HEI STATEMENT
Synopsis of Research Report 103

The Nature of Chromosomal Alterations and How They Are Induced by Benzene in Mice and Humans

INTRODUCTION

Exposure to high levels of benzene is associated with the development of leukemia and other blood disorders, but the effects of exposure to low levels of benzene are not well understood. In the 1990s, the Health Effects Institute initiated its Air Toxics Research Program to address uncertainties about the health effects that may result from exposure to ambient levels of benzene and other air toxics derived from mobile sources. One of the goals of this program was to develop and validate biomarkers of benzene exposure.

Benzene can induce changes in the structure and function of chromosomes, although the relevance of these findings to the development of clinical conditions has not been fully established. HEI funded Dr David Eastmond to investigate two related approaches to determining whether such chromosomal changes could be used as biomarkers of benzene exposure in mice and humans. HEI also thought that Eastmond's study would provide useful data to compare benzene's effects in two species.

APPROACH

The first part of the study involved detecting chromosomal alterations in cells using a modification of a molecular cytogenetic technique known as fluorescence in situ hybridization (FISH). Eastmond used two different fluorescently labeled DNA sequences ("tandem labeled probes") that would bind to unique regions of particular chromosomes. This approach, if successful, may be better than other cytogenetic methods for estimating benzene's effects because it is potentially highly sensitive and may be useful in large population studies. It could also provide information about how different chromosomal alterations arise. Eastmond and colleagues evaluated the frequency of such chromosomal aberrations in the erythrocytes (red blood cells) from the bone marrow of mice exposed to various doses of benzene (50 to 450 mg/kg of body weight per day) and for different exposure durations (2, 6, or 12 weeks). The investigators also tested aberrations in chromosomes 1 and 9 of peripheral blood cells from two groups of humans occupationally exposed to benzene who were matched with control subjects. One exposed population comprised 44 Chinese workers who were either currently being exposed to median levels of 31 parts per million (ppm) benzene, or had formerly been exposed to such high levels that they had become "benzene poisoned." The other exposed population was made up of 17 Estonian workers; 12 subjects were in benzene production (exposed to about 1.3 ppm) and 5 were operating a coke oven (exposed to about 0.3 ppm benzene).

The second part of Eastmond's proposal was to determine whether benzene or its metabolites affect DNA indirectly, acting through the nuclear enzyme topoisomerase II. This enzyme plays a key role in maintaining the chromosomal structure, so inhibiting topoisomerase II function might lead to chromosomal damage or to the development of aberrations. The investigators tested a number of benzene metabolites in vitro to assess their inhibitory effects on the purified human enzyme and on the enzyme's activity in a human cell line. They also tested whether administering benzene orally to mice would inhibit the enzyme's activity in vivo. This part of the study was expected to provide novel information about what mechanisms may be relevant to the carcinogenic effects of benzene, which are not well understood.

RESULTS AND INTERPRETATION

Eastmond and colleagues addressed several important goals in their study. Using tandem labeled fluorescent probes they demonstrated that they could detect some types of benzene-induced chromosomal

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr David Eastmond of the University of California, Riverside. The following Research Report contains both the detailed Investigators' Report and a Commentary on the study prepared by the Institute's Health Review Committee.

alterations in mice and humans. Controlled exposure studies in mice suggested that benzene-induced increases in chromosomal alterations in bone marrow erythrocytes are dependent on both dose and duration of exposure. By contrast, the results of human biomonitoring were not as clearcut: Chromosomal alterations in the highly exposed population of Chinese workers did not differ from control levels, but the smaller group of Estonian workers who were exposed to lower levels did show chromosomal changes related to benzene exposure. A number of reasons may explain why the investigators found higher numbers of aberrations in the chromosomes of Estonian workers than in Chinese workers. For example, an agent or agents distinct from benzene in the Estonian work environment (such as polycyclic aromatic hydrocarbons) may be a factor; differences in lifestyle (such as diet or medications) may be influential; or an unusual dose-response curve for benzene, in which lower doses would induce higher numbers of aberrations, is an option. An additional explanation is that these two groups of workers could express different types of enzymes that may metabolize benzene along distinct pathways to harmful or less harmful metabolites. The binding of the fluorescent DNA probes to cells is also likely to be critically influenced by the way in which the slides of cell samples are prepared. Because slides for the two studies were prepared in different countries, it is quite probable that differences in preparation conditions might also have affected the results. Thus, although the results obtained by Eastmond and his colleagues indicate the feasibility of the approach tested, they also underline important limitations in the use of the tandem labeled FISH assay in large human studies.

These investigators were the first to show that benzene administration to mice in vivo, and some benzene metabolites or potential metabolites in vitro, can inhibit the nuclear enzyme topoisomerase II. These findings suggest a potential mechanism by which benzene may induce genotoxic and carcinogenic effects. Because the results of the in vitro assay of topoisomerase II activity were not linear in the dilution range tested, however, the assay cannot be used at present as an indicator of early benzene effects.

The investigators also were able to conduct initial tests of new biomarkers of benzene exposure and effects in humans. Additional studies will help to determine whether using FISH with tandem probes or measuring topoisomerase II activity will be useful biomarkers for assessing ambient or occupational exposures to benzene.

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