the Luc activity. Interestingly, DB[*a,h*]A appeared to be a potent inducer common to Luc and SEAP activities. On the other hand, DB[*a,c*]A and Chr appeared to be strong inducers, particularly of Luc activity. Until now, much research has been done to assess the inducibility of CYP1A1/1A2 after exposure to PAHs.[36] Of these, DB[*a,h*]A was classified as a strong CYP1A1/1A2 inducer. Exposure of HepG2 cells to these compounds produced marked induction in CYP1A1 and CYP1A2 mRNA levels and enzymatic activities.[37] In addition, the reporter gene system utilized a stable reporter cell line and also indicated high response to DB[*a,h*]A. DB[*a,c*]A also caused significant increase of CYP1A1 and CYP1A2 mRNA levels in HepG2 cells, but these levels were lower than the levels of induction by DB[*a,h*]A. In CYP1A2, DB[*a,c*]A was as high as the mRNA induction level by 3-MC. These observations are consistent with our dual reporter assay results. On the other hand, although Chr produced a maximum increase in the Luc activity, this result was different from previous investigations.[38,39] In general, the four-ring PAHs such as Chr have a weaker tendency for induction than five-ring PAHs such as DB[*a,h*]A and DB[*a,c*]A.[38,39] This discrepancy might be due to difference in experimental conditions and the ability to metabolize Chr. Overall, these results show that the stable cell line results in high reporter activities at relatively low concentration (1 μM) that were easily used to detect CYP1A1/1A2 induction. However, B[*a*]P, a typical CYP1A1/1A2 inducer, did not induce reporter activity at the concentration of 1 μM, so we treated the same compound at a concentration of 3 μM. We found that B[*a*]P induced reporter activity and that the pattern of reporter activity for other PAHs was similar to that at the concentration of 1 μM. Thus, the stable cell line might be available to assess interactions between environmental pollutants such as PAHs and therapeutic agents.

Corchero *et al.* reported that the *CYP1A1* and *CYP1A2* genes are separated by a 23-kb segment that contains no other open reading frame. The *CYP1A1* and *CYP1A2* genes are in head-to-head orientation, revealing that the 5′-flanking region is common between the 2 genes.[11] These genes have been reported to be simultaneously regulated through common regulatory elements.[14] To our knowledge, this is the first report in which the stable cell line contained the 23-kb full-length regulatory region and could be used to simultaneously assess transcriptional activation of the *CYP1A1* and *CYP1A2* genes. Results obtained from the stable cell line could be used to rapidly and accurately predict the CYP1A1/1A2 induction. In addition, although the molecular mechanism of AhR-mediated activation has been extensively studied in CYP1A1, the same in CYP1A2 remains unclear. In this regard, the 23-kb full-length regulatory region in this stable cell line may be valuable for elucidating the precise mechanism of CYP1A2 induction.

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Original Article

Malondialdehyde-modified low density lipoprotein (MDA-LDL)-induced cell growth was suppressed by polycyclic aromatic hydrocarbons (PAHs)

Hiroyuki Suzuki, Takamitsu Sasaki, Takeshi Kumagai, Shuhei Sakaguchi and Kiyoshi Nagata

Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aobaku, Sendai, Miyagi 981-8558, Japan

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ABSTRACT — Malondialdehyde-modified low-density lipoprotein (MDA-LDL) and oxidized LDL (Ox-LDL), which accelerate the pathogenesis of arteriosclerosis, are thought to be involved in parthenogenesis caused by smooth muscle cell proliferation. In this study, we investigated the suppression mechanism of polycyclic aromatic hydrocarbons (PAHs) on the growth of an MDA-LDL-induced human acute monocyte leukemia suspension cell line (THP-1 cells). We found that PAHs suppressed MDA-LDL-induced THP-1 cell growth. Cotreatment with benzo[*a*]pyrene (BaP) or 3-methylchoranthrene (3-MC) decreased MDA-LDL-induced THP-1 cell growth, whereas treatment with benzo[*e*]pyrene (BeP) or pyrene, which is not a ligand for the arylhydrocarbon receptor (AhR), did not decrease THP-1 cell growth. Our findings clearly demonstrated that THP-1 cell growth, which was suppressed by PAHs, was restored by the addition of α-naphtoflavone, which is a partial antagonist to AhR. Moreover, it was shown that cotreatment with MDA-LDL and BaP markedly induced the expression of human cytochrome P4501A1 (hCYP1A1) messenger ribonucleic acid (mRNA) and significantly induced the expressions of p53 and p21 mRNAs. In support of these findings, AhR small interfering RNA suppressed the induced level of p21 mRNA and by BaP and the overexpression of hCYP1A1 significantly induced levels of p21 mRNA. On the other hand, the uptake rate of [^{14}C]BaP into cells was increased more significantly by cotreatment with MDA-LDL than by treatment with [^{14}C]BaP alone. These results strongly suggest that the suppression of MDA-LDL-induced THP-1 cell growth is caused by the increased uptake of PAHs, which strongly activate the AhR signal pathway accompanying DNA damage.

Key words: AhR, hCYP1A1, MDA-LDL, PAHs, p21

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are common environmental pollutants; exposure to PAHs has been suspected to be associated with the occurrence of pulmonary diseases. Benzo[*a*]pyrene (BaP) is a well-studied PAHs found in many environmental complex mixtures, including cigarette smoke and air pollution. BaP is highly mutagenic and must be metabolically activated to exert its carcinogenic potential (Miller and Ramos, 2001). In addition, BaP induces metabolic enzymes such as human cytochrome p4501A1/2 (hCYP1A1/2) and hCYP1B1 via arylhydrocarbon receptor (AhR) (Harrigan *et al.*, 2006; Jonsson *et al.*, 2006; Willett *et al.*, 2006). Thus, these hCYPs are involved in BaP-induced carcinogenesis (Galván *et al.*, 2005; Kim *et al.*, 1998; Harrigan *et al.*, 2004).

The pathogenesis of arteriosclerosis, now generally considered to be an inflammatory disease, is well characterized, but the initiating event is unknown (Ross, 1999). Individuals who smoke are at increased risk for developing arteriosclerosis and associated clinical events (Chouraki *et al.*, 2008; Antoniades *et al.*, 2008; Iribarren *et al.*, 1999; LaCroix *et al.*, 1991). These findings, combined with data from clinical studies, indicate that the pathogenesis of arteriosclerosis is accelerated through a number of different mechanisms, which collectively contribute to increased risk of developing the disease. The subendothelial accumulation of foam cells, which are primarily derived from monocytes and macrophages through

Correspondence: Hiroyuki Suzuki (E-mail: aube@msd.biglobe.ne.jp)

the uptake of oxidized LDL (Ox-LDL), plays a key role in the initiation of arteriosclerosis (Steinberg and Witztum, 1990). It is well known that the LDL receptor, which is down-regulated when intracellular cholesterol levels are increased, is not involved in the intracellular accumulation of cholesterol in foam cells. However, macrophages avidly accumulate Ox-LDL via scavenger receptors that are unresponsive to intracellular cholesterol levels (Goldstein and Brown, 1977; Ylä-Herttuala *et al.*, 1991). Furthermore, Ox-LDL induces proliferation of monocytes and macrophages, smooth muscle cells, and other cells (Heery *et al.*, 1995; Zhao *et al.*, 2005; Matsumura *et al.*, 1997; Martens *et al.*, 1998; Hamilton *et al.*, 1999).

Generally, the pathogenesis of arteriosclerosis is accelerated through MDA-LDL and Ox-LDL. Interestingly, MDA-LDL was reportedly involved in atherogenesis by causing smooth muscle cell proliferation (Ozer *et al.*, 1993), which was generated by the uptake of MDA-LDL in macrophages via scavenger receptors; PAHs have also been reported to induce arteriosclerosis (Iwano *et al.*, 2005). However, the interaction between PAHs and MDA-LDL has not been clarified. Therefore, in this study, we investigated the suppression mechanism of MDA-LDL-induced THP-1 cell growth by PAHs to clarify the association between PAHs and MDA-LDL.

MATERIALS AND METHODS

Chemicals

BaP, β-naphthoflavone (β-NF), benzo[*e*]pyrene (BeP), pyrene, and α-naphthoflavone (α-NF) were purchased from Tokyo Chemical Industry Corporation (Tokyo, Japan). 3-Methylcholanthrene (3-MC) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade and purchased from commercial sources.

Cell culture

THP-1 (human acute monocytic leukemia) cells were obtained from Japan Human Health Sciences Foundation (Tokyo, Japan) and HepG2 (human hepatoma cell lines) cells were obtained from Riken Bioresource Center (Ibaraki, Japan). THP-1 cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium from Invitrogen (Carlsbad, CA, USA) supplemented with 5% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin. HepG2 cells were cultured in Dulbecco's modified Eagle medium (DMEM) from Wako (Osaka, Japan) containing 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, 0.25 µg/ml amphotericin B, and 0.1 mM nonessential amino acids. THP-1 cells were seeded onto 96-well plates (3×10^4 cells/well) and 24-well plates (1×10^6 cells/well) from Becton Dickinson (Heidelberg, Germany). THP-1 cells were incubated with 0.1 ml (96-well plate for cell proliferation) and 0.5 ml (24-well plate for cell proliferation and incorporation of [^{14}C] BaP) of the medium containing PAHs (BaP, 3-MC, BeP, and pyrene): β-NF, MDA-LDL, 0.1% DMSO, or cotreatment with PAHs and MDA-LDL. The HepG2 cells were seeded onto 24-well plates at 5×10^4 cells/well for 24 hr, and used for a virus infection experiment.

Recombinant adenovirus

Adenovirus-expressing human *CYP1A1* and *CYP1A2* (Ad-hCYP1A1 and Ad-hCYP1A2) were done with the AdEasy™ System (Quantam Biotechnologies, Montreal, Canada) according to a previous report (Aoyama *et al.*, 2009). AdCont (AxCALacZ), which expresses β-galactosidase, was provided by Dr. Izumi Saito (Tokyo University) and used as a control adenovirus. The titer of the recombinant adenovirus, 50% titer culture infection dose ($TCID_{50}$), and multiplicity of infection (MOI) were determined as reported previously (Kamiyama *et al.*, 2007).

Infection of recombinant adenovirus

The HepG2 cells were incubated with 0.1 ml of a medium containing a recombinant adenovirus for 1 hr followed by the addition of 0.9 ml of medium and further culturing for 96 hr. Seventy-two hr after the adenovirus infection, the HepG2 cells were treated with 0.01 µM and 0.1 µM BaP or 0.1% DMSO for 24 hr.

Modification of LDL by malondialdehyde

Malondialdehyde was generated at room temperature by rapid acid hydrolysis (0.2 ml of 12 M HCl) of tetramethoxypropane (Nacalai Tesque, Kyoto, Japan). Then, 4.6 ml of 0.1 M sodium phosphate buffer (pH 6) was added to this reaction medium and the solution was adjusted to pH 6 with 10 M NaOH. Synthesized MDA was subsequently added to freshly prepared LDL (Chemicon, Tokyo, Japan) (2 mg/ml) and incubated at a final concentration of 0.02 mM for 3 hr at 37°C. The reaction was stopped by dialysis against phosphate-buffered saline (PBS) (pH 7.6) for 24 hr at 4°C. The extent of modification was determined by a colorimetric thiobarbituric acid assay (Fogelman *et al.*, 1980; Patton and Kurtz, 1951).

Tritiated thymidine incorporation assay

Proliferation of THP-1 cells was determined by the incorporation of [^{3}H] thymidine into cellular DNA. Briefly, 10 µl of 10 µCi/ml methyl [^{3}H] thymidine (80 Ci/mmol, Amersham Bioscience) was added to each well of

a 24-well plate and incubated for the final 18 hr in each experiment. The medium was then aspirated, cells were washed with ice-cold 10% trichloroacetic acid to precipitate DNA and unincorporated labeled thymidine was removed. Cells were dissolved in 0.1 M NaOH to hydrolyze the acid-insoluble material. Radioactivity was measured using a liquid scintillation counter (Beckman Counter LS 6500 AT, Fullerton, CA, USA).

WST-8 growth assay

THP-1 proliferation was determined by WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-dinitrophenyl)-2H-tetrazolium, monosodium salt] assays using a Cell Counting Kit-8 (Donjindo, Kumamoto, Japan). Briefly, 10 µl of WST-8/1-methoxy-phenazine methosulfate solution was added to each well of a 96-well plate and incubated for 2 hr at 37°C. The absorbance at 450 nm was then measured with a multiwell spectrophotometer (MPR-A4i; Toso, Tokyo, Japan).

[^{14}C]BaP incorporation assay

BaP incorporation into cellular DNA was determined by incorporation of [^{14}C]BaP. Briefly, 2 µl of [^{14}C]BaP (60 mCi/mmol, Amersham Bioscience) was added to each well of a 24-well plate. The medium was then aspirated, cells were washed with ice-cold 10% trichloroacetic acid to precipitate DNA and unincorporated labeled BaP was removed. Cells were dissolved in 0.1 M NaOH to hydrolyze the acid-insoluble material. Radioactivity was measured using a liquid scintillation counter.

Quantitative real-time polymerase chain reaction (PCR)

Total ribonucleic acid (RNA) was extracted from the cells using TRIzol according to the manufacturer's protocol. The cDNA was synthesized from 1 µg of total RNA using an oligo dT-adaptor and avian myeloblastosis virus ExScript reverse transcriptase (Takara, Shiga, Japan). Quantitative real-time PCR was performed using a Thermal Cycler Dice™ TP800 (Takara). The primer sequences used in this study were as follows: hCYP1A1: sense (5′-GGCCACTTTGACCCTTACAA-3′) and anti-sense (5′-CAGGTAACGGAGGACAGGAA-3′); hCYP1A2: sense (5′-GTTCCTGCAGAAAACAGTCCA-3′) and anti-sense (5′-CTGTGCTTGAACAGGGCAC -3′); AhR: sense (5′-GCACGAGAGGCTCAGGTTATCA-3′) and anti-sense (5′-GTGCATTAGACTGGACCCAAGTC-3′); p53: sense (5′-CTGTCCCTTCCCACAAAACC-3′) and anti-sense (5′-CCACTCGGATAAGATGCT-3′); p21: sense (5′-CGACTGTGATGCGCTAATGG-3′) and anti-sense (5′-CCAGTGGTGTCTCGGTGACA-3′); and glyceraldehyde-3-phosphate dehydrogenase (GAPDH): sense (5′-TCGGAGTCAACGGATTTGGTCGTA-3′) and anti-sense (5′-ATGGACTGTGGTCATGAGTCCTTC-3′). PCR was performed using a SYBR® Premix Ex Taq™ (Takara). The reaction was carried out under the following conditions: 50°C for 2 min, 95°C for 2 min, followed by 40 cycles at 95°C for 5 sec, and 60°C for 30 sec. To confirm the amplification specificity, the PCR products were subjected to a melting curve analysis. The mRNA expression levels of the hCYP1A1, hCYP1A2, AhR, p53, and p21 in each sample were normalized to that of GAPDH.

Small interfering RNA

Double-strand siRNAs (25-mer) that target the AhR and control siRNA that target estrogen receptor α (ERα) were designed and synthesized by Invitrogen. The corresponding target mRNA sequences for the siRNAs were as follows: AhR siRNA, UUAAGUCGGUCU-CUAUGCCGCUUGG; ERα siRNA, UAGUCAUUG-CACACUGCACAGUAGC. HepG2 cells were transfected with siRNA when cells reached 30-50% confluence (48-well plate), according to the manufacturer's protocol. Briefly, gene-specific siRNA oligomers (20 nM) and 0.4 µl/well lipofectamine RNAiMAX were diluted in 40 µl/well DMEM (Invitrogen). After 20-min incubation at room temperature, the complexes were added to the cells in a final volume of 240 µl medium. Forty-eight hr after the siRNA transfection, the HepG2 cells were treated with 0.01 µM and 0.1 µM BaP or 0.1% DMSO for 48 hr.

Preparation of S9 fraction

HepG2 cells were washed with ice-cold PBS (without $CaCl_2$ and $MgCl_2$) and then removed with a cell scraper. The cells were suspended in 0.25 M sucrose/1 mM EDTA/10 mM Tris HCl buffer (pH 7.4). The sonicated cells were then homogenized under ice-cold conditions. After centrifugation of the homogenated cells at 9,000 × *g* for 20 min at 4°C, the supernatant (S9) was collected. Protein content was determined by using a Protein Assay Kit (Bio-Rad, Tokyo, Japan) according to the manufacturer's protocol, with bovine serum albumin as the standard.

7-Ethoxyresorufin O-deethylase (EROD) assays

CYP1A activity was determined using an EROD assay according to the methods of Burke and Mayer (1974) with the following modifications. The reaction mixture contained 1 µM 7-ethoxyresorufin (Sigma), S9 fraction (200 µg of S9 protein), and 0.1 M potassium phosphate buffer (pH 7.4) in a final volume of 0.5 ml. After preincubation of the mixture at 37°C for 5 min, 0.5 mM NADPH

(Wako) was added to initiate the reaction, and the mixture was further incubated for 10 min. The reaction was terminated by the addition of 2 ml of ice-cold methanol, and the samples were then centrifuged at 9,000 × *g* for 10 min. The metabolite, resorufin, was measured using Powerscan®HT (Dainippon Sumitomo Pharma, Osaka, Japan) (excitation and emission wavelengths of 530 nm and 585 nm, respectively).

Statistical analysis

Data are expressed as the mean ± standard (S.D.) error from three determinations. Statistical comparison of two groups was performed using a two-tailed Student's *t*-test. *P*-values less than 0.05 were considered to be statistically significant.

RESULTS

Effects of PAHs on MDA-LDL-induced THP-1 cell growth

We examined the effects of MDA-LDL on THP-1 cells growth. THP-1 cell growth increased nearly 30% compared to the control by treatment with 0.4 mg/ml MDA-LDL. We further investigated whether PAHs had an effect on MDA-LDL-induced THP-1 cell growth. Cotreatment with BaP or 3-MC and MDA-LDL reduced MDA-LDL-induced THP-1 cell growth (Fig. 1-A). On the other hand, cotreatment with BeP or pyrene, which does not activate the AhR, and MDA-LDL did not reduce MDA-LDL-induced THP-1 cell growth (Fig. 1-B). Or, that the experiments with Ox-LDL yielded similar results to those observed in MDA-LDL; however, we examined the role of the MDA-LDL in this study because the results of MDA-LDL were clearer than the results of Ox-LDL.

Effects of α-NF or β-NF on suppression of MDA-LDL-induced THP-1 cell growth by BaP

Among the PAHs, BaP and 3-MC activate AhR-mediated signal transduction, but pyrene and BeP do not. To clarify whether AhR-mediated signal transduction is involved in the suppression of MDA-LDL-induced THP-1 cell growth by BaP, we examined the effect of α-NF, a partial antagonist to AhR, on [^{3}H] thymidine incorporation. Cotreatment with BaP and MDA-LDL reduced [^{3}H] thymidine incorporation. The suppression of [^{3}H] thymidine incorporation into THP-1 cells was clearly restored by the addition of 1 μM α-NF (Fig. 2-A). β-NF is a potent agonist to AhR, but not to PAHs, and does not cause cell damage. To clarify the involvement of AhR in suppression of MDA-LDL-induced THP-1 cell growth, we further examined whether β-NF, as well as BaP, suppresses MDA-LDL-induced THP-1 cell growth. Co-treatment with β-NF and MDA-LDL did not reduce the MDA-LDL-induced THP-1 cell growth (Fig. 2-B).

Effect of MDA-LDL and BaP on hCYP1A1 mRNA expression

To further clarify whether AhR-mediated signal transduction was activated by BaP, we examined the effect of cotreatment with MDA-LDL and BaP on the expression of hCYP1A1 mRNA. hCYP1A1 is a BaP-activating enzyme, and the expression was induced by activation of the AhR pathway. Cotreatment with MDA-LDL and BaP markedly induced the expression of hCYP1A1 mRNA (28-fold). In contrast, BaP treatment also induced the expression, but at a low level (9.8-fold) (Fig. 3).

The role of MDA-LDL in BaP activation in THP-1 cells

Together with the above results, cotreatment with BaP and MDA-LDL was predicted to cause strong BaP activation due to the acceleration of BaP incorporation into the cells. We next examined the incorporation of BaP into THP-1 cells. Incorporation of [^{14}C]BaP with MDA-LDL into THP-1 cells increased 5.6-fold compared to the results from treatment with [^{14}C]BaP alone (Fig. 4).

Analysis of the suppression mechanism of MDA-LDL-induced THP-1 cell growth by BaP

Together with these results, induced hCYP1A1 is predicted to transform BaP to an activated form causing DNA damage and, consequently, increased levels of tumor suppressor proteins such as p53 and p21, the proliferation inhibitor protein. Therefore, we next measured p53 and p21 mRNA levels by cotreatment with MDA-LDL and BaP. As expected, cotreatment with BaP and MDA-LDL significantly induced the expression of p53 (2.6-fold) and p21 (1.9-fold) mRNAs compared to that of the control. Treatment with BaP alone did not induce the expression of mRNAs (Fig. 5).

Effect of knockdown of AhR and overexpression of hCYP1A1 on the expression level of p21 mRNAs

To determine the influence of the AhR pathway or metabolic activation of BaP by hCYP1A1 on the expression level of p21 mRNA, we performed knockdown of the AhR using AhR siRNA and overexpression of hCYP1A1 using an hCYP1A1-expressing adenovirus (Ad-hCYP1A1). It was difficult to overexpress the recombinant protein in THP-1 cells through a cDNA expression vector. Therefore, in this study, we used HepG2 cells and a hCYP1A1-

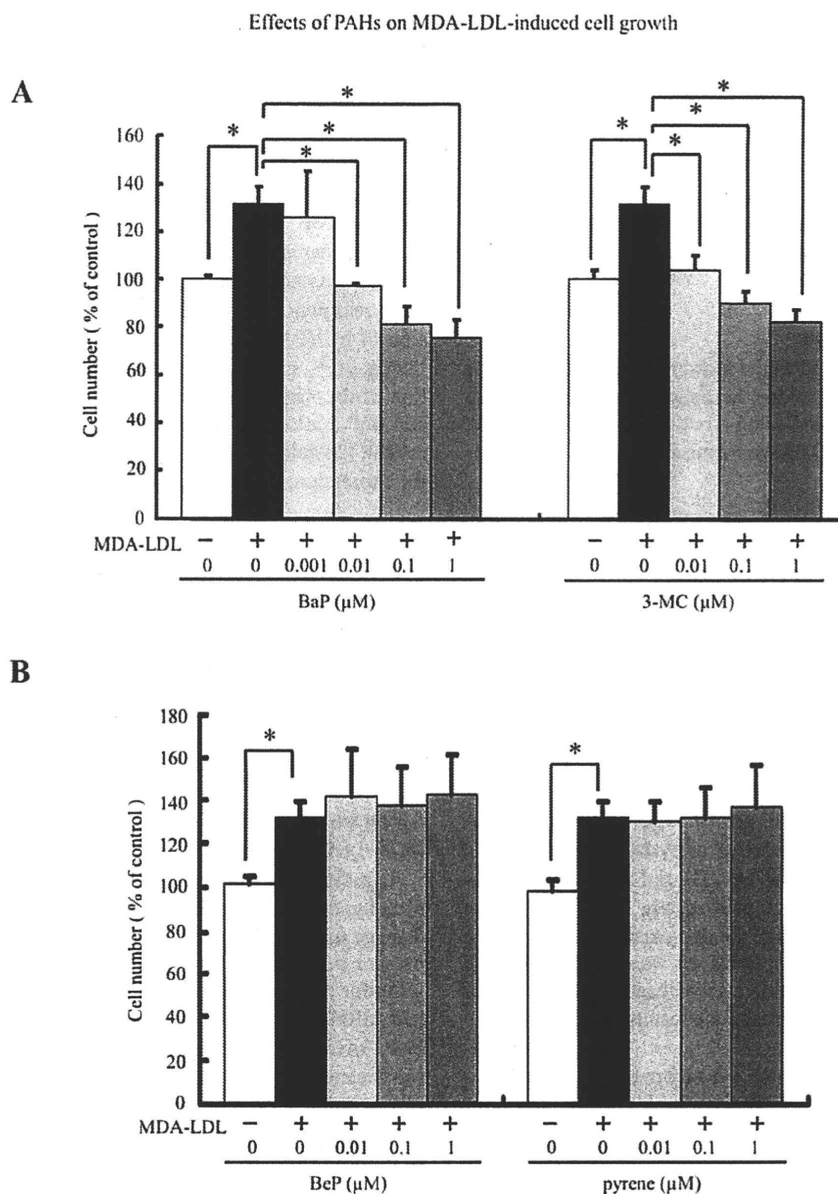

Fig. 1. Effects of BaP and 3-MC (A) and BeP and pyrene (B) on MDA-LDL-induced THP-1 cell growth. THP-1 cell proliferation was assessed by a WST-8 assay. Concentrations of MDA-LDL and PAHs were 0.4 mg/ml and 0.01-1 μM, respectively. Data show the mean ± S.D. of at three independent experiments. $^*p < 0.001$.

expressing adenovirus. As a result, the levels of AhR and CYP1A1 mRNA were downregulated by 73% and more than 50%, respectively, compared with the control (Fig. 6-A). The level of p21 mRNA was induced in a dose-dependent manner by the addition of BaP compared with the control (1.7- to 2.0-fold) in 48-hr cultured cells. However, when AhR siRNA was introduced into cells, p21 mRNA was not induced in 48-hr cultured cells (Fig. 6-

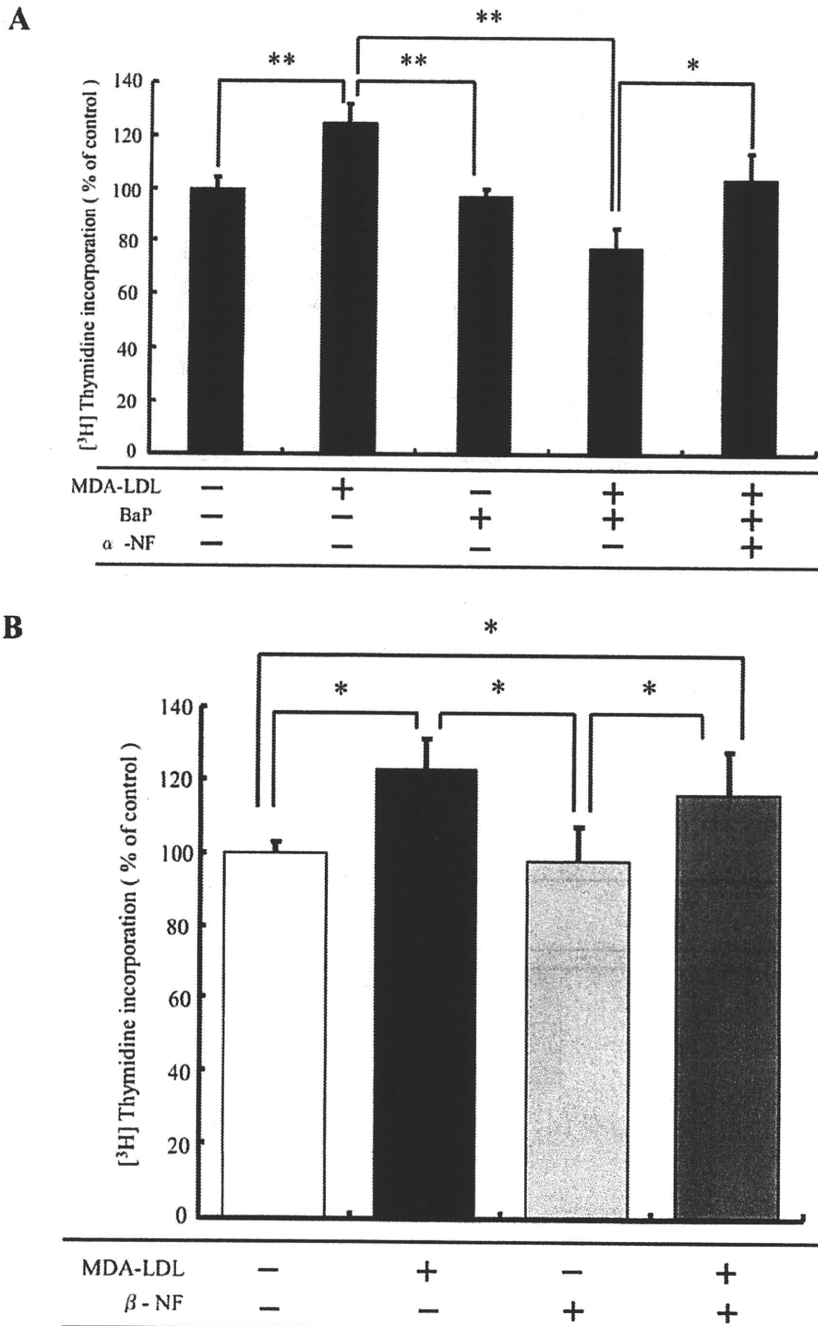

Fig. 2. Effects of α-NF (A) and β-NF (B) on BaP suppression of MDA-LDL-induced THP-1 cell growth. THP-1 cell proliferation was assessed by a [^{3}H] thymidine incorporation assay. Concentrations of BaP, MDA-LDL, α-NF, and β-NF were 0.1 μM, 0.4 mg/ml, 0.1 μM, and 0.1 μM, respectively. Data show the mean ± S.D. of at three independent experiments. $^{*}p < 0.01$; $^{**}p < 0.001$.

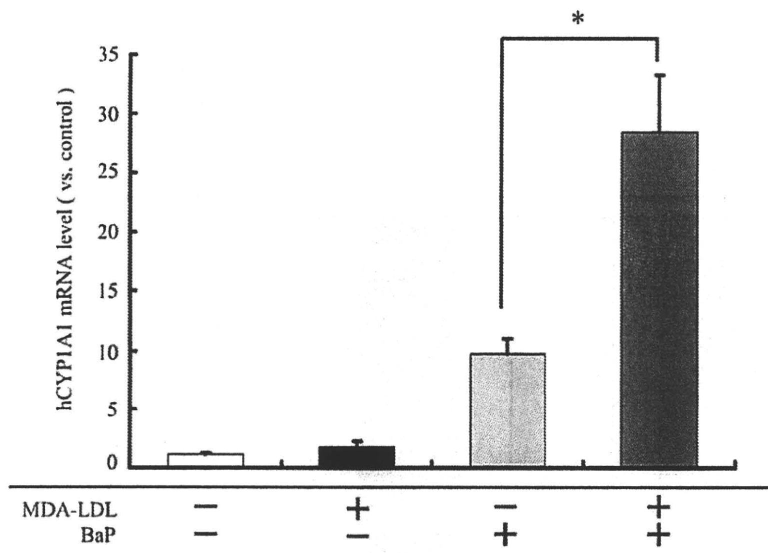

Fig. 3. Expression of hCYP1A1 mRNA in THP-1 cells. THP-1 cells were incubated with DMSO, BaP, MDA-LDL, or MDA-LDL along with BaP for 12 hr. Total RNA was prepared 12 hr later as described in the experimental procedures. The mRNA expression of the target genes was analyzed by real-time RT-PCR, and the mRNA levels of the target gene were normalized to that of GAPDH. Concentrations of MDA-LDL and BaP were 0.4 mg/ml and 0.1 μM, respectively. Data show the mean ± S.D. of at three independent experiments. $^{*}p < 0.01$.

B). Moreover, p21 mRNA was induced in a dose-dependent manner by treatment with Ad-hCYP1A1 and BaP in 24-hr cultured cells (Fig. 6-C) but not with Ad-hCYP1A2 and BaP. Typical catalylic activities for hCYP1A1 and hCYP1A2 were detected with EROD. EROD activities of overexpressed hCYP1A1 and hCYP1A2 were 2.0 and 2.39 times higher, respectively, than that of the control (Fig. 6-D).

DISCUSSION

Macrophage proliferation is recognized as an important factor of pathogenesis in the development of arteriosclerosis. Studies from several research groups have shown that Ox-LDL induced macrophage proliferation (Matsumura *et al.*, 1997; Martens *et al.*, 1998; Hamilton *et al.*, 1999). Interestingly, PAHs have been known to induce arteriosclerosis (Curfs *et al.*, 2005; van Grevenynghe *et al.*, 2004), and several observations support the hypothesis that the aggravation of arteriosclerosis is caused by PAHs coexiting with Ox-LDL such as MDA-LDL. However, it is unclear as to how arteriosclerosis is promoted by PAHs. Therefore, we expected that MDA-LDL-induced cell growth is supported by the presence of PAHs. In the present study, we used THP-1 cells to examine whether MDA-LDL-induced cell growth was caused by PAHs. Contrary to expectations, our results demonstrated that cotreatment of BaP or 3-MC with MDA-LDL decreased MDA-LDL-induced THP-1 cell growth (Fig. 1-A). Interestingly, we found that there were differences between the ligands (BaP or 3-MC) and nonligands (pyrene or BeP) of the AhR (Figs. 1-A and B). In addition, we conducted experiments using an AhR partial antagonist (α-NF) and agonist (β-NF) other than PAHs to determine whether depressed MDA-LDL-induced THP-1 cell growth in the AhR could be found under the same conditions. It is of interest that α-NF clearly restored the synergistic action by the combination of BaP and MDA-LDL (Fig. 2-A), but β-NF did not depress MDA-LDL-induced THP-1 cell growth. It seems that activation of the AhR pathway is involved in the depression of MDA-LDL-induced THP-1 cells growth; however, involvement of another mechanism after activation of the AhR pathway was predicted. Next, we designed an experiment to investigate the expression level of hCYP1A1 mRNA. Higher expression of mRNA was observed after cotreatment of

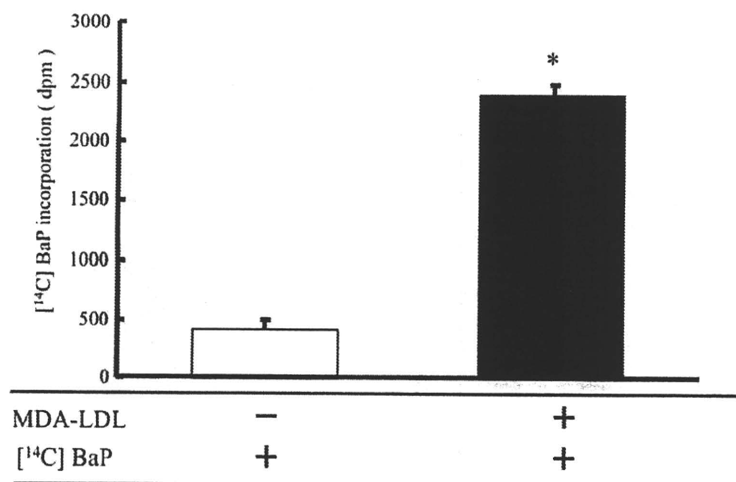

Fig. 4. Effect of MDA-LDL on [^{14}C]BaP incorporation into THP-1 cells. THP-1 cells were incubated with either [^{14}C]BaP or BaP and MDA-LDL for 24 hr. Incorporation of BaP was assessed by a [^{14}C]BaP incorporation assay. Concentrations of [^{14}C]BaP and MDA-LDL were 0.1 μCi/ml and 0.4 mg/ml, respectively. Data show the mean ± S.D. of at three independent experiments. $^{*}p < 0.001$ compared with [^{14}C]BaP alone.

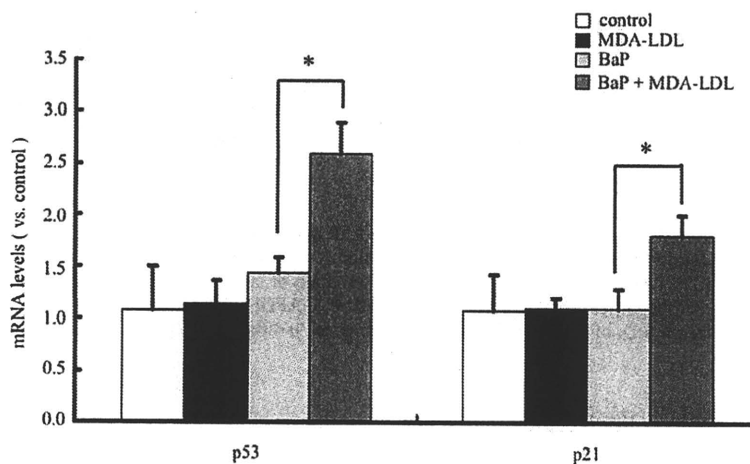

Fig. 5. Expression of p53 and p21 mRNAs in THP-1 cells. THP-1 cells were incubated with DMSO, BaP, MDA-LDL, or MDA-LDL along with BaP for 12 hr. Total RNA was prepared 12 hr later as described in the experimental procedures. The mRNA expression of the target genes was analyzed by real-time RT-PCR, and the mRNA levels of the target gene were normalized to that of GAPDH. Concentrations of MDA-LDL and BaP were 0.4 mg/ml and 0.1 μM, respectively. Data show the mean ± S.D. of at three independent experiments. $^{*}p < 0.001$.

BaP and MDA-LDL than after treatment with BaP alone (Fig. 3). These findings suggest that the influence of PAHs on THP-1 cell growth is likely to be through AhR, and strong BaP activation is predicted to be due to the acceleration of BaP incorporation into cells.

BaP, as well as other PAHs, is poorly soluble in water.

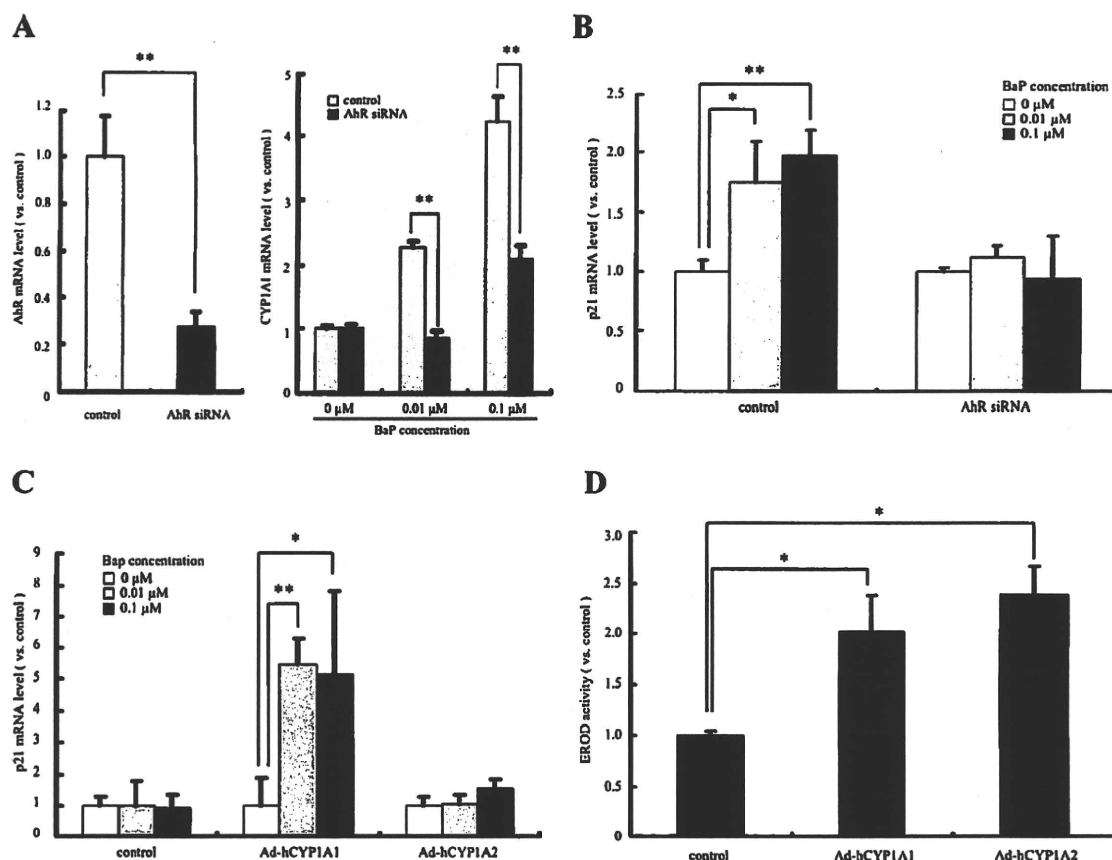

Fig. 6. (A) Effects of AhR siRNA on the expression levels of the AhR and CYP1A1 mRNA in HepG2 cells. Effects of AhR siRNA (B) and overexpression of hCYP1A1 (C) on the expression levels of p21 mRNA in HepG2 cells. (D) EROD activity of hCYP1A1 and hCYP1A2 in HepG2 cells. The mRNA expression of target genes was analyzed by real-time RT-PCR, and the mRNA levels of the target gene were normalized to that of GAPDH. EROD activity compared to the control is shown. Data show the mean ± S.D. of at three independent experiments. *$p < 0.05$; **$p < 0.001$.

In plasma, BaP partitions readily incorporate into plasma lipoproteins (Avigan, 1959; Shu and Nichols, 1979). The entry of BaP into cells from plasma lipoproteins has been shown to be via diffusion and to be nonmediated by a transfer process (Remsen and Shireman, 1981; Plant *et al.*, 1985). Therefore, we investigated the uptake of BaP into THP-1 cell using [^{14}C]BaP. Interestingly, the uptake rate of [^{14}C]BaP was increased by cotreatment with MDA-LDL compared to treatment with [^{14}C]BaP alone (Fig. 4). The relatively slow rate of BaP uptake into cells suggests that the lipophilic properties of PAHs would likely result in extremely slow permeation into the tissues. It is important to understand the mechanism by which PAHs enter cells.

In a recent study, Iseki *et al.* (2005) suggested that the AhR in Leydig cells mediated growth inhibition through p21, which is a major transcriptional target of the tumor suppressor p53. Furthermore, p21 is known to play an important role in cell cycle control by interacting with the cyclin-dependent kinase complexes. Finally, the cell cycle may be arrested after the p53/p21 pathway is induced by DNA damage (Iwano *et al.*, 2006). Therefore, in the next experiment, we measured the expression levels of p53 and p21 mRNAs and found that expression of p53 and p21 mRNAs was significantly increased after cotreatment with BaP and MDA-LDL (Fig. 5). Our findings also indi-

cated that suppression of MDA-LDL-induced THP-1 cells growth by BaP may be attributable to apoptosis (Fig. 5). However, this finding is not compatible with the results of flow cytometry (data not shown). Furthermore, as mentioned previously, β-NF is a typical hCYP1A1 inducer that does not contribute to the suppression of MDA-LDL-induced THP-1 cell growth. These findings suggest that metabolites of BaP by hCYP1A1 induced through AhR activation may cause DNA damage. The cell cycle arrest was caused by p53/p21 activation through DNA damage. These results were strongly supported by the AhR siRNA experiment. AhR siRNA restored p21 mRNA expression level as shown in Fig. 6-B. The metabolic activation of BaP by hCYP1A1 could be a necessary step for suppression of MDA-LDL-induced THP-1 cell growth. Recently, knockout experiments of CYP1A1 have shown that CYP1A1 is not involved in BaP toxicity and was reported to be a detoxication enzyme (Uno *et al.*, 2006; Endo *et al.*, 2008). However, our overexpression experiment of hCYP1A1 using an hCYP1A1-expressing adenovirus further supported our findings. The reason for the discrepancy is not clear at this time but could be attributed to differences in the experimental conditions.

In conclusion, suppression of MDA-LDL-induced THP-1 cell growth by BaP may be due to accelerated incorporation of BaP. Thereafter, the hCYP1A1 gene is strongly induced by activation of the AhR by incorporated BaP. It seems that MDA-LDL may play an important role, at least in part, in that incorporation. In addition, BaP was metabolized to the activated form by strongly induced hCYP1A1. Subsequently, DNA damage was caused by the activated BaP, and the p53/p21 pathway was strongly activated. It is, therefore, possible that MDA-LDL-induced THP-1 cell growth was suppressed by PAHs.

Our findings suggest that compounds involved in arteriosclerosis are easily incorporated into cell via MDA-LDL, and further investigation is needed.

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BRIEF COMMUNICATION

*HLA-B*1511* is a risk factor for carbamazepine-induced Stevens-Johnson syndrome and toxic epidermal necrolysis in Japanese patients

***Nahoko Kaniwa, *Yoshiro Saito, †Michiko Aihara, ‡Kayoko Matsunaga, *Masahiro Tohkin, *Kouichi Kurose, §Hirokazu Furuya, ¶Yukitoshi Takahashi, #Masaaki Muramatsu, **Shigeru Kinoshita, ‡Masamichi Abe, ¶Hiroko Ikeda, #Mariko Kashiwagi, #Yixuan Song, **Mayumi Ueta, **Chie Sotozono, †Zenro Ikezawa, and *Ryuichi Hasegawa, for the JSAR research group[1]**

*Division of Medicinal Safety Sciences, National Institute of Health Sciences, Tokyo, Japan; †Department of Environmental Immunodermatology, Yokohama City University Graduate School of Medicine, Yokohama, Japan; ‡Department of Dermatology, Fujita Health University School of Medicine, Toyoake, Japan; §Department of Neurology, Neuro-Muscular Center, National Oomuta Hospital Oomuta, Japan; ¶Shizuoka Institute of Epilepsy and Neurological Disorders, National Epilepsy Center, Shizuoka, Japan; #Molecular Epidemiology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan; and **Department of Ophthalmology, Kyoto Prefectural University of Medicine, Kyoto, Japan

Summary

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are rare but life-threatening severe cutaneous adverse reactions. Recently, strong associations of *HLA-B*1502* with carbamazepine-induced SJS/TEN have been found in Han Chinese patients. These associations have been confirmed in several Asian populations, excluding Japanese. SJS patients carrying *HLA-B*1508, HLA-B*1511*, or *HLA-B*1521*, which are members of the HLA-B75 type along with *HLA-B*1502*, were detected in studies in India and Thailand. In the current study, we genotyped the *HLA-B* locus from 14 Japanese typical and atypical SJS/TEN patients in whom carbamazepine was considered to be involved in the onset of adverse reactions. Although there were no *HLA-B*1502* carriers, four patients had *HLA-B*1511*. Our data suggest that *HLA-B*1511*, a member of *HLA-B75*, is a risk factor for carbamazepine-induced SJS/TEN in Japanese.

KEY WORDS: HLA-B*1502, HLA-B75, Serotype.

Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) are severe adverse drug reactions (ADRs) with mucosal and cutaneous disorders, and often are accompanied by high fever and systemic complications. Although incidence is low, SJS and TEN are life-threatening and their mortalities are estimated at 5% and 30%, respectively. On the basis of summarized spontaneous reports of severe ADRs to the Ministry of Health, Labor and Welfare (MHLW) from 2006 to 2008, the incidence of SJS/TEN in Japan can be calculated as 3.4 patients per million per year (approximately 430 cases annually), and major causative drugs are allopurinol and carbamazepine.

As for carbamazepine-induced SJS/TEN, involvement of *HLA-B*1502* in Han Chinese SJS/TEN patients has been reported (Chung et al., 2004), and has been confirmed in Asians in Hong Kong (Man et al., 2007), Europe (Lonjou et al., 2006), Thailand (Locharernkul et al., 2008), and India (Mehta et al., 2009). However, no association between *HLA-B*1502* and carbamazepine-related SJS/TEN was detected in our previous study with seven Japanese SJS/TEN patients (Kaniwa et al., 2008). Therefore, we extended the investigation to explore other biomarkers in Japanese SJS/TEN patients who were administered carbamazepine.

Methods

Patients

The ethics committee of each participating institute of the JSAR (Japan Severe Adverse Reactions) research group approved this study. Written informed consent was obtained from each patient. Fifteen unrelated Japanese patients who were prescribed carbamazepine before the onset of SJS/TEN were recruited from participating institutes or through

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Address correspondence to Nahoko Kaniwa, PhD, Division of Medicinal Safety Sciences, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. E-mail: nkaniwa@nihs.go.jp

[1]The JSAR (Japan Severe Adverse Reactions) research group: the representative of the research group is Nahoko Kaniwa at National Institute of Health Sciences, and all authors are members of the research group.

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a nationwide blood sampling network in Japan operated by the National Institute of Health Sciences in cooperation with the MHLW and the Federation of Pharmaceutical Manufacturers' Association of Japan. Patient characteristics are summarized in Table 1. Seven patients were included in our previous report (Kaniwa et al., 2008), and two patients were in another study (Ikeda et al., 2009). Twelve patients were diagnosed as definite SJS or TEN and three patients were diagnosed as probable SJS due to atypical or mild symptoms by the JSAR research group experts. This diagnosis was based on criteria proposed by Bastuji-Garin et al. (1993) using a standardized case report form including medicinal records, disease progress, and involvement of systemic complications as well as treatment. Severity of ocular complication was scored as follows: 0, no involvement; 1, only hyperemia of bulbar and palpebral conjunctiva; 2, pseudomembrane formation; 3, defect of conjunctival or corneal epithelia.

HLA-B typing

High-resolution *HLA-B* typing was performed by a sequence-based method using SeCore B Locus Sequencing kit (Invitrogen Corp., Brown Deer, WI, U.S.A.) and an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, U.S.A.). Genomic DNA (250 ng) was used for PCR amplification and sequencing exons 2, 3, and 4. HLA-B haplotype was estimated with the Assign SBT software (version 3.2.7b; Conexio Genomics, Applecross, WA, Australia).

Statistical analysis

*HLA-B*1511* allele frequency reported by Tanaka et al. was used as the control frequency (Tanaka et al., 1996). Fisher's exact test was conducted using JMP ver. 7.0.1 (SAS Institute Japan, Co., Ltd., Tokyo, Japan) to calculate the odds ratio and its 95% confidence interval (CI).

Results

Demographics, symptomatic state, coadministered drugs with carbamazepine, and *HLA-B* diplotypes of 15 patients are summarized in Table 1. However, Patient 12 was excluded from the following statistical analyses because zonisamide was a more likely causative drug. Involvement of carbamazepine in the onset of SJS/TEN could not be excluded for the remaining 11 definite SJS/TEN patients and three probable SJS patients.

In contrast to data on Han Chinese (Chung et al., 2004) and Thai populations (Locharernkul et al., 2008), *HLA-B*1502* was not detected in this work. However, two patients with definite SJS/TEN and two patients with probable SJS carried *HLA-B*1511*. The allele frequencies of *HLA-B*1511* in the SJS/TEN groups were compared with the allele frequency in a Japanese population reported by Tanaka et al. (1996) (n = 493) instead of that in carbamazepine-tolerant patients, because the incidence of SJS/TEN in Japan is very low (three per million/year). Allele frequencies of *HLA-B*1511* increased significantly in the SJS/TEN group regardless of the exclusion or inclusion of probable SJS patients [0.0909 (2 of 22) and 0.143 (4 of 28), respectively) than in the Japanese population (0.01), and the odds ratios were 9.76 ($p = 0.0263$, CI 2.01–47.5) and 16.3 ($p = 0.0004$, CI 4.76–55.6), respectively. No patients with *HLA-B*1511* had severe ocular complications.

Discussion

Recently, *HLA-B*1502* involvement has been reported in carbamazepine-induced SJS/TEN in Southern Asian patients (Chung et al., 2004; Man et al., 2007; Locharernkul et al., 2008; Mehta et al., 2009) and patients of Asian ancestry living in Europe (Lonjou et al., 2006). Although we did not detect SJS/TEN patients receiving carbamazepine who carried *HLA-B*1502*, we did find four patients carrying *HLA-B*1511*. *HLA-B*1511* and *HLA-B*1502* belong to the same HLA-B75 serotype. Other major members of HLA-B75 are *HLA-B*1508, HLA-B*1515,* and *HLA-B*1521.* Mehta et al. (2009) have investigated the association between *HLA-B*1502* and carbamazepine-induced SJS using eight Indian patients. Although in their study most patients (six of eight) did carry *HLA-B*1502*, one patient was homozygous *HLA-B*1508*. Tassaneeyakul et al. (2010)have also performed a case–control study using 42 CBZ-induced SJS/TEN patients and 42 carbamazepine-tolerant controls in a Thai population. In their study, 37 SJS/TEN patients carried *HLA-B*1502* and the very strong association of *HLA-B*1502* with SJS/TEN was again confirmed. Although the statistical significance was not examined, two patients carrying heterozygous *HLA-B*1521* and one patient carrying heterozygous *HLA-B*1511* were detected, suggesting that not only *HLA-B*1502* but also some subfamilies of serotype HLA-B75 are involved in the onset of carbamazepine-induced SJS/TEN.

Allele frequencies of individual HLA genotypes in worldwide populations obtained from various studies are shown at Allelefrequencies.net (Middleton et al., 2003). Table 2 summarizes the population allele frequencies of representative types of HLA-B75 in various ethnic groups. In Han Chinese, Thai and Indians, carriers of *HLA-B*1502*, *HLA-B*1521*, and *HLA-B*1508* are at high risk of carbamazepine-induced SJS/TEN, although *HLA-B*1502* is mainly involved. A comparable allele frequency of *HLA-B*1511* (higher than 3.8%) to that of *HLA-B*1502* in Han Chinese in Beijing has been reported recently by Yang et al. (Yang et al., 2010). Because the allele frequency of *HLA-B*1511* is higher than that of *HLA-B*1502* in Japanese and Koreans, carriers of the former may more easily be detected in association studies than carriers of the latter in northeast Asian populations. *HLA-B*1521* can be a risk

Table 1. Backgrounds and HLA-B diplotypes of Japanese carbamazepine-related SJS/TEN patients

									Coadministered drugs		HLA-B diplotype	
ID[a]	ADR type	Sex/Age	Severity score in ophthalmic disorders	Highest BT (°C)	Total area of blistering skin (%)	Systemic complications	Result of DLST to CBZ	Period of onset for CBZ (days)	Drug name	DLST result/period of onset	High resolution	Low resolution
1 (1)	TEN	M/73	1	>39	20	Neutropenia	–	14	Potassium citrate/sodium citrate hydrate	–/4 days	**1511**/4801	**B75**/B48
						Liver dysfunction			Allopurinol	–/>5 years		
									Etizolam	–/>5 years		
									Sodium pravastatin	–/>5 years		
2 (5)[b]	SJS	F/6	At least 1[c]	>37.0	<10%	GI tract disturbance	Not tested	9	None		4006/5101	B61/B51
3 (6)[b]	SJS	F/52	At least 1[c]	Unknown	<10%	Neutropenia Liver dysfunction	Not tested	14	Zonisamide	Not tested/ 346 days	4601/5901	B46/B59
4	SJS	M/52	0	38	1	GI tract disturbance Neutropenia Liver dysfunction Renal dysfunction	Not tested	51	Tegafur/gimeracil/oteracil potassium	Not tested/38 days	0702/5201	B7/B52
5	SJS	M/32	1	39	5	Liver dysfunction	Not tested	42	None		4002/5401	B60/B54
6 (2)	SJS	F/42	3	>39	5	GI tract disturbance	–	Shorter than 34	Sodium diclofenac	–/1 year	4001/5201	B60/B52
									L-carbocysteine	–/1 year		
									Cefteram pivoxil	–/4 days		
									Olopatadine hydrochloride	Not tested/ unknown		
7	SJS	F/64	At least 1[c]	>37.0	10	Liver dysfunction	+	13	Mecobalamin	Not tested/13 days	**1511**/4002	**B75**/B60
8 (3)	SJS	M/45	3	>37.0	5	Liver dysfunction	Not tested	49	None		4801/5601	B48/B56
9 (4)	SJS	M/54	0	<37.0	0.5	None	+	34	None		1501/3501	B62/B35
10	TEN	M/38	3	40.3	40	Liver dysfunction	+	15	Troxipide	–/8 days	1302/4403	B13/B44
									Levofloxacin hydrate	–/15 days		
									Mecobalamin	–/9 days		
									Acyclovir	–/9 days		
11 (7)	TEN	M/17	3	39.7	20	Respiratory involvement	+	5	Zonisamide	+/33 days	4601/5601	B46/B56
						Neutropenia			Amoxicillin hydrate	+/1 day		
						Liver dysfunction			Promethazine methylenedisalicylate	Not tested/1 day		
12[d]	SJS	M/6	1	Unknown	<10%	Liver dysfunction	–	145	Zonisamide	+/24 days	**1511**/4006	**B75**/B61
13	Probable SJS	F/54	Unknown	<37.0	>10%	Liver dysfunction	Not tested	22	Sodium pravastatin	Not tested/ unknown	4006/4403	B61/B44
									Nifedipine	Not tested/81 days		
									Etizolam	Not tested/15 days		
									Lansoprazole	Not tested/46 days		
									Sodium risedronate hydrate	Not tested/46 days		
14	Probable SJS	F/36	At least 1[c]	Unknown	5	None	+	15	Timiperone	Not tested/1 day	1301/**1511**	B13/**B75**
15	Atypical SJS	F/65	1	37.4	0.1	None	+	9	None		**1511**/3501	**B75**/B35

BT, body temperature; DLST, drug lymphocyte stimulation test; CBZ, carbamazepine.
[a]Number in parentheses is ID # from our previous study (Kaniwa et al., 2008).
[b]These patients were also included in Ikeda et al. (2010)
[c]Ophthalmic complications were observed, but severity was unknown.
[d]This patient was excluded from statistical analyses due to likely zonisamide-induced SJS.

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Table 2. Population allele frequencies of individual types of HLA-B75 in various ethnic groups

	Population allele frequencies reported in allelefrequencies.net website[a]				
Ethnic group	*HLA-B*1502*	*HLA-B*1515*	*HLA-B*1521*	*HLA-B*1508*	*HLA-B*1511*
Japanese	0.001	Data unavailable	Data unavailable	Data unavailable	**0.004–0.008**[b,c]
Koreans	0.002	0.000	0.000	0.000	0.020
Han Chinese	**0.019–0.124**[b]	0.010	0.000–0.002	0.005–0.015	0.000–0.017[d]
Thai	**0.061–0.085**[b]	Data unavailable	**0.007–0.010**[b]	0.010	**0.010**[b]
Indians	**0.000–0.060**[b]	Data unavailable	Data unavailable	**0.005–0.033**[b]	Data unavailable
Caucasians (West Europe)	0.000	0.000	0.000	0.000–0.004	0.000–0.003
Caucasians (East Europe)	0.000	0.000	0.000	0.000–0.009	0.000
Sub-Saharan Africans	0.000	0.000–0.008	Data unavailable	0.000	0.000
Hispanics	0.000	0.004–0.008	0.000	0.000–0.006	0.000
Arabians	0.000	0.000	0.000	0.000–0.007	0.000
Australian aborigine	0.000–0.007	Data unavailable	0.026–0.135	Data unavailable	Data unavailable

[a]New Allele Frequency Database: http://www.allelefrequencies.net/ (Middleton et al., 2003).
[b]SJS/TEN patients carrying the allele shown in the second row have been reported in the study using an ethnic group shown in the first column.
[c]The frequency of 0.1 was reported by Tanaka et al. (1996).
[d]Higher value than 0.038 in Han Chinese in Beijing was recently reported by Yang et al. (2010).

factor for carbamazepine-induced SJS/TEN for Thai and Australian aborigine. Interestingly, HLA-B75 has not been detected in carbamazepine-induced SJS/TEN Caucasian patients (Lonjou et al., 2006). This may be due to extremely low allele frequencies or no existence of HLA-B75 subfamilies.

*HLA-B*1502* has been reported to have associations with SJS/TEN caused by other aromatic antiepileptic drugs such as phenytoin and lamotrigine in Han Chinese and Thai (Man et al., 2007; Locharernkul et al., 2008). In this study we detected a patient carrying *HLA-B*1511* whose causative drug was probably zonisamide, an aromatic antiepileptic drug. Therefore, *HLA-B*1511* may be also involved in the onset of SJS/TEN induced by other aromatic antiepileptic drugs as well as *HLA-B*1502*, although further investigation is needed.

The odds ratio of *HLA-B*1511* for SJS/TEN obtained in this study was low in comparison with those observed in Thai, Indians, and Han Chinese in Taiwan (25.5, 71.4, and 25.04 respectively) (Chung et al., 2004; Locharernkul et al., 2008; Mehta et al., 2009). One reason for this may be the low allele frequency (<0.01) of *HLA-B*1511* among the Japanese. The administration of multiple drugs to Japanese patients may also contribute to the low odds ratio. Indeed, on average, more than three drugs were administered to the patients in this study. We concluded that patients receiving multiple drugs developed SJS/TEN due to carbamazepine by comparing the periods of latency of the individual drugs prior to SJS/TEN onset. However, we cannot completely exclude the possibility of other causative drugs. Another possibility is that *HLA-B*1502* is more prone than *HLA-B*1511* to cause carbamazepine-induced SJS/TEN. Carbamazepine or its metabolites may covalently (Weltzien et al., 1996) or noncovalently (Wu et al., 2007; Yang et al., 2007) bind more easily to the HLA-B*1502 protein or its binding peptide.

There are no SJS/TEN patients carrying *HLA-B*1511* who had severe ocular complications. This result coincides with the previous report that none of the 71 SJS/TEN patients with ocular surface complications had *HLA-B*1511* (Ueta et al., 2008).

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Disclosure

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. None of the authors has any conflict of interest to disclose.

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Review

Prediction of Severe Adverse Drug Reactions Using Pharmacogenetic Biomarkers

Masahiro TOHKIN[1,*], Akihiro ISHIGURO[1,2], Nahoko KANIWA[1], Yoshiro SAITO[1], Kouichi KUROSE[1] and Ryuichi HASEGAWA[1]

[1]*Division of Medicinal Safety Science, National Institute of Health Sciences, Tokyo, Japan*
[2]*Office of Safety II, Pharmaceuticals and Medical Devices Agency, Tokyo, Japan*

Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: Severe adverse drug reactions (ADRs) are a major issue for drug therapy because they can cause serious disorders and be life-threatening. Many severe ADRs appear to be idiosyncratic and unpredictable. Genetic factors may underlie susceptibility to severe ADRs, and identification of predisposing genotypes may improve drug therapy by facilitating prescreening of carriers for specific genetic biomarkers. In this review, we clarify the current status of ADRs in Japan from open ADR data sources. Then, we introduce recent progress in the field of pharmacogenetic biomarkers for severe cutaneous ADRs, liver injury, and statin-induced myopathy. Key challenges for discovery of predictable risk alleles for these severe ADRs are also discussed.

Keywords: drug-induced liver injury; drug-induced myopathy; human lymphocyte antigen; Stevens-Johnson syndrome; toxic epidermal necrolysis

Introduction

Severe adverse drug reaction (ADR) is a major reason for failure of new drug development and withdrawal of approved drugs from the market. The classical pharmacological classification of ADRs by Rawlins and Thompson distinguished two types of severe ADRs.[1] Type A reactions are dose-dependent and predictable on the basis of the drug's known pharmacological actions. Type A reactions are relatively common and include hypoglycemia induced by diabetic drugs and bleeding induced by warfarin, an oral anti-coagulant. By contrast, type B reactions are idiosyncratic, unpredictable from the pharmacological action of the drug, and are not necessarily dose-dependent. These type B reactions make up approximately 10–15% of all ADRs and include severe cutaneous disorders, such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN), and drug-induced liver injury (DILI) caused by various drugs.

The aim of this review is to provide an update on the current understanding of pharmacogenetic analysis related to severe ADRs, especially severe cutaneous ADRs, DILI, and statin-induced myopathy; great progress has been recently observed in pharmacogenetic biomarkers of these ADRs, and this should facilitate early-stage detection of severe ADRs. Therefore, pharmacogenetic biomarkers of ADRs hold promise for reducing severe ADRs and pave the way for creating more affordable pharmaceuticals.

Domestic case reports for severe adverse drug reactions in Japan

Domestic cases of severe ADRs are reported to the Pharmaceuticals and Medical Devices Agency (PMDA) by pharmaceutical companies based on the Pharmaceutical Affairs Law in Japan. The ADR report in Japan includes information on suspicious drugs; ADR diagnoses, which are expressed using the Medical Dictionary for Regulatory Activities preferred terms (MedDRA-PT); and patient background such as gender, age, and concomitant use of other drugs. Because the quotation frequency of MedDRA-PTs in ADR reports reflects the number of ADR events, the quotation frequency provides basic information for estimating the event frequency of each ADR. Ac-

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**To whom correspondence should be addressed:* Masahiro TOHKIN, Ph.D., *Division of Medicinal Safety Science, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan. Tel. +81-3-3700-9789, Fax. +81-3-3700-9788, E-mail: tohkin@nihs.go.jp*
The URL of the Drug Safety Information website of the Pharmaceuticals and Medical Devices Agency is "http://www.info.pmda.go.jp/fukusayou/menu_fukusayou_attention.html" and the URL of material from the Committee on Safety of Drugs of the Ministry of Health, Labour and Welfare is "http://www.mhlw.go.jp/shingi/yakuji.html#anzen".

Table 1. Accumulated number of each adverse drug reaction term in cases reported from April 2004 to February 2009

Classification of accumulated frequency of each ADR term	Number of ADR terms in each classification	Representative ADR terms concerning SJS/TEN, hepatotoxicity, and rhabdomyolysis*
>1,000	30	Hepatic function abnormal (4,866), Liver disorder (3,936), Rhabdomyolysis (1,648), Stevens-Johnson syndrome (1,202)
500–1,000	26	Toxic epidermal necrolysis (751), Jaundice (715)
100–499	221	Hepatitis fulminant (406), Hepatitis acute (388), Oculomucocutaneous syndrome (324), Hepatitis (310), Cholestasis (184)
<100	4,491	
Total	4,768	

Based on the open data source MHLW website: http://www.mhlw.go.jp/shingi/yakuji.html#anzen
*Accumulated frequency is listed in parentheses.

Table 2. Number of case reports concerning SJS/TEN for fiscal years 2005 to 2008

Year (fiscal) Molecular entities	2005			2006			2007			2008		
	SJS	TEN	Sum	SJS	TEN	Sum	SJS	TEN	Sum	SJS	TEN	Sum
Allopurinol	14	12	26	22	8	30	15	11	27	33	18	51
Carbamazepine	17	5	22	17	4	21	24	6	30	26	10	36
Diclofenac	9	5	14	8	2	10	6	1	7	7	9	16
Loxoprofen	9	2	11	11	5	16	7	7	14	12	9	21
Phenobarbital	5	5	10	2	4	6	6	0	6	8	4	12
Non-pyrines	6	4	10	2	4	6	1	9	10	2	4	6
Zonisamide	9	1	10	3	2	5	1	4	5	9	6	15
Acetaminophen	1	4	5	1	5	6	1	12	13	3	10	13
Mortality (rate, %)	13 (6)	41 (27)		14 (5)	41 (30)		17 (7)	36 (23)		12 (4)	50 (26)	
Total	223	151	374	271	136	407	260	156	416	289	189	478

Based on the open data source PMDA drug safety information website: http://www.info.pmda.go.jp/fukusayou/menu_fukusayou_attention.html

cording to material from the Committee on Safety of Drugs of the Ministry of Health, Labour and Welfare (MHLW, see footnote for URL address), the total number of MedDRA-PTs was 4,768 terms cited among 168,045 ADR events from domestic cases reported to PMDA from April 2004 to February 2009. As shown in **Table 1**, the number of MedDRA-PTs cited more than 100 times among 168,045 ADR events was 277. SJS/TEN, hepatotoxicity, and rhabdomyolysis are considered to be major ADRs in Japan because the frequently cited top 277 MedDRA-PTs included most ADR terms concerning SJS/TEN, hepatotoxicity, and rhabdomyolysis. In particular, accumulated quotation frequency of abnormal hepatic function (4,866) and liver disorder (3,936) ranked second and third, followed by interstitial pneumonia (5,190).

Major severe cutaneous ADRs, SJS and TEN, are life-threatening skin disorders which are often accompanied by high fever and systemic complications.[2,3] SJS/TEN incidence is generally very low and more than 100 different causative drugs have been reported.[4-6] We counted the event number for major suspected drugs in domestic cases concerning SJS and TEN from April 2005 to March 2009 based on the open data source of PMDA, and the results are shown in **Table 2**. Allopurinol, an anti-hyperuricemia drug, is the most frequently reported drug for SJS and TEN (**Table 2**). Many cases were reported with anticonvulsant drugs including carbamazepine (CBZ), phenobarbital, and zonisamide and non-steroidal anti-inflammation drugs (NSAID), which include diclofenac, loxoprofen, non-pyrines, and acetaminophen (**Table 2**). Although it is difficult to calculate the exact incidence of SJS and TEN in Japan, the Japanese mortality rates of SJS and TEN based on domestic cases were about 4–7% and 23–30%, respectively, which were in accordance with rates reported in other populations[7,8] (**Table 2**). Therefore, the mortality rate calculated by ADR reports can be a useful reference to estimate the mortality rate of severe ADRs such as SJS and TEN.

The liver is a common target for drug toxicity due to its pivotal role in drug metabolism. Moreover, any drug has the potential to cause liver injury.[9] Therefore, hepatotoxicity is the most common ADR, causing drug withdrawals and post-marketing regulatory decisions and