Dean Hartley

JOURNAL OF TOXICOLOGY AND ENVIRONMENTAL HEALTH



olume 16
Number 5
985

JTEHD6 16(5) 665-725 (1985)
ISSN: 0098-4108

QUINONES AS TOXIC METABOLITES OF BENZENE

Richard D. Irons

Chemical Industry institute of Toxicology, Research
Triangle Park, North Carolina

Occupational exposure to benzene has long been associated with toxicity to the blood and bone marrow, including lymphocytopenia, pancytopenia, aplastic anemia, acute myelogenous leukemia, and possibly lymphoma. A variety of studies have established that benzene itself is not the toxic species but requires metabolism to reactive intermediates. The bioactivation of benzene is complex. Both primary and secondary oxidation of benzene and its metabolites are mediated via cytochronie P-450 in the liver, although the role of secondary metabolism in the bone marrow is not clear. Toxicity is associated with the dihydroxy metabolites, hydroquinone and catechol, which concentrate in bone marrow. Hydroquinone and its terminal oxidation product, p-benzoquinone, have been demonstrated to be potent suppressors of cell growth in culture. Suppression of lymphocyte blastogenesis by these compounds is a sulfyhdryl-dependent process and occurs at concentrations that do not result in cell death, or in detectable alterations in energy metabolism, intracellular glutathione concentration, or protein synthesis. Recent studies suggest that these compounds and other membrane-penetrating sulfyhdryl alkylating agents, such as N-ethylmaleimide and cytochalasin A, and endogenous regulatory molecules, such as soluble immune response suppressor (SIRS), interfere with microtubule assembly in vitro and selectively interfere with microtubule-dependent cell funcitons at identical concentrations. These agents appear to react with nucleophilic sulfhydryl groups essential for guanosine triphosphdte binding to tubulin that are particularly sensitive to sulfhydryl-alkylating agents.

BACKGROUND

Repeated exposure to benzene at high concentrations results in a variety of blood dyscrasias in humans and experimental animals. These include lymphocytopenia, thrombocytopenia, and pancytopenia or aplastic anemia (Goldwater, 1941; Irons et al., 1979; Laskin and Goldstein, 1977; Latta and Davies, 1941). Several retrospective mortality and case control studies have indicated that chronic exposure to benzene at concentrations of approximately 100 ppm or greater results in an increase in the incidence of acute myelogenous leukemia (AML) and possibly lymphoma in humans (Aksoy et al., 1974; Decoufle et al., 1983; Infante et al., 1977; Viana and Polan, 1979; Vigliani, 1976). Mice chronically exposed to high concentrations of benzene exhibit increased incidence of a variety of tumors depending on the route of administration; however, no experimental model for benzene-induced AML has

Requests for *reprints* should be *sent* to Dr. *R.* D. *Irons.* CIIT, **P.O. Box** *12137,* Research Triangle **Park,** North *Carolina* 27709.

Journal of Toxicology and *Environmental* Health, 16:673–678, 1985
Copyright © 1985 by Hemisphere Publishing Corporation

yet been established (National Toxicology Program, in preparation; Cronkite et al., 1984; Maltoni and Scarnato, 1979). The significance, with respect to human health, of exposure to lower concentrations of benzene (1–10 ppm) is currently the subject of much controversy.

BENZENE METABOLISM

It is now generally accepted that benzene toxicity requires metabolism; however, the bioactivation of benzene is complex, Fig. 1. Phenol (PH) is the major metabolite and is formed by oxidation of benzene primarily by the hepatic cytochrome P-450 system (Gonasun et al., 1973). The principal secondary metabolites of benzene, hydroquinone and catechol, are also formed in liver, the former by hydroxylation of phenol and the latter by dehydrogenation of benzene dihydrodiol (Jerina et al., 1968; Tunek et al., 1980). Suppression of cell growth and function in bone marrow and the lymphoid system correlates with the concentration of hydroquinone (HQ) and catechol (CT), which accumulate in bone marrow and lymphoid tissue following exposure to benrene (Greenlee and Irons, 1981; Greenlee et al., 1981a; Irons et al.,

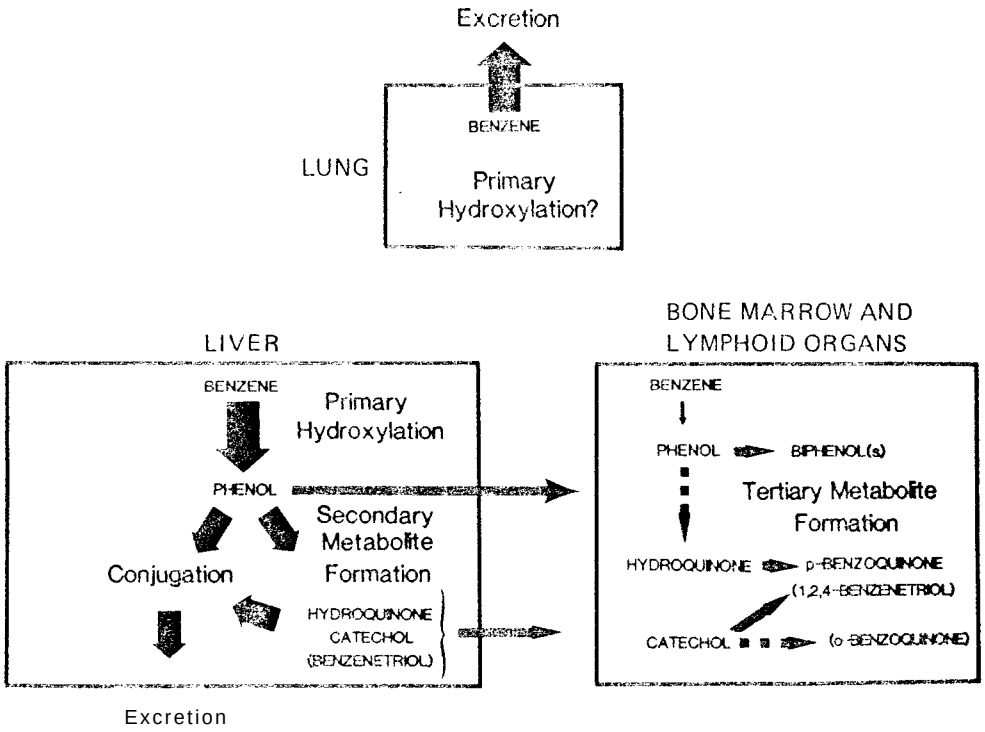

FIGURE 1. Schematic representation summarizing current understanding of the disposition and bioactivation of benzene.

1980; Wierda and Irons, 1982). Although bone marrow possesses a limited capacity to metabolize benrene, it is insufficient to at count for the concentration of its metabolites concentrating in this tissue (Irons et al., 1980). Phenol, however, is readily metabolized in bone marrow by a myeloperoxidase-mediated pathway (Sawahata et al., 1985). The relative contribution of edch of these pathways to the production of bone marrow toxicity following benzene exposure is unclear; however, the quinone metabolites of benzene have proven to be toxic to bone marrow and lymphoid cells both in vivo and in vitro (Irons et al., 1981; Pfeifer and Irons, 1981; Pfeifer and Irons, 1982; Wierda and Irons, 1982).

Quinones are formed by the oxidation of hydroquinone or catechol. Hydroquinone spontancously oxidizes under physiologic conditions in vitro to form *p*-benzoquinone (p-BQ), forming the *p*-semiquinone intermediate in the reaction (Greenlee et al., 1981b; It-oils et al., 1981). Catechol, on the other hand, does not appreciably autooxidize under similar conditions. Further metabolism of CT, presumably via cytochrome P-450, is required foi its conversion to 1,2,4-benzenetriol. CT can be oxidized to the highly reactive ortho quinone by tyrosinase in vitro; however, the significance of this or other enzyme-catalyzed reactions in vivo is unclear (Irons and Neptun, 1980). The significance of autooxidation in the bioactivation of HQ in vivo is not entirely understood, but it is clear that the oxidation of HQ or CT can proceed independently via enzyme-mediated pathways such as myeloperoxidase. Putative mechanisms of toxicity for either tHQ or 1,2,4 BT include free radical formation via superoxide, covalent binding of the semiquinones to DNA, RNA, or other cellular macromolecules, or, alternatively, direct alkylation of sulfhydryl (SH) groups by p-BQ or its orthohydroxy derivative. Studies conducted in our laboratory over the past several years indicate that the latter mechanism figures prominently in the suppression of cell growth associated with tHQ arid p-BQ.

Benzene metabolites have been shown to produce a variety of alterations in bone marrow and lymphoid cells in vivo or in vitro, including reductions in cellularity in bone marrow and blood, suppression of lymphocyte function, and sister chromatid exchanges (Irons e; al., 1979; Morimoto and Wolff, 1980; Pfeifer and Irons, 1981; Pfeifer and Irons, 198%; Wierda arid Irons, 1982). The significance of these alterations with respect to bone marrow damage or leukemogenesis is unclear; however, potent suppression of cell growth arid blastogenesis by these compounds suggest this mechanism to be of importance with respect to bone marrow arid lymphoid suppression and aplastic anernia.

The respective ability *of* PH, HQ, and CT to suppress cell growth in vitro correlates well with their capacity to undergo autooxidation. Neither PH nor CT suppress lymphocyte growth or Function at concentrations that do not result in cell death (10^{-3} arid 10^{-4} *M*, respectively!.

On the other hand, HQ suppresses mitogen-induced lymphocyte blastogenesis at micromolar concentrations, and p-BQ is approximately twice as potent as HQ (Irons et al., 1981; Pfeifer and Irons, 1981; 1982; 1983). A relatively unique characteristic common to HQ and p-BQ is that they are effective at concentrations that do not otherwise appear cytotoxic. For example, p-BQ suppresses blastogenesis at concentrations that do not result in a loss of plasma membrane integrity, reduction of cellular ATP concentration, or a suppression in basal protein synthesis. This effect is SH-dependent and can be protected against by the addition of low-molecular-weight SH compounds, such as 2-mercaptoethanol, cysteine, or dithiothreitol, but not other nucleophiles. Furthermore, analogous experiments using prototype cell-membrane-permeating and nonpermeating SH-reagents [e.g., *N*-ethylmaleimide (NEM), dithiobis-2-nitrobenzoic acid, cytochalasin A) indicate that suppression of blastogenesis involves internal cellular SH groups critical for lymphocyte blastogenesis. The most likely explanation of these findings involves the SH-alkylating properties of p-BQ, in which a highly polarized saturated carbon-carbon bond is subject to attack by nucleophilic intracellular SH groups. The most sensitive thiol groups do not appear to be associated with glutathione (GSH) or SH-dependent enzymes because blastogenesis is essentially inhibited at concentrations of p-BQ or cytochalasin A that do not appreciably alter intracellular GSH or the activity of a variety of SH-dependent enzymes.

Previous studies have revealed benzene exposure in vivo to be associated with a block or arrest of already proliferating bone marrow cells in the G2/M phase ot the cell cycle (irons et al., 1981; Muirhead et al., 1980). This cell cycle arrest together with the apparent SH selectivity of p-BQ and HQ led us to examine tubulin as a potential macromolecular target for these compounds. Microtubule integrity is known to be required not only For spindle formation and division but also for regulation of surface receptor movement and normal signal transduction at the cell surface (Irons et al., 1983; Irons et al., 1981; Pfeifer and Irons, 1983). Tubulin has previously been shown to possess extremely nucleophilic SH groups that are required for guanosine triphosphate (GTP) binding during the process ot microtubule assembly (Mann et al., 1974). Subsequently, we demonstrated that a variety of prototype SH-alkylating coinpounds, including *HQ*, p-BQ, *N*-ethylmaleimide, and cytochalasin A, interfere with microtubule-deperident cell functions, microtubule organization in whole cells, and microtubule assembly in vitro (Irons and Septun, 1980; Pfeifer and Irons, 1983). All ot these events are observed at concentrations of these compounds that are not overtly cytotoxic. Furthermore, microtubule assembly is effectively blocked at concentrations of these compounds that do not interfere with other traditionally ineasured SH-depeiident processes within the cell. Explanations for this exquisite sensitivity of microtu-

bules to SH-alkylating agents may be forthcoming from recent studies in which we have demonstrated that the T suppressor cell lymphokine, soluble immune response suppressor (SIRS), suppresses cell growth and function by interfering with SH-dependent microtubule function in a manner analogous to SH-alkylating agents (Irons et al., 1984). It is therefore possible that highly nucleophilic SH groups essential for GTP binding to tubulin serve as a control site for the regulation of microtubule assembly directly, and consequently for microtubule-dependent processes such as blastogenesis, secretion, and cell division. This site appears to be particularly sensitive to SH-alkylating reagents, such as the simple *p*-quinones, that may mimic the effects of SIRS at concentrations for which their potential for participation in redox phenomena are not apparent.

REFERENCES

Aksoy, M., Erdem, S., and Dincol, G. 1974. Leukemia in shoe workers exposed chronically to benzene. *Blood* 44:837–841.

Cronkite, E. P., Bullis, J. E., Inoue, T., and Drew, R. T. 1984. Benzene inhalation produces leukemia in mice. *Toxicol. Appl. Pharmacol.* 75:358–361.

Decoufle, P., Blattner, W., and Blair, A. 1983. Mortality among chemical workers exposed to benzene and other agents. *Environ. Res.* 30:16–25.

Goldwater, L. J. 1941. Disturbances in the blood following exposure to benzol. *J. Lab. Clin. Invest.* 26:957–973.

Gonasun, L. M., Witmer, C., Kocsis, J. J., and Snyder, R. 1973. Benzene metabolism in mouse liver microsomes. *Toxicol. Appl. Pharmacol.* 26:398–406.

Greenlee, W. F., and Irons, R. D. 1981. Modulation of benzene-induced lymphocytopenia in the rat by 2,4,5,2',4',5'-hexachlorobiphenyl and 3,4,3',4'-tetrachlorobiphenyl. *Chem. Biol. Interact.* 33:345–360.

Greenlee, W. F., Gross, E. A., and Irons, R. D. 1981a. Relationship between benzene toxicity and the disposition of ^{14}C-labeled benzene metabolites in the rat. *Chem. Biol. Interact.* 33:285–299.

Greenlee, W. F., Sun, J. D., and Bus, J. S. 1981b. A proposed mechanism of benzene toxicity: Formation of reactive intermediates from polyphenol metabolites. *Toxicol. Appl. Pharmacol.* 59:187–195.

Infante, P. F., Rinsky, R. A., Wagoner, J. K., and Young, R. J. 1977. Leukemia in benzene workers. *Lancet* 2:76–78.

Irons, R. D., and Neptun, D. A. 1980. Effects of the principal hydroxy-metabolites of benzene on microtubule polymerization. *Arch. Toxicol.* 45:297–305.

Irons, R. D., Heck, H. d'A., Moore, B. J., and Muirhead, K. A. 1979. Effects of short-term benzene administration on bone marrow cell cycle kinetics in the rat. *Toxicol. Appl. Pharmacol.* 51:399–409.

Irons, R. D., Dent, J. G., Baker, T. S., and Rickert, D. E. 1980. Benzene is metabolized and covalently bound in bone marrow *in situ*. *Chem. Biol. Interact.* 30:241–245.

Irons, R. D., Neptun, D. A., and Pfeifer, R. W. 1981. Inhibition of lymphocyte transformation and microtubule assembly by quinone metabolites of benzene: Evidence for a common mechanism. *J. Reticuloendothel. Soc.* 30:359–372.

Irons, R. D., Pfeifer, R. W., Aune, T. M., and Pierce, C. W. 1984. Soluble immune response suppressor inhibits microtubule function *in vivo* and microtubule assembly *in vitro*. *J. Immunol.* 133:2032–2036.

Jerina, D., Daly, J., Witkop, B., Zaltzman-Nirenberg, P., dnd Undenfriend, S. 1968. Role of the arene oxide-oxepin system in the metabolism of aromatic substrates. I. *In vitro* conversion of benzene oxide to a premercapturic acid and a dihydrodiol. *Arch. Biochem. Biophys.* 128:176–183.

Laskin, S., and Goldstein, B. D., eds. 1977. *Benzene toxicity: A critical review. J. Toxicol Environ.* Health Suppl. 2.

Latta, J. S., and Davies, L. T. 1941. Effects on the blood and hemopoietic organs ot the albino rat of repeated administration of benzene. *Arch. Pathol.* 31:55–67.

Maltoni, C., and Scarnato, C. 1979. First experimental demonstration of the carcinogenic effects of benzene. *Med. Lav.* 70:332–357.

Mann, K., Ciesel, M., Fasold, H., and Haase, W. 1974. Isolation of native microtubules from porcine brain and characterization of SH groups essential for polymerization at the CTP binding sites. *FEBS Lett.* 92:45–48.

Morimoto, K., and Wolff, 5. 1980. Increase in sister chroinatid exchanges and perturbations of cell division kinetics in human lymphocytes by benrene metabolites. Cancer Res. 40:1189–1193.

Muirhead, K. A., Irons. R. D., Bruins, R., and Horank, P. K. 1980. A rabbit bone marrow model system fur the evaluation of cytotoxicity: Characterization of normal bone marrow cell cycle parameters by flow cytometry. *Cytochemistry* 28:526–532.

National Toxicology Program. Carcinogenesis studies of benrcne in B6C3F1 mice. U.S. Tech. Rep, Ser., Department of Health and Human Services, in press.

Pfeifer, R. W., and Irons, R. D. 1981. Inhibition of lectin stimulated agglutination and mitosis by hydroquinone: Reactivity with intracellular sulfhydryl groups. *Exp. Mol. Pathol.* 35:189–198.

t'icifer. R. W., and Irons, R. D. 1982. Effect of benzene metabolites on PHA-stimulated lymphopoiesis in rat bone marrow. J. *Reticuloendothel.* Soc. 31:155–170.

Pfiefer. R. W., and ons, R. D., 1983. Alteration of lymphocyte function by quinones through sulfhydryl-dep ndent disruption of microtubule assembly. *Int. J. Immunopharmacol.* 5:463–470.

Sawahata, T., Rickert, D. E., and Greenlee, W. F. 1985. Metabolism of benzene and its metabolites in bone marrow. In Toxicology *of the Blood and Bone* Marrow, ed. R. D. Irons. pp. 141–148. New York: Raven.

Tunek. A., Plat, K. L., Pryzybylski, M., arid Oesch, F. 1980. Multistep metabolic activation of benzene. Effect of superoxide dismutase on covalent binding *to* microsomal macromolecules, and identification of glutathione conjugates using high pressure liquid chromatography and field desorption mass spectrometry. *Chem. Biol. Interact.* 33:1–17.

Viana, N. J., and Polan, A. 1979. Lymphomas and occup itional benzene exposure. *Lancet* i:1394–1395.

Vigliani, E. C. 1976. Leukemia associated with benzene exposure. *Ann. N. Y. Acad. Sci.* 271:134–151.

Wierda, D., arid Irons, R. D. 1982. Hydroquinone and catechol reduce the frequency of progenitor B lymphocytes *in mouse* spleen and bone marrow. *Immunopharmacology* 4:41–54.