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Concentration- and time-dependent genotoxicity profiles of isoprene monoepoxides and diepoxide, and the cross-linking potential of isoprene diepoxide in cells

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ABSTRACT

Isoprene, a possible carcinogen, is a petrochemical and a natural product being primarily produced by plants. It is biotransformed to 2-ethenyl-2-methyloxirane (IP-1,2-O) and 2-(1-methylethenyl)oxirane (IP-3,4-O), both of which can be further metabolized to 2-methyl-2,2′-bioxirane (MBO). MBO is mutagenic, but IP-1,2-O and IP-3,4-O are not. While IP-1,2-O has been reported being genotoxic, the genotoxicity of IP-3,4-O and MBO, and the cross-linking potential of MBO have not been examined. In the present study, we used the comet assay to investigate the concentration- and time-dependent genotoxicity profiles of the three metabolites and the cross-linking potential of MBO in human hepatocyte L02 cells. For the incubation time of 1 h, all metabolites showed positive concentration-dependent profiles with a potency rank order of IP-3,4-O > MBO > IP-1,2-O. In human hepatocellular carcinoma (HepG2) and human leukemia (HL60) cells, IP-3,4-O was still more potent in inducing DNA breaks than MBO at high concentrations (>200 μM), although at low concentrations (≤200 μM) IP-3,4-O exhibited slightly lower or similar potency to MBO. Interestingly, their time-dependent genotoxicity profiles (0.5–4 h) in L02 cells were different from each other: IP-1,2-O and MBO (200 μM) exhibited negative and positive profiles, respectively, with IP-3,4-O lying in between, namely, IP-3,4-O-caused DNA breaks did not change over the exposure time. Further experiments demonstrated that hydrolysis of IP-1,2-O contributed to the negative profile and MBO induced cross-links at high concentrations and long incubation times. Collectively, the results suggested that IP-3,4-O might play a significant role in the toxicity of isoprene.

Abbreviations: DEB, 1,2,3,4-diepoxybutane; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; HepG2, human hepatocellular carcinoma cells; HL60, human leukemia cells; IMDM, Iscove's Modified Dulbecco's Medium; IP-1,2-O, 2-ethenyl-2-methyloxirane; IP-3,4-O, 2-(1-methylethenyl)oxirane; MBO, 2-methyl-2,2′-bioxirane; mCPBA, *m*-chloroperoxybenzoic acid; MMS, methyl methanesulfonate; MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide; PBMCs, peripheral blood mononuclear cells; SD, standard deviation; %Tail DNA, percentage of DNA in the tail.

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1. Introduction

Isoprene (2-methyl-1,3-butadiene), the 2-methyl analog of human carcinogen 1,3-butadiene, is an important petrochemical that is primarily used in the manufacture of synthetic rubber. It is also a natural product that is produced by plants, animals [1,2], bacteria [3], and humans [4,5].

Isoprene is a possible carcinogen. Animal toxicology studies have indicated that it is carcinogenic to mice [6] but is very weakly carcinogenic to rats [7]. Isoprene is classified as "possibly carcinogenic to humans (group 2B)" by the International Agency for Research on Cancer [8] and as "reasonably anticipated to be a human carcinogen" by the U.S. National Toxicology Program [9]. However, it has not been classified as a human carcinogen due to lack of epidemiological data.

The environmental sources of isoprene include natural and anthropogenic ones. Emissions from plants are the primary source of isoprene in the atmosphere; the quantities of emissions from plants exceed those produced synthetically by approximately 300-fold [2]. In fact, isoprene is the single largest biogenic nonmethane hydrocarbon emitted into the Earth's atmosphere; the annual global emission is estimated to be $\sim 6 \times 10^{11}$ kg [10]. The major anthropogenic sources are combustion processes, including biomass burning, tobacco smoking, and automobile exhaust [9,11,12]. Thus, like 1,3-butadiene, isoprene is also ubiquitous in the environment; its concentration in U.S. ambient air ranges from 1 to 21 ppb and generally is less than 10 ppb [9].

Human exposure to isoprene is largely caused by its generation through endogenous processes, because it is the major endogenously produced hydrocarbon [1] and is abundant in human breath at concentrations in the range of 50–1000 ppb [13]. Nonetheless, smoking significantly increases human exposure to isoprene because tobacco smoke is the primary source of isoprene in indoor air [9,14]. Isoprene is one of the major hazardous volatile organic compounds in cigarette smoke; its total yield (~800 μg/cigarette) is the second largest among 14 hazardous volatile organic compounds [12]. It is ranked third (after 1,3-butadiene and acetaldehyde) with respect to cancer hazards stemming from smoking by the World Health Organization on the basis of its abundance in cigarette smoke and its animal carcinogenicity [15].

Similarly to 1,3-butadiene, isoprene undergoes oxidative metabolism, which is primarily mediated by cytochrome P450 2E1, followed by P450 2B6 [16], to produce two isomeric monoepoxides, 2-ethenyl-2-methyloxirane (i.e., isoprene-1,2-oxide, IP-1,2-O) and 2-(1-methylethenyl)oxirane (i.e., isoprene-3,4-oxide, IP-3,4-O). Both monoepoxides can be further metabolized to the diepoxide, 2-methyl-2,2′-bioxirane (MBO) (Fig. 1) [16–19]. The epoxides can be hydrolyzed by epoxide hydrolase to form the corresponding diols or epoxydiols, or can be conjugated with glutathione [16,20,21]. For the two monoepoxides, IP-1,2-O is the major metabolite and IP-3,4-O is the minor one (~20%) [16–18].

Isoprene itself is not mutagenic as examined by the Ames test, even after metabolic activation using rat liver microsomes [22]. Unlike the monoepoxide of

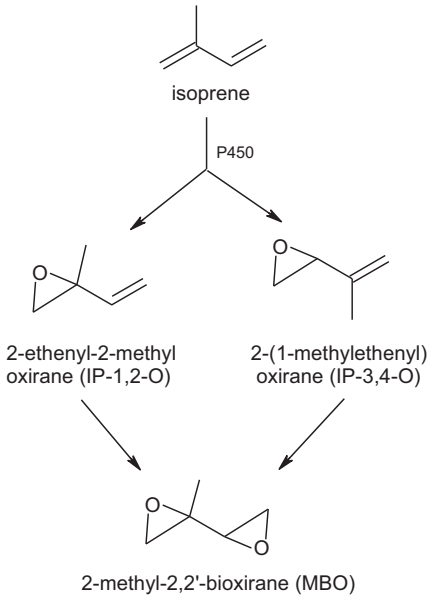

Fig. 1. The metabolism pathways of isoprene.

1,3-butadiene, IP-1,2-O and IP-3,4-O are non-mutagenic [23,24]. However, MBO was found to be as mutagenic as 1,2,3,4-diepoxybutane (DEB) [23,24], the diepoxide of 1,3-butadiene. On the other hand, isoprene itself did not induce strand breaks in the absence of metabolic activation but did so with metabolic activation in peripheral blood mononuclear cells (PBMCs) and human leukemia cells (HL60) as evaluated by the comet assay [25]. This may indicate that isoprene metabolites are genotoxic. Indeed, IP-1,2-O has been found to be genotoxic in PBMCs and HL60 [25,26]. However, so far the genotoxicity of IP-3,4-O and MBO has not been examined yet.

In comparison with 1,3-butadiene, the presence of the extra methyl group in isoprene has a profound influence on properties of its metabolites, including reactivity, mutagenicity, etc., because the methyl group causes steric hindrance and also introduces an asymmetric factor in these molecules. Due to the asymmetry, there exist two monoepoxides that show quite distinct reactivity in some reactions. For instance, IP-1,2-O is easily hydrolyzed, whereas IP-3,4-O (and also MBO) is much more resistant to hydrolysis. Their half-lives at physiological pH and temperature are 1.25 and 73 h (46 h for MBO), respectively [23,24]. The difference between IP-1,2-O and IP-3,4-O is especially great in the acid-catalyzed hydrolysis; the hydrolysis rate constant of IP-1,2-O is 10,000-fold larger than that of IP-3,4-O [27]. For MBO, the asymmetry renders the reactivity of the two oxirane rings different, which was thought to result in suppressed cross-linking potential of MBO compared to DEB (however, the studies were conducted through the reactions between MBO and model compounds (valine methyl ester or purines) and the cross-linking potential of MBO has not been examined in cells or in vivo) [2].

Due to the presence of reactive oxirane moieties in these molecules, IP-1,2-O and IP-3,4-O are alkylating agents