HEALTH EFFECTS INSTITUTE

STATEMENT

Synopsis of Research Report 150

Relative Mutagenicity of Stereoisomers of Butadiene Metabolites in Human Cells

BACKGROUND

1,3-butadiene (BD) is used extensively in the chemical industry (e.g., for synthetic rubber production) and is also present in motor vehicle exhaust and cigarette smoke. It is listed by the U.S. Environmental Protection Agency as a mobile-source air toxic and is classified as a human carcinogen when inhaled. Studies in rodents have shown that mice and rats differ in their sensitivity to BD, with mice developing tumors at much lower exposure concentrations than rats. A key research and risk assessment question has been which species is likely to be more relevant to what might happen in humans. Species differences in tumor induction by BD have been attributed to differences in its metabolism. The major metabolites of interest are 1,2-epoxy-3-butene (BDO), 1,2,3,4-diepoxybutane (BDO_2), and 1,2-dihydroxy-3,4-epoxybutane (BDO-diol). Studies have shown that these compounds differ in their ability to damage DNA in exposed animals or in cultured cells. In these studies, mostly racemic mixtures that contained all stereoisomers (spatially different forms of the same chemical structure) of each BD metabolite were tested. Limited work with specific stereoisomers has provided some evidence of a possible role of stereochemistry in the metabolism of BD and in the formation of DNA adducts, but none of those studies evaluated the mutagenicity of the individual stereoisomers.

In response to the Walter A. Rosenblith New Investigator Award Request for Applications (RFA 00-2), Dr. Ryan Q. Meng proposed to determine the cytotoxicity and mutagenicity of the stereoisomers of three major butadiene metabolites in the TK6 human lymphoblastoid cell line. The HEI Research Committee recommended the study for funding because they thought that Dr. Meng was a promising investigator and that the proposal was very relevant to the HEI research program on air toxics because it addressed an important question regarding the mutagenicity of BD metabolites.

APPROACH

The study had two major aims: (1) to synthesize the nine stereoisomers of the three main BD metabolites and (2) to evaluate the cytotoxicity and mutagenicity of each stereoisomer of each BD metabolite in cells exposed in vitro. Mutagenicity was evaluated at two genes: the hypoxanthine-guanine phosphoribosyltransferase (*HPRT*) and thymidine kinase (*TK*) genes. A secondary aim was to characterize the types of mutations induced by BDO_2 stereoisomers at the *HPRT* gene. The synthesis of the stereoisomers was conducted at the University of Texas Medical Branch in Galveston, Texas. The investigators assessed the purity of each compound by evaluating various analytic parameters such as gas chromatography–mass spectrometry and nuclear magnetic resonance spectra, melting point, and optical rotation. Stereoisomers result from the asymmetric bonding of some atoms to a carbon atom, resulting in "left hand"(*S*) and "right hand" (*R*) forms, which differ in their three-dimensional configuration and optical activity but are chemically identical. BDO has two stereoisomers: (2*R*) and (2*S*). BDO_2 has three stereoisomers: (2*R*,3*R*), (2*S*,3*S*), and *meso* (a compound that is not optically active). BDO-diol has four stereoisomers: (2*R*,3*R*), (2*R*,3*S*), (2*S*,3*R*), and (2*S*,3*S*).

The cytotoxicity and mutagenicity of each stereoisomer was evaluated in the TK6 human

This Statement, prepared by the Health Effects Institute, summarizes a research project funded by HEI and conducted by Dr. Ryan Q. Meng of Battelle Toxicology Northwest, Richland, Washington, and colleagues. The complete report, *Mutagenicity of Stereochemical Configurations of 1,3-Butadiene Epoxy Metabolites in Human Cells* (© 2010 Health Effects Institute), can be obtained from HEI or our Web site (see next page).

lymphoblastoid cell line. Cells were exposed in culture to different doses of each stereoisomer for 24 hours. Cytotoxicity, or percent survival, at each dose was calculated as the percentage of cells that form colonies relative to control cells (with the control value set at 100%). Mutations were examined in the *HPRT* and the *TK* genes. Cells with mutations at the *HPRT* gene grew in a medium containing 6-thioguanine, while cells with mutations at the *TK* gene were grown in the presence of trifluorothymidine; normal cells cannot grow in these media. The frequency of mutations was calculated as the percentage of cells that grew in the presence of the selection agent relative to the percentage that grew in normal medium (without the agent).

In separate experiments, Meng and colleagues determined the spectrum of the mutations at the *HPRT* gene induced by BDO_2 using amplification of the *HPRT* DNA and analysis of the DNA by ultraviolet light. This method primarily detects large deletions.

RESULTS AND INTERPRETATION

The authors successfully synthesized the nine stereoisomers with sufficient yield to conduct the proposed assays. The purity of the stereoisomers was determined to be at least 98% to 99.9%.

A key and novel finding was that the (2*R*,3*S*)-BDO-diol stereoisomer was about 30-fold more cytotoxic and mutagenic than the other three BDO-diol stereoisomers. The dose-related mutagenic responses for the stereoisomers of BDO and BDO_2 were not statistically different. (2*R*,3*S*)-BDO-diol was 5-to-10-fold less mutagenic than any of the BDO_2 stereoisomers, but 10-to-20-fold more mutagenic than the BDO stereoisomers.

The analysis of the types of mutation induced by the stereoisomers of BDO_2 showed a higher frequency of deletions in cells exposed to each stereoisomer of BDO_2 relative to unexposed cells. While appropriately carried out, these experiments did not provide any new insights relating to the mechanism of action of BD. A more detailed analysis of the sizes of the deletions would be helpful to determine whether the deletion spectra of the mutants differ.

In its independent evaluation of the study, the Review Committee thought that the investigators had conducted a well-designed and novel study that combined chemistry and molecular biology to address a relevant research question regarding the mutagenicity of specific stereoisomers of the key BD metabolites. They thought the investigators provided convincing data on the purity of the stereoisomers, determined using a variety of analyses. The study's key finding that one of the four stereoisomers of BDO-diol was responsible for most of the mutagenicity of this metabolite is interesting and could explain some of the possible species differences in the mutagenicity of BD. The study also suggests that the species differences in susceptibility to BD that have been noted in other work are not likely to be related to the stereochemistry of BDO and BDO_2. The caveat to these conclusions is that the normal target cells for BD in rodents may detoxify the different BD metabolites at very different rates, thus leading to steady-state levels of one metabolite or its stereoisomers that are higher than those of others. The results of this study using a cell line and individual stereoisomers provide justification for follow-up studies that consider the kinetics of the formation and distribution of BDO-diol stereoisomers in rodents and humans and that have endpoints relating to both mutagenicity and carcinogenicity.

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