Chemico-Biological Interactions 279 (2018) 10–20

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Facilitation of 9,10-phenanthrenequinone-elicited neuroblastoma cell apoptosis by NAD(P)H:quinone oxidoreductase 1

Toshiyuki Matsunaga[a,*], Kyoko Kamase[a], Hiroaki Takasawa[a], Yukiko Yamaji[a], Satoshi Endo[a], Ossama El-Kabbani[b], Akira Ikari[a]

[a] Laboratory of Biochemistry, Gifu Pharmaceutical University, Gifu, 501-1196, Japan
[b] Nagoya University Graduate School of Medicine, Nagoya 466-8550, Japan

ARTICLE INFO

Keywords:
9,10-Phenanthrenequinone
Neuroblastoma cell
Apoptosis
Reactive oxygen species
Antioxidant property
NAD(P)H:quinone oxidoreductase 1

ABSTRACT

9,10-Phenanthrenequinone (PQ), a major quinone component in diesel exhaust particles, is considered to provoke damage of respiratory and vascular cells through highly producing reactive oxygen species (ROS), but little is known about its pathophysiological role in neuronal cell damage. In this study, we found that incubation with 1,2-naphthoquinone, 1,4-naphthoquinone and PQ, major quinone components in diesel exhausts, provokes apoptosis of human neuroblastoma cell lines. SK-N-SH cell treatment with a lethal concentration of PQ facilitated ROS production within 6 h. The treatment also promoted formation of 8-hydroxy-deoxyguanosine, p53 activation, elevation of Bax/Bcl-2 ratio, lowering of mitochondrial membrane potential, and resultant activation of caspase-9 and caspase-3, inferring that ROS production, DNA damage and mitochondrial dysfunction are crucial processes of the PQ-triggered SK-N-SH cell apoptosis. The PQ treatment of SK-N-SH cells elevated the level of 4-hydroxynonenal (HNE), a cytotoxic reactive aldehyde generated from lipid peroxidation. The treatment with PQ and HNE also decreased cellular levels of total and reduced glutathiones, and the damage elicited by HNE was ameliorated and deteriorated by pretreating with cell-permeable glutathione analog and the depletor, respectively. Moreover, the treatment with PQ and HNE decreased the proteasomal proteolytic activities, suggesting a contribution of decrease in the antioxidant abilities to the ROS-mediated neuroblastoma cell apoptosis. Our comparative analyses of 17 cells showed a positive correlation between the PQ reductase and NAD(P)H:quinone oxidoreductase 1 (NQO1) activities. In addition, overexpression and knockdown of NQO1 augmented and lowered, respectively, the ROS production through PQ redox-cycling and the quinone toxicity. Furthermore, the treatment with PQ and HNE up-regulated the NQO1 expression. Taken together, PQ exposure produces large amounts of ROS in neuroblastoma cells via NQO1 up-regulation and resultant acceleration of its redox-cycling, followed by activation of the ROS-dependent apoptotic mechanism.

1. Introduction

Diesel exhaust particle (DEP), a major constituent of urban air pollution, is a microparticle in an exhaust gas that was diffused in the environment by diesel fuel combustion. Owing to its small size (less than 10-μm diameter), DEP inhaled is speculated to be capable of easily passing from the respiratory tract into the circulation, and thereby exerting its injurious actions in various organs. Although the lung is one of the most preferentially targeted organs on inhalation of the particle [1,2], cerebral nerve and cardiovascular systems are also regarded as potential targets in case-crossover and exposure studies conducted to evaluate the association with air pollutants [3,4]. In experiments using isolated rat brain capillaries, it is found that DEP alters blood-brain barrier function through oxidative stress and proinflammatory cytokine production [5]. Based on the report, exposure to DEP at its low concentrations less than 5 μg/mL appears to alter the expression and transport activity of the key transporter, P-glycoprotein. On the other hand, fetal exposure of mice to the particle affects the emotional behaviors associated with the serotonergic and dopaminergic systems in the mouse brain [6]. These findings have suggested that low

Abbreviations: AKR, aldo-keto reductase; AMC, 7-amido-4-methylcoumarin; ATM, ataxia telangiectasia mutated kinase; BSO, DL-buthionine-(*S*,*R*)-sulfoximine; CBR, carbonyl reductase; CuZn-SOD, copper-zinc superoxide dismutase; DCFH-DA, 6-carboxy-2′,7′-dichloro-dihydrofluorescein diacetate; DEP, diesel exhaust particle; DPBS, Dulbecco's phosphate-buffered saline; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); FBS, Fetal bovine serum; GSH, reduced glutathione; HNE, 4-hydroxynonenal; NAC, *N*-acetyl-L-cysteine; NQ, naphthoquinone; NQO1, NAD(P)H:quinone oxidoreductase 1; MG132, *Z*-Leu-Leu-Leu-CHO; PMSF, phenylmethanesulfonyl fluoride; 8-OH-dG, 8-hydroxy-deoxyguanosine; PQ, 9,10-phenanthrenequinone; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction; siRNA, small-interfering RNA; XR, L-xylulose reductase

* Corresponding author. Laboratory of Biochemistry, Gifu Pharmaceutical University, 1-25-4 Daigaku-nishi, Gifu, 501-1196, Japan.
E-mail address: matsunagat@gifu-pu.ac.jp (T. Matsunaga).

https://doi.org/10.1016/j.cbi.2017.10.028
Received 6 September 2017; Received in revised form 3 October 2017; Accepted 30 October 2017

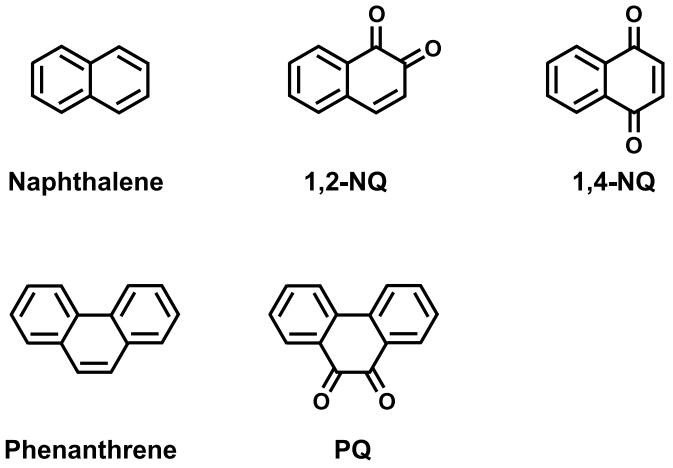

Fig. 1. Structures of naphthalene, 1,2-naphthoquinone (1,2-NQ), 1,4-NQ, phenanthrene and 9,10-phenanthrenequinone (PQ).

concentrations of DEP elicit encephalic damages in addition to onset and exacerbation of cerebrovascular and neurodegenerative diseases. Indeed, other animal experiments revealed that exposure to urban pollution induces inflammatory and stress protein brain responses and Alzheimer's disease-like pathologic alterations [7,8]. In addition, upon cell-based experiments, treatment with DEP was shown to selectively elicit damage in dopaminergic neurons through the phagocytic activation of microglial NADPH oxidase and consequent oxidative insult [9]. These findings would thus raise concern about severe cranial nerve disorders associated with chronic exposure to DEP.

DEP consists of carbonaceous nuclei and a vast number of organic components such as polycyclic aromatic hydrocarbons, nitroaromatic hydrocarbons, heterocyclics, aldehydes and quinones [10,11]. Among the components, the abundant and potent toxicants are 9,10-phenanthrenequinone (PQ), 1,2-naphthoquinone (1,2-NQ) and 1,4-NQ (Fig. 1), whose cytolethal effects are mainly attributed to the generation of reactive oxygen species (ROS) via the redox-cycles and the subsequent ROS-dependent pathways [12–14], closely mimicking the toxic mechanisms of DEP [15]. Therefore, it is suggested that the three quinones play crucial roles in pathogenic mechanisms triggered by DEP. Of the three cytotoxic quinones, one of the most extensively studied is a tricyclic quinone PQ, which is highly produced during biological and photo-oxidative reactions of phenanthrene [16]. The ROS production caused by PQ is assumed to be mediated by its redox-cycling, which is initiated by two-electron reduction of the quinone into its hydroquinone 9,10-dihydroxyphenanthrene by some NADPH-dependent reductases [12]. The resultant hydroquinone is rapidly converted into its semiquinone radical through the nonenzymatic dispropotionation with PQ and then followed by regenerating PQ, in parallel with the ROS generation from molecular oxygen. Thus, exposure to small amount of PQ is thought to excessively generate ROS and to consequently cause severe health hazards. Literature revealed contributions of cytochrome P450 reductase and carbonyl reductase (CBR) 1 in the redox-cycling of PQ [12,17]. In addition, we previously showed that CBR4 [18], L-xylulose reductase (XR) [19] and two members (AKR1C3 and AKR1B10) in the aldo-keto reductase (AKR) superfamily [20,21] participate in acceleration of the redox-cycling and consequently activate the ROS-dependent apoptotic signaling. NAD(P)H:quinone oxidoreductase 1 (NQO1) is well accepted as a superoxide anion scavenger that protects from cytotoxicity elicited by ROS [22,23]. The enzyme is reported to convert quinones into their corresponding hydroquinones in a NADPH-linked two-electron reduction [24,25], which is, however, the same as that in initial step of the redox-cycling of PQ catalyzed by the above oxidoreductases. Therefore, NQO1 is assumed to be involved in the PQ-mediated redox-cycling, which leads to the cytotoxicity of PQ.

2. Materials and methods

2.1. Materials

PQ was obtained from Nacalai tesque (Kyoto, Japan); naphthalene, 1,2-NQ and 1,4-NQ were from Tokyo Chemical Industry (Tokyo, Japan); and phenanthrene, 2-hexenal and 2-nonenal were from Wako Pure Chemical Industries (Osaka, Japan). *N*-Acetyl Asp-Glu-Val-Asp 7-amido-4-methylcoumarin (AMC), *N*-acetyl Leu-Glu-His-Asp-AMC, *N*-succinyl-Leu-Leu-Val-Tyr-AMC, *t*-butyloxycarbonyl-Leu-Arg-Arg-AMC, *N*-acetyl-L-cysteine (NAC), DL-buthionine-(*S*,*R*)-sulfoximine (BSO), glutathione ethyl ester, *Z*-Leu-Leu-Leu-CHO (MG132) and dicumarol were procured from Sigma-Aldrich (St. Louis, MO). 5,5′,6,6′-Tetrachloro-1,1′,3,3′- tetraethylbenzimidazolylcarbocyanine iodide (JC-1), 8-hydroxy-deoxyguanosine (8-OH-dG) ELISA kit, 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), bicinchoninic acid protein assay reagent and enhanced chemiluminescence substrate system were purchased from Calbiochem (La Jolla, CA), Japan Institute for the Control of Aging (Shizuoka, Japan), Molecular probes (Rockland, ME), Pierce (Rockford, IL) and Amersham Biosciences (Piscataway, NJ), respectively. Acrolein and copper-zinc superoxide dismutase (CuZn-SOD) were products of Jannsen Chica (Beerse, Belgium) and Ube Industries (Tokyo, Japan), respectively. 4-Hydroxynonenal (HNE) was synthesized as described previously [26]. All other chemicals were of the highest grade that could be obtained commercially.

2.2. Cell culture and transfection

Human neuroblastoma SK-N-SH (RIKEN Cell Bank, Tsukuba, Japan) and bronchial BEAS-2B cells (American Type Culture Collection, Manassas, VA) were cultured in Eagle's minimum essential medium alpha modifications and Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12, respectively, supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL) and streptomycin (100 μg/mL) in type-I collagen-coated dishes at 37 °C in a humidified incubator containing 5% CO_2. Other cells (neuroblastoma A172, leukemia HL60, Molt4 and U937, lung H23 and A549, liver HepG2, osteoblastic Saos2, colon RKO, HCT15 and HT29, breast MCF7 and cervical Hela) were obtained from American Type Culture Collection, except that neuroblastoma TGW and gastric MKN45 cells were obtained from Health Science Research Resources Bank (Osaka, Japan), and all these cells were maintained in Dulbecco's-modified Eagle's medium supplemented with 10% FBS and the antibiotics before usage of experiments.

For preparation of cells that overexpress NQO1, the mammalian pGW1 expression vector harboring the cDNA for human NQO1 was constructed by means of the subcloning technique. The cDNA was amplified by PCR using the following primers from cDNA isolated from human aortic endothelial cells. The forward primer, 5′-CCCCG*GTCGAC*gccacc<u>ATG</u>GTCGGCAGAAGAGCACTG-3′, contains a *Sal*-I site, a Kozak sequence and a start codon, which are shown in italic, small, and underlined letters, respectively. The reverse primer, 5′-CCCC*AAGCTT*<u>TCA</u>TTTTCTAGCTTTGATCTGGTTG-3′, is complementary to the C-terminal sequence of NQO1 containing a *Hind*-III site and a stop codon, which are shown in italic and underlined letters, respectively. The amplified cDNA was subcloned at the *Sal*-I and *Hind*-III sites into the pGW1 expression vectors, and the sequence of the insert was then verified by DNA sequencing using a CEQ2000XL DNA sequencer (Beckman Coulter, Fullerton, CA). The transfection of the expression vector carrying cDNAs for AKR1B10 [27], AKR1C3 [20] and CBR1 [28] was performed as described previously. The expression vector was transfected into SK-N-SH and BEAS-2B cells using Lipofectamine 2000 when the cells were grown to 90% confluence in microplates or dishes. The empty vector was similarly transfected into the cells, which were used as control cells. For NQO1 knockdown, SK-N-SH cells were transfected with either small-interfering RNA (siRNA) targeting NQO1 or the non-target scramble sequence (GE Dharmacon,