Antiandrogenic activity of diesel exhaust particulates

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Antiandrogenic Activity of Diesel Exhaust Particulates

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Abstract – We collected diesel exhaust particulates (DEPs) emitted from the diesel-powered vehicles and prepared DEP extracts (DEPEs). While DEPEs did not exhibit any androgenic effect in two cell-based assay methods, a luciferase reporter gene assay in PC3/AR human prostate cancer cells and a yeast two-hybrid assay, they exerted antiandrogenic effect in each assay. The antiandrogenic effects of DEPEs were due in part to the constituents with AhR agonistic activity and to the constituents with AR antagonistic activity.

I. Introduction

The main air pollutants in urban areas of Japan and Europe are nitrogen dioxide and suspended particulate matter (SPM). The greater part of SPM is derived from vehicle emission. The number of diesel-powered vehicles has been increasing in Japan and Europe because of the diesel engine's greater efficiency and lower cost compared with gasoline engines. Diesel-powered vehicles emit some 30-100 times more particles than do gasoline-powered vehicles, and consequently diesel exhaust particulates (DEPs) comprise most of SPM in the urban atmosphere. DEP contains diverse organic compounds such as polycyclic aromatic hydrocarbons (PAHs). While the principal concern regarding exposure to DEP has been the risks for cancers, especially for lung cancer, and chronic respiratory diseases, more recently DEP has also attracted attention in terms of endocrine disrupting effects.

Androgens have a pivotal role in the development and maintenance of the male reproductive system. An important endocrine disrupting effect of DEP is their potential adverse impact on the male reproductive-system functions. It has been reported that inhalation of diesel exhaust (DE) caused the dysfunction of male reproductive system in rats and mice [1,2]. Further, a study showed that the dysfunction was more pronounced in the total DE exposure group compared to the filtered DE exposure group [2]. These findings raise the possibility that DEP contributes to the dysfunction. DEP contains numerous chemicals, *e.g.* PAH which exhibited antiestrogenic effect. It seems likely that some DEP constituents can interact with androgen receptor (AR) and/or other nuclear transcription factors. In fact, PAHs having four or more rings have been reported to be constituents of DEP and they antagonize androgen action through activation of aryl hydrocarbon receptor (AhR) [3]. It is thus important to clarify the DEP constituents implicated in the dysfunction of male reproductive system and the molecular mechanisms of their action.

While several environmental chemicals with the androgenic or antiandrogenic properties have been identified [4], there is no study on androgenic and antiandrogenic activities of DEP. Here we report results of the first study on the androgenic and antiandrogenic effects of DEP extracts (DEPEs) emitted from diesel-powered vehicles under daily use in order to obtain knowledge on the constituents involved in DE-induced dysfunction of male reproductive systems. In this study, androgenic and antiandrogenic effects of DEPEs were assayed in two cell systems. One is human prostate carcinoma PC3/AR cells transiently transfected with a prostate specific antigen (PSA) gene promoter driven luciferase expression vector pGLPSA5.8 [5]. The other is recombinant yeast cells which express β-galactosidase in response to androgens by two-hybrid mechanism [6].

II. Materials and Methods

A. DEP collection and DEPE preparation

Three diesel-engine vehicles in daily use, a car (made in Japan, 2,500 cc, direct injection type, 1996 model), a bus (made in Japan, 4,160 cc, direct injection type, 1990 model) and a truck (made in Japan, 7,410 cc, direct injection type, 1989 model) were used under idling conditions with commercial light oil (JIS No.2). DEP was collected on glass-fiber filters (Pallflex T60A20) by a low-volume air sampler. DEP samples were ultrasonically extracted with benzene/ethanol (3:1) and the extracts were reconstituted in ethanol to give an extract concentration of 10 mg/mL. The extract samples originated from the car, bus and truck were designated as EC, EB and ET, respectively. A filter blank sample designated as FB was prepared similarly from new unused filters by reconstituting the extract in the smallest volume of ethanol used for reconstitution of DEPE samples.

B. PC3/AR luciferase assay

PC3/AR cells were transiently transfected with a luciferase expressing plasmid. The vector transfected to PC3/AR cells was an AR-regulated PSA promoter-driven luciferase expressing plasmid pGLPSA5.8, an AhR-regulated cytochrome P450 (CYP) 1A1 promoter-driven luciferase expressing plasmid pLUC1A1 or a constitutively luciferase expressing plasmid pGL3-control. The cells were treated with 0.2% (v/v) ethanol (blank), 10 pM 5α-dihydrotestosterone (DHT control), FB or a DEPE sample alone or in combination with 10 pM DHT, α-naphthoflavone or SKF-525A for 24 h. The DEPE sample and other reagent solutions were added to the medium at 0.1% (v/v). The final ethanol concentration in the medium was adjusted at 0.2% (v/v). After the treatment, the cells were lysed and luciferase activity was measured and normalized to protein concentration. Luciferase activities obtained on pGLPSA5.8, pLUC1A1 and pGL3-control vectors were designated as Luc_AR, Luc_AhR and Luc_Control activity, respectively.

C. Yeast two-hybrid assay

A transformant of the yeast *Saccharomyces cerevisiae*, Y190 strain for two-hybrid assay of androgenic activity is designated as Y190_AR. Yeast cells were transformed with yeast two-hybrid system control vectors, pGBKT7-53 and pGADT7-T, using a lithium acetate method and grown on SD medium lacking tryptophan and leucine to yield a transformant that constitutively expresses β-galactosidase. This transformant was designated as Y190_p53-SV40LT. Yeast cells were treated with 1.1% (v/v) ethanol (blank), 0.5 nM DHT (DHT control), FB or a DEPE sample alone or in combination with 500 pM DHT for 4 h. DEPE sample and DHT solutions were added to medium at 1% and 0.1% (v/v), respectively. The final ethanol concentration in medium was adjusted at 1.1% (v/v). After treatment, a portion of cell suspension solution was withdrawn and measured for absorbance at 620 nm as an indication of cell density. β-Galactosidase activity was measured and normalized to absorbance at 620 nm (cell density). β-Galactosidase activities obtained from Y190_AR and Y190_p53-SV40LT cells were designated as β-Gal_AR and β-Gal_Control activity, respectively.

III. Results and Discussion

A. Androgenic and antiandrogenic effects of DEPE samples

First of all, FB did not significantly affect any activities in all following experiments, suggesting that the influence of the constituents, which were derived from filters, was negligible (Fig.1).

While all DEPE samples did not induce Luc_AR activity in the absence of DHT (Fig.1A), they significantly depressed DHT-induced Luc_AR activity in the presence of DHT in a concentration-

dependent manner (Fig.1B). To address whether DEPE samples lowered general gene transcription of PC3/AR cells, we measured Luc_Control activity (Fig.1C). Since DEPE samples did not significantly affect the Luc_Control activities, the suppression of DHT-induced Luc_AR activity of DEPE samples was found to be the antiandrogenic effect (Fig.1B). The antiandrogenic effect was greater in the following order: ET > EB > EC (Fig.1B).

B. Contribution of the metabolites of DEPE samples

DEPE samples elevated CYP1 family enzyme activity. First, the contribution of DEPE metabolites on the antiandrogenic effect was examined. While co-treatment of PC3/AR cells with SKF-525A, a non-selective inhibitor of CYP enzymes, did not affect any activities, it enhanced the antiandrogenic effect of DEPE samples, indicating that the antiandrogenic effect is mainly caused by intact species of DEPE constituents.

C. Contribution of the constituents with AhR agonistic activity

Next, since CYP1, especially CYP1A1, activity was regulated by AhR and AhR agonists exhibited antiandrogenic effect, the contribution of AhR agonistic constituents on the antiandrogenic effect of DEPE samples was examined. While co-treatment of PC3/AR cells with α-naphthoflavone, an AhR antagonist, did not affect any activities, it lowered the DEPE-induced Luc_AhR activity and reversed the antiandrogenic effects of DEPE samples. Further, as a result of evaluation of AhR agonistic and antiandrogenic activity of 16 PAHs with four or more rings as candidates for AhR agonists: pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*j*]fluoranthene, benzo[*a*]pyrene, benzo[*e*]pyrene, benzo[*b*]chrysene, perylene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene, dibenzo[*a,e*]pyrene, coronene, 3-methylcholanthrene and 7,12-dimethylbenz[*a*]anthracene, and the other: β-naphthoflavone in PC3/AR cells, it was revealed that antiandrogenic activity is significantly correlated with AhR agonistic activity in PC3/AR cells. These results suggest that the antiandrogenic effect of DEPE samples is due in part to the constituents acting as AhR agonists.

D. Contribution of the constituents capable of binding to AR

On the other hand, we used a yeast two-hybrid assay to examine the only AR-mediated androgenic or antiandrogenic effects of DEPE samples. In the absence of DHT, all DEPE samples did not induce β-Gal_AR activity in Y190_AR recombinant yeast cells (Fig.1D). In the presence of DHT, DEPE samples significantly inhibited DHT-induced β-Gal_AR activity in Y190_AR cells in a concentration-dependent manner (Fig.1E). Since DEPE samples did not significantly affect the β-Gal_Control activities in Y190_p53-SV40LT cells (Fig.1F), the depression of DHT-induced β-Gal_AR activity of DEPE samples was found to be the AR antagonistic effect (Fig.1E). The antagonism was greater in the following order: ET > EB > EC (Fig.1E). DEPE samples elicited only antiandrogenic effects in recombinant yeast cells, which express β-galactosidase in response to androgen, without AhR. All these findings show that DEPE samples exhibit significant antiandrogenic effect in cell-based transcription assay and that this effect is due in part to the constituents with AR antagonist activity.

IV. Summary and Conclusions

The present study demonstrated that DEPE samples exhibit significant antiandrogenic effect in PC3/AR human prostate carcinoma cells and that these effect are due in part to the constituents with AhR agonist activity and to the constituents with AR antagonist activity. Furter DEP was shown to be a possible environmental factor affecting male reproductive functions. Further study is necessary to identify other DEP constituents with antiandrogenic activity and clarify their mechanisms of action.

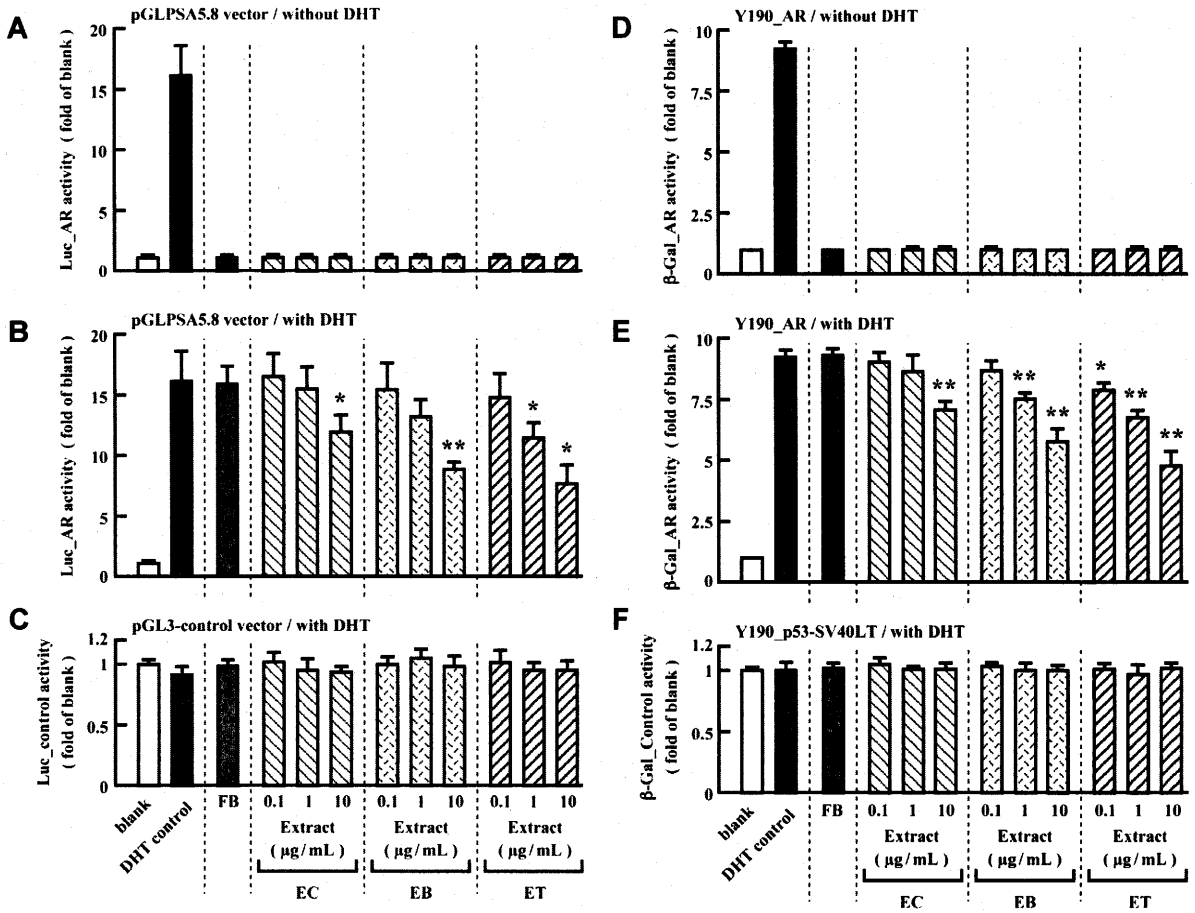

Fig. 1. Antiandrogenic activity of DEPE samples. *Left*, PC3/AR luciferase assay for androgenic (A), antiandrogenic (B), cytotoxic (C) effects of DEPE samples. *Right*, Yeast two-hybrid assay for androgenic (D), antiandrogenic (E), cytotoxic (F) effects of DEPE samples. *,**Significantly different from each DHT control ($p<0.05$ and $P<0.01$, respectively).

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