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Original Contribution

Depurinating naphthalene–DNA adducts in mouse skin related to cancer initiation

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ABSTRACT

Naphthalene has been shown to be a weak carcinogen in rats. To investigate its mechanism of metabolic activation and cancer initiation, mice were topically treated with naphthalene or one of its metabolites, 1-naphthol, 1,2-dihydrodiolnaphthalene (1,2-DDN), 1,2-dihydroxynaphthalene (1,2-DHN), and 1,2-naphthoquinone (1,2-NQ). After 4 h, the mice were sacrificed, the treated skin was excised, and the depurinating and stable DNA adducts were analyzed. The depurinating adducts were identified and quantified by ultraperformance liquid chromatography/tandem mass spectrometry, whereas the stable adducts were quantified by ^{32}P-postlabeling. For comparison, the stable adducts formed when a mixture of the four deoxyribonucleoside monophosphates was treated with 1,2-NQ or enzyme-activated naphthalene were also analyzed. The depurinating adducts 1,2-DHN-1-N3Ade and 1,2-DHN-1-N7Gua arise from reaction of 1,2-NQ with DNA. Similarly, the major stable adducts appear to derive from the 1,2-NQ. The depurinating DNA adducts are, in general, the most abundant. Therefore, naphthalene undergoes metabolic activation to the electrophilic *ortho*-quinone, 1,2-NQ, which reacts with DNA to form depurinating adducts. This is the same mechanism as other weak carcinogens, such as the natural and synthetic estrogens, and benzene.

Introduction

Naphthalene is a component of coal tar products and moth repellents. It is used extensively in the production of plasticizers, resins, insecticides, and surface-active agents [1]. It is a ubiquitous pollutant found mainly in ambient air and to a minor extent in effluent water. The major contributor of naphthalene in air is fossil fuel combustion, but a significant amount is also released as a pyrolytic product of mainstream and side-stream tobacco smoke. Naphthalene was found in nearly 40% of human fat samples [2] and 75% of human breast milk samples [3]. These facts indicate that the population of the United States is exposed to naphthalene, which is included as one of 189 hazardous air pollutants under the Clean Air Act Amendments of 1990 (Title III) of the Environmental Protection Agency [4].

Chronic inhalation of naphthalene (10 or 30 ppm) by mice led to diverse effects, including inflammation of the nose, metaplasia of the olfactory epithelium, and hyperplasia of the respiratory epithelium [5–7]. No neoplastic effects in male mice were found, but female mice showed a slight increase in alveolar/bronchiolar adenomas and carcinomas at the highest exposure level. More recently, the U.S. National Toxicology Program conducted a 2-year bioassay study with rats exposed to doses of 10, 30, or 60 ppm naphthalene [8,9], showing a concentration-dependent increase in adenomas of the respiratory epithelium of the nose and neuroblastomas of the olfactory epithelium. These results in rodent studies have raised concerns about naphthalene as a potential human carcinogen [1,10].

The toxicity of naphthalene depends on its metabolic activation (Fig. 1). Studies conducted *in vitro* and *in vivo* demonstrated that the first step in the metabolic conversion of naphthalene is the cytochrome P450-dependent formation of the 1,2-epoxide (Fig. 1) [5,11–16]. This compound is unstable at physiological pH [13,14] and can either react with glutathione to form glutathione conjugates or convert to the metabolites 1-naphthol by chemical isomerization or naphthalene 1,2-dihydrodiol (1,2-DDN) by epoxide hydrolase [15,16]. Conversion to 2-naphthol can occur after β-elimination of the sulfated/glucuronidated conjugate of naphthalene 1,2-dihydrodiol (not shown in Fig. 1). 1,2-DDN [17–19] or 1-naphthol [20] can be further metabolically oxidized to 1,2-dihydroxynaphthalene (1,2-DHN) or its oxidized product, 1,2-naphthoquinone (1,2-NQ). 1,2-NQ is thought to be the metabolite that binds covalently to proteins [20,21]. In a recent publication, we reported the predominant formation of depurinating adducts (Fig. 1) after reaction of 1,2-NQ or enzyme-activated 1,2-DHN with DNA [22].

In this article we report our results from topical treatment of SENCAR mice with naphthalene at two dose levels. In addition, mice

Abbreviations: Ade, adenine; 1,2-DDN, 1,2-dihydro-1,2-dihydroxynaphthalene; 1,2-DHN, 1,2-dihydroxynaphthalene; dN3p, 2′-deoxynucleoside 3-monophosphate; EDTA, ethylenediamine tetraacetic acid disodium salt; Gua, guanine; IACUC, Institutional Animal Care and Use Committee; 1,2-NQ, 1,2-naphthoquinon; 4-OHE$_2$, 4-hydroxyestradiol; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; TBE, Tris-boric acid-EDTA buffer; TEMED, *N,N,N′,N′*-tetramethylethylenediamine; UPLC-MS/MS, ultraperformance liquid chromatography–tandem mass spectrometry.

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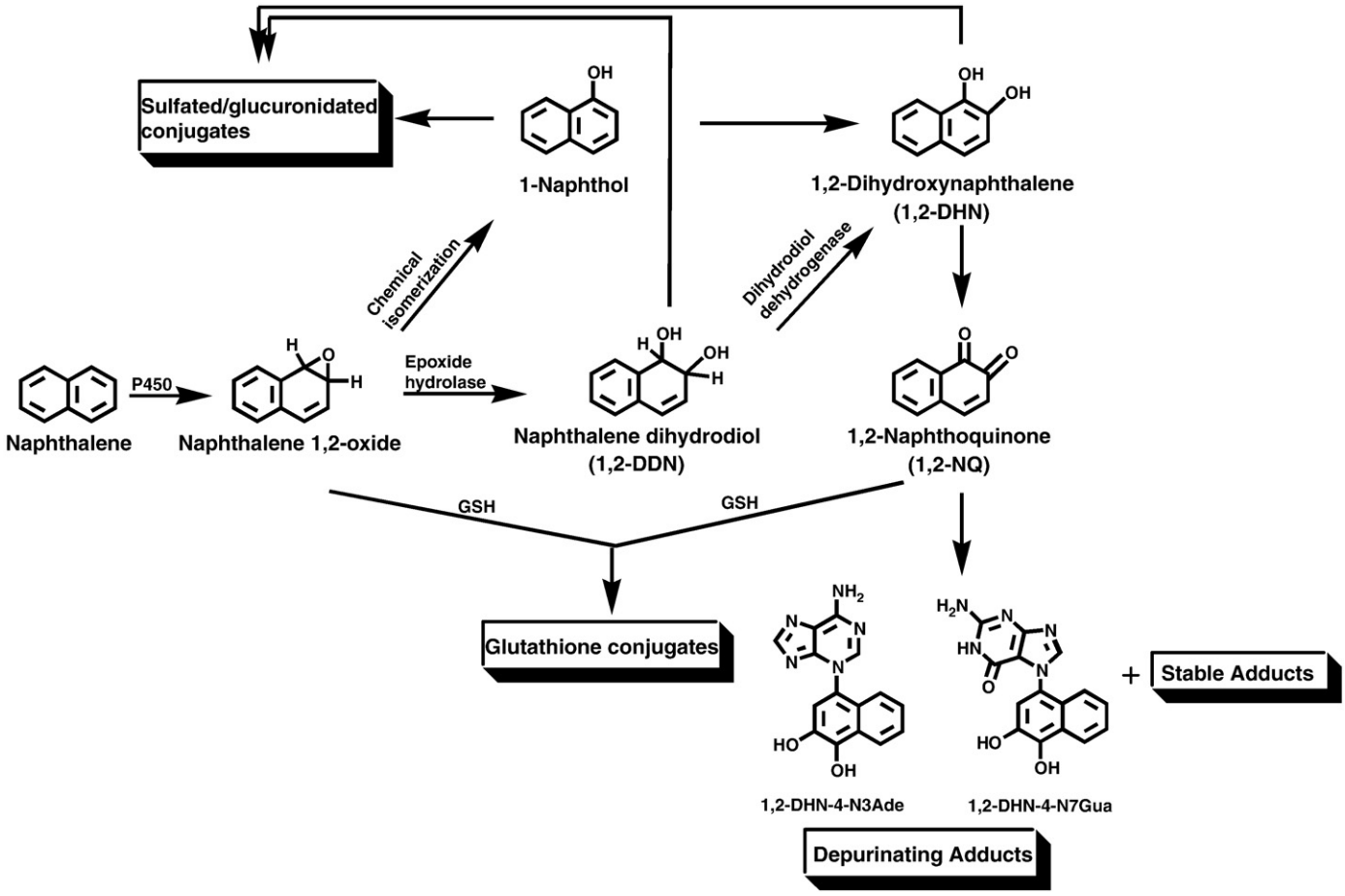

Fig. 1. Pathways of naphthalene metabolism.

were treated with metabolites of naphthalene, 1-naphthol, 1,2-DDN, 1,2-DHN, and 1,2-NQ, and formation of naphthalene–DNA adducts was measured. The depurinating adducts were identified and quantified by ultraperformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) by using the standard synthesized adducts, 1,2-DHN-4-N3Ade and 1,2-DHN-4-N7Gua, whereas the unknown stable adducts were measured by the ^{32}P-postlabeling technique. These findings are critical for understanding the metabolic activation of naphthalene in the initiation of cancer.

Materials and methods

Chemicals, reagents, and enzymes

1,2-NQ, 1-naphthol, DMSO, boric acid, and $NaBH_4$ were purchased from Aldrich Chemical Co. (Milwaukee, WI). 1,2-DHN and 1,2-DDN were prepared by reacting 1,2-NQ with $NaBH_4$ as described previously [23]. Proteinase K, Tris (Sigma 7–9), EDTA, 2′-deoxyguanosine 3-monophosphate (dG3p), 2′-adenosine 3-monophosphate (dA3p), 2′-cytidine 3-monophosphate (dC3p), thymidine 3-monophosphate (dT3p), NADPH, and SDS were purchased from Sigma (St. Louis, MO). Histological grade acetone (Fisher Scientific) was used to prepare solutions for treatment of mouse skin.

SENCAR mouse skin treatment

Forty-nine female SENCAR mice (NCI) at 6 weeks of age were housed in the Eppley Animal Facility. When the mice were 8 weeks old, a dorsal area of the skin was shaved and groups of 4 or 5 mice were treated with the compounds. Two groups were treated with each compound and dose. Naphthalene (1200 or 500 nmol) or its metabolite (500 nmol) was dissolved in acetone to deliver a 50-μl application on the mouse skin. The treated areas were outlined immediately after the application. Four hours later, the mice were euthanized according to IACUC guidelines, and the treated areas of the skins were excised, placed together for each treatment group in 50-ml tubes, and stored at -20°C. For each set of skins, the epidermis of the mouse skin was isolated, minced, and ground together in liquid N_2. Approximately 10% of the ground epidermis was used for analyzing chromosome-bound stable DNA adducts by the ^{32}P-postlabeling technique, as described below. The remaining epidermis was processed for analyzing depurinating adducts, as described in the following section.

Sample preparation for analysis of depurinating adducts

Samples of ground epidermis were weighed and suspended in 15 ml of Tris buffer composed of 20 ml of 1 M Tris (pH 8.0), 20 ml of 0.5 M EDTA, and 1 ml of 10% SDS plus distilled water to a total volume of 100 ml. The suspension was homogenized by using a Tissue Tearor (Model 587370, BioSpec Product, Bartlesville, OK). The homogenate was incubated with proteinase K (10 mg dissolved in 1 ml of 1 M Tris, pH 8.0) at 37°C for 5 h. The resulting viscous liquid was cooled on ice, extracted with hexane to remove fat, and finally treated with 2 vol of ethanol. The precipitated material was removed by centrifuging at high speed for 5 min, and the supernatant was removed and evaporated in a Jouan RC1010 centrifuge evaporator (Jouan Inc.). The residue was suspended in 1 ml of CH_3OH/H_2O (1/1), filtered through a 0.2-μm acrodisc syringe filter (Fisher Scientific) directly into a 2-ml Eppendorf tube, and evaporated to about 200 μl. The solution