Brief Communication

3,4-Epoxy-1-butene, a Reactive Metabolite of 1,3-Butadiene, Induces Somatic Mutations in Xpc-null Mice


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Xpc-null (Xpc<sup>−/−</sup>) mice, deficient in the global genome repair subpathway of nucleotide excision repair (NER-GGR), were exposed by intraperitoneal (IP) injection to a 300 mg/kg mutagenic dose of 3,4-epoxy-1-butene (EB), to investigate NER’s potential role in repairing butadiene (BD) epoxide DNA lesions. Mutagenic sensitivity was assessed using the Hprt assay. Xpc<sup>−/−</sup> mice were significantly more sensitive to EB exposure, exhibiting an average 2.8-fold increase in Hprt mutant frequency (MF) relative to those of exposed Xpc<sup>+</sup>/+(wild-type) mice. As a positive control for NER-GGR, additional mice were exposed by IP injection to a 150 mg/kg mutagenic dose of benzo[a]pyrene (B[a]P). The Xpc<sup>−/−</sup> mice had MFs 2.9-fold higher than those of exposed Xpc<sup>+</sup>/+ mice. These results suggest that NER-GGR plays a role in recognizing and repairing some of the DNA adducts formed following in vivo exposure to EB. Additional research is needed to examine the response of Xpc<sup>−/−</sup> mice, as well as other NER-deficient strains, to inhaled BD. Furthermore, it is likely that alternative DNA repair pathways also are involved in restoring genomic integrity compromised by BD-epoxide DNA damage. Collaborative studies are currently underway to address these critical issues. Environ. Mol. Mutagen. 47:67–70, 2006. © 2005 Wiley-Liss, Inc.

Key words: epoxybutene; benzo[a]pyrene; Hprt; nucleotide excision repair; Xpc; butadiene

INTRODUCTION

Olefins mediate their genotoxic effects through epoxide metabolites. One model olefin, 1,3-butadiene (BD), is bio-transformed in vivo to three reactive epoxides. These include an initial epoxide, epoxybutene (EB), and two secondary epoxides, diepoxybutane (DEB) and epoxybutenediol (EBD). All are documented mutagens, with DEB presumably representing the ultimate mutagen [reviewed by Jackson et al., 2000].

Comparatively little is known about the recognition and repair of the epoxide-DNA adducts that represent the pre-mutagenic lesions. A number of modified nucleotides and dinucleotides have been detected in vivo and in vitro, including reaction products resulting from rearrangements of unstable primary lesions. These include alkylated bases, predominantly purines, and interstrand crosslinks [Selzer and Elfarra, 1996, 1999; Tretyakova et al., 1998; Koc et al., 1999; Koivisto et al., 1999; Solomon, 1999; Park and Tretyakova, 2004; Zhang and Elfarra, 2004]. Mutational spectra in cultured cells, as well as mice and humans exposed to BD or BD-epoxides, reveal increases in specific point mutations (A/T→T/A transversions and A/T→G/C transitions) and deletions ranging from simple frameshifts to entire gene regions [Ma et al., 2000; Recio et al., 2001; Meng et al., 2004]. Studies examining the mutagenicity of synthesized lesions in vitro indicate that...
the alkylpurines and their subsequent reaction products may produce point mutations, and an intranastand crosslink may produce deletions [Carmichael et al., 2000a,b; Rodriguez et al., 2001; Kanuri et al., 2002].

Because of the broad spectrum of DNA adducts and mutations caused by the BD-epoxides, multiple DNA repair pathways are likely involved in maintaining genomic integrity following exposure. This includes nucleotide excision repair (NER), which may be essential for the repair of crosslinked adducts that are principally formed by DEB [Friedberg et al., 1995]. To examine the role that NER has in this process, Xpc-null (Xpc−/−) mice, deficient in NER global genome repair (NER-GGR), were exposed to EB by injection. These mice were then examined for the induction of somatic mutations, using the Hprt assay. Increased Hprt mutant frequencies (MFs) in Xpc−/− mice, compared with Xpc+/+ mice, would indicate that NER-GGR is responsible, in part, for maintaining genomic integrity compromised by BD-epoxides.

**MATERIALS AND METHODS**

Xpc+/+ and Xpc−/− C57BL/6 female mice [Cheo et al., 1997] were exposed by intraperitoneal (IP) injection to a 300 mg/kg mutagenic dose of 3,4-epoxy-1-butene (EB; 98% pure, cat. no. 12,757-4, Sigma-Aldrich, St. Louis, MO). Three injections of 100 mg/kg EB were delivered at 48-hr intervals. Xpc+/+ littermates were randomly assigned to treatment groups. Age-matched Xpc−/− mice were obtained from Charles River Laboratories (CRL, Wilmington, MA) and were treated with saline alone as injection controls. For positive NER controls, additional mice were exposed by IP injection to 150 mg/kg benzo[a]pyrene (B[a]P; cat. no. B1760, Sigma-Aldrich) dissolved in dimethylsulfoxide (DMSO). Mice serving as vehicle controls were injected with DMSO alone. Three injections of 50 mg/kg B[a]P were delivered at 48-hr intervals. All mice were treated at 5–6 weeks of age and were maintained for 4 weeks after exposure to EB, before performing the Hprt assay [Skopek et al., 1992; Meng et al., 1998; Wickliffe et al., 2003]. Mice treated with B[a]P were housed for 10 weeks before conducting the Hprt assay. All experiments were conducted in accordance with the Animal Care and Use Committee's standards, at the University of Texas Medical Branch, under protocol 880202402. Mice were provided water and rodent chow (Prolab RMH 2500, LabDiet, Brentwood, MO) ad libitum and maintained on a 12 hr light–dark cycle.

**Statistical Analysis**

Hprt MFs were analyzed by univariate ANOVA followed by post hoc mean comparisons (Bonferroni-corrected), using the SPSS program (SPSS, Chicago, IL). An α < 0.05 was used to determine statistical significance.

**RESULTS AND DISCUSSION**

Cloning efficiencies, proportion of 6-thioguanine-resistant clones, and Hprt MFs for each mouse used in this study are presented in Table I. Two mice (M07 and M08)
were excluded from the study. M07 had a cloning efficiency of only 0.001, and M08 died immediately following the first injection of 100 mg/kg EB.

3,4-Epoxy-1-butene Exposure

Mice deficient in NER-GGR were more sensitive than Xpc\(^{+/+}\) mice to the mutagenic potential of EB, following exposure. The Xpc\(^{-/-}\) mice had significantly higher Hprt MFs (\(P < 0.05\)) than those of Xpc\(^{+/+}\) mice exposed to EB and control mice (both Xpc\(^{-/-}\) and Xpc\(^{+/+}\)) exposed to saline alone (Fig. 1). The average 2.8-fold increase in Hprt MFs in Xpc\(^{-/-}\) mice relative to the MFs of Xpc\(^{+/+}\) mice is similar to that observed in a recent experiment in which mice deficient in microsomal epoxide hydrolase (Ephx1-null) were exposed to 240 mg/kg EB (data unpublished). In that experiment, we observed a significant (\(P < 0.05\)) 2.9-fold increase in Hprt MFs in Ephx1-null mice, compared with the MFs of exposed Xpc\(^{+/+}\) mice. This suggests that, at these approximate levels, EB-exposed mice that are incapable of recognizing and repairing the BD-epoxide DNA lesions that are NER-GGR substrates are as sensitive to the genotoxic effects of BD as Ephx1-null mice that are incapable of detoxifying the BD-epoxides themselves. In this study, exposed Xpc\(^{-/-}\) mice exhibited Hprt MFs that were 2.7-fold higher than those of unexposed Xpc\(^{+/+}\) mice.

Benzo[a]Pyrene Exposure

B[a]P is metabolized to a reactive epoxide that forms bulky DNA adducts that are classic substrates for NER. Therefore, we exposed Xpc\(^{-/-}\) and Xpc\(^{+/+}\) mice, by IP injection, to a mutagenic level of B[a]P. All B[a]P-exposed mice exhibited significantly higher Hprt MFs than those of both Xpc\(^{-/-}\) and Xpc\(^{+/+}\) mice injected with vehicle (DMSO) alone (Fig. 2). As hypothesized, exposed Xpc\(^{-/-}\) mice were considerably more sensitive to B[a]P, compared with their Xpc\(^{+/+}\) counterpart mice. Xpc\(^{-/-}\) mice exhibited Hprt MFs that were 2.9-fold higher than those of exposed Xpc\(^{+/+}\) mice and at least 30-fold higher than those of unexposed control mice (Fig. 2). Considering the
mutagenic response to the BD-epoxide EB, it was suggested that BD-epoxides are comparatively weak mutagens and/or that alternative DNA repair pathways are efficiently removing premutagenic lesions in the Xpc<sup>−/−</sup> mice.

SUMMARY

This study indicates that exposure to EB results in the formation of DNA adducts that are recognized and repaired by NER-GGR, but it remains unclear as to which adducts are the NER-GGR substrates. Since mice are capable of generating all of the reactive BD-epoxide intermediates from EB, it is reasonable to assume that DEB, formed in vivo, may be the specific epoxide responsible for generating these substrates. Additional studies are needed to address this hypothesis.

The relevant experiments that are now necessary to better understand the recognition and repair of BD-induced DNA lesions should involve inhalation exposures to BD itself. These future studies, which would allow for the complete in vivo biotransformation of BD, will better address the genotoxicity and enhanced sensitivity associated with deficiencies in DNA repair. Characterizing DNA adduct formation in DNA repair-deficient mice and examining mutation induction and mutational spectra in reporter genes, such as Hprt, following exposure to BD will answer important questions regarding formation, recognition, and repair of BD-induced DNA lesions.

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REFERENCES


