Stimulation of Human Monocytic THP-1 Cells by Metabolic Activation of Hepatotoxic Drugs

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Summary: Drug-induced liver injury (DILI) is thought to be involved in the participation of drugs that either directly affect the cell viability or elicit an immune response. However, there is limited information about the immune responses induced by drugs, including those drugs that are metabolically activated. In this study, we constructed an in vitro assay system to assess the involvement of immune-related factors induced by metabolic activation of drugs. To investigate whether CYP3A4-mediated metabolism of 10 hepatotoxic drugs is associated with immune-related responses, human monocytic leukemia THP-1 cells were co-incubated with CYP3A4 Supersomes. Cluster of differentiation (CD) 86 and CD54 expression levels on THP-1 cells were upregulated by treatment with albendazole and amiodarone (AMD), respectively, in the presence of CYP3A4. Additionally, N-desethylamiodarone (DEA), a major metabolite of AMD, upregulated the CD54 expression of THP-1 cells with CYP3A4. The release of interleukin (IL)-8 and tumor necrosis factor (TNF) α from THP-1 cells was significantly increased by the treatment of AMD or DEA with CYP3A4. Similarly, IL-8 and TNFα were also upregulated by the treatment of AMD and DEA with human liver microsomes, but were inhibited by adding ketoconazole to the cell culture. In this study, we first report that albendazole, AMD and DEA activate immune reaction when metabolically activated.

Keywords: adverse drug reactions; bioactivation; monocytes/macrophages; CYP3A4; drug-induced hepatotoxicity; inflammation

Introduction

Drug-induced liver injury (DILI) is a rare but serious adverse reaction to a large number of pharmaceutical drugs and the most common reason for restrictions or withdrawal from the market. Most such drugs are known to produce reactive metabolites after metabolic activation. It has been suggested that activation of the innate immune system by metabolic activation of drugs is involved in the pathogenesis of the immune-mediated drug-induced liver injury as one of the causes.

Cytochrome P450 (CYP) plays a prominent role in metabolic activation, resulting in the generation of a reactive metabolite. One of the CYP isoforms, CYP3A4, is the predominant isoform in liver and metabolizes more than 50% of the clinical drugs commonly used. On the other hand, CYP3A4 is one of the important enzymes involved in the metabolic activation of various drugs causing drug-induced liver injury.

There have been some reports about the immune responses caused by metabolic activation of drugs. For example, it has been reported that halothane is metabolized to trifluoroacetyl radicals by CYP2E1 and these covalently bind to target macromolecules. Sulfamethoxazole and its reactive metabolite nitroso sulfamethoxazole stimulate dendritic cells, resulting in the generation of co-stimulatory signals required to initiate a primary immune response. However, such drugs causing immune response after metabolic activation remain largely undefined.

Recently, the involvement of the immune system in drug-induced liver injury has been suggested using in vivo mouse models. Inflammatory reactions in the liver are induced by the activation of innate immune cells, such as monocytes, macrophages and Kupffer cells. Activated monocytes and
macrophages release large amounts of pro-inflammatory cytokines and chemokines, including tumor necrosis factor (TNF) α and interleukin (IL)-8. TNFα triggers the release of a cascade of other cytokines that recruit and activate lymphocytes and macrophages. IL-8 exhibits multiple effects on neutrophils, including the induction of lysosomal enzyme release, increase in the expression of adhesion molecules, and rapid infiltration. In a mouse model of halothane-induced liver injury, it was shown that the production of TNFα, IL-1β, IL-6 and IL-8 and neutrophil infiltration in liver play a critical role in immune-mediated liver injury.

In recent years, human monocytic cell lines have been used to examine the inflammatory responses mediated by drugs withdrawn from the market. In human monocytic THP-1 cells, the mRNA expression levels and/or the release of pro-inflammatory cytokines and chemokines were increased by treatment with troglitazone or ximelagatran. Additionally, our previous research revealed that albendazole and terbinafine also stimulate THP-1 cells resulting in the upregulation of the cell surface markers cluster of differentiation 86 and CD54 and the release of pro-inflammatory cytokines and chemokines from THP-1 cells in the presence or absence of CYP3A4 or human liver microsomes (HLM).

Materials and Methods

Materials: Albendazole and nitrofurantoin were obtained from Sigma-Aldrich (St. Louis, MO). Albendazole sulfoxide was obtained from Toronto Research Chemicals (Ontario, Canada). β-NADPH was from Oriental Yeast (Tokyo, Japan). Diclofenac, desipramine, nefazodone, tacrine and terbinafine were from Wako Pure Chemicals (Osaka, Japan). AMD and lefunomide were obtained from LKT Labs (St. Paul, MN) and Alexis Biochemicals (San Diego, CA), respectively. DEA (2-butyl-3-benzofuranyl 4-[2-(monoethylamino)ethoxy]-3,5-diiodophenyl ketone, a main metabolite of AMD) was kindly provided by Taisho Pharmaceutical (Tokyo, Japan). Human CYP3A4 Supersomes (recombinant cDNA-expressed P450 enzymes prepared from a baculovirus insect cell system) (testosterone 6β-hydroxylase activity is 22,059 pmol/mg × min) and control Supersomes and pooled HLM (n = 50, testosterone 6β-hydroxylase activity is 5,700 pmol/mg × min) were purchased from BD Gentest (Woburn, MA). The specific catalytic activities of each CYP isoform in these microsomes were provided by the manufacturer. Primers were commercially synthesized at Hokkaido System Sciences (Sapporo, Japan). All other reagents were of the highest grade commercially available.

Cell culture and drug treatment: Human monocytic leukemia cell line THP-1 was obtained from Riken Gene Bank (Tsukuba, Japan). THP-1 cells were cultured in RPMI 1640 medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA), and were maintained at 37°C under an atmosphere of 5% CO2. THP-1 cells were seeded at a density of 1 × 10⁶ cells/well in 24-well plates with the medium containing the indicated concentration of the drugs and 1 mM β-NADPH and 15 nM human CYP3A4 Supersomes, or control Supersomes or 0.3 mg/mL microsomal protein of human livers and then incubated at 37°C. The selected drugs have all been shown to be substrates of CYP3A4, albeit not exclusive to CYP3A4. The final concentration of dimethyl sulfoxide (DMSO) in the medium was 0.1%. Heat-inactivated (treated at 56°C for 30 min) HLM were used as a control.

Flow cytometry using monoclonal antibodies (mAbs): The expression of CD86 and CD54 on the THP-1 cells was measured as previously described with some modifications. In brief, cells were harvested 24 h after the treatment and washed twice with fluorescence-activated cell sorting (FACS) buffer (phosphate-buffered saline containing 1% bovine serum albumin). Cell surface staining was performed using the following fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies (mAbs): anti-human CD54 (clone; 6.5B5) from DAKO (Glostrup, Denmark), anti-human CD86 (clone; Fun-1) from BD Pharmingen (San Diego, CA); and FITC-labeled mouse IgG1 (clone; MOPC-21) from BD Pharmingen. Cells were incubated with the above mAbs at 6 µl/3 × 10⁵ cells/50 µl for anti-human CD54 mAb. In addition, FITC-labeled mouse IgG1 as an isotype control was used at a dilution of 3 µl/3 × 10⁵ cells/50 µl. Cells were incubated with these mAbs for 30 min at 4°C. After washing and resuspension with FACS buffer, the surface marker expression was measured and analyzed using FACS Calibur and Cell Quest Pro software (BD Biosciences, San Jose, CA). Propidium iodide (PI) was used at a concentration of 0.625 µg/mL, after which dead cells were gated out and the mean fluorescence intensity for each marker was recorded for a total of 10,000 living cells. The relative mean fluorescence intensity represented as percentage was calculated by taking the ratios of the mean fluorescence intensity of treated cells to that of controls. Cell viability was determined by the PI assay. If cell viability was less than 50%, the expression levels of this tested concentration was not calculated and included in the evaluation because of diffuse labeling cytoplasmic structures due to cell membrane destruction.

Enzyme-linked immunosorbent assay (ELISA): The pro-inflammatory cytokine TNFα and the chemokine IL-8 are reported to be sensitive markers in THP-1 cells. TNFα and IL-8 in cell supernatants were measured by Human TNFα or IL-8 ELISA Ready-SET-GO™ ELISA Kit (Shinya ENDO, et al. Copyright © 2012 by the Japanese Society for the Study of Xenobiotics (JSSX))
Real-time reverse transcription–polymerase chain reaction (RT-PCR): Total RNA was extracted from THP-1 cells with RNAsiso (Takara Bio, Shiga, Japan) according to the protocol supplied by manufacturer. The primers used in this study were human IL-8 (forward: 5'-CAGCCTTCTGATTCTCGAC-3', reverse: 5'-AGACAGAGCTCTTCCATCAG-3') and human TNFα (forward: 5'-CTTCTGCTGTGCATTTGGAG-3', reverse: 5'-GCTACAGCGCTTGTACCTCG-3'). The IL-8 and TNFα mRNA levels were normalized with human glycer-aldehyde 3-phosphate dehydrogenase (GAPDH) mRNA (forward: 5'-CCATGAGAATGATGACAACACGCG-3', reverse: 5'-TGGGTGGCAATGTGCGATTTGCC-3'). The reverse transcription process and real-time RT-PCR were performed as described previously.22

Quantification of AMD, DEA and DiDEA using high-performance liquid chromatography (HPLC): Quantification of AMD and DEA was performed according to our previous method with slight modifications.26 After treatment with 30 µM AMD or 20 µM DEA in the presence or absence of CYP3A4 for 12 and 24 h, 5 µM tolbutamide as an internal standard (IS) was added after the incubation and the THP-1 cells were collected. Cells were lysed by three freeze-thaw cycles. The reaction mixture was extracted with 4 mL of dichloromethane for 1 min. The organic layer was transferred to a clean test tube and was evaporated under a gentle stream of nitrogen at 40°C. The residue was redissolved in 100 µL of mobile phase, and an 80-µL portion was subjected to HPLC. HPLC analysis was performed using an L-7100 pump (Hitachi, Tokyo, Japan), 712 WISP intelligent sample processor (Waters, Tokyo, Japan), Chromatograph-integrator D-2000 (Hitachi), and CTO-6A column oven (Shimadzu, Kyoto, Japan) with a Capcell Pak CN UG120 (4.6 x 150 mm; 5 mm) column (Shiseido, Tokyo, Japan). The eluent was monitored at 240 nm using an SPD-6A UV detector (Shimadzu). The mobile phase was 32% acetonitrile, 1% acetic acid, and 0.2% diethylamine. The flow rate was 1.0 mL/min, and the column temperature was 35°C. Under these conditions, retention times of IS, di-N-desethylamiodarone (DiDEA), DEA and AMD were 3.5, 6.5, 9.5, and 13.0 min, respectively.

Cell viability assay: Cell viability was evaluated by the PI assay. After incubation, cells were washed twice with FACS buffer, then stained with PI (0.625 µg/mL), and the cell viability was measured using FACSCalibur and Cell Quest Pro software (BD Biosciences). Total events for living cell counting were 10,000.

Statistical analysis: Data are expressed as mean ± SD. Comparison of 2 groups was made with an unpaired, two-tailed Student’s t-test. Comparison of multiple groups was made with ANOVA followed by a Dunnett or Tukey test. A value of p < 0.05 was considered statistically significant.

### Table 1. Effects of various hepatotoxic drugs on cell viability of THP-1 cells in the PI assay

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (µM)</th>
<th>Cell viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CYP3A4 (-)</td>
<td>CYP3A4 (+)</td>
</tr>
<tr>
<td>0.1% DMSO</td>
<td>—</td>
<td>100.0 ± 1.5</td>
</tr>
<tr>
<td>Albenzoate</td>
<td>50</td>
<td>84.3 ± 2.1</td>
</tr>
<tr>
<td>Albenzoate sulfoxide</td>
<td>50</td>
<td>85.9 ± 2.6</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>30</td>
<td>90.4 ± 8.5</td>
</tr>
<tr>
<td>Desethylamiodarone</td>
<td>20</td>
<td>91.6 ± 2.3</td>
</tr>
<tr>
<td>Desipramine</td>
<td>30</td>
<td>97.3 ± 3.1</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>100</td>
<td>90.7 ± 1.8</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>100</td>
<td>81.0 ± 2.7</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>20</td>
<td>98.7 ± 2.3</td>
</tr>
<tr>
<td>Nefazodone</td>
<td>10</td>
<td>97.5 ± 2.4</td>
</tr>
<tr>
<td>Tacrine</td>
<td>100</td>
<td>100.9 ± 1.4</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>20</td>
<td>88.1 ± 5.5</td>
</tr>
<tr>
<td>Terbinafine</td>
<td>100</td>
<td>77.5 ± 5.7</td>
</tr>
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</table>

Data represent the mean ± SD (n = 3).

### Results

**Effect of CYP3A4 on various hepatotoxic drugs assessed by the expression of CDs in human monocytic THP-1 cells:**

To investigate whether metabolic activation of hepatotoxic drugs affects human monocytic cells, THP-1 cells were treated with the indicated concentrations (Table 1) of various hepatotoxic drugs for 24 h in the presence or absence of CYP3A4 Supersomes and then the expressions of CD86 and CD54 on the THP-1 cells were measured by FACS. The cell viability of THP-1 cells treated with these drugs was 70% or more (Table 1), which is consistent with the other report.27 In the presence of CYP3A4, CD86 expression in THP-1 cells was significantly increased by the treatment with albenzol and compared with control Supersomes but not by treatment with albenzol sulfoxide, the active metabolite of albenzol (Fig. 1A).28 The CD54 expression in THP-1 cells was significantly increased by the treatment with AMD and DEA, active metabolites of AMD, in the presence of CYP3A4, compared with control Supersomes (Fig. 1B). None of the other hepatotoxic drugs showed the effect of CYP3A4 metabolism on the expression of CD86 and CD54. These results suggested that albenzol, AMD and DEA have the ability to increase the CD expression levels leading to the activation of the immune responses. For the subsequent studies, the CYP3A4-dependent metabolism of AMD and DEA was focused on because AMD showed the highest activation and the major metabolite of AMD also activates the CD of THP-1 cells.

**Dose-dependent changes of the CD54 expression in THP-1 cells treated with AMD or DEA in the presence or absence of CYP3A4:** To investigate whether CYP3A4-dependent metabolism of AMD and DEA at low concentrations can affect the CD54 expression, THP-1 cells were treated with AMD or DEA at the indicated concentrations with or without CYP3A4 Supersomes and then the expressions of CD54 were measured by FACS. The CD54 expression in THP-1 cells treated with AMD or DEA in the presence of CYP3A4-supersomes was significantly increased compared with those treated with AMD or DEA in the absence of CYP3A4-supersomes (Fig. 1C).29
concentration for 24 h in the presence or absence of CYP3A4, and then the expression of CD54 was measured using FACS. As shown in Figure 2, AMD and DEA increased the CD54 expression in the presence of CYP3A4 compared with the control. With the CYP3A4 treatment, the expression of CD54 in THP-1 cells was significantly increased in an AMD dose-dependent manner and, to a lesser extent, increased CD54 expression induced by DEA.

Time-dependent changes of the mRNA expression and the release of IL-8 and TNFα in THP-1 cells treated with AMD or DEA in the presence or absence of CYP3A4: The time-dependent changes of the CYP3A4-dependent metabolism by AMD on the IL-8 and TNFα levels in THP-1 cells were investigated. When treated with 30 µM AMD in the presence of CYP3A4, the mRNA expression levels and the release of IL-8 and TNFα in THP-1 cells were significantly increased after incubation for 3 to 24 h compared with the control (Fig. 3). The mRNA expression levels of IL-8 were increased in a time-dependent manner, and were most increased after 12 h-incubation (Fig. 3A). The highest increase of the mRNA expression and release of TNFα was shown at 12 h- and 6 h-incubation (Figs. 3B and 3D).

When treated with 20 µM DEA in the presence of CYP3A4, the mRNA expression of IL-8 was most increased at 3 h-incubation (Fig. 4A). The highest increase of the mRNA expression levels and release of TNFα was shown at 6 h-incubation (Figs. 4B and 4D). To investigate the effect of AMD metabolism, the incubation time of 12 h was selected for the subsequent study to measure the formation of AMD, DEA and DiDEA. To investigate whether there were cytotoxic effects on THP-1 cells caused by the leakage of intracellular cytokines and chemokines, a cell viability assay was performed in THP-1 cells. At 24 h-incubation, AMD and DEA had slight cytotoxic effects on the THP-1 cells (Supplemental Fig. 1).

Quantification of metabolites of AMD and DEA using HPLC: The formation of the metabolites of AMD by CYP3A4 Supersomes was measured by HPLC. The concentrations of AMD, DEA and DiDEA were determined 12 and 24 h after the treatment with 30 µM AMD (Table 2) or 20 µM DEA (Table 3) in the presence or absence of
CYP3A4. The metabolites of AMD, DEA and DiDEA were detected only in the presence of CYP3A4 Supersomes 24 h after AMD treatment (Table 2). DiDEA was detected in the presence of CYP3A4 Supersomes after 24 h treatment with AMD and in the presence of CYP3A4 Supersomes after 12 and 24 h treatment with DEA (Table 3). The AMD concentrations decreased time-dependently after 24 h exposure, but the DEA concentrations slightly decreased after 24 h exposure, and thus DiDEA was likely not efficiently biotransformed. In contrast, incubation with control Supersomes did not generate detectable amounts of the metabolites with either AMD or DEA.

Table 2. Metabolism of AMD by CYP3A4 Supersomes for 12-h- and 24-h-incubation

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>AMD (µM)</th>
<th>DEA (µM)</th>
<th>DiDEA (µM)</th>
<th>AMD (µM)</th>
<th>DEA (µM)</th>
<th>DiDEA (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>33.4 ± 8.0</td>
<td>ND ND</td>
<td>ND ND</td>
<td>16.0 ± 1.4</td>
<td>ND ND</td>
<td>ND ND</td>
</tr>
<tr>
<td>12</td>
<td>27.6 ± 2.1</td>
<td>ND ND</td>
<td>ND ND</td>
<td>19.4 ± 3.1</td>
<td>ND ND</td>
<td>ND ND</td>
</tr>
<tr>
<td>24</td>
<td>28.6 ± 2.6</td>
<td>ND ND</td>
<td>ND ND</td>
<td>19.2 ± 6.5</td>
<td>ND ND</td>
<td>ND ND</td>
</tr>
</tbody>
</table>

Table 3. Metabolism of DEA by CYP3A4 Supersomes for 12-h- and 24-h-incubation

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>AMD (µM)</th>
<th>DEA (µM)</th>
<th>DiDEA (µM)</th>
<th>AMD (µM)</th>
<th>DEA (µM)</th>
<th>DiDEA (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>ND ND</td>
<td>16.0 ± 1.4</td>
<td>ND ND</td>
<td>ND ND</td>
<td>17.5 ± 3.6</td>
<td>ND ND</td>
</tr>
<tr>
<td>12</td>
<td>ND ND</td>
<td>19.4 ± 3.1</td>
<td>ND ND</td>
<td>ND ND</td>
<td>15.3 ± 5.0</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>24</td>
<td>ND ND</td>
<td>19.2 ± 6.5</td>
<td>ND ND</td>
<td>ND ND</td>
<td>19.2 ± 6.2</td>
<td>0.5 ± 0.3</td>
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</table>

THP-1 cells were treated with 30 µM AMD for 12 and 24 h in the presence or absence of CYP3A4 (15 pmol/mL). Data represent the mean ± SD (n = 3). ND, not detectable.

THP-1 cells were treated with 20 µM DEA for 12 and 24 h in the presence or absence of CYP3A4 (15 pmol/mL). Data represent the mean ± SD (n = 3). ND, not detectable.
and TNFα were significantly increased after 12 h- to 24 h-incubation and 6 h- to 12 h-incubation compared with the control, respectively (Figs. 5A and 5B). When treated with 20 µM DEA in the presence of HLM, the mRNA expression of IL-8 in THP-1 cells was significantly increased after 12 h- to 24 h-incubation compared with the control, whereas TNFα was significantly increased at 12 h compared with the control (Figs. 5C and 5D).

Effects of ketoconazole (KCZ) on the mRNA expression of IL-8 in THP-1 cells treated with AMD and DEA in the presence or absence of HLM: To confirm the effects of the metabolic activation by CYP3A4, the IL-8 mRNA expression was investigated because it showed the highest sensitivity in Figure 5. The effect of KCZ, a specific and potent CYP3A4 inhibitor, on the expression of IL-8 in THP-1 cells treated with AMD and DEA in the presence of HLM was investigated. As shown in Figure 6, the mRNA expression of IL-8 was upregulated by the treatment with AMD and DEA with HLM, and these effects were completely suppressed by the co-treatment with KCZ.

Discussion

It has been suggested that the activation of the innate immune system by metabolic activation of drugs is involved in immune-mediated drug-induced liver injury as one of the causes. However, limited information is available about the immune responses caused by metabolic activation. Inflammatory reactions in liver are induced by the activation of immune cells such as monocytes, macrophages and Kupffer cells. Human monocytic THP-1 cells are suggested to be useful for predicting sensitizers by measuring the cell surface molecules CD86, a protein that provides signals necessary for T cell activation and survival, and CD54, a protein known as inter-cellular adhesion molecule 1 (ICAM-1). A previous report indicated that PMA/TNFα-treated THP-1 cells did not show a perceptible up-regulation of the expression of CD54 or CD86 with either 2,4-dinitrochloro-benzene (DNCB) or DMSO treatment. In our experiments, PMA-treated THP-1 cells showed no difference in the sensitivity to the drug. Moreover, our previous research revealed that albendazole and terbinafine directly stimulate THP-1 cells resulting in IL-8 and TNFα release, suggesting the involvement of an immune-mediated pathway in DILI. Therefore, we used CD86 and CD54 expression and the release of IL-8 and TNFα as markers for predicting the activation of the immune reaction of drugs in this study. In the present study, we investigated whether bioactivation of hepatotoxic drugs stimulates THP-1 cells, resulting in
upregulation of cell surface CD markers and the release of pro-inflammatory cytokines and chemokines in the presence or absence of CYP3A4 or HLM.

First, we investigated the effect of CYP3A4-mediated metabolism on the expression of CDs in THP-1 cells by using FACS analyses. Ten drugs that are already known to cause hepatotoxicity in humans and to be associated with metabolic activation by CYP3A4 were selected. The production of active metabolites of albendazole, albendazole sulfoxide, in human liver is mediated via both FMO and CYP, principally CYP3A4.29 The major metabolite of AMD, DEA, was reported to cause cytotoxicity in HepG2 cells and rat hepatocytes at lower concentrations than the parent drug, AMD.30 DEA is produced mainly by CYP3A4 as well as CYP2C8 in humans.26 As shown in Table 1, we carefully followed the cell viability to determine the drug concentration that would be less than that showing the typically decreased cell viability by the parent drugs. However, for this kind of in vitro assay, it is recommended to perform it at a concentration up to 30 times the clinically efficacious concentration or 100 µM.31 The 10 drugs selected in this study were reported to be metabolically activated by CYP3A4, but many drugs showed negative responses in terms of the expression of CD86 and CD54 in the presence of CYP3A4. The reason why many drugs showed no upregulation of the CDs expression could be explained by the following three points. First, the involvement of metabolic activation was hardly observed.

Fig. 5. Time-dependent changes in mRNA levels and release of IL-8 and TNFα in THP-1 cells after exposure to 30 µM AMD and 20 µM DEA in the presence of HLM

THP-1 cells were treated with 30 µM AMD and 20 µM DEA in the presence of 0.3 mg/mL HLM for 3, 6, 12, or 24 h. The mRNA expression levels of IL-8 (A, C) and TNFα (B, D) in THP-1 cells were measured by real-time RT-PCR analysis. The levels of IL-8 or TNFα mRNA were normalized to the levels of GAPDH mRNA. Data represent the mean ± SD (n = 3). *p < 0.05 and **p < 0.01, compared with CYP3A4 (−) groups at each time point.

Fig. 6. Effects of KCZ on the mRNA expression levels of IL-8 in THP-1 cells treated with AMD and DEA in the presence of HLM

THP-1 cells were treated with 30 µM AMD and 20 µM DEA in the presence of 0.3 mg/mL HLM with or without 1 µM KCZ for 24 h. The mRNA expression levels of IL-8 in THP-1 cells were measured by real-time RT-PCR analysis. The levels of IL-8 or TNFα mRNA were normalized to the levels of GAPDH mRNA. Data represent the mean ± SD (n = 3). **p < 0.01, compared with CYP3A4 (−) groups.
because of the greater effect of the parent drug, such as albendazole and terbinafine. Second, CYP3A4 is not the main or most potent enzyme in the metabolic activation of drugs such as diclofenac and leflunomide. Third, as the most plausible reason, metabolic activation of drugs affects toxicity by two different mechanisms: direct cellular damages to parenchymal cells and activation of immune cells. Many of the drugs investigated in this study actually exhibited direct cellular toxicity to cells, such as HepG2 cells and hepatocytes, as we previously reported. However, the concentration of drugs did not prominently cause the decreasing cell viability in THP-1 cells (Table 1). This is thought to be due primarily to the differences in sensitivity among the cells.

The CD86 expression in THP-1 cells was significantly increased by the treatment with albendazole in the presence of CYP3A4 compared with control Supersomes, but not by the metabolite albendazole sulfoxide (Figs. 1A and 1B). This result is consistent with our previous report that albendazole sulfoxide did not increase pro-inflammatory cytokine release from THP-1 cells directly. However, albendazole stimulated the expression of CD86, and there was further increase by CYP3A4 but not CYP34, whereas AMD and DEA stimulated the expression of CD54, and there was further increase by CYP3A4 but not CYP34 (Figs. 1A and 1B). To explain these results, it has been suggested that phenotypic changes through signal transduction pathways and a cross talk between MAP kinase signaling pathways differ according to the drug/chemical.

CD54 and IL-8 are considered to be good markers for the activation of dendritic cells and THP-1 cells by sensitizers. In the present assay system, we first demonstrated that AMD and DEA incubated with CYP3A4 stimulated THP-1 cells, resulting in significant increases in the CD54 expression and IL-8 and TNFα release (Figs. 3 and 4). Similar metabolic activation was obtained by using HLM instead of CYP3A4 Supersomes (Figs. 5 and 6), indicating that the result could be extrapolated to in vivo. From these lines of data, the present assay system could evaluate the activation of immune-related cells with a parent drug and metabolites concurrently.

AMD has been reported to cause liver injury, but many experimental reports suggested that tissue accumulation of the parent compound as well as that of metabolites eventually results in cellular toxicity due to impaired mitochondrial function. However, several case reports suggested different mechanisms of liver injury by AMD, including immune-mediated centrilobular necrosis, neutrophilic infiltration and portal inflammation. In vitro studies also revealed that high CYP3A4 activity is a risk factor for the hepatotoxicity associated with AMD. In 1996, Futamura reported that AMD caused an increase in the production of pro-inflammatory cytokine IL-1α, IL-1β and TNFα from alveolar macrophages. We also previously reported that albendazole and structurally similar drugs have the ability to stimulate the release of IL-8 and TNFα.

In conclusion, we first revealed that AMD and DEA were metabolically activated by CYP3A4, resulting in the upregulation of CD54 expression and IL-8 and TNFα release from THP-1 cells. It is suggested that metabolic activation of AMD elicits the inflammatory response in monocytes and macrophages, which might partially contribute to immune-mediated liver injury. Moreover, we constructed a cell-based system for investigating the immune response by drugs including metabolic activation. This assay system is useful for assessing the metabolic activation by CYPs or HLM and may be beneficial for predicting risks for immune-mediated liver injury by drugs in preclinical drug development.

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