Antiandrogenic activity of diesel exhaust particle extracts emitted from diesel-engine vehicles : Role of aryl hydrocarbon receptor agonists

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学位論文要旨

ABSTRACT

Air in urban areas is heavily polluted with diesel exhaust particles (DEP). The constituents and their action mechanisms responsible for the antiandrogenic effects of diesel exhaust particle extracts (DEPEs) were studies in human prostate carcinoma PC3/AR cells transiently transfected with prostate specific antigen (PSA)-promoter luciferase expression vector. The present study revealed that the antiandrogenic effects of DEPEs were due in part to polycyclic aromatic hydrocarbons (PAHs) and their action was mediated by aryl hydrocarbon receptor (AhR). Further, the effects of DEPEs were enhanced by a higher engine load which resulted in DEPEs with elevated AhR agonistic and androgen receptor (AR) antagonistic activities. The present study also suggest that an AhR-mediated mechanism of AhR agonists, *e.g.* PAHs, to antagonize the AR is to inhibit the formation of AR transcription complex which caused by the interaction of AhR/AhR nuclear translocator (ARNT) complex with the second XRE counted from the transcription start site of PSA promoter in PC3/AR cells.

INTRODUCTION

The main air pollutants in urban areas of Japan and Europe are nitrogen dioxide and suspended particulate matter (SPM). In Japan and Europe, the greatest portion of SPM, which is feared to adversely affect human health, is diesel exhaust particles (DEP). Aryl hydrocarbon receptor (AhR) agonists, *e.g.* polycyclic aromatic hydrocarbons (PAHs) having four or more rings, have been reported to be constituents of DEP and they antagonize androgen action through activation of AhR. Therefore, it was thought that the antiandrogenic effects of diesel exhaust particle extracts (DEPEs) were caused by not only the constituents which directly affect androgen receptor (AR) and also the constituents which activate AhR. This study is focused on the AhR-mediated antiandrogenic effects of DEPEs and the molecular mechanism of the effects of AhR agonists. The effects of DEPEs and AhR agonists were evaluated on the basis of prostate specific antigen (PSA) promoter-induced luciferase expression in PC3/AR wild-type human AR-expressing prostate carcinoma cells.

1. Antiandrogenic Activity of Diesel Exhaust Particle Extracts in PC3/AR Human Prostate Carcinoma Cells

In chapter 1, the author reports results of the first study on the androgenic and antiandrogenic effects of extracts of DEP emitted from diesel-powered vehicles under daily use. The author collected DEP emitted from three diesel-engine vehicles, a car, a bus and a truck, and prepared DEPE samples, designated as EC, EB and ET, respectively. The androgenic and antiandrogenic effects of the DEPE samples were examined by a luciferase reporter assay in human prostate carcinoma PC3/AR cells transiently transfected with a PSA promoter-driven luciferase expression vector pGLPSA5.8. PC3/AR is a subline of human prostate carcinoma PC3 transformed to stably express wild-type human AR. While DEPE samples did not exhibit any androgenic effect, they exerted antiandrogenic effect, inhibiting 5α-dihydrotestosterone (DHT)-induced luciferase activity at an extract concentration of 10 μg/mL. The antiandrogenic effect was greater in the following order: ET > EB > EC. Co-treatment

of PC3/AR cells with SKF-525A, a non-selective inhibitor of cytochrome P450 (CYP) enzymes, enhanced the antiandrogenic effect, indicating that the antiandrogenic effect is caused by intact species of DEPE constituents. The antiandrogenic effect of DEPE samples was reversed by α-naphthoflavone, an AhR antagonist. The antiandrogenic activity of a DEPE sample correlated with its AhR agonist activity assayed in PC3/AR cells transiently transfected with CYP1A1 gene promoter-driven luciferase expression vector pLUC1A1. Equimolar mixtures of ten PAHs having four or more rings, structures found in the DEPEs, showed significant antiandrogenic effects and AhR agonist activity at concentrations equivalent to those found in DEPE samples. Further, DEPE samples elicited only antiandrogenic effects in recombinant yeast cells which express β-galactosidase in response to androgen. A competitive AR binding assay showed that AR-binding constituents exist in DEPE samples, indicating that greater part of AR-binding constituents in DEPEs are AR antagonists. 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 AhR agonist activity including PAHs and to the constituents with AR antagonist activity.

2. Variation of Antiandrogenic Activity of Diesel Exhaust Particle Extracts under Different Engine Loads

In chapter 2, the author reports results from the first study to examine the alteration of androgenic and antiandrogenic activities of DEPE samples associated with several engine conditions on actually running diesel-engine vehicle. The author collected DEP samples emitted from a diesel-engine truck under different conditions of engine loads (0, 50 and 75% of a maximum engine load) and vehicle speeds (20, 50 and 80 km/h), and prepared DEPE samples, designated as L0, L50 and L75 based on the engine load values, and S20, S50 and S80 based on the vehicle speed values. The androgenic and antiandrogenic activities of the DEPE samples were examined using a PSA promoter-luciferase reporter gene assay in PC3/AR cells. While all DEPE samples did not exhibit androgenic effects, the antiandrogenic effects were enhanced by higher engine

load but not by higher vehicle speed (Fig. 1). In this study, significant correlations between antiandrogenic and AhR agonistic activities were demonstrated in PC3/AR cells by 16 polycyclic aromatic compounds and β-naphthoflavone (Fig. 2). Yeast two-hybrid assay and CYP1A1 promoter-luciferase reporter gene assay showed that the antiandrogenic constituents acting as AR antagonists and AhR agonists were increased by only the higher engine load. In conclusion, the antiandrogenic effects of DEPE samples were enhanced by a higher engine load which resulted in DEPE samples with elevated AhR agonistic and AR antagonistic activities.

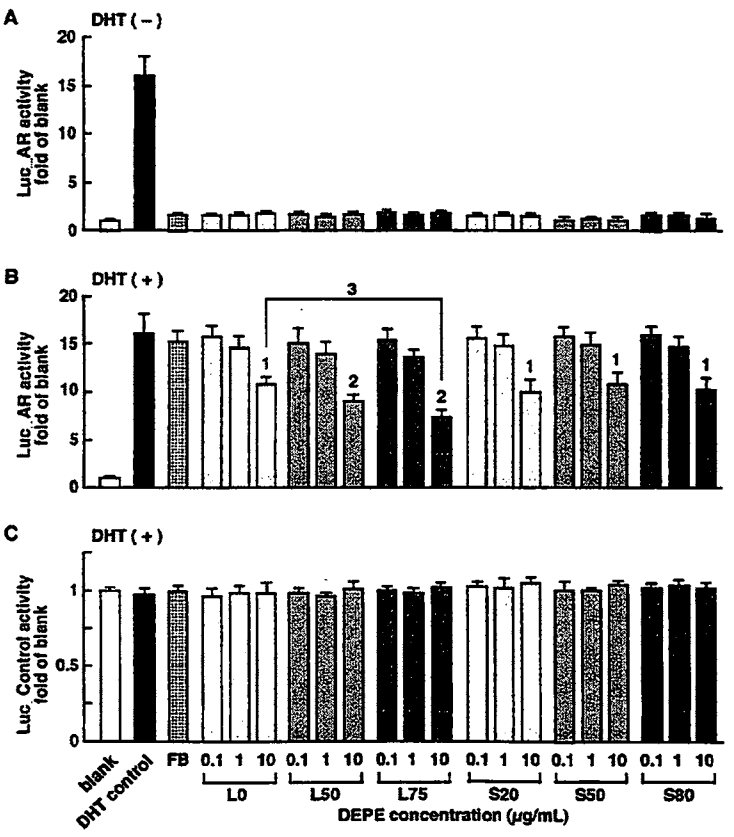

Figure 1. DEPE have antiandrogenic activity. PC3/AR cells were transiently transfected with pGLPSAp5.8 (A and B) or pGL3-control vector (C). Cells were treated with 0.2% ethanol (blank), 10 pM DHT (DHT control), filter blank (FB) or DEPE samples at indicated concentration in the absence or presence of 10 pM DHT for 24 h and then luciferase activity was measured. The effect of DEPE as androgen agonists (A), androgen antagonists (B) and on pGL3-control luciferase as an index of cytotoxicity (C) is shown. Each column and vertical bar represent the mean and SD, respectively, of three separate extract samples. [1] and [2]Significantly different ($p < 0.05$ and $p < 0.01$) from DHT control, respectively; [3]Significantly different ($p < 0.05$) from L75 at 10 μg/mL.

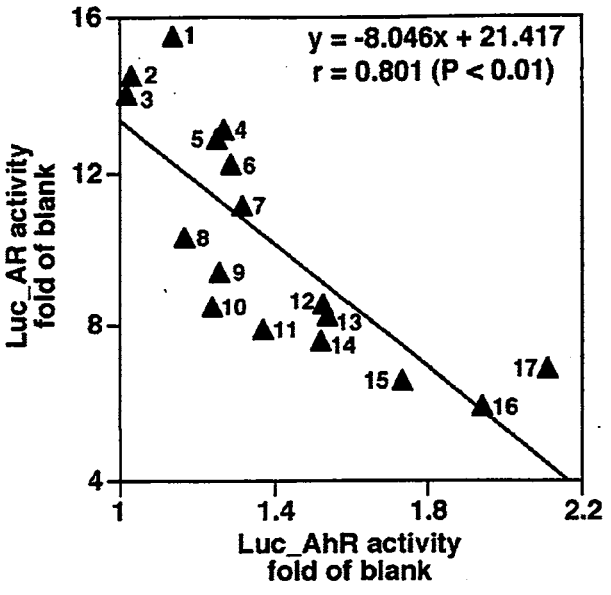

Figure 2. Comparison of antiandrogenic (Luc_AR) and AhR agonistic activity (Luc_AhR) of PAHs. PC3/AR cells were transiently transfected with pGLPSAp5.8 or pLUC1A1 and treated with 0.2% ethanol (blank), 10 pM DHT (DHT control) or 10 pM DHT—0.1 μM PAHs or β-naphthoflavone for 24 h and then luciferase activity was measured. Each numbered point represents the Luc_AR and Luc_AhR activity measured for the following compounds; 1: benzo[*j*]fluoranthene, 2: dibenzo[*a,e*]pyrene, 3: pyrene, 4: chrysene, 5: indeno[1,2,3-*cd*]pyrene, 6: dimethylbenz[*a*]anthracene, 7: benzo[*b*]fluoranthene, 8: benzo[*b*]chrysene, 9: perylene, 10: benzo[*k*]fluoranthene, 11: coronene, 12: benzo[*e*]pyrene, 13: benz[*a*]anthracene, 14: benzo[*ghi*]perylene, 15: benzo[*a*]pyrene, 16: 3-methylcholanthrene, 17: β-naphthoflavone. Linear regression, equation in graph, indicates a statistically significant ($p < 0.01$) negative correlation between antiandrogenic and AhR agonistic activity.

3. Molecular Mechanism of Antiandrogenic Activity of Aryl Hydrocarbon Receptor Agonists on Prostate Specific Antigen Promoter

In chapter 3, the author reports the results of the first study on the participation of XRE located in promoter region of the human PSA gene in the antiandrogenic effects of AhR agonists. PSA promoter has eight xenobiotic responsive element (XRE) core motifs (Fig. 3). In this study, it was investigated that molecular mechanism of antiandrogenic activity of AhR agonists, *e.g.* benzo[*a*]pyrene (BaP), in PC3/AR cells transiently transfected with several mutants of PSA promoter–luciferase reporter plasmids. BaP did not show antiandrogenic activity in the cells transfected with pGLPSAX2m plasmid deleted or mutated the second XRE (X2) counted from the transcription start site (Fig. 4). These results suggest that the antiandrogenic activity of BaP has been only due to the binding of AhR/AhR nuclear translocator (ARNT) complex to the second XRE from the transcription start site on PSA promoter in PC3/AR cells.

Figure 3. Mapping of the human PSA gene in the luciferase reporter gene construct.

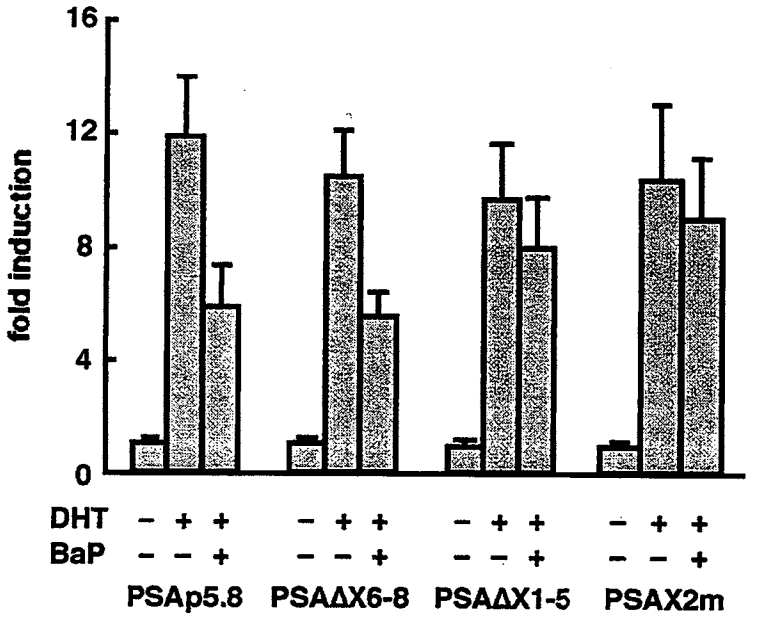

Figure 4. Effects of deletions or mutations of XREs on the antiandrogenic effects of AhR agonists. PC3/AR cells were transiently transfected with pGLPSAp5.8, ΔX6-8, ΔX1-5 and X2m, and treated with 0.2% ethanol (blank), 10 pM DHT, 10 pM DHT—1 μM BaP for 24 h and then luciferase activity was measured as fold of blank. Each column and vertical bar represent the mean and SD, respectively, of four separate cultures.

学位論文審査結果の要旨

［審査経過］平成16年1月26日の第1回審査委員会で審査方針を決定した。基礎学力を確認し、各委員による面接と諮問を行い、1月29日に口頭発表（最終試験）を行った。終了後に開催した最終審査委員会において協議した結果、次の結論を得た。

［審査結果］今日、都市や幹線道路周辺の地域を中心にディーゼル車排気粉じん（DEP）による大気汚染が深刻化しており、肺がんやぜん息の増加に対する寄与ばかりでなく、その内分泌撹乱作用についても懸念されている。本研究では、種々の運転条件で捕集したDEPから抽出物（DEPE）を調製し、ヒト由来の培養細胞及び酵母におけるレポータージーンアッセイでアンドロゲン様作用／抗アンドロゲン作用を評価して以下の知見を得た。1）DEPEが抗アンドロゲン作用を示し、その作用の一部はアンドロゲン受容体（AR）のアンタゴニストとして作用する成分、また一部はアリル炭化水素受容体（AhR）のアゴニストとして作用する成分に基づく、2）エンジン負荷が高いほど、ARアンタゴニスト及びAhRアゴニスト依存性の抗アンドロゲン作用が強くなる、3）AhRアゴニストの抗アンドロゲン作用は、活性化されたAhRがアンドロゲン応答遺伝子の転写調節領域のXREに結合してアンドロゲン依存性の転写を阻害することに基づく可能性がある。本研究は、主要な大気汚染物質の一つであるDEPに含まれる抗アンドロゲン物質とその作用機構に関する初めての知見を得たものであり、また、大気汚染の規制や対策を考える上でも有用な知見を提供しており、審査委員会は博士（薬学）に値すると判定した。