Estrogenic and Anti-androgenic Activity of Nitrophenols in Diesel Exhaust Particles (DEP)

Shinji Taneda,*a Yoki Mori,b Kazuyuki Kamata,b Hideyuki Hayashi,b Chie Furuta,c Chunmei Li,c,d Koh-ichi Seki,a Akiyo Sakushima,a Shin Yoshino,b Kouya Yamaki,b Gen Watanabe,c Kazuyoshi Taya,c,d and Akira K. Suzukiab

*PM2.5/DEP Research Project, National Institute for Environmental Studies; 16–2 Onogawa, Tsukuba 305–8506, Japan; Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido; Ishikari-Tobetsu 061–0293, Japan; Laboratory of Veterinary Physiology, Tokyo University of Agriculture and Technology; 3–5–8 Saiwai-cho, Fuchu, Tokyo 183–8509, Japan; Department of Basic Veterinary Sciences, The United Graduate School of Veterinary Sciences, Gifu University; 1–1 Yanagido, Gifu 501–1193, Japan; Central Institute of Isotope Science, Graduate School of Medicine, Hokkaido University; Kita-ku, Kita 15, Nishi 7, Sapporo 060–0815, Japan; Faculty of Pharmaceutical Sciences, Kyushu University of Health and Welfare; 1714–1 Yoshino-machi, Nobeoka 882–8508, Japan; and Kobe Pharmaceutical University; 4–19–1 Motoyamakita, Higashinada-ku, Kobe 658–8558, Japan.

Received January 20, 2004; accepted March 8, 2004; published online March 10, 2004

We recently isolated 4-nitrophenol, 2-methyl-4-nitrophenol, 3-methyl-4-nitrophenol, and 4-nitro-3-phenylphenol from diesel exhaust particles (DEP) and identified them as vasodilators. Because these compounds are alkylphenolic derivatives that might mimic hormones, we evaluated their estrogenic activity by human estrogen receptor (hER)-yeast screen assay. All of these nitrophenol derivatives except 2-methyl-4-nitrophenol exhibited estrogenic activity. Some estrogenic compounds are also anti-androgenic, so we measured the anti-androgenic activity of the same compounds by human androgen receptor (hAR)-yeast screen assay. We found anti-androgenicity in all four nitrophenols. Nitrophenols in DEP possess not only vasodilatory activity but also estrogenic and anti-androgenic activity.

Key words nitrophenol; estrogenic activity; anti-androgenic activity; diesel exhaust particle

Environmental levels of a number of endocrine-disrupting chemicals have been increasing. These chemicals affect human health by interacting with hormone receptors and thus mimicking normal endocrine functions.1–6 In recent studies, both diesel exhaust (DE) and diesel exhaust particles (DEP) have been reported to exert toxic effects on both the male and female reproductive systems. These compounds suppress spermatogenesis in mice7 and rats8; in one study, serum testosterone levels and the weights of accessory sex glands increased significantly in F344 male rats exposed to DE for 8 months.9 In addition, pregnant C57BL mice injected with diesel exhaust particulate extract (DEPE) showed a significant increase in the rate of abortions,10 and DEPE increases the uterine weight and myometrial contractility in this strain of mice.11 These results suggest that chemical substances present in diesel exhaust emission cause disturbance of endocrine systems. Meek12 reported that dichloromethane extracts of DEP could act as activating ligands for estrogen receptors, and Taneda et al.13,14 reported that crude DEP, as well as successively extracted hexane, benzene, dichloromethane, and methanol fractions, showed estrogenic activity on heR-yeast screen assay. Mori et al.15 reported that 4,6- and 2,8-dimethylidibenzothiophenes isolated from DEP had estrogenic activity in heR-yeast assay. However, the specific compound that is responsible for this phenomenon remains unclear.

We recently isolated the nitrophenol derivatives 4-nitrophenol, 2-methyl-4-nitrophenol, 3-methyl-4-nitrophenol, and 4-nitro-3-phenylphenol from DEP and showed that they had vasodilatory activity.16,17 These compounds captured our attention because it has long been known that alkylphenols are associated with estrogenic activity.1,2 In receptor-binding studies, alkylphenols have been shown to interact directly with the estrogen receptors of rainbow trout and to act in an identical way to 17β-estradiol (E2) in stimulating receptor transcription.2,3 Furthermore, some estrogenic compounds are known to possess anti-androgenic activity.18 The alkylphenols of DEP are likely to be associated with both estrogenicity and anti-androgenicity.

We therefore evaluated the estrogenic and anti-androgenic activity of the nitrophenols with alkylphenolic structures that we had previously isolated from DEP.

MATERIALS AND METHODS

Reagents We purchased 4-nitrophenol (PNP), 2-methyl-4-nitrophenol (4-nitro-o-cresol, PNOC), and 3-methyl-4-nitrophenol (4-nitro-m-cresol, PNMC) from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan), and we synthesized the 4-nitro-3-phenylphenol (PNMPP) by the method described previously.17 All other reagents used were of the purest grade commercially available.

Human Estrogen Receptor and Human Androgen Receptor Yeasts Human estrogen receptor (hER)-yeast and human androgen receptor (hAR)-yeast were kindly provided by Prof. John P. Sumpter of Brunel University, Uxbridge, U.K. These strains were developed in the Genetics Department at Glaxo Wellcome, plc. (Stevenage, Herts, U.K.).

The DNA sequences of either hERα or hAR were integrated stably into the genome of a strain of the yeast Saccharomyces cerevisiae. The yeast also contained expression plasmids carrying estrogen- or androgen-responsive elements that regulated the expression of the reporter gene lacZ (encoding the enzyme β-galactosidase).

Assay of Estrogenic Activity Estrogenic activity was...
determined by recombinant hER-yeast screen according to the method of Routledge and Sumpter,3) with slight modifications, as described previously.19) Color development of the medium was measured at an absorbance of 540 nm, and also at 620 nm, to allow for subsequent correction for turbidity (a measure of growth rate of the yeast cell). Corrected value = chemical absorbance at 540 nm – (chemical absorbance at 620 nm – blank absorbance at 620 nm).

Assay of Anti-androgenic Activity To determine anti-androgenic activity, 5α-dihydrotestosterone (DHT) was added to the assay medium at a background concentration of 1.25 × 10^{-9} M (approximately 65% response), and androgenic activity in the presence of each sample in hAR-yeast plates were measured at 32 °C, 48 h and the plates were removed at 28 °C, 24 h, giving a total incubation of 72 h.

Statistical Analysis Each value was expressed as the mean ± S.E. (n=6). Analysis of variance (ANOVA) was used to evaluate the results. When ANOVA was significant, any difference between groups was assessed by means of ANOVA followed by Scheffé’s F-test. Estrogenic activity was compared with control (baseline) value. Anti-androgenic activity was compared with the background DHT value.

RESULTS AND DISCUSSION

We used the alkylphenolic derivatives that we had originally isolated from DEP as vasodilators to examine the ability of nitrophenols to mimic sex hormones. First, we evaluated their estrogenic activity by hER-yeast screen assay.

Figure 1 shows the estrogenic activity of the nitrophenols. Estrogenic activity was observed in PNP, PNOC and PNMC. PNMPP showed an inverted U-shaped response, indicating that at high concentrations of PNMPP was toxic to yeast cells. We consider to these cytotoxicities originate from the phenolic skeleton of PNMPP. PNOC did not possess estrogenic activity. The ER binding activity of PNP and PNMC has been confirmed before by analysis with the MultiCASE expert system.20)

Routledge and Sumpter have reported that the position (para > meta > ortho) of the alkyl group affects estrogenicity.4) PNOC is considered to inhibit connection of an OH-group with the estrogen receptor by bonding of the methyl group to an ortho position.

Sohoni and Sumpter have reported that several environmental estrogens are also anti-androgens.18) We therefore measured the anti-androgenic activity of our nitrophenol compounds. We found anti-androgenic activity in all four compounds tested. (Fig. 2) The responses of all the nitrophenols at high concentrations were lower than baseline (control). This is because the increase in β-galactosidase concentration in the control hAR-yeast was faster than in the nitrophenols at high concentrations. Also, with all nitrophenols, suppression of yeast cell growth was observed at high concentrations. We speculate that these cytotoxicities originate from the cresol skeleton or the phenolic skeleton of these compounds.

The present findings also are important from the environmental perspective, as DE emissions are ubiquitous in the environment, and remarkable amount of DEP are exhausted into the air that we breath. For example, in Japan 58902 tons (t),21) in the United States of America 111530 t,22) in England (UK) 37000 t,23) and in EURO, the highest of 240000 t are emitted each year and this an amount that can not be ignored. The amount of PNP, PNOC, PNMC and PNMPP that are included in 1 kg of DEP are 15, 34, 28 and 15 mg, respectively.17) The environmental concentrations of these compounds are not well known since the research of the isolation of the compounds found in DEP has just been begun.
In addition, PNP and PNMC are known degradation products of the insecticides parathion and fenitrothion, respectively. Fenitrothion is used widely in many countries and PNMC is accumulating in the air. Parathion and fenitrothion inhibit DHT binding to the androgen receptor in cytoplasmic extract from the rat ventral prostate and in transfected HepG2 human hepatoma liver cells, respectively. Furthermore, Sohoni et al. have determined by hAR-yeast assay that parathion and fenitrothion possess androgenic and anti-androgenic activity and fenitrothion inhibits the stimulatory effect of testosterone propionate on Cowper’s gland in the rat.

It was found that nitrophenols isolated from DEP possessed not only vasodilatory activity but also estrogenic and anti-androgenic activity. Furthermore, our results suggest that, as a result of diesel exhaust emission or the degradation of various pesticides, the accumulation of nitrophenols (including PNP and PNMC) in the air and soil could have serious deleterious effects on wildlife and human health though disturbance of endocrine and cardiovascular functions.

Experiments on the estrogenic and anti-androgenic activity of these compounds in rats and Japanese quail (Coturnix japonica) are now under way.

Acknowledgments  We thank Glaxo Wellcome for developing the yeast screens, which, with permission, was given to us by Prof. John P. Sumpter of Brunel University, U.K.

REFERENCES