-2 from Helix pomatia (EC 3.2.1.31) (with glucuronidase activity ≥100,000 units/mL and sulphatase activity ≤7500 units/mL), 2-bromohypoxanthine and 4,5-diamino-6-hydroxy-2-sulphanylpyrimidine (>85%) were from Sigma–Aldrich (Czech Republic). Other chemicals were of analytical or synthetic grade and were used as received.

Qualitative LC/MS analyses as well as quantitative determination of N2HPOG were carried out on a Thermo Scientific LXQ linear trap mass spectrometer in tandem with a Janeiro LC system consisting of two Rheos 2200 pumps and CTC PAL autosampler. For quantitative determination of N2HPG a triple quadrupole system was used consisting of a Micromass Quattro PremierXE (Waters Micromass, USA) interfaced with Agilent 1200 Series binary gradient pump (Agilent, USA) and CTC PAL Analytics Autosampler (Switzerland). NMR spectra were taken on a Varian Gemini 300 MHz spectrometer.

2.2. Preparation of authentic standards

N^2-(4-Hydroxyphenyl)guanine (N2HPG) was prepared as described in the literature (Wright and Dudycz, 1984). N^2-(4-Hydroxyphenyl)-8-oxoguanine (N2HPOG) was prepared in three synthetic steps from 4,5-diamino-6-hydroxy-2-sulphanylpyrimidine. Its reaction with urea gave 6,8-dihydroxy-2-sulphanylpurine (Noell and Robins, 1959), which was subsequently reacted with bromine to give 2-bromo-6,8-dihydroxypurine (Beaman et al., 1962). Finally, 2-bromo-6,8-dihydroxypurine (250 mg, 1 mmol) was refluxed with 4-aminophenol (360 mg, 3.3 mmol) in 7 mL of methoxyethanol for 2.5 h. The reaction mixture was then diluted with 7 mL of water and concentrated by evaporation of approx. a half of the solvent in a vacuum. A precipitate formed on standing overnight in a refrigerator was filtered off, washed subsequently with water, acetone and diethyl ether and dried over P_4O_{10}. The product (240 mg, 87%) was obtained as a beige powder.

^{1}H NMR (DMSO-d_6): δ = 6.68 (d, J = 8.1 Hz, 2H, C2′–H and C6′–H); 7.23 (d, J = 8.1 Hz, 2H, C3′–H and C5′–H); 8.23 (s, 1H, NH); 9.20 (s, 1H, NH); 10.4 (s, 2H, NH); 10.95 (s, 1H, OH). ^{13}C NMR (DMSO-d_6): δ = 101.3 (C5); 115.9 (C2′ and C6′); 123.2 (C3′ and C5′); 130.5 (C1′); 148.1 (C4′); 150.6 and 151.5 (C2 and C4); 153.6 and 154.2 (C6 and C8). ESI-MS: m/z 260 $(M+H)^+$; MS^2: m/z 260 → 243 $(MH-OH)^+$; 232 $(MH-CO)^+$; 258 $(M-H)^-$; MS^2: m/z 258 → 215 $(M-H-NHCO)^-$; 165 $(M-H-C_6H_5O)^-$; 145; 133.

2.3. Animal treatment

Adult male Wistar rats, average weight 300 ± 8 g (mean ± S.D.) were placed individually into glass metabolic cages with free access to pelleted food and water. To enhance diuresis, sucrose (8 mg/mL) was added to the drinking water. Control urine was collected for 24 h. N2HPG dissolved in DMSO at a concentration of 1 mg/mL was administered to three animals by a single *ip* injection of 2 mL/kg, i.e., at a dose of 2 mg/kg. One control animal was injected with 2 mL/kg of DMSO. Urine was then collected for 2 consecutive days at 24 h intervals. During sample collection the urine was filtered through a gauze filter to remove pieces of faeces and crumbs of food pellets. The walls of the metabolic cages were rinsed with distilled water and result-