Chemico-Biological Interactions xxx (2010) xxx–xxx

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Analysis of hydroquinone and catechol in peripheral blood of benzene-exposed workers

P.J. Kerzic[a,b], W.S. Liu[a], M.T. Pan[a], H. Fu[c], Y. Zhou[d], A.R. Schnatter[d], R.D. Irons[a,b,e,*]

[a] Fudan-Cinpathogen Clinical and Molecular Research Center, Shanghai, China
[b] Cinpathogen Inc., Shanghai, China
[c] Fudan University School of Public Health, Shanghai, China
[d] ExxonMobil Biomedical Sciences, East Annandale, NJ, USA
[e] University of Colorado Denver, Denver, CO, USA

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Benzene
Hydroquinone
Catechol
Metabolism
Blood

ABSTRACT

We have developed a gas chromatography–mass spectrometry method for analysis of benzene (BZ) metabolites in human urine and blood. Here we describe peripheral blood concentrations of hydroquinone (HQ[1]) and catechol (CAT[2]) in total, protein-bound, and unbound (free) forms obtained from BZ-exposed factory workers and controls. Total and unbound metabolites were directly measured in independent experiments, while bound forms were calculated as [total] – [unbound]. In this subset of a larger study, breathing zone benzene, toluene, and xylene were measured for the duration of a workshift, and end-shift blood samples taken from 143 subjects and controls. Potential lifestyle and environmental influences were assessed by questionnaire and bioassay, and single nucleotide polymorphisms in xenobiotic metabolizing enzymes NQO1, MPO, CYP2E1, and GSTT1 were also analyzed for potential contribution to differences in blood metabolite concentration. Total CAT, bound CAT, total HQ, and bound HQ correlated well with benzene exposure, while unbound CAT and HQ displayed no correlation. Nearly all of the metabolites found in blood were bound to protein (CAT 96–99+%, HQ 78–92+%), and when the ratio of bound to unbound metabolites were compared in subsets of exposed workers, the increase in blood metabolite concentration was nearly all due to an increase in the protein-bound molecule. These findings suggest that a threshold for conjugation does not exist within the exposure spectrum studied (0.01–78.8 mg/m^3). This method demonstrates the feasibility of analyzing benzene metabolites in human blood, and should allow for further investigation of the health effects of benzene and its metabolites.

1. Introduction

Benzene is a widely used industrial chemical with multiple uses in many industries, and long-term exposure to high concentrations of benzene has been associated with adverse health effects including hematopoietic disorders such as acute myelogenous leukemia [1,2]. A number of methods of tracking potential benzene exposure in the occupational setting have been employed, including passive air monitoring, urinalysis of benzene metabolites, and parent compound excretion and exhalation [3–5]. Also, specific protein adducts have been identified that may represent exposures over time periods longer than several days [6–8]. Using urinary metabolite and benzene excretion as source data, together with extensive knowledge of human and animal pharmacokinetics and pharmacodynamics, computer models have been developed to predict the molecule's biological fate [9]. However, little is known about the total concentrations of major benzene metabolites in blood following occupational exposure to benzene.

It is understood that metabolism is required for benzene hematotoxicity, and the enzymes and bioactivation involved in benzene metabolism have been widely studied [10]. First metabolized by CYP2E1 to benzene oxide, the molecule can undergo multiple fates involving activity by epoxide hydrolase, chemical rearrangement, or further activity by CYP2E1 to form the major metabolites, hydroquinone (HQ), catechol (CAT), and phenol (PH). Minor pathways result in the formation of S-phenyl mercapturic acid (SPMA) and the open-ring t,t-muconic acid (ttMA) [11]. Nearly all of this activity occurs in the liver, and the metabolites formed circulate as either protein-bound species or in free form to the bone marrow,

Abbreviations: BZ, benzene; CAT, catechol; HQ, hydroquinone; GC–MS, gas chromatography–mass spectrometry; NQO1, NAD(P)H:quinone oxidoreductase; MPO, myeloperoxidase; CYP2E1, cytochrome p450 2E1; GSTT1, glutathione S-transferase theta 1.

* Corresponding author at: 399 Cai Lun Rd., 1st Floor, Building 5, Shanghai 201210, China. Tel.: +86 21 6163 3871; fax: +86 21 6163 3870.
E-mail addresses: ann.louden@ucdenver.edu, Richard.Irons@Cinpathogen.com (R.D. Irons).

[1] Formula: Free HQ = total HQ – bound HQ.
[2] Formula: Free CAT = total CAT – bound CAT.

0009-2797/$ – see front matter © 2010 Published by Elsevier Ireland Ltd.
doi:10.1016/j.cbi.2009.12.010

where myeloperoxidase (MPO) oxidizes HQ and CAT to form reactive quinone species [12]. These reactive quinones are thought to be ultimately responsible for toxicity through binding with cellular macromolecules or generation of oxidative stress [13,14].

Biomarker studies have attempted to identify markers of benzene exposure, and most have included genetic components that take into account polymorphisms in xenobiotic metabolizing enzymes that may significantly alter enzyme activity [15,16]. Nearly all biomarker studies of late have focused on urinary excretion of benzene metabolites, searching for a marker of sufficient specificity and sensitivity to identify (and potentially quantify) benzene exposures over a wide range of concentrations. While the presence of major metabolites HQ and CAT in urine correlate well with benzene exposure, neither can be assumed to be specific due to their presence in high concentrations in subjects not exposed to benzene, as well as the fact that they are ubiquitous metabolites from components of multiple sources, including cigarette smoke, automobile exhaust, and dietary intake [17–19]. Minor metabolites such as ttMA and SPMA display better correlations with benzene exposure in many studies, but both are present in very low concentrations in urine and there are few methods for the simultaneous analysis of multiple benzene metabolites [20]. There have been very few attempts to use blood metabolite concentrations as biomarkers of benzene exposure, most likely due to assumed high background levels in unexposed individuals as well as lack of suitable methods for analysis. This has resulted in a major gap in knowledge regarding our understanding of the mechanism of benzene toxicity, since target organ toxicity is dependent upon metabolite transportation through the blood to the bone marrow, where toxic effects occur.

The present study was undertaken to assess blood concentrations of major benzene metabolites HQ and CAT, in total amounts, free, and protein-bound form, after occupational exposure to benzene. We developed a method for analysis of HQ and CAT in blood that is easy to use, precise and accurate. Direct measurement of total and free metabolites were made and protein-bound was defined as [total] – [free]. We also administered lifestyle and work history questionnaires to identify variables such as cigarette smoking, and identified the genotypes of study subjects regarding multiple enzymes responsible for benzene metabolism.

2. Materials and methods

2.1. Chemical reagents

Internal standards ($[^2H_6]$CAT and $[^2H_6]$HQ) were purchased from Cambridge Isotope Laboratories (Andover, MA). All other chemical reagents were purchased from Sinopharm (Shanghai, China).

2.2. Study subjects and sample collection

Blood and urine samples were collected from 143 individuals working at a rubber product manufacturing and finishing facility located near Shanghai, China. Exposure to benzene, xylene, and toluene was measured using 3 M vapor monitors throughout the workday, and the monitors were analyzed by gas chromatography using NIOSH standard procedure 4000. All subjects gave their informed consent using forms and procedures approved by the University of Colorado Multiple Institutional Review Board (COMIRB) and the Fudan University Institutional Review Board, as well as questionnaires dealing with personal and occupational history and lifestyle habits. Subjects were included in the study if they completed an informed consent form and questionnaire, and provided urine and blood samples. All samples were collected after work shifts had ended. Urine samples were collected and stored at 4 °C until transportation to the laboratory, where they were stored at −20 °C. Blood samples were transported to the laboratory in anticoagulant-coated tubes at room temperature, where DNA extraction was performed and blood aliquots (1 ml) were stabilized by addition of 0.5% ascorbic acid prior to freezing at −80 °C prior to metabolite analysis.

2.3. Measurement of blood metabolites

Internal standards were added to thawed blood (25 μl of: CAT 1.6 μg/ml, HQ 3.2 μg/ml) and after mixing, the solution was extracted with extraction buffer (acetonitrile 1: toluene 3: MTBE 4) to remove free metabolites. After removing the organic layer, 25 μl of internal standards and 100 μl concentrated HCl was added. This solution was mixed and incubated for 1 h at 95 °C to hydrolyze protein-bound metabolites, extracted again, and the two organic layers were combined for analysis of total HQ and CAT after dehydration with Na_2SO_4 and drying to a small volume at 70 °C under nitrogen gas. For analysis of free HQ and CAT, the first organic layer was taken through the assay without hydrolysis of protein-bound metabolites. All metabolites were derivatized using pentafluorobenzyl bromide for 30 min at 70 °C, and the solution was extracted with hexane. The organic layer was reduced to dryness under nitrogen gas, and metabolites were dissolved in toluene for analysis.

2.4. Analytical instrumentation and GC–MS conditions

Metabolite analysis was performed using a PerkinElmer Autosystem XL GC/Turbo MS with an autosampler, fitted with a J&W DB-5MS 3 m × 0.25 mm × 0.25 μm column (Agilent Technologies). The temperatures of the injector and interface were set at 200 °C and all 1 μl injections were splitless. The temperature of the CI source was also set at 200 °C. The helium flow rate was set at 1 ml/min, and the GC oven temperature was held at 80 °C for the first minute and then increased to 285 °C at a rate of 15 °C per min. The oven was held at this final temperature for an additional 8 min. The following characteristic ions were monitored: CAT m/z 293 and 289; HQ m/z 293 and 289.

2.5. Gene polymorphism analyses and cotinine measurement

Urinary cotinine, a metabolite of nicotine, was measured by ELISA to validate responses to questionnaires regarding smoking status according to manufacturer's instructions (Bio-Quant, San Diego, CA).

Genomic DNA was isolated from blood using a Qiagen QIAamp DNA isolation kit (Qiagen, Chatsworth, CA) according to the manufacturer's directions. Briefly, proteinase K was added to EDTA-treated peripheral blood, followed by the addition of Qiagen lysis buffer. After lysis at 70 °C, ethanol was added and the resulting solution was passed over an appropriately sized QIAamp DNA isolation column and spun. The columns were then washed with wash buffers, and the DNA was eluted with distilled water. A spectrophotometer was used to measure the absorbance of the DNA-containing eluate at 260 and 280 nm, and the concentration and purity of the DNA was recorded. Several aliquots of DNA were prepared and stored at −80 °C for future use.

Presence of a single nucleotide polymorphism (SNP) was determined by restriction fragment length polymorphism (RFLP) analysis. A list of genes studied, the primers and restriction enzymes used is included below. For each RFLP method, several positive and negative samples were subjected to DNA sequencing to verify the accuracy of the method.

Please cite this article in press as: P.J. Kerzic, et al., Analysis of hydroquinone and catechol in peripheral blood of benzene-exposed workers, Chem. Biol. Interact. (2010), doi:10.1016/j.cbi.2009.12.010

Gene	Primers	Polymorphism/result
*NQO1*2*	F: 5′-TCCTCAGAGTGGCATTCTGC-3′ R: 5′-TCTCCTCATCCTGTACCTCT-3′	C609T Gain of HinfI site
MPO	F: 5′-GGTATAGGCACACAATGGTGAG-3′ R: 5′-GCAATGGTTCAAGCGATTCTTC-3′	G463A Loss of AciI site
CYP2E1	F: 5′-CCAGTCGAGTCTACATTGTCA-3′ R: 5′-TTCATTCTGTCTTCTAACTGG-3′	C1019T Loss of RsaI site
GSTT1	F: 5′-TTCCTTACTGGTCCTCACATCTC-3′ R: 5′-TCACCGGATCATGGCCAGCA-3′	Null genotype No PCR product
β-globin	F: 5′-CAACTTCATCCACGTTCACC-3′ R: 5′-GAAGAGCCAAGGACAGGTAC-3′	*GSTT1* control

2.6. Method validation

Control samples of blood from unexposed, non-smoking volunteers spiked with known concentrations of metabolites were included in each batch of samples prepared for analysis for quality control purposes. Standard curves were prepared by spiking thawed control blood (0–500 ng/ml CAT and HQ) and then carrying the samples through the assay as described. No blood samples were found to contain CAT or HQ outside of the linear range of the standard curves. Assay precision was determined by analyzing duplicate samples from subjects as well as repeated analysis of control samples across all batches. Limits of detection and quantitation for CAT and HQ in blood could not be estimated due to high background levels found in unexposed controls.

2.7. Statistical analysis

Statistical analysis was performed using SAS version 9.2, with HQ and CAT as dependent variables. Natural logarithm of metabolite concentrations and airborne benzene measurements was used to improve data distributional properties.

3. Results

3.1. Assay precision, accuracy and linearity

Estimates of CVs for metabolites in blood were 10.94% for HQ and 4.86% for CAT. Accuracy was measured to be 7.77% for HQ and 11.96% for CAT. Limits of detection could not be estimated due to the presence of significant background concentrations for both metabolites in unexposed, non-smoking controls. Standard curves were linear ($r^2 \geq 0.98$) up to 1000 ng/ml for CAT and HQ.

3.2. Correlation between blood metabolites and benzene exposure

Blood samples and exposure data were collected from 143 individuals, and analyzed for benzene exposure and total blood metabolite concentrations for HQ and CAT. A second analysis for free metabolites was performed using an abbreviated method that did not include hydrolysis of protein-bound molecules (see Section 2) and a separate set of standard curves and quality control samples. This analysis included only 100 individuals, because the volumes of blood samples collected were not sufficient to permit re-analysis of all subjects. Benzene, toluene, and xylene were monitored, and for all individuals in this study benzene was the predominant solvent subjects were exposed to. Demographic characteristics of study subjects can be found in Table 1. The majority of subjects were male, and the majority were smokers. Fig. 1 displays the total metabolite concentrations for HQ and CAT versus same-day benzene exposure for all 143 subjects, and indicates increasing metabolite concentrations for both HQ and CAT with increasing benzene exposure. A separate measurement of free HQ and CAT was performed on a subset of 100 individuals (Fig. 2). These results do not indicate an

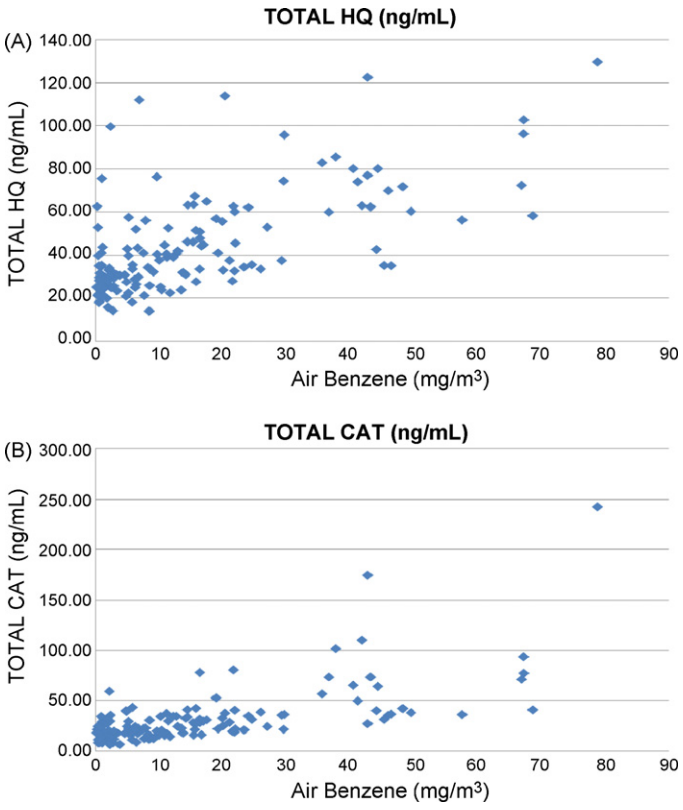

Fig. 1. Relationship between benzene exposure and total blood metabolite concentrations. Total HQ (A) and CAT (B) in blood samples from 143 study subjects are plotted against same-day benzene exposure.

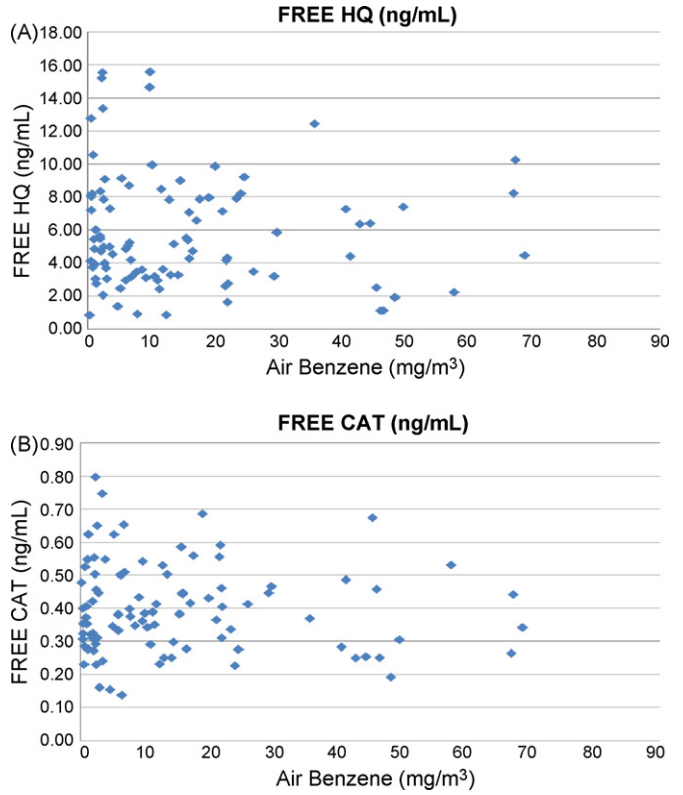

Fig. 2. Relationship between benzene exposure and free blood metabolite concentrations. Free HQ (A) and CAT (B) in blood samples from 100 study subjects are plotted against same-day benzene exposure.

Please cite this article in press as: P.J. Kerzic, et al., Analysis of hydroquinone and catechol in peripheral blood of benzene-exposed workers, Chem. Biol. Interact. (2010), doi:10.1016/j.cbi.2009.12.010

Table 1
Demographic characteristics of study subjects.

	Male/female	Age ± SD (range)	BMI ± SD (range)	Smokers by questionnaire (per day ave, range)	Exposure range (mg/m^3)
Unexposed	5/2	47.2 ± 4.05 (43–52)	23.0 ± 2.62 (19.4–26.1)	5 (18, 15–20)	0.01–0.1
Exposed	100/36	45.4 ± 5.4 (30–57)	23.3 ± 3.01 (17.1–30.4)	81 (16.8, 3–40)	0.19–78.8
Total	105/38	45.5 ± 5.3 (30–57)	23.3 ± 2.98 (17.1–30.4)	86 (16.8, 3–40)	0.01–78.8

upward trend of metabolite concentration with increasing same-day benzene exposure. Protein-bound metabolites, calculated as the difference between total and free concentrations, are shown in Fig. 3, and indicate increasing blood concentrations for both HQ and CAT with increasing exposure.

Correlation coefficients for all metabolite data are displayed in Table 2. Total metabolites, as well as protein-bound metabolites, correlated well with benzene exposure, while free metabolites showed no correlation with increasing benzene exposure. The best correlations with exposure were seen with total HQ and protein-bound HQ. There was a near perfect correlation between bound and total metabolites for both HQ and CAT.

3.3. Metabolite percentage of total and ratio

Exposure data and correlations indicate that free metabolites do not make up a significant proportion of the increase in total metabolites seen with increasing exposure. To examine this we separated subjects into exposure subgroups and analyzed the percentage of total metabolites that were comprised of protein-bound molecules (Fig. 4A). Nearly all CAT was found in the protein-bound form across the entire range of exposures studied (0.01–78.8 mg/m^3), with very little increase in the percentage bound at higher exposures. In contrast, the majority of HQ was found to be protein-bound in unexposed subjects, and the proportion bound increased with increasing benzene exposure (Fig. 4A). The fraction of protein-bound metabolites does not decrease with increasing exposure, indicating that, there is no threshold for metabolite conjugation over the exposure range studied. We further analyzed the ratio of bound to free metabolites, and similar trends were seen (Fig. 4B). There was an increase in the proportion of bound/free CAT with increasing exposure, although the concentrations of unbound CAT were much lower than HQ in all subjects.

3.4. Metabolite correlations among smokers and non-smokers

Cigarette smoking has been identified as a confounding variable in analysis of benzene metabolites, because the byproducts of inhaled tobacco smoke are a major source of HQ and CAT. In order to evaluate the potential impact of cigarette smoking on the exposure-metabolite relationship, we analyzed correlations between blood metabolites and exposure in smokers versus non-smokers, as well as in the exposure subgroups described above (Table 3). We hypothesized that smoking might confound blood metabolite analysis in individuals exposed to low concentrations of benzene, but segregation of smokers versus non-smokers in higher exposure groups would not result in significant differences in blood metabolite con-

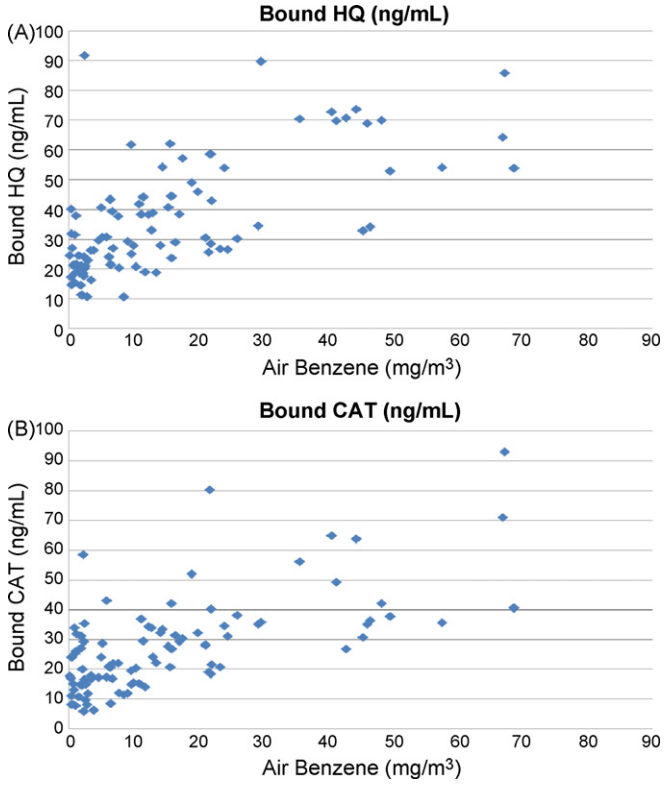

Fig. 3. Relationship between benzene exposure and protein-bound blood metabolite concentrations. Protein-bound HQ (A) and CAT (B) in 100 study subjects, calculated from the difference between total and free metabolite concentrations, are plotted against same-day benzene exposure.

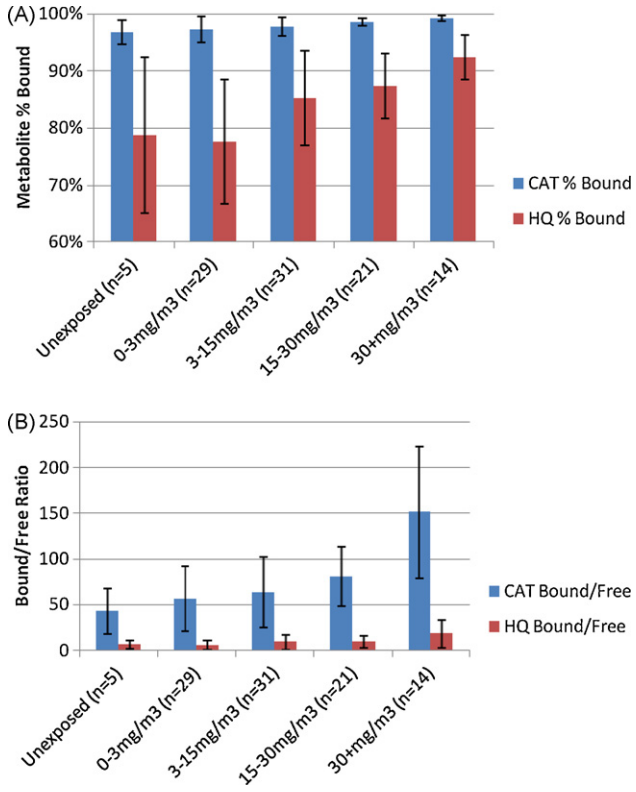

Fig. 4. Benzene metabolite percentage and ratio by exposure group. Study subjects (n = 100) were divided into exposure groups and the percentage of metabolites bound to protein (A) and the ratio of bound to free metabolites (B) were calculated.

Please cite this article in press as: P.J. Kerzic, et al., Analysis of hydroquinone and catechol in peripheral blood of benzene-exposed workers, Chem. Biol. Interact. (2010), doi:10.1016/j.cbi.2009.12.010

Table 2
Correlations between blood metabolite concentrations and benzene exposure. Pearson correlation coefficients, p values, and number of samples are shown for log-transformed data.

	Total HQ	Total CAT	Bound HQ	Bound CAT	Free HQ	Free CAT
Benzene exposure	0.61587 $p < 0.0001$ $n = 143$	0.60362 $p < 0.0001$ $n = 143$	0.66803 $p < 0.0001$ $n = 100$	0.59063 $p < 0.0001$ $n = 100$	−0.04738 $p = 0.6397$ $n = 100$	−0.06589 $p = 0.5148$ $n = 100$
Total HQ		0.64242 $p < 0.0001$ $n = 143$	0.97613 $p < 0.0001$ $n = 100$	0.66632 $p < 0.0001$ $n = 100$	0.27045 $p = 0.0065$ $n = 100$	−0.10539 $p = 0.2967$ $n = 100$
Total CAT			0.66653 $p < 0.0001$ $n = 100$	0.99983 $p < 0.0001$ $n = 100$	0.08688 $p = 0.3901$ $n = 100$	−0.13392 $p = 0.1841$ $n = 100$
Bound HQ				0.66549 $p < 0.0001$ $n = 100$	0.07840 $p = 0.4381$ $n = 100$	−0.13655 $p = 0.1755$ $n = 100$
Bound CAT					0.08379 $p = 0.4072$ $n = 100$	−0.14768 $p = 0.1426$ $n = 100$
Free HQ						0.09801 $p = 0.3320$ $n = 100$

centrations. Among all subjects, there were significantly higher concentrations of free HQ found in the blood of smokers when compared to non-smokers, although this parameter did not correlate with benzene exposure. Only in the low exposure group (0–3 mg/m^3) were differences found among metabolites that also correlated with benzene exposure, such as total CAT and protein-bound CAT and HQ. The impact of cigarette smoking was not evident in individuals exposed to benzene above 3 mg/m^3 except in the 15–30 mg/m^3 group, in which non-smokers were found to have higher concentrations of total and protein-bound HQ.

3.5. Metabolite correlations by genotype

The formation of benzene metabolites after exposure can be influenced by a variety of factors, and single nucleotide polymorphisms have received much attention in this regard. In order to determine if some genotypes altered correlations between exposure and metabolite concentration, we identified the genotypes of several key metabolizing enzymes (CYP2E1, NQO1, MPO, and GSTT1) in all subjects and correlated metabolites and benzene exposure by genotype, as well as by combinations of genotypes we expect to have similar effects on toxicity (Table 4). For example, we expect MPO GG and NQO1 vt/vt to lead to higher levels if 1,4 and 1,2 benzoquinone, which are more toxic than HQ or CAT, and have defined the combination of these genotypes as unfavorable. Similarly, CYP2E1 CC and GSTT1 (negative) fall into the same unfavorable classification due to their influences on activation and detoxification. Favorable classification was determined by the combination of NQO1 wt/wt or wt/vt and MPO AA or AG, along with the combination of CYP2E1 C1C2 or C2C2 and GSTT1 (positive). Other combinations were defined as neutral. Blood metabolite concentrations were not significantly different in any subgroup defined by genotype alone or in combination with others (data not shown), though in some cases the correlations differed. NQO1

Table 3
Metabolite concentrations in smokers and non-smokers. Metabolites were measured in blood samples from subjects segregated by smoking status assessed by questionnaire and validated by urinary cotinine ELISA ($n = 137$), with further analysis after separating subjects into exposure subgroups (number of subjects in parantheses).

	Total HQ	Total CAT	Free CAT	Free HQ	Bound HQ	Bound CAT
All subjects						
Non-smokers (57)	47.153	32.118	0.414	4.798	37.785	26.911
Smokers (80)	40.787	30.496	0.399	6.298	33.672	27.015
p	0.101	0.426	0.280	0.008	0.182	0.469
0–3 mg/m^3						
Non-smokers (15)	27.679	14.321	0.359	4.902	15.896	12.045
Smokers (25)	32.824	22.785	0.428	6.854	27.047	23.150
p	0.173	0.001	0.064	0.038	0.003	0.0001
3–15 mg/m^3						
Non-smokers (16)	37.595	21.036	0.475	4.585	30.697	19.563
Smokers (29)	37.347	23.941	0.364	5.719	31.779	22.329
p	0.484	0.157	0.015	0.171	0.384	0.207
15–30 mg/m^3						
Non-smokers (13)	59.146	29.628	0.445	4.970	51.900	32.691
Smokers (17)	45.328	34.653	0.426	6.182	37.034	33.408
p	0.037	0.177	0.376	0.133	0.040	0.452
30+ mg/m^3						
Non-smokers (13)	69.396	68.781	0.386	4.755	59.866	48.108
Smokers (9)	79.806	76.397	0.327	6.703	67.210	50.019
p	0.157	0.363	0.213	0.188	0.156	0.417

Please cite this article in press as: P.J. Kerzic, et al., Analysis of hydroquinone and catechol in peripheral blood of benzene-exposed workers, Chem. Biol. Interact. (2010), doi:10.1016/j.cbi.2009.12.010

Table 4
Correlations between blood metabolite concentrations and same-day benzene exposure by genotype (number of subjects in parentheses).

	Total CAT	Total HQ	Free CAT	Free HQ	Bound CAT	Bound HQ
CYP2E1						
C1C1 (84)	0.560†	0.565†	−0.027	−0.096	0.599†	0.595†
C1C2 and C2C2 (59)	0.755†	0.710†	−0.181	−0.024	0.786†	0.785†
NQO1						
vt/vt (28)	0.784†	0.714†	−0.063	0.117	0.902†	0.786†
wt/vt (71)	0.681†	0.643†	−0.015	−0.102	0.632†	0.582†
wt/wt (44)	0.531†	0.562†	−0.210	−0.208	0.456†	0.707†
MPO						
AA and AG (38)	0.776†	0.781†	−0.092	−0.298	0.623†	0.714†
GG (105)	0.605†	0.569†	−0.058	−0.051	0.655†	0.658†
GSTT1						
Negative (71)	0.695†	0.600†	−0.230	−0.265	0.516†	0.548†
Positive (72)	0.621†	0.671†	0.020	0.049	0.729†	0.733†
CYP + GST1						
Unfavorable (43)	0.554†	0.464*	−0.295	−0.280	0.490¶	0.504¶
Neutral (68)	0.703†	0.706†	0.064	0.052	0.684†	0.704†
Favorable (32)	0.716†	0.682†	−0.224	0.021	0.791†	0.788†
MPO + NQO1						
Unfavorable (22)	0.786†	0.812†	−0.072	0.218	0.925†	0.823†
Neutral (89)	0.548†	0.518†	−0.069	−0.140	0.515†	0.594†
Favorable (32)	0.802†	0.829†	−0.633	−0.238	0.705*	0.803†

¶ $p \leq 0.01$.
* $p \leq 0.001$.
† $p \leq 0.0001$.

allele status had a significant impact on total CAT and HQ, as well as protein-bound CAT, with individuals carrying two copies of the variant allele (NQO1 vt/vt) having higher correlations with exposure. Carriers of the GSTT1 null allele had lower correlations between exposure and the protein-bound forms of both CAT and HQ. Subjects defined as having unfavorable CYP2E1 and GSTT1 genotypes displayed decreased exposure correlations with total as well as bound CAT and HQ. Carriers of MPO and NQO1 genotypes defined as neutral displayed decreased exposure correlations of total and bound metabolites compared to both favorable and unfavorable genotype combinations.

4. Discussion

The identification of specific metabolites in blood or blood protein-conjugated molecules possesses several advantages over analysis of urinary metabolites for the study of biomarkers. Blood protein adducts typically have a much longer half-life than urinary metabolites, potentially allowing for biomarker studies of exposures covering longer periods of time [21]. Benzene exposure has been associated with cysteinyl, hemoglobin, and albumin adducts in previous studies [6–8,22], although it is unclear which, if any, endogenous and dietary sources may have contributed to their formation. These studies identify molecules that have undergone further tertiary metabolism beyond HQ and CAT and may represent accumulated biomarkers and/or repeated exposures. These are useful for studies involving exposure over periods of days to several weeks or interrupted exposure scenarios. Ideally, a biomarker of toxicant exposure would appear at very low concentrations in unexposed individuals, and in the current study, as in previous studies referenced above, the metabolites that correlate best with exposure (protein-bound and total HQ and CAT) appear in relatively high concentrations in unexposed individuals. This is the case for a number of proposed biomarkers of benzene exposure, due to the variety and prevalence of sources of exposure other than occupational benzene. Analysis of the kinetics of protein-bound HQ and CAT formation and elimination would be of value to identify the most useful range of measurements and duration of exposures for these compounds. The use of blood metabolites as biomarkers of exposure rather than urinary metabolites has the advantage of providing information concerning bioactivation, kinetics of metabolism and distribution rather than elimination. Therefore, correlation of blood metabolites with urinary metabolites across an exposure range is likely to be more informative than analysis of either alone.

Several studies have identified lifestyle influences on urinary benzene metabolite concentrations, and the data presented here are consistent with earlier results. Factors known to influence HQ and CAT (and other metabolites) in urine include exposure to passive and active cigarette smoke, automobile exhaust, phenolic compounds in tea and other dietary sources [23–25]. The minor urinary benzene metabolites, SPMA and ttMA, which have correlated the best with exposure across a broad range of concentrations, do not appear to be related to multiple dietary influences, though ttMA may in some cases be influenced by preservative intake or other environmental factors [26]. Nevertheless, the mechanisms of ttMA formation remain unclear, thus the potential influences of genetic and other confounding variables cannot be assessed. Conversely, production of blood CAT and HQ occurs early in the process of benzene metabolism, and the steps involved are relatively well known. Data presented here demonstrate a positive influence of smoking on total blood CAT and both bound HQ and CAT, although this influence is only present at low exposures (<3 mg/m^3). Interestingly, non-smokers in the 15–30 mg/m^3 group had higher concentrations of total and bound HQ in blood, suggesting that smoking may have varied effects on individuals exposed to BZ at different levels. Alternatively, other factors such as lifestyle influences may have an effect. Further analysis of other potential behavioral influences will be presented in future publications.

The potential for genetic factors to influence production of benzene biomarkers has been well studied, albeit virtually exclusively using urinary metabolites as endpoints. The data presented here is consistent with most previously published reports, in that the concentrations of HQ and CAT are not significantly influenced by

Please cite this article in press as: P.J. Kerzic, et al., Analysis of hydroquinone and catechol in peripheral blood of benzene-exposed workers, Chem. Biol. Interact. (2010), doi:10.1016/j.cbi.2009.12.010

genetic factors, although some genotypes display better correlations with exposure than others. NQO1 is considered to have an important influence on genetic susceptibility to benzene poisoning [27]. In the present study, carriers of two copies of the NQO1 vt allele display better correlations with exposure for multiple endpoints than either heterozygous individuals or carriers of wild type alleles. Clearly, the influence of specific genetic markers, as well as the potential interplay with other genes and environmental factors, requires further study. Our results confirm that these analyses should take into account both urinary and blood metabolite concentrations as end points.

Conflict of interest

R.D.I. has received consulting fees from law firms in cases involving benzene. All other authors declare no competing financial interests.

Acknowledgements

The authors would like to extend our appreciation to Ann Louden for manuscript and clerical assistance. The CMRC received funds from the Benzene Health Research Consortium consisting of ExxonMobil, BP Amoco, Chevron-Texaco, Conoco-Philips and Shell Chemical Companies. This work was supported in part by Cinpathogen, Inc.

References

[1] World Health Organization, International Agency for Research on Cancer. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, IARC Press, Lyon, France, 1997.
[2] Agency of Toxic Substances and Disease Registry (ATSDR), Toxicological profile for benzene, Draft for public comment, Oak Ridge National Laboratory, 1987.
[3] C.N. Ong, B.L. Lee, Determination of benzene and its metabolites: application in biological monitoring of environmental and occupational exposure to benzene, J. Chromatogr.: Biomed. Appl. 660 (1994) 1–22.
[4] S. Ghittori, L. Maestri, M.L. Fiorentino, M. Imbriani, Evaluation of occupational exposure to benzene by urinalysis, Int. Arch. Occup. Environ. Health 67 (1995) 195–200.
[5] S. Lagorio, R. Crebelli, R. Ricciarello, L. Conti, I. Iavarone, A. Zona, S. Ghittori, A. Carere, Methodological issues in biomonitoring of low level exposure to benzene, Occup. Med. 48 (1998) 497–504 (Oxford).
[6] K. Yeowell-O'Connell, N. Rothman, M.T. Smith, R.B. Hayes, G. Li, S. Waidyanatha, M. Dosemeci, L. Zhang, S. Yin, N. Titenko-Holland, S.M. Rappaport, Hemoglobin and albumin adducts of benzene oxide among workers exposed to high levels of benzene, Carcinogenesis 19 (1998) 1565–1571.
[7] S.M. Rappaport, S. Waidyanatha, Q. Qu, R. Shore, X. Jin, B. Cohen, L.C. Chen, A.A. Melikian, G. Li, S. Yin, H. Yan, B. Xu, R. Mu, Y. Li, X. Zhang, K. Li, Albumin adducts of benzene oxide and 1,4-benzoquinone as measures of human benzene metabolism, Cancer Res. 62 (2002) 1330–1337.
[8] Y.S. Lin, R. Vermeulen, C.H. Tsai, S. Waidyanatha, Q. Lan, N. Rothman, M.T. Smith, L. Zhang, M. Shen, G. Li, S. Yin, S. Kim, S.M. Rappaport, Albumin adducts of electrophilic benzene metabolites in benzene-exposed and control workers, Environ. Health Perspect. 115 (2007) 28–34.
[9] S. Scheding, M. Loeffler, S. Schmitz, H.-J. Seidel, H.-E. Wichmann, Hematotoxic effects of benzene analyzed by mathematical modeling, Toxicology 72 (1992) 265–279.
[10] D. Ross, The role of metabolism and specific metabolites in benzene-induced toxicity: evidence and issues, J. Toxicol. Environ. Health A 61 (2000) 357–372.
[11] M.R. Lovern, C.E. Cole, P.M. Schlosser, A review of quantitative studies of benzene metabolism, Crit. Rev. Toxicol. 31 (2001) 285–311.
[12] V.V. Subrahmanyam, P. Kolachana, M.T. Smith, Metabolism of hydroquinone by human myeloperoxidase: mechanisms of stimulation by other phenolic compounds, Arch. Biochem. Biophys. 286 (1991) 76–84.
[13] R.D. Irons, Quinones as toxic metabolites of benzene, J. Toxicol. Environ. Health 16 (1985) 673–678.
[14] Y. Shen, H.M. Shen, C.Y. Shi, C.N. Ong, Benzene metabolites enhance reactive oxygen species generation in HL60 human leukemia cells, Hum. Exp. Toxicol. 15 (1996) 422–427.
[15] G.J. Morgan, M.T. Smith, Metabolic enzyme polymorphisms and susceptibility to acute leukemia in adults, Am. J. Pharmacogenomics 2 (2002) 79–92.
[16] M. Sorensen, H. Skov, H. Autrup, O. Hertel, S. Loft, Urban benzene exposure and oxidative DNA damage: influence of genetic polymorphisms in metabolism genes, Sci. Total Environ. 309 (2003) 69–80.
[17] R. Gopalakrishna, Z.-H. Chen, U. Gundimeda, Tobacco smoke tumor promoters, catechol and hydroquinone, induce oxidative regulation of protein kinase C and influence invasion and metastasis of lung carcinoma cells, PNAS 91 (1994) 12233–12237.
[18] L Wallace, Environmental exposure to benzene: An update, Environ. Health Perspect. 104 (Suppl. 6) (1996) 1129–1136.
[19] T.A. McDonald, N.T. Holland, C. Skibola, P. Duramad, M.T. Smith, Hypothesis:, Phenol and hydroquinone derived mainly from diet and gastrointestinal flora activity are causal factors in leukemia, Leukemia 15 (2001) 10–20.
[20] S. Waidyanatha, N. Rothman, G. Li, M.T. Smith, S. Yin, S.M. Rappaport, Rapid determination of six urinary benzene metabolites in occupationally exposed and unexposed subjects, Anal. Biochem. 327 (2004) 184–199.
[21] W.E. Bechtold, J.K. Willis, J.D. Sun, W.C. Griffith, T.V. Reddy, Biological markers of exposure to benzene: *S*-phenylcysteine in albumin, Carcinogenesis 13 (1992) 1217–1220.
[22] D.E. Nerland, W.M. Pierce Jr., Identification of *N*-acetyl-*S*-(2,5-dihydroxyphenyl)-L-cysteine as a urinary metabolite of benzene, phenol, and hydroquinone, Drug Metab. Dispos. 18 (6) (1990) 958–961.
[23] B. Javelaud, L. Vian, R. Molle, P. Allain, B. Allemand, B. Andre, F. Barbier, A.M. Churet, J. Dupuis, M. Galand, F. Millet, J. Talmon, C. Touron, M. Vaissiere, D. Vechambre, M. Vieules, D. Viver, Benzene exposure in car mechanics and road tanker drivers, Int. Arch. Occup. Environ. Health 71 (1998) 277–283.
[24] S. Fustinoni, D. Consonni, L. Campo, M. Buratti, A. Colombi, A.C. Pesatori, M. Bonzini, P.A. Bertazzi, V. Foa, S. Garte, P.B. Farmer, L.S. Levy, M. Pala, F. Valerio, V. Fontana, A. Desideri, D.F. Merlo, Monitoring low benzene exposure: comparative evaluation of urinary biomarkers, influence of cigarette smoking, and genetic polymorphisms, cancer epidemiology, Biomark. Prevent. 14 (2005) 2237–2244.
[25] A.P. DeCaprio, The toxicology of hydroquinone—relevance to occupational and environmental exposure, Crit. Rev. Toxicol. 29 (1999) 283–330.
[26] T. Ruppert, G. Scherer, A.R. Tricker, F. Adlkofer, Trans, trans-muconic acid as a biomarker of non-occupational environmental exposure to benzene, Int. Arch. Occup. Environ. Health 69 (1997) 247–251.
[27] M.T Smith, Benzene, NQO1, and genetic susceptibility to cancer, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 7624–7626.

Please cite this article in press as: P.J. Kerzic, et al., Analysis of hydroquinone and catechol in peripheral blood of benzene-exposed workers, Chem. Biol. Interact. (2010), doi:10.1016/j.cbi.2009.12.010