

Review

Health Risk Assessment of Air Pollutants: Air Pollutant Genotoxicity and Its Enhancement by Suppression of Phase II Drug-metabolizing Enzymes

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In Japan, to reduce the health risks associated with hazardous air pollutants, Environmental Quality Standards have been set for certain chemicals in ambient air, and national or local government and industry are required to ensure that the concentrations of those chemicals remain below the Environmental Quality Standards. Guideline Values have also been set to reduce health risks resulting from hazardous air pollutants in the atmosphere. Whether carcinogenicity has a threshold or not is an important factor for risk assessments and for setting Environmental Quality Standards and Guideline Values for carcinogens. In the “Guidelines on Health Risk Assessment Methods for Hazardous Air Pollutants”, carcinogenic air pollutants are proposed to be grouped according to judgment of whether carcinogenicity has a threshold. However, factors for determining the existence, and actual value of threshold of carcinogenicity have not yet been identified. Our research group believes that susceptibility to genotoxic carcinogens is a determinant of carcinogenic threshold, and that metabolic activation of mutagens, excision DNA repair, translesional DNA synthesis, and apoptosis are the main factors determining susceptibility. We have investigated metabolic activation (especially the level of phase II-drug metabolizing enzymes) as a factor in susceptibility to genotoxic carcinogens. Our studies of mice deficient in *Nrf2* (a gene of a transcription factor for inducing phase II enzymes) suggest that expression of phase II enzymes prevents the induction of certain mutations caused by genotoxic carcinogens such as benzo[a]pyrene.

Key words: carcinogen, environmental quality standard, guideline value, knockout mouse, *Nrf2*

Introduction

Various chemicals are emitted into atmospheric, aquatic, and soil environments from sources such as factories and manufacturing plants, and from houses and automobiles. The chemicals emitted into the environment include not only chemical products (man-made chemicals such as toluene and benzene), but also unintended products such as polycyclic aromatic

hydrocarbons (PAHs) and dioxins generated by the burning of fossil fuels and as by-products of manufacturing processes. These emissions are suspected to have adverse effects on humans. To protect society from the hazards of environmental chemicals, health risk assessment of chemicals is now required. For example, benzo[a]pyrene (BaP), a typical product of the combustion of fossil fuels and organic matter, is recognized as a potent mutagen and carcinogen. The World Health Organization evaluated the virtually safe dose (VSD, 1/100,000 cancer risk with life-time exposure) of BaP in ambient air to be 0.1 ng/m³ as a surrogate of carcinogenic PAHs (1). The UK government’s Department for Environment, Food and Rural Affairs set the air quality standard of BaP at 0.25 ng/m³ (2). In 2010, the annual average concentration of BaP in urban air in Japan was between 0.02 and 1.7 ng/m³ (3), and it showed that in parts of urban area of Japan the concentration of BaP in ambient air exceeds the UK air quality standard.

Environmental Quality Standards and Guideline Values as Measures to Reduce Environmental Risk

To reduce the health risks associated with environmental emissions, setting Environmental Quality Standards (EQSs) and conducting environmental monitoring are the two major measures adopted by the Japanese government’s Ministry of the Environment (4). EQSs have been set for chemicals in ambient air, water, and soil, and national or local government and industry are required to take actions to keep the environmental concentrations of those chemicals below the EQS. Furthermore, to reduce the health risks associated with hazardous air pollutants, in addition to EQSs, Guideline Values (GVs) have been set to help evaluate the

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results of ongoing air pollution monitoring and assess the efforts of the business sector to reduce emissions. Both EQSs and GVs are determined according to health risk assessments based on data from epidemiological studies or inhalation experiments conducted in experimental animals. National or local governments conduct environmental monitoring and analyze the concentrations of hazardous chemicals in the environment to assess whether the EQSs and GVs are being maintained (5).

Setting Guideline Values for Hazardous Air Pollutants

In Japan, 23 hazardous air pollutants are classified as Substances Requiring Priority Action, for which EQSs or GVs setting have been planned, and the environmental monitoring is performed. These chemicals have been shown to have adverse effects, such as carcinogenicity or neurotoxicity, on human health, and their amounts of release in ambient air are recorded by the Japanese Pollutant Release and Transfer Registration (PRTR) system (6). EQSs for air have been set for 5 substances (benzene, trichloroethylene, tetrachloroethylene, dioxins, and dichloromethane), and GVs for 8 substances were set between 2003 and 2010 (Table 1). The risk was assessed for setting these EQSs and GVs mainly based on epidemiological studies—especially on studies of inhalation in the work place. The EQS for benzene and GVs for vinyl chloride, nickel compounds, 1,3-butadiene, and arsenic and its inorganic compounds were determined from unit risks derived from epidemiological data (7). However, since the high level inhalation of chemicals to workers has been prevented due to improvements in workplace environments, there will soon be possibly a lack of environmental epidemiological findings of occupational diseases caused by exposure of

chemicals, meaning that it will become more and more difficult to determine EQSs and GVs on the basis of epidemiological data. In recent decade, it has become necessary to assess health risks of hazardous air pollutants by using the findings from research in experimental animals. For example, our research group determined the unit risk of 1,2-dichloroethane for setting the GV at the level of 1/100,000 lifetime cancer risk with fitting a mathematical model to a data set of cancer incidence following lifetime inhalation in rats (8). In case of chloroform, the nasal effects and kidney cancer induced by inhalation in mice were selected as the endpoints, and the GV was determined on the basis of approach of a reference dose which is obtained from dividing No-observed adverse effect level [NOAEL] by an uncertainty factor (7). EQSs or GVs are now under consideration for 10 substances requiring priority action (acetaldehyde, ethylene oxide, beryllium, BaP, formaldehyde, manganese, chromium [III], methyl chloride, chromium [VI], and toluene) (legend of Table 1). These include human carcinogens (group 1 of the International Agency for Research on Cancer classification: ethylene oxide, beryllium, BaP, formaldehyde, chromium [VI]). To determine the EQS or GV for hazardous air pollutants, whether animal experimental data are available for the risk assessment of these carcinogens is an important issue, and we need to consider whether the carcinogenicity of substance has a threshold.

Whether Carcinogenicity Has a Threshold or Not is Important When Assessing the Risk

The health risks of carcinogens are generally assessed as if these substances have no threshold. According to the World Health Organization's Environmental Health Criteria 210 (9), for most types of toxic effect (i.e., organ-specific, neurological or behavioral, immunologi-

Table 1. List of substances requiring priority action for which environmental quality standards (EQSs) and guideline values (GVs) were set

Substances requiring priority actions	Set (year)	EQS ($\mu\text{g}/\text{m}^3$)	GV ($\mu\text{g}/\text{m}^3$)	Endpoint	Evaluation method
Benzene	1996	3		Cancer (leukemia)	Epidemiology
Trichloroethylene	1996	200		Neural effects	Epidemiology
Tetrachloroethylene	1996	200		Neural effects	Epidemiology
Dioxins	1999	0.6×10^{-6} *		Reproductive toxicity	Experiment (rat)
Dichloromethane	2000	150		Neural effects	Epidemiology
Acrylonitrile	2003		2	Chronic effects	Epidemiology
Vinyl chloride	2003		10	Cancer (liver)	Epidemiology
Mercury	2003		0.04	Neural effects	Epidemiology
Nickel	2003		0.025	Cancer (lung)	Epidemiology
Chloroform	2006		18	Nasal effects, Cancer (kidney)	Experiment (mouse)
1,2-dichloroethane	2006		1.6	Cancer (leukemia)	Experiment (rat)
1,3-butadiene	2006		2.5	Cancer (leukemia)	Epidemiology
Arsenic	2010		0.006	Cancer (lung)	Epidemiology

*Derived from tolerable daily intake evaluated on the basis of a diet-intake study. Substances for which EQS and GV have not been set: Acetaldehyde, Ethylene oxide (International Agency for Research on Cancer classification group 1), Beryllium (group 1), Benzo[a]pyrene (group 1), Formaldehyde (group 1), Manganese, Chromium (III), Methyl chloride, Chromium (VI) (group 1), Toluene.

cal, non-genotoxic carcinogenesis, reproductive, or developmental) it is generally considered that there is a dose or concentration below which adverse effects will not occur (i.e., a threshold). However, for other types of toxic effects, it is assumed that there is some probability of harm at any level of exposure (i.e., that no threshold exists); this currently applies primarily for mutagenicity and carcinogenicity. Nevertheless, thresholds have been recognized to exist for the carcinogenicity of some chemicals. In 2012, the National Institute for Environmental Studies drafted a set of guidelines titled “Guidelines on Health Risk Assessment Methods for Hazardous Air Pollutants” to help set EQSs and GVs (10). In these guidelines, judgment of whether carcinogenicity has a threshold depends on 1) whether a carcinogenic chemical is genotoxic or not, and 2) the extent to which genotoxicity is involved in carcinogenicity. According to these criteria, it was proposed to group carcinogenic air pollutants; (i) Cases in which the carcinogenicity of a chemical involves genotoxicity: the chemical is determined to be carcinogenic without a threshold and the unit risk is sought with an appropriate mathematical model. (ii) Cases in which carcinogenic chemicals are not genotoxic, or in which it is conjectured that genotoxicity is not involved in the carcinogenicity of a chemical: the chemical is determined to be carcinogenic with a threshold and the NOAEL is determined. In assessments of whether genotoxicity is involved in the carcinogenicity of chemicals, comprehensive judgments should be made after having determined with certainty the genotoxic mechanism and the carcinogenic mode of action of the chemical in question in both humans and animals.

Level of Phase II Drug-metabolizing Enzymes Is a Possible Determinant of Threshold

As reviewed above, there are different approaches to the risk assessment of carcinogens (unit risk approach vs. reference dose approach), depending on whether a carcinogenic threshold exists. However, the factors for determining the existence of a threshold and then actual threshold value have not been well described. Our research group believes that susceptibility to genotoxic carcinogens is a determinant of carcinogenic threshold, and that metabolic activation of mutagens, excision DNA repair, translesional DNA synthesis, and apoptosis are the main factors determining susceptibility. We have investigated metabolic activation—especially the level of expression of phase II-drug metabolizing enzymes—as a factor in susceptibility to genotoxic carcinogens. Gene expression of phase I drug-metabolizing enzymes (mono-oxidases) induced by PAHs is regulated by the ligand-dependent transcription factor, aryl hydrocarbon receptor (AhR). Nrf2 is an essential transcription factor for constitutive or inducible expression

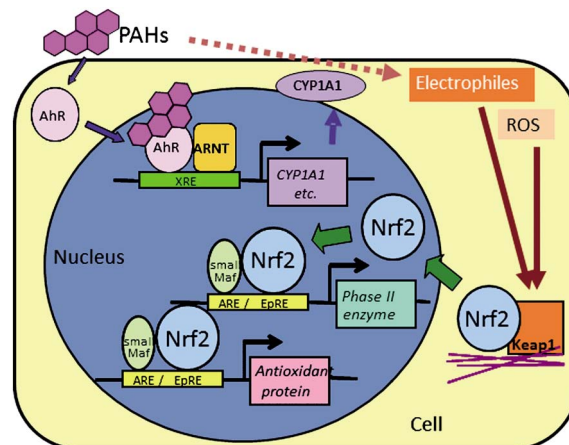


Fig. 1. PAH-induced gene expression of phase I and phase II drug-metabolizing enzymes regulated by AhR and Nrf2 transcription factors.

of phase II drug-metabolizing enzymes (conjugating enzymes) (Fig. 1); Dr. Masayuki Yamamoto (Tohoku University, Japan) has intensively investigated this issue (11). AhR sequesters a PAH such as BaP, which is then translocated into the nucleus. It is then bound to a *cis*-element on the gene as a heterodimer together with ARNT. This results in upregulation of expression of the genes encoding cytochrome P450 1A1 and other related cytochrome P450s. In contrast, Nrf2 is located in the cytoplasm as a heterodimer with Keap1, and release from Keap1 is triggered by electrophiles such as the reactive intermediates of PAHs or reactive oxidative species (11). The released Nrf2 is also translocated into the nucleus, and together with a small Maf protein it binds to antioxidant responsive element/electrophile responsive element, thus upregulating expression of the genes encoding phase II drug-metabolizing enzymes such as glutathione S-transferase (GST), which catalyzes the conjugation of the reactive intermediates of PAHs with glutathione and accelerates the excretion of conjugates and other related antioxidant proteins.

Enhanced Mutagenesis in Phase II Enzyme-suppressed Conditions

We hypothesized that exposure to PAHs increases the levels of DNA adducts and the frequency of mutations in *Nrf2*-knockout (*Nrf2*-KO; *Nrf2*(-/-)) mice. In fact, in the lungs of *Nrf2*-KO mice, expression of the GSTs A1/2 and P1/2 is suppressed compared with in the wild type (12), showing that *Nrf2*-KO mice are good model animals for assessing how the mutagenicity of PAH is enhanced in phase II enzyme-deficient conditions. We previously observed that the level of DNA adducts was increased in rat lung by exposure to urban air (13). Furthermore, the level of DNA adducts and the frequency of DNA mutation are elevated in the rat lung

by exposure to diesel exhaust (DE) as a model air pollutant containing various carcinogenic or mutagenic PAHs (14). We next attempted to reveal how DNA adduct formation was accelerated in the lungs of *Nrf2*-KO mice (15) exposed to DE. Exposure to DE at a concentration of 3 mg/m³ suspended particulate matter caused a significant increase in bulky DNA adduct levels in the lungs of both *Nrf2*-KO and *Nrf2*(+/-) mice, which were used as *Nrf2*-bearing mice. DNA adduct levels in *Nrf2*-KO mice exposed to DE increased to approximately 2.3 times those in *Nrf2*(+/-) mice exposed to DE (16). Accumulation of the oxidative DNA adduct 8-oxoguanine and the occurrence of severe hyperplasia were observed in the bronchial epidermis of *Nrf2*-KO mice following DE exposure. *Nrf2* plays a protective role against mutagenic PAHs and reactive oxygen species generated by DE in the lung (16). Next, we examined whether BaP enhanced *in vivo* mutagenesis in the lungs of *Nrf2*-KO mice. To determine mutation frequency in *Nrf2*-KO mice, we produced *Nrf2*-KO *gpt* delta mice by mating *Nrf2*-KO mice and the *gpt* delta mice (17), which carry the guanine phosphoribosyltransferase (*gpt*) gene that acts as a target gene for detecting *in vivo* mutagenesis of the genomic DNA (12). The frequency of mutation is increased in *Nrf2*-KO mice without BaP treatment (Fig. 2), indicating that constitutive expression of phase II enzymes prevents spontaneous mutation in the lung. Intratracheal instillation of BaP increased the frequency of mutation in the lungs of *Nrf2*(+/-) and *Nrf2*-KO mice, respectively, to 3.1- and 6.1-fold that in control *Nrf2*(+/-) mice, showing that *Nrf2*-KO mice are more susceptible to genotoxic mutagens. Among the base substitutions, G:C to T:A transversion, which is a predominant mutation induced by BaP treatment and 8-

oxo-guanine formation, was markedly elevated in *Nrf2*-KO mice by BaP treatment. The frequency of BaP-induced mutations is probably enhanced by the suppression of phase II enzymes in *Nrf2*-KO mice, but oxidative stress may also contribute to this enhancement.

Conclusion

Nrf2 helps prevent mutations *in vivo*, and expression of phase II drug-metabolizing enzymes may act to protect genomic DNA against certain types of mutations induced by PAHs such as BaP. Our results also show that transgenic rodents designed for detecting mutations are potentially good model systems for evaluating the *in vivo* mutagenicity of environmental chemicals and pollutants for assessment of cancer risk. However, further studies are required to establish a methodology for assessing whether a threshold exists in a particular carcinogenic risk.

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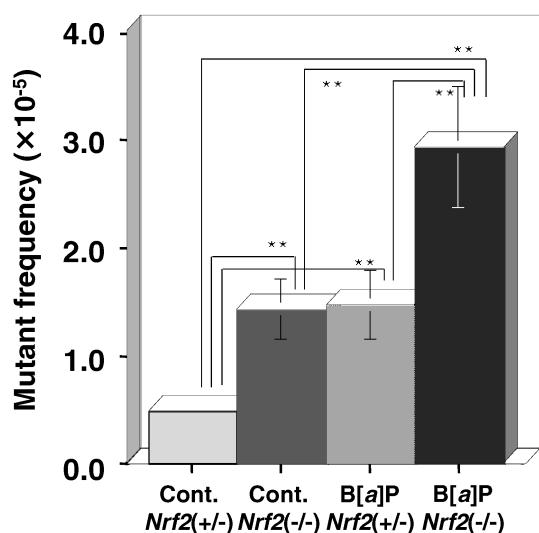


Fig. 2. Frequencies of mutation in the lungs of *Nrf2*-KO (*Nrf2*(-/-)) and *Nrf2*(+/-) *gpt* delta mice treated with benzo[a]pyrene (B[a]P) and untreated (Cont.) (12). ***p* < 0.01.

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