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Naphthalene cytotoxicity in microsomal epoxide hydrolase deficient mice

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HIGHLIGHTS

- mEH metabolites may not be driving the overall toxicity of NA for known tumor sites.
- The mEH pathway may be partially responsible for cytotoxicity in extrapulmonary airways.
- Acute exposure to naphthalene causes cytotoxicity in all airway generations at 5 ppm.
- Female mice may be more susceptible to naphthalene acute toxicity than males.

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ABSTRACT

Naphthalene (NA) is a ubiquitous pollutant to which humans are widely exposed. 1,2-Dihydro-1,2-dihydroxynaphthalene (NA-dihydrodiol) is a major metabolite of NA generated by microsomal epoxide hydrolase (mEH). To investigate the role of the NA-dihydrodiol and subsequent metabolites (i.e. 1,2-naphthoquinone) in cytotoxicity, we exposed both male and female wild type (WT) and mEH null mice (KO) to NA by inhalation (5, 10, 20 ppm for 4 h). NA-dihydrodiol was ablated in the KO mice. High-resolution histopathology was used to study site-specific cytotoxicity, and formation of naphthalene metabolites was measured by HPLC in microdissected airways. Swollen and vacuolated airway epithelial cells were observed in the intra- and extrapulmonary airways of all mice at and below the current OSHA standard (10 ppm). Female mice may be more susceptible to this acute cytotoxicity. In the extrapulmonary airways, WT mice were more susceptible to damage than KO mice, indicating that the metabolites associated with mEH-mediated metabolism could be partially responsible for cytotoxicity at this site. The level of cytotoxicity in the mEH KO mice at all airway levels suggests that non-mEH metabolites are contributing to NA cellular damage in the lung. Our results indicate that the apparent contribution of mEH-dependent metabolites to toxicity differs by location in the lung. These studies suggest that metabolites generated through the mEH pathway may be of minor importance in distal airway toxicity and subsequent carcinogenesis from NA exposure.

1. Introduction

Naphthalene (NA) is a widespread pollutant that is currently classified as a possible human carcinogen, though this classification is under review (IARC, 2002; USEPA, 1998). The results of the NTP (National Toxicology Program) chronic rodent bioassays for NA found significant, dose-dependent increases in alveolar adenomas in the lungs of female mice, and in adenomas and neuroblastomas of the nasal epithelium in rats (Abdo et al., 2001, 1992). Primary sources of NA include biomass burning (Kakareka and Kukharchyk, 2003), automobile emissions (USEPA, 1986) and tobacco smoke (Schmeltz et al., 1976; Witschi et al., 1997);

Abbreviations: P450, cytochrome P450; GSH, glutathione; GST, glutathione transferase; KO, knockout; mEH, microsomal epoxide hydrolase; NA, naphthalene; NQ, naphthoquinone; OSHA, Occupational Safety and Health Administration; WT, wild type.

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however, NA has also been found in food (Kobayashi et al., 2008) and ground water. In the National Human Adipose Tissue Survey, 40% of the study population had detectable levels of NA in adipose tissue (Stanley, 1986). Another study found that 75% of a lactating female population had measurable levels of NA in milk (Pellizzari et al., 1982).

The toxicity of NA is cytochrome P450 dependent, with reactive metabolites produced by enzymes in both the lung and the liver (Buckpitt and Warren, 1983). In the lung, nonciliated bronchiolar Club (formerly known as Clara) cells express the highest amounts of P450 (Plopper et al., 1987) and are severely affected by NA toxicity (Buckpitt et al., 1995). Recurrent cycles of cytotoxicity and proliferation are thought to be the driving force behind formation of mouse lung tumors (National Toxicology Program, 1992; West et al., 2001) and rat nasal tumors (Long et al., 2003) following chronic exposure to NA because tumors form in respiratory tissues that exhibit high acute toxicity. However, it is unknown which metabolite(s) drive(s) this toxicity. A summary of NA metabolism to potentially toxic metabolites is diagrammed in Fig. 1.

In mice, the bioactivation of NA by P450 enzymes (CYP2A5 and CYP2F2) generates a 1,2-epoxide (Li et al., 2011). Glutathione (GSH) conjugates can form from the epoxide, and are eliminated in urine as mercapturic acids (Pakenham et al., 2002). NA 1,2-epoxide can also be metabolized by microsomal epoxide hydrolase (mEH) to NA-dihydrodiol (Kitteringham et al., 1996). When GSH is depleted, it is possible that the mEH pathway may be favored. Previous studies demonstrated that severe GSH depletion increases NA toxicity in the respiratory tract (Phimister et al., 2004; Plopper et al., 2001). It has been speculated that this increased toxicity and the resulting tumorigenesis of NA is mediated by the formation of a 1,2-naphthoquinone (NQ) through the mEH pathway. In fact, Bogen et al. suggests that the most recent EPA evaluation of NA hinged on the interpretation of bioassays that suggested that 1,2-NQ is genotoxic (Bogen et al., 2008). However, the toxicity could also result from increased levels of NA epoxide. 1,2-NA epoxide can form adducts with protein (Waidyanatha et al., 2002). In animal models, the extent to which specific metabolic pathways (to epoxides, quinones, and diols) contribute to cytotoxicity is unknown. Studies indicating the potential genotoxicity of NQs are either in vitro (Flowers-Geary et al., 1996; National Toxicology Program, 1992) or have not evaluated critical tumor sites, such as the lung. Recent studies (Saeed et al., 2007; Saeed et al., 2009) demonstrated the formation of both depurinated and stable NA DNA adducts in vitro and in skin painting studies but the relevance of the painting studies is uncertain because skin is not a known target tissue for NA cytotoxicity. NA metabolite-derived DNA adducts have not been demonstrated in the respiratory system of any species yet. There is a current need for information relating specific in vivo NA metabolism pathways to NA toxicity so that risk from exposure can be understood.

In the current study, we investigated the role of mEH-mediated metabolites (ie 1,2-NQ) in cytotoxicity. To do this, we used mEH knockout (KO) mice (Miyata et al., 1999). These mice have been used previously to investigate the role of mEH in benzene-induced toxicity (Bauer et al., 2003) and 7,12-dimethylbenz[a]anthracene-induced tumorigenicity (Miyata et al., 1999). The aims of this study were to determine (1) if mEH metabolites are of importance in the overall toxicity of NA at known tumor sites, (2) if cytotoxicity of NA is dose-dependent in mEH KO mice, and (3) if NA toxicity differs between intra- and extrapulmonary airways in mEH KO mice. To explore these questions, we exposed both male and female wild type (WT) and mEH null mice (KO) to NA by inhalation (5, 10, 20 ppm; 4 h). These data will inform future studies of NA risk assessment.

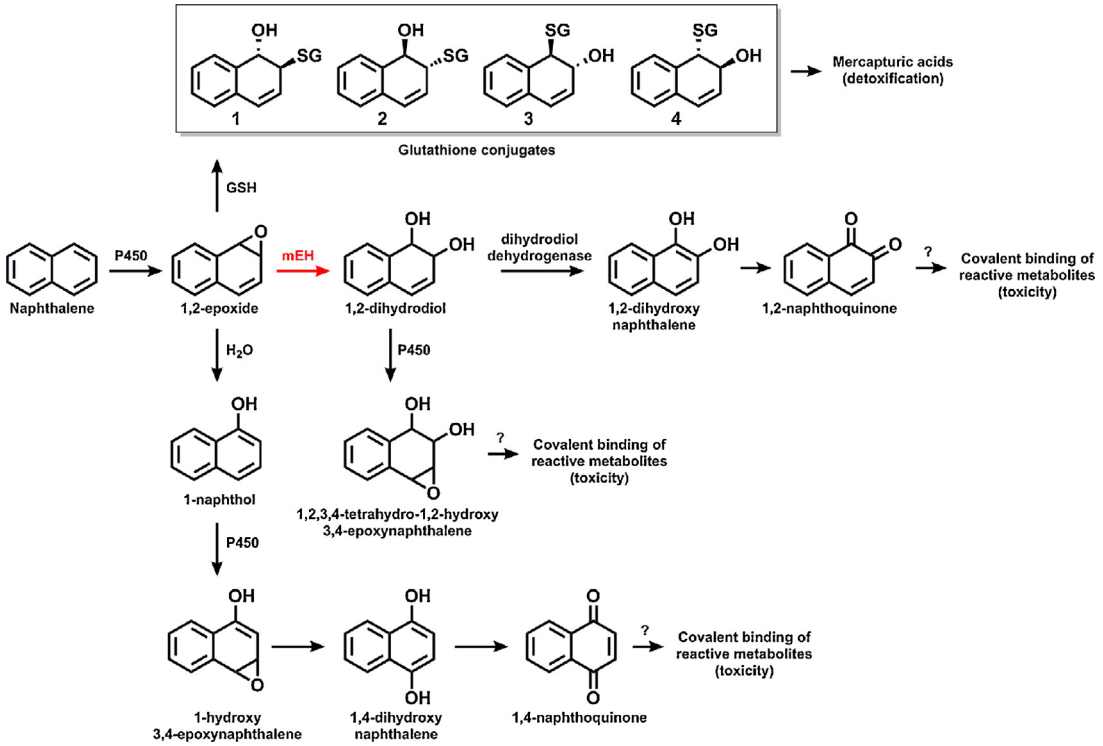

Fig. 1. Naphthalene metabolism to plausible toxic metabolites. Quantifiable metabolites are the GSH conjugates, 1,2 NA-dihydrodiol, and 1-naphthol. GSH conjugates are usually associated with detoxification while the mEH pathway is associated with toxicity.