Oxidative Stress-Dependent Cellular Toxicity and Cytoprotection during Exposure to 9,10-phenanthraquinone, a Component of Diesel Exhaust Particles

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Quinones are ubiquitously present in the environment. This review summarizes the cellular toxicity of 9,10-phenanthraquinone (9,10-PQ), a component of diesel exhaust particles, and the cytoprotective mechanism of the nuclear factor erythroid 2-related factor 2 (Nrf2)/Kelch-like ECH-associated protein 1 (Keap1) system against oxidative stress exerted by 9,10-PQ.

Key words —— quinone, oxidative stress, nuclear factor erythroid 2-related factor 2, cytoprotection, detoxification

INTRODUCTION

Epidemiologic studies show that long exposure to airborne particulate matter resulting from the combustion of fossil fuels correlates closely with the incidence of cancer and respiratory and cardiovascular diseases. Complexes consisting of a huge number of chemicals make identification of the causative chemicals difficult. However, the results of many studies support the idea that oxidative stress contributes to the disorders caused by airborne particle matter. Therefore we focused on components that have the ability to exert oxidative stress in diesel exhaust particles (DEP), which account for a large majority of airborne particle matter. Half of the organic phase of DEP extracted with dichloromethane consists of chemicals derived from phenanthrene, a three-ring polycyclic aromatic hydrocarbon (PAH). Since a one-electron reduction potential value of 9,10-phenanthraquinone (9,10-PQ) was found to be $-124$ mV, this o-quinone derived from phenanthrene appears to act as an efficient electron acceptor, resulting in the production of reactive oxygen species (ROS) in cells.1)

OXIDATIVE STRESS CAUSED BY 9,10-PQ

Quinones are produced from PAH by photooxidation2) or by biological metabolism.3) PAHs are oxidized to anti-diol-epoxides through PAH trans-dihydrodiols by cytochrome P450s (CYP) and epoxide hydrolase. PAH trans-dihydrodiols are converted to quinones through dihydroquinones by dihydrodiol dehydrogenases (aldo-keto reductases; AKRs). PAH quinones are reduced to dihydroquinones through semiquinone radicals. Quinones ubiquitously exist as biological factors (e.g., coenzyme Q in the electron transport chain) or as environmental chemicals.

Quinones are electron acceptors that easily react with biological nucleophiles such as proteins, lipids, or DNA. The chemical effects of quinones on proteinous thiols are generally either covalent binding and/or redox cycling. 9,10-PQ exclusively undergoes redox cycling with thiols, but interestingly not with monothiols but with di thiols.4) A thiol is contained in a proteinous cysteine residue. As biological di thiols, the antioxidative protein thioredoxin or the intracellular antioxidant α-dihydrolipoic acid should be target molecules of 9,10-PQ. The redox cycling of 9,10-PQ is identified by detecting the production of superoxide and the one-electron reducing form, the semiquinone radical (9,10-PQ$^{·−}$), consumption of thiols and molecular oxygen, and...
a constant amount of 9,10-PQ. The production of superoxide exceeds the expected amount of stoichiometry. Superoxide is easily converted to hydrogen oxide or hydroxyl radical. Propagation of these ROS is thought to be a potent cause of oxidative stress by 9,10-PQ. 9,10-PQ oxidized cellular proteins and led to cell death.\(^5,6\)

DETOXIFICATION PATHWAY OF 9,10-PQ

In the detoxification of 9,10-PQ, quinones must be reduced to dihydroquinones to conjugate with glutathione, glucuronic acid, or sulfate. At least two types of enzyme need to be cooperatively involved in two-electron reduction and conjugation. Representative two-electron reductases are NAD(P)H:quinone reductase (NQO1) and AKRs isozymes. Some dihydroquinones are stable and inactive, although others are not. 9,10-PQH\(_2\) is classified as the latter, a group of unstable dihydroquinones. In terms of chemical properties, 9,10-PQH\(_2\) is as powerful as 9,10-PQ. When cells are exposed to 9,10-PQ, a product is excreted into the extracellular space. An unknown product of 9,10-PQ was identified as monoglucuronide of 9,10-dihydroxyphenanthrene (PQHG) by comparison with an authentic sample.\(^7\) This ultimate metabolite lost the ability to exert oxidative stress like 9,10-PQ because it could not undergo redox cycling. Taken together, the results show that glucuronidation of 9,10-PQH\(_2\) occurs using uridine 5’-diphosphate glucuronosyltransferase (UGT), followed by two-electron reduction of 9,10-PQ.

CYTOPROTECTION BY THE TRANSCRIPTION FACTOR Nrf2

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that belongs to the cap’n’collar (CNC) family. The molecular mechanisms of Nrf2 activation have been well charac-

Fig. 1. Toxification and Detoxification Pathways of 9,10-PQ and the Nrf2/Keap1 System
In unstressed conditions, Nrf2 is rapidly degraded through the ubiquitin-proteasome system by Kelch-like ECH-associated protein 1 (Keap1)-Cullin3 E3 ligase. The half-life of the protein is approximately 20 min. DLG (weak site) and ETGE (strong site) motifs in Nrf2 are recognized and retained by the DC domain of Keap1. A “hinge and latch model” was proposed. The cytoplasmic protein Keap1 has 25 cysteines in a molecule. Some of the reactive thiols are targets of a variety of chemicals. Such modification of the Keap1 thiols causes activation of Nrf2, as confirmed by nuclear localization from the cytoplasm of this transcription factor. Diethyl malate (DEM), tert-butyl hydroquinone (tBHQ), and sulforaphane (an ingredient of broccoli sprouts) are known to be typical Nrf2 activators. These chemicals are electrophiles that react with cysteine residues in Keap1. In cellular experiments, 9,10-PQ also activates Nrf2 at a weakly toxic dose to the same extent as DEM (Taguchi et al., unpublished data). Some cells exposed to 9,10-PQ activate Nrf2 to upregulate the downstream genes. Although the mechanism by which 9,10-PQ activates Nrf2 remains to be elucidated, we speculate that 9,10-PQ directly reacts with proximal thiols in Keap1 as reported previously or ROS during redox cycling of 9,10-PQ in the cells might oxidize Keap1 thiols, thereby dissociating Nrf2 from Keap1 (Fig. 1). Some Nrf2-target gene products contribute to the detoxification of 9,10-PQ, such as NQO1, AKRs, and UGTs, and to antioxidation, such as heme oxygenase-1 (HO-1) and glutamate-cysteine ligase catalytic subunit (GCLC). Furthermore, 9,10-PQ exhibits weaker toxicity in primary hepatocytes isolated from hepatocyte-specific Keap1 conditional knockout mice with constitutively activated Nrf2 compared with wild-type mice, suggesting that Nrf2 activation plays a protective role in PQ toxicity.

**REFERENCES**


