Induction of CYP4F3 In White Blood Cells from Benzene-Poisoning Patients and Human HL60 Cells

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Summary

Exposure to benzene elicits a range of hematotoxicity from leukopenia to leukemia. We used microarray to analyze differential gene expression in the white blood cells (WBC) from 7 patients diagnosed with occupational benzene poisoning compared with 7 matched controls. All patients exhibited elevated expression of CYP4F3, a leukotriene B_4 (LTB_4) ω-hydroxylase critical in the inactivation of LTB_4 in polymorphonuclear leukocytes (PMN), with the fold of induction between 3 and 71. CYP4F3 was also induced in cultured promyolocytic leukemia cells (HL-60) by a benzene metabolite, phenol, similarly to *all-trans* retinoic acid (ATRA). Induction of CYP4F3 may play a role in benzene hematotoxicity and serve as a biomarker of benzene exposure and toxicity.

Introduction

Benzene is widely used in industries as a general purpose solvent and in the synthesis of other chemicals. Exposure to benzene occurs worldwide to workers in shoe making, automobile repair, oil industry, shipping, and chemical manufacturing, as well as to the general population from cigarette smoking, gasoline vapor, and automobile exhaust. Chronic exposure to benzene typically results in bone marrow toxicity that often manifests decreased peripheral blood cell counts initially, but may progress to pancytopenia, aplastic

anemia, and ultimately, myelogenous leukemia [1]. Benzene undergoes metabolic activation in the liver to form multiple metabolites that exhibit biological activities. CYP2E1 is critical in catalyzing the initial and subsequent oxygenation of benzene giving rise to benzene oxide, phenol, hydroquinone, catechol, *E,E*-muconic acid and other metabolites. Mice lacking CYP2E1 had relatively low levels of benzene metabolites and were resistant to benzene hematotoxicity compared with the wild type [2]. Other enzymes including mEH (EPHX1), NQO1, and GSTs participate in the formation of the metabolites. In humans, polymorphisms of these enzymes were found to associate with the urine levels of benzene metabolites. The molecular events governing the development of benzene-induced toxicity and cancer are not well understood. The chronic and progressive nature of benzene hematotoxicity and tumorigenecity suggests genomic reprogramming that would result in aberrant gene expression during the development of myelotoxicity and leukemia by benzene. Few studies have been conducted to address this issue.

Materials and Methods

Seven female workers from a shoe manufacture factory and a chemical production company in the Xinxiang City, China, were diagnosed with varying degrees of benzene poisoning by the local authority. All patients exhibited reduction of peripheral blood cell counts. The concentrations of benzene that these patients were exposed to at the work place ranged from 10 to 200 mg/m^3 at the time of diagnosis. Seven local workers who did not have occupational benzene exposure histories and were matched with the patients on age, gender, geographical location, year of working experience, educational level, and history of smoking and alcoholic drinking, were chosen as controls. No patients or controls had occupational histories of exposure to other hematotoxic chemicals or radiation and were not exposed to chemotherapy within 15 days prior to the study. All procedures involving human subjects including information and sample collection, sample analysis, and informed consent were approved by the institutional review board of Wuhan University Medical Center, Wuhan, China. Ten milliliters of peripheral blood were collected from the patients and controls. White blood cells were prepared from fresh blood samples using the Polymorphprep solution (AXIS-SHIELD, PoC AS, Oslo, Norway). Total RNA was prepared using the Trizol reagents (Invitrogen, Carlsbad, CA, USA). The cDNA microarray was performed using the CSC-GE-80 microarray chip with cDNAs representing 8,064 human expressed genes, following the procedures provided by the chip manufacturer (Shenzhen Chipscreen Biosciences Ltd., Shenzhen, Guandong, China). Each chip contained a total of 384 genes as positive and negative controls, internal standards, and external standards. A ratio of larger than 2 or less than 0.5 was considered significantly different between the patient and the control samples. The human promyelocytic leukimia HL-60 cells were obtained from ATCC (Manasus, VA, USA). The cells were cultured in IMDM (Invitrogen, Calsbad, CA, USA) with 10% fetal bovine serum at 37°C and 5% CO_2. HL-60 cells

were seeded in a 6-well plate at a density of $1x10^5$ cells/ml and were treated with benzene, phenol, and hydroquinone for 4 days. *All-trans*-retinoic acid (ATRA, 1 μM, 4 days) was used as a positive control. Real-time PCR was carried out with probes that distinguish CYP4F3 and CYP4F3B mRNAs. Flow cytometry was performed with anti-CYP4F3 antibodies from Abnova (Taipei, Taiwan).

Results

The microarray results revealed altered expressions of multiple genes involved in apoptosis, DNA repair, immune function, and drug metabolism in the peripheral WBC of patients with benzene poisoning compared with those of healthy controls [3]. In this report, we analyzed the expression profiles of CYPs. Expression of CYP4F3 (LTB_4 ω-hydroxylase) was elevated in all patients. Fold of induction varied from 3 to 71 with a geometric mean of 9-fold (Table 1). CYP1A1 and 1B1, two PAH-inducible forms of CYPs, were elevated in 4 and 2 patients, respectively. CYP2B6 and CYP51 were down regulated in one patient, whereas CYP27A1 was up-regulated in one patient but was down-regulated in 3 patients.

To confirm that benzene indeed induces CYP4F3, we analyzed induction of CYP4F3 in HL-60 cells, a human promyolocytic leukemia cell line. Both CYP4F3 and CYP4F3B (a splice variant of CYP4F3) mRNAs were expressed in HL-60 cells at low but detectable levels by RT-PCR (Figure 1). ATRA, which is known to induce CYP4F3 in human leukocytes and HL-60 cells,

Table 1 Fold of induction of CYP4F3 mRNA expression in chronic benzene poisoning workers relative to matched controls

Worker number	Diagnosis	Fold of up-regulation
1	Suspicious benzene poisoning	2.96
2	Moderate level benzene poisoning	11.8
3	Aplastic anemia	3.92
4	Medium level benzene poisoning	22.2
5	Medium level benzene poisoning	3.94
6	Severe level benzene poisoning	2.96
7	Moderate level benzene poisoning	70.6
Averaged fold of induction		9.012 (1.22)*

*: Geometric mean (standard deviation)

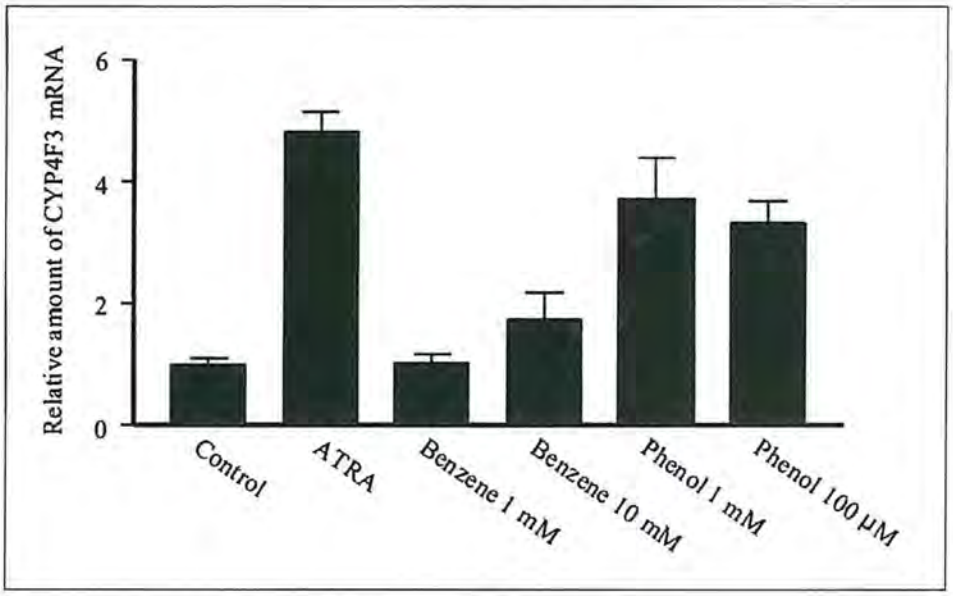

Figure 1 Induction of CYP4F3 in human promyelocytic leukemia HL-60 cells by real-time PCR.

induced CYP4F3 mRNA significantly at 1 μM (4 days). Benzene at a high concentration (10 mM, 4 days) only modestly induced CYP4F3; but phenol at 1 mM and 100 μM (4 days) induced both genes significantly. Real-time quantitative PCR revealed that benzene at 10 mM induced CYP4F3 by 2-fold; phenol at 1 mM and 100 mM induced 4F3 by 4 and 3.5-fold, comparable to the induction by ATRA (~5 fold) (Figure 1). In addition, phenol at both concentrations induced 4F3B by ~9 and 6-fold, respectively, but ATRA induced 4F3B only slightly (~2-fold). Benzene had no apparent inductive effect on 4F3B mRNA expression. Induction of the CYP4F3 protein was confirmed using immuno fluorescent flow cytometry. ATRA induced the CYP4F3 protein at 1 μM as expected. Phenol induced the CYP4F3 protein in a concentration-dependent manner at 10 and 100 μM, but induction was reduced at higher concentrations of phenol (10 mM). Induction by phenol was also time-dependent with high inductions during 4 and 6 days of treatment. The findings indicate that the benzene metabolite, phenol, induced CYP4F3 at both mRNA and protein levels in human leukocytes during benzene poisoning.

Conclusions

We utilized cDNA microarray to analyze the gene expression profiles in the peripheral white blood cells (WBC) from seven female workers diagnosed

with occupational benzene poisoning in comparison with seven matched control subjects with no histories of occupational benzene exposure. The results revealed altered expression of a number of CYP genes. In particular, the expression of CYP4F3, which encodes LTB_4 w-hydroxylase in WBC [4], was elevated in all patients. Moreover, CYP4F3 was shown to be induced in human promyelocytic leukinia HL-60 cells by the benzene metabolite, phenol, at both mRNA and protein levels. The findings implicate induction of CYP4F3 in the development of benzene hematotoxicity and suggest it as a potential biomarker for occupational benzene poisoning.

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