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Some insights into the mode of action of butadiene by examining the genotoxicity of its metabolites

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Abstract

1,3-Butadiene (BTD) is an important commodity chemical and air pollutant that has been shown to be a potent carcinogen in mice, and to a lesser extent, a carcinogen in rats. To better assess butadiene's carcinogenic risk to humans, it is important to understand its mode of action and how this relates to differences in responses among species. In a series of in vitro experiments, lymphocytes from rats, mice, and humans were exposed to 3,4-epoxy-1-butene (EB) or 1,2:3,4-diepoxybutane (DEB) for 1 h at the G_0 stage of the cell cycle, stimulated to divide, and cultured to assess the ability of these metabolites to induce sister chromatid exchange (SCE) and chromosome aberrations (CAs). EB induced no increases in SCEs or CAs in the cells from the three species. DEB was a potent SCE- and CA-inducer, with the results being similar in each rodent species. The response for SCEs seen in the human cells was more complex, with genetic polymorphism for glutathione-*S*-transferases (GST) possibly modulating the response. The single cell gel electrophoresis assay was used on genetically engineered V79 cell lines to investigate a possible influence of GST status. Experiments were also conducted to investigate the reason for EB's failure to induce SCEs or CAs in G_0 cells. The results indicate that EB-induced DNA damage was repaired before DNA synthesis in unstimulated lymphocytes, but EB caused a large increase in SCEs if actively cycling cells were treated. Thus, the results indicate that DEB damage is persistent in G_0 cells, and DEB is a much more potent genotoxicant than EB. The carcinogenic effect of butadiene will most likely depend on the degree to which DEB is produced and reaches target tissues, and to a lesser extent on the ability of EB to reach actively dividing or repair deficient cells.

Keywords: Butadiene; Clastogenicity; DNA damage and repair; Diepoxybutane; Mode of action; Monoepoxybutene; Sister chromatid exchange

1. Introduction

1,3-Butadiene (BTD) is an important commodity chemical used in the manufacture of rubber, plastics, and various resins. There is significant human exposure to BTD at the workplace, as an air pollutant from internal combustion engines, and in cigarette smoke. BTD is a potent carcinogen in mice [1] but less so in rats [2]. IARC considers it a probable human carcinogen [3] while the US EPA classifies it as a human carcinogen when exposure is by inhalation [4].

The rodent carcinogenicity of BTD is not in doubt. However, understanding how BTD induces cancer is important, not only from a regulatory point of view where determining how rodent carcinogenicity relates to its potency in humans is an important question, but also from a strictly scientific perspective by determining how species' differences in sensitivities to genotoxic carcinogens may occur.

In this paper, we discuss some of our previously published work on the genotoxicity of two of the

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primary metabolites of BTD: 3,4-epoxy-1-butene (EB) and 1,2:3,4-diepoxybutane (DEB) and place it in a new context to examine how DNA repair, cell replication, and glutathione-*S*-transferase (GST) polymorphisms may influence the potency of BTD. We will do this by addressing the following questions:

(1) Are there innate sensitivity differences among primary lymphocytes of mice, rats, and humans in their responses to EB and DEB?
(2) Does GSTT1 or GSTM1 affect the response of cells to DEB?
(3) How do DNA damage, DNA repair, and cell cycle stage affect the level of cytogenetic damage?

(Much of the research described in the following pages has previously been published. We refer the readers of this overview to the original publications for experimental details.)

2. Question #1: Are there innate sensitivity differences among primary lymphocytes of mice, rats, and humans in their responses to EB and DEB?

It is well recognized that the metabolism of BTD in mice produces significantly more of the highly reactive DEB than is produced in rats [5], and many researchers believe this accounts for the much greater carcinogenic potency of BTD in mice as compared to rats. However, to our knowledge, no one had previously addressed the question of whether or not the primary cells of these species and those of humans are equally sensitive to the DNA-damaging effects of EB and DEB. We investigated

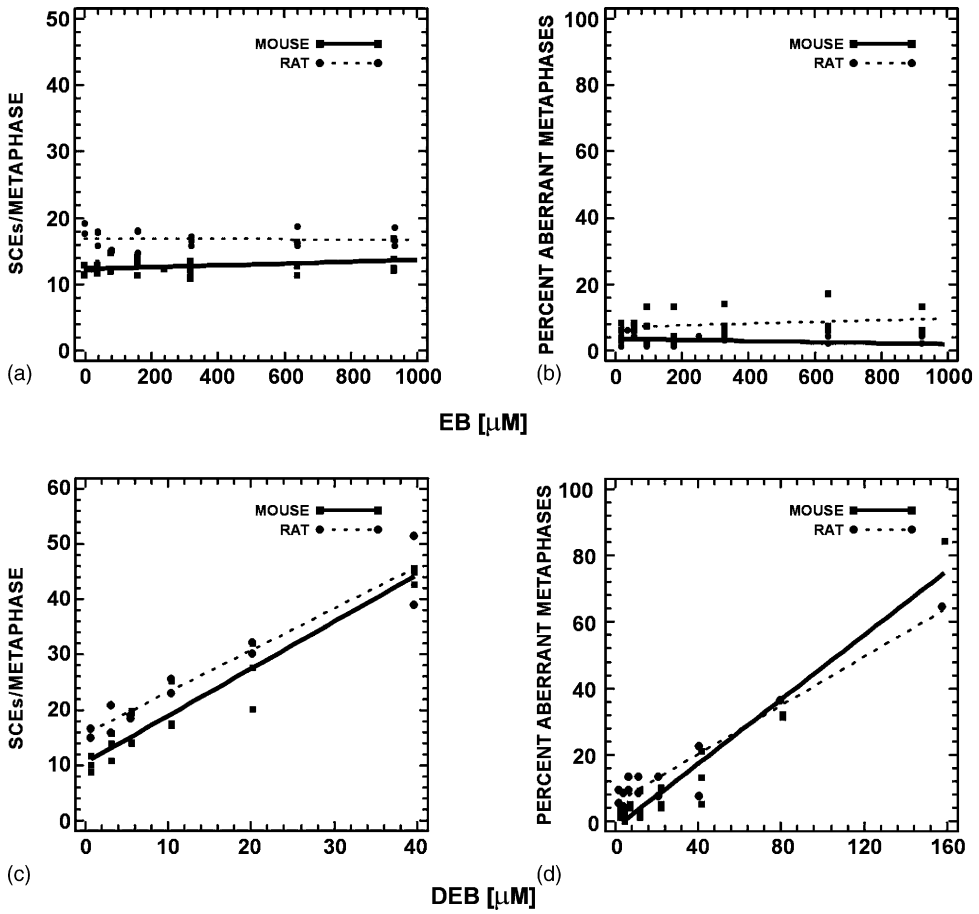

Fig. 1. The effect of EB (a and b) or DEB (c and d) on mouse and rat G_0 lymphocytes treated in vitro for 1 h, washed, stimulated with mitogen to divide, and scored for SCEs (a and c) or CAs (b and d). The slopes of the regression lines for DEB are 0.84 and 0.74 SCEs/μM and 0.47 and 0.36 aberrant cells/μM, for the mouse and rat, respectively. Each point is a datum from an individual culture, and the graphs are the composite from two to three independent experiments. First-division metaphases were scored for CAs and second-division metaphases for SCEs. Induced aberrations were almost exclusively chromatid-type. Modified from Ref. [6].