Benzene, a carcinogen that induces chromosomal breaks, is strongly associated with leukemias in humans. Possible genetic determinants of benzene susceptibility include proteins involved in repair of benzene-induced DNA damage. The catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), encoded by Prkdc, is one such protein. DNA-PKcs is involved in the nonhomologous end-joining (NHEJ) pathway of DNA double-strand break (DSB) repair. Here we compared the toxic effects of benzene on mice (C57BL/6 and 129/Sv) homozygous for the wild-type Prkdc allele and mice (129/SvJ) homozygous for a Prkdc functional polymorphism that leads to diminished DNA-PK activity and enhanced apoptosis in response to radiation-induced damage. Male and female mice were exposed to 0, 10, 50, or 100 ppm benzene for 6 h/d, 5 d/week for 2 weeks. Male mice were more susceptible to benzene toxicity compared with females. Hematotoxicity was evident in all male mice but was not seen in female mice. We observed similar, large increases in both micronucleated erythrocyte populations in all male mice. Female mice had smaller but significant increases in micronucleated cells. The p53-dependent response was induced in all strains and genders of mice following benzene exposure, as indicated by an increase in p21 mRNA levels in bone marrow that frequently corresponded with cell cycle arrest in G2/M. Prkdc does not appear to be a significant genetic susceptibility factor for acute benzene toxicity. Moreover, the role of NHEJ, mediated by DNA-PK, in restoring genomic integrity following benzene-induced DSB remains equivocal.


This study investigated benzene-induced neoplasia in CBA/Ca mice, with special emphasis on hematopoietic tissues. Ten-week-old male CBA/Ca mice were exposed to 300 ppm benzene via inhalation for 6 hr/day, 5 days/week, for 16 weeks and held 18 months after the last exposure. There were 125 benzene-exposed and 125 sham-exposed mice. Malignant lymphoma was a statistically significant cause of early mortality in the benzene-exposed mice. Fourteen benzene-exposed mice developed lymphoma (lymphoblastic, lymphocytic, or mixed) as compared to only 2 sham-exposed mice. Benzene-exposed mice also developed preputial gland squamous cell carcinomas (60% in benzene-exposed vs 0% in sham-exposed) and had an increased incidence of lung adenomas (36% vs 14%). Moderate to marked granulocytic hyperplasia was present in benzene-exposed animals, with a 36% incidence in the bone marrow and 6% in the spleen, as compared to the sham-exposed with 8 and 0%, respectively. Interpretation of the granulocytic response as a direct effect of benzene was complicated by the presence of inflammation in the mice. Although inhaled benzene was clearly carcinogenic in CBA mice, it did not induce granulocytic leukemia.


Long-term inhalation exposure of benzene has been shown to cause hematotoxicity and an increased incidence of acute myelogenous leukemia in humans. The progression of benzene-induced hematotoxicity and the features of the toxicity that may play a major role in the leukemogenesis are not known. We report the hematological consequences of benzene inhalation in B6C3F1 mice exposed to 1, 5, 10, 100, and 200 ppm benzene for 6 hr/day, 5 days/week for 1, 2, 4, or 8 weeks and a recovery group. There were no significant effects on hematopoietic parameters from exposure to 10 ppm benzene or less. Exposure of mice to 100 and 200 ppm benzene reduced the number of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, and most blood parameters. Replication of primitive progenitor cells in the bone marrow was increased during the exposure period as a compensation for the cytotoxicity induced by 100 and 200 ppm benzene. In mice exposed to 200 ppm benzene, the primitive progeni or cells maintained an increased percentage of cells in S-phase through 25 days of recovery compared with controls. The increased replication of primitive progenitor cells in concert with the reported genotoxicity induced by benzene provides the components necessary for producing an increased incidence of lymphoma in mice. Furthermore, we propose this mode of action as a biologically plausible mechanism for benzene-induced leukemia in humans exposed to high concentrations of benzene.
Benzene toxicity is attributed to its metabolism, which is primarily mediated by the ethanol-inducible cytochrome P450 2E1 isoform (CYP2E1). The present study investigated the myelotoxicity and urinary concentrations of major benzene metabolites in adult CD-1 male mice treated with low levels of benzene vapors, ethanol, or a combination of the two. Groups of ethanol-treated (5% in a Lieber-DeCarli liquid diet, 3 weeks) or pair-fed control mice were exposed to 10 ppm benzene, 6 h per day, 5 days per week for 2 weeks, starting from the second week of ethanol administration. On the last day of treatment, the number of early and late erythroid progenitors (BFU-E and CFU-E) was reduced by 55%, while the number of granulocyte/macrophage progenitors (CFU-GM) was reduced by 36% in benzene-treated mice. Ethanol lowered the CFU-E, BFU-E, and CFU-GM colony formation by 33%, 28%, and 12%, respectively. In animals coexposed to benzene and ethanol, the CFU-E colony counts were decreased by 70%, the BFU-E by 80%, and the CFU-GM by 45%. Phenol (Ph), hydroquinone (HQ), catechol (Cat), and trans,trans-muconic acid (MA) were measured by HPLC-UV in urine samples collected weekly during the last 6-h benzene/air exposure session. In benzene-exposed mice urinary metabolite levels peaked at the end of the first week of treatment (µg/kg body weight (bw)): Ph: 4931 +/- 1055; Cat: 109 +/- 17; HQ: 784 +/- 137; MA: 534 +/- 92) and significantly decreased at the end of the second week (µg/kg bw: Ph: 3909 +/- 984; Cat: 82 +/- 24; HQ: 337 +/- 72; MA: 235 +/- 55). In mice given benzene and ethanol, the urinary levels of Ph, Cat, HQ, and MA were significantly lower than those measured in the group given benzene alone. The urinary levels of Ph and Cat showed a decreasing trend, again, from the first to the second week of benzene exposure. These data indicate that chronic ethanol ingestion exacerbates benzene myelotoxicity and, in addition, reduces the urinary excretion of benzene metabolites in mice, suggesting that the influence of ethanol intake should be considered carefully in biomonitoring benzene exposure.


Benzene Hemoglobin Adducts in Mice and Rats: Characterization of Formation and Physiological Modeling. SUN, J. D., MEDINSKY, M. A., BIRNBAUM, L. S., LUCIER, G., AND HENDERSON, R. F. (1990). Fundam. Appl. Toxicol 15, 468-475. Benzene is a myelotoxin and a human leukemogen. Humans are exposed to this compound, both occupationally and environmentally. This study was conducted to determine whether formation of benzene-derived adducts with blood hemoglobin (Hb) can be used as a biomarker of exposure to benzene. B6C3F1 mice and F344/N rats were given 0.1 to 10,000 µmol [14C]benzene/kg body wt, orally. Twenty-four hours later, animals were euthanized, and globin was isolated from blood samples. The globin was analyzed by liquid scintillation spectrometry for the presence of [14C]benzene-de-rived adducts. Hb adduct formation was linear with respect to dose for amounts of up to 500 µmol [14C]benzene/kg body wt, for both rodent species. Within this linear dose-response range, mice formed adducts from [14C]benzene approximately 3.5 times less efficiently (0.022 +/- 0.010 (pmol adducts/mg globin)/(µmol/kg body wt dose)) than did rats (0.076 +/- 0.014 (pmol adducts)/(µmol/kg body wt dose)). Benzene-derived Hb adducts also accumulated linearly when mice and rats were given up to three daily doses of 500 µmol [14C]benzene/kg body wt. These data were used to develop a physiological model for benzene-derived Hb
adduct formation. Both first-order and saturable pathways for adduct formation were incorporated. The results showed that the model simulated the levels of Hb adducts in both mice and rats after oral exposures to benzene and predicted the levels of Hb adducts present after inhalation exposure. These studies suggest that Hb adducts might be useful biomarkers for human exposures to benzene.


Benzene is a ubiquitous pollutant and known human leukemogen. Benzene can be enzymatically bioactivated to reactive intermediates that can lead to increased formation of reactive oxygen species (ROS). ROS formation can directly induce DNA double-strand breaks, and also oxidize nucleotides that are subsequently converted to double-strand breaks during DNA replication that can be repaired through homologous recombination, which is not error-free. Therefore increased DNA double-strand-break levels may induce hyper-recombination, which can lead to deleterious genetic changes. To test the hypothesis that benzene and its metabolites can initiate hyper-recombination and to investigate the potential role of ROS, a Chinese hamster ovary (CHO) cell line containing a neo direct repeat recombination substrate (CHO 3-6), was used to determine whether benzene or its metabolites phenol, hydroquinone, catechol, or benzoquinone initiated increased homologous recombination and whether this increase could be diminished by the coincubation of cells with the antioxidative enzyme catalase. Results demonstrated that cells exposed to benzene (1, 10, 30, or 100 {micro}M) for 24 h did not exhibit increased homologous recombination. Increased recombination occurred with exposure to phenol (1.8-, 2.6-, or 2.9-fold), catechol (1.9-, 2-, 5-, or 3.2-fold), or benzoquinone (2.7-, 5.5-, or 6.9-fold) at 1, 10, and 30- {micro}M concentrations, respectively, and with exposure to hydroquinone at 10 and 30 {micro}M concentrations (1.5-1.9-fold; p < 0.05). Studies investigating the effects of catalase demonstrated that increased homologous recombination due to exposure to phenol, hydroquinone, catechol, or benzoquinone (10 {micro}M) could be completely abolished by the addition of catalase. These data support the hypothesis that increased homologous recombination mediates benzene-initiated toxicity and supports a role for oxidative stress in this mechanism.


Benzene can induce hematotoxicity and leukemia in humans and mice. Since a review of the literature shows that the CYP2E1 knockout mouse is not known to possess any benzene toxicity, the metabolism of benzene by CYP2E1 in the liver is regarded to be prerequisite for its cytotoxicity and genotoxicity, although the mechanism is not fully understood yet. Because it was found some years ago that benzene was also a substrate for CYP1A1, we investigated the involvement of the aryl hydrocarbon receptor (AhR) in benzene hematotoxicity using AhR wild-type (AhR+/+), heterozygous (AhR+/−), and homozygous (AhR−/−) male mice. Interestingly, following a 2-week inhalation of 300 ppm benzene (a potent dose for leukemogenicity), no hematotoxicity was induced in AhR−/− mice. Further, there were no changes in cellularity of peripheral blood and bone marrow (BM), nor in levels of granulocyte-macrophage colony-forming units in BM. This lack of hematotoxicity was associated with the lack of p21 overexpression, which was regularly seen in the wild-type mice following benzene inhalation. Combined treatment with two major benzene metabolites, phenol and hydroquinone, induced hemopoietic toxicity, although it was not known whether this happened due to a surprising lack of expression of CYP2E1 by AhR knockout, or due to a lack of other AhR-mediated CYP enzymes, including 1A1 (i.e., a possible alternative pathway of benzene metabolism). The former possibility, evaluated in the present study, failed to show a significant relationship between AhR and the expression of CYP2E1. Furthermore, a subsequent evaluation of AhR expression after benzene inhalation tended to show higher but less significant expression in the liver, and none in the BM, compared with sham control. Although this study failed to identify the more likely of the above-mentioned two possibilities, the study using AhR knockout mice on benzene inhalation presents the unique possibility that the benzene toxicity may be regulated by AhR signaling.