

A Metabolic Activation Mechanism of 7H-Dibenzo[*c,g*]carbazole via *O*-Quinone. Part 2: Covalent Adducts of 7H-Dibenzo[*c,g*]carbazole-3,4-dione with Nucleic Acid Bases and Nucleosides

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7H-Dibenzo[*c,g*]carbazole (DBC) is a potent multispecies, multisite carcinogen present in the environment. The metabolic activation pathways of DBC are not completely known. It is hypothesized that DBC may be metabolically activated by oxidation to the reactive Michael acceptor *o*-quinones, which can form stable and depurinating DNA adducts. The synthesis of DBC-3,4-dione has been previously reported by this research group. In the present article, we describe the synthesis and chemical structural elucidation of nine DBC-nucleic acid adducts produced from reactions of DBC-3,4-dione with Ade, Cyt, 2'-deoxyguanosine (dGuo), 2'-deoxycytidine (dCyd), and Guo. Adducts were isolated from reaction mixtures by HPLC and analyzed using MS including elemental compositions and collision-activated dissociation (CAD), ¹H NMR, and two-dimensional chemical shift correlation spectroscopy (COSY) NMR. The adducts, 7-[3,4-dione-DBC-1-yl]-Ade, *N*¹-[3,4-dione-DBC-1-yl]-Cyt, 5-[3,4-dione-DBC-1-yl]-Cyt, two conformational isomers of *N*²-[3,4-dihydroxy-DBC-1-yl]-dGuo, and two conformational isomers of *N*²-[3,4-dihydroxy-DBC-1-yl]-Guo, were characterized. Two adducts from reactions of DBC-3,4-dione with dCyd were identified by MS but not fully characterized by NMR due to instability of the adducts. Under similar conditions, the reactions of DBC-3,4-dione with Gua and 2'-deoxyadenosine (dAdo) did not result in an identifiable adduct. Liver DNA adducts from mice treated topically with DBC-3,4-dione (100 μg) in dimethyl sulfoxide/acetone (15/85, 100 μL) were identified with ³²P-postlabeling. The major adduct chromatographically matched one of the adducts formed from livers of DBC-treated mouse (adduct 3) using identical conditions.

Introduction

As an environmental nitrogen heteropolycyclic aromatic compound (NAH),¹ 7H-dibenzo[*c,g*]carbazole (DBC) has been shown to be a potent multispecies, multisite carcinogen in experimental animals with both local and systemic effects (1, 2). To elucidate the metabolic activation mechanisms and the ultimate carcinogenic metabolite(s) of DBC, extensive research has been carried out. Unlike the polycyclic aromatic hydrocarbons (PAHs), DBC metabolism in vitro and in vivo produces mainly monohydroxylated derivatives rather than dihydrodiols (1–5). However, the predominant metabolites produced, 5-hydroxy-DBC and 3-hydroxy-DBC (3-OH-DBC), were not as sarcomagenic by subcutaneous injection as the parent DBC (1, 6, 7). DBC metabolism was also expected

to occur through N-hydroxylation, as is the case of carcinogenic aromatic amines (8, 9). After acetylation in situ to trap the unstable monohydroxylated metabolites generated in the DBC incubation mixture with rat liver microsomes, there was no *N*-hydroxyl-DBC detected (10). Although DBC, which has a relatively low ionization potential (11), can form depurinated DNA adducts through radical-cations by one-electron oxidation (12), this was found to be a minor metabolic pathway in mouse liver and lung (13). An *o*-quinone was also tentatively assigned as a minor component in DBC metabolism (3). However, no further examination was carried on at that time due to the lack of information on the role of *o*-quinone in metabolic activation of PAH and reference standards. Studies in our laboratories have demonstrated that 3-OH- and 4-OH-DBC produced higher levels of DNA adducts in mouse skin, lung, and liver than the parent compound. We thus proposed that 3-OH-DBC is a proximate metabolite (14, 15), which needs further activation to an ultimate electrophile and/or redox-active form that is capable of covalently binding with DNA or causing oxidative DNA damage. Referring to the relatively well-documented metabolic activations of estrogens and homo-PAHs (16, 17), we hypothesize that 3-OH-/4-OH-DBC may be further bioactivated to yield DBC-*o*-quinones via DBC-3,4-catechol, as ultimate carcinogenic metabolites

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¹ Abbreviations: DBC, 7H-dibenzo[*c,g*]carbazole; 3-OH-DBC, 3-hydroxy-DBC; 4-OH-DBC, 4-hydroxy-DBC; PAH, polycyclic aromatic hydrocarbon; NAH, nitrogen heterocyclic aromatic hydrocarbon; HR-MS, high-resolution mass spectroscopy; ESI, electrospray ionization; CAD, collision-activated dissociation; COSY, two-dimensional chemical shift correlation spectroscopy; DMSO, dimethyl sulfoxide; DMF, dimethylformamide; dGuo, 2'-deoxyguanosine; dCyd, 2'-deoxycytidine; dAdo, 2'-deoxyadenosine.