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The role of CYP 3A4 and 1A1 in amiodarone-induced hepatocellular toxicity☆

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Abstract

Amiodarone is a widely used potent antiarrhythmic for the treatment of cardiac disease; however, its use is often discontinued due to numerous adverse effects, including hepatotoxicity. To investigate the role of drug metabolism in this liver toxicity, amiodarone and its major metabolite desethylamiodarone were incubated with HepG2 cells overexpressing a series of cytochrome P450 (CYP) isoforms. Significantly higher cytotoxicity of amiodarone was observed in HepG2 cells overexpressing CYP3A4 or CYP1A1, compared with that observed in empty vector transduced control cells. Further, higher levels of the more potent hepatotoxic metabolite desethylamiodarone were detected in CYP3A4 or CYP1A1 expressed cells. The CYP3A4 inhibitor ketoconazole and the CYP1A1 inhibitor α -naphthoflavone drastically inhibited the metabolism of amiodarone to desethylamiodarone. Along with the inhibition of CYP1A1 or CYP3A4, the cytotoxicity of amiodarone was significantly reduced. These data indicate that the metabolism of amiodarone to desethylamiodarone by CYP1A1 or CYP3A4 plays an important role in the hepatocellular toxicity of amiodarone.

Keywords

Amiodarone; Desethylamiodarone; Amiodarone metabolism; Cytochrome P450s (CYPs); HepG2 cells; HepG2 expressing CYP

1. Introduction

Amiodarone (2-*n*-butyl-3-[3,5 didiodo-4-diethylaminoethoxybenzoyl]-benzofuran), an iodine-containing benzofuran derivative, is a commonly used potent class III antiarrhythmic for the treatment of a wide spectrum of cardiac arrhythmias (Waldhauser et al., 2006). However, the use of this antiarrhythmic drug is often restricted by its numerous side effects,

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Conflict of interest

None.

such as pulmonary toxicity (Sobol and Rakita, 1982), hepatotoxicity (Lewis et al., 1989), and thyroid toxicity (Harjai and Licata, 1997). Although the pulmonary toxicity is the most serious adverse effect of amiodarone, more than 20% of patients have been reported to have hepatic injury as indicated by elevations in serum aminotransferases (Lewis et al., 1989).

The mechanisms of amiodarone's toxicity are not fully understood. The systemic toxic effects are presumed to be related to phospholipid accumulation in target tissues. Alveolar macrophages with phospholipid-packed lysosomes were enlarged in lung tissue, and amiodarone is also highly concentrated in hepatic lysosomes (Lewis et al., 1989). It has also been reported that impaired mitochondrial function contributes to the hepatic toxicity of amiodarone (Zahno et al., 2011).

Many studies suggested that the metabolism of amiodarone is involved in its hepatic toxicity (Shayeganpour et al., 2006; Waldhauser et al., 2006). Amiodarone is eliminated primarily by metabolism following oral administration (Latini et al., 1984). In humans, amiodarone is rapidly metabolized to mono-N-desethylamiodarone and di-N-desethylamiodarone by cytochrome P450 (CYP)-catalyzed N-deethylation (Flanagan et al., 1982; Ha et al., 2005; Zahno et al., 2011). Both amiodarone and its major metabolite desethylamiodarone are highly lipophilic and can accumulate in multiple tissues, especially the highly perfused and lipid-rich liver (Lewis et al., 1989).

Various CYP isoforms have been reported to be involved in the metabolism of amiodarone in humans (Elsherbiny et al., 2008, 2010; Ohyama et al., 2000b; Zahno et al., 2011). CYP1A1, 2C8, 2D6, and 3A4 showed potential in converting amiodarone to desethylamiodarone *in vitro* using recombinant CYP isoenzymes (Elsherbiny et al., 2008). Ohyama et al. (2000b) reported that amiodarone can inactivate CYP3A4 and that desethylamiodarone can inhibit CYP1A1, 1A2, 2B6, and 2D6 in microsomes from β -lymphoblastoid cells. Zahno et al. (2011) further demonstrated that human CYP3A4 played an important role in the mitochondrial toxicity of amiodarone, making metabolism by CYP3A4 a major risk factor in amiodarone's liver toxicity.

To characterize better the role of human CYP isoforms in mechanisms related to drug-induced liver toxicity and to identify the responsible CYPs, we previously generated a comprehensive set of HepG2-derived cell lines that stably express fourteen individual CYPs (1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 3A4, 3A5 and 3A7) and demonstrated the usefulness of these cells in evaluating CYP-mediated toxicity of drugs (Xuan et al., 2015). Our preliminary data showed that amiodarone remarkably decreased cell viability in HepG2 cells overexpressing CYP1A1 or CYP3A4 (Xuan et al., 2015). To study further the role of CYP1A1 and CYP3A4 in the metabolism of amiodarone, the major metabolite desethylamiodarone was quantified in these HepG2 cells after the treatment with amiodarone. The significant contribution of CYP1A1 and 3A4 to the hepatotoxicity of amiodarone was also confirmed using the CYP1A1- and CYP3A4-specific inhibitors α -naphthoflavone and ketoconazole.

2. Materials and methods

2.1. Chemicals and reagents

Dulbecco's Modified Eagle's Medium (DMEM), amiodarone hydrochloride, formic acid, dimethyl sulfoxide (DMSO), α -naphthoflavone, and ketoconazole were purchased from Sigma-Aldrich (St. Louis, MO). Desethylamiodarone hydrochloride, amiodarone-d4 hydrochloride, and desethylamiodarone-d4 hydrochloride were obtained from Toronto Research Chemicals Inc. (Toronto, ON, Canada). Blastocidin S hydrochloride was purchased from Life Technologies (Grand Island, NY). Human microsomes were obtained from BioreclamationIVT (Baltimore, MD). Water and acetonitrile were LC-MS grade and obtained from Fisher Scientific (Pittsburgh, PA).

2.2. Cell culture

The human hepatic cell line HepG2 was purchased from American Type Culture Collection (ATCC; Manassas, VA). HepG2 cells were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, 100 μ g/ml streptomycin, and 0.25 μ g/ml fungizone. Stably CYP overexpressing HepG2 cells were maintained in the above growth medium supplemented with 2.5 μ g/ml blastocidin. All cells were grown at 37°C in a humidified atmosphere with 5% CO₂. Cells were seeded at a density of $3\text{--}5 \times 10^5$ cells/ml in 96-well tissue culture plates or 60-cm tissue culture dishes and were allowed to adhere for 24 h prior to treatment with drugs or vehicle (Xuan et al., 2015).

2.3. Cytotoxicity assay

At therapeutic doses, the mean plasma concentration of amiodarone in patients is approximately 1.2 μ M and peak concentrations can reach 3.2 μ M (Lafuente-Lafuente et al., 2009). Studies have revealed that amiodarone attains high concentrations in various tissues, especially in fat and liver tissues (Adams et al., 1985). The estimated amiodarone concentration in liver is 24 μ M (Lafuente-Lafuente et al., 2009; Weiss, 1999). The tissue disposition of amiodarone is believed to be associated with lipid-rich inclusion bodies that can act as traps for the lipophilic amiodarone (Weiss, 1999). Therefore, clinically relevant amiodarone concentrations of 6.25 to 100 μ M were used for cytotoxicity evaluation and metabolite detection in both our previous study (Xuan et al., 2015) and the current study. Cell viability was determined at the end of a 6- or 24-h drug exposure period using the CellTiter-Glo® Luminescent Cell Viability Assay (Promega Corporation, Madison, WI) as described previously (Xuan et al., 2015).

2.4. Amiodarone and desethylamiodarone quantification

At the end of treatment, cells were harvested by digesting with 0.25% trypsin and washed 3 times with PBS. After adding 200 μ l of Nanopure water, the cells were lysed by three freeze and thaw cycles and then subjected to centrifugation. The supernatant was extracted using five volume of acetonitrile containing 100 ng/ml of the two deuterated internal standards, vortexed, and sonicated for 20 min. Samples were centrifuged at 12,000 rpm for 5 min and the supernatant was loaded into sample vials for HPLC-MS analysis. Samples were injected onto a Waters e2695 Alliance HPLC System coupled with a Waters ACQUITY QDa Mass

Detector. Amiodarone, desethylamiodarone, and their deuterated internal standards were eluted on a Waters XBridge BEH-C8 column (3.0×75 mm, 2.5 µm) at 4°C with mobile phases of LC–MS grade water (A) and acetonitrile (B), both containing 0.1% formic acid, at a flow rate of 0.5 ml/min. Elution started with 10% solvent B for 0.5 min, followed by a linear gradient elution of 10–90% solvent B in 14.5 min, returning solvent B to 10% in 0.5 min, and maintained for 4.5 min to re-equilibrate the column. The eluate was detected by mass spectrometry with an electrospray ion source operating in the positive ion mode (ESI⁺) using single ion recording (SIR). The monitored (M + H)⁺ ions were *m/z* 646 for amiodarone, *m/z* 650 for amiodarone-d4, *m/z* 618 for desethylamiodarone, and *m/z* 622 for desethylamiodarone-d4. Amiodarone and desethylamiodarone were quantified using calibration curves ranging from 1 ng/ml up to 10 µg/ml using Waters Empower 3 software. The protein concentration of sample supernatants was measured with a Millipore Direct Detect spectrometer (Darmstadt, Germany) following the manufacturer's instructions. The final results were expressed as µg analyte/mg protein.

2.5. Microsomal incubation

Microsomal incubation was carried out in 200 µl of 0.1 M potassium phosphate buffer (pH 7.4) containing 1 mg/ml human liver microsomes, 5 mM MgCl₂, 1 mM NADPH, and 12.5 µM amiodarone for 60 min at 37°C. The metabolism of amiodarone in the presence of CYP inhibitor was conducted under the same conditions. The CYP inhibitor α-naphthoflavone or ketoconazole was pre-incubated for 10 min in the reaction mixture; then 12.5 µM amiodarone was added. After incubation for 60 min, five volumes of ice-cold acetonitrile extraction solvent were added to quench the reaction. The extracted supernatants were analyzed using HPLC-QDa mass spectrometry.

2.6. Statistical analysis

All data are expressed as mean±SD. Statistical analyses were carried out using one-way ANOVA, followed by Dunnett's post hoc test, or unpaired Student's *t* test in GraphPad Prism 5 (San Diego, CA). The level of statistical significance was set at *p* < 0.05.

3. Results

3.1. Cytotoxicity of amiodarone on HepG2-CYP overexpressing cells

To investigate the metabolism of hepatotoxic drugs, we developed a panel of metabolically competent HepG2 cell lines by lentiviral-mediated transduction of fourteen human CYP isoforms. The cytotoxic effects of amiodarone were assessed in these established cell lines after treatment with 12.5 µM of amiodarone for 6 h or 24 h. As shown in Fig. 1, the cell viability was slightly decreased in CYP1A1 and CYP3A4 overexpressing HepG2 cells after 6-h exposure although no statistical significance was observed at this short-term time point; however, cell viability was decreased significantly following amiodarone exposure in HepG2 cells overexpressing CYP1A1 or CYP3A4 compared to control HepG2/empty vector cells at 24 h. These data indicate that the metabolism of amiodarone by these two CYP enzymes contributes to the drug's hepatocellular toxicity.

3.2. Cytotoxicity of desethylamiodarone versus amiodarone on HepG2 cells

The lower cell viability observed in HepG2 cells overexpressing CYP1A1 or CYP3A4 may be attributed to the combined cytotoxicity arising from both the parent amiodarone and any possible CYP-mediated metabolites. To investigate this possibility, a direct comparison was made between the cytotoxicity of amiodarone *versus* its primary metabolite desethylamiodarone in HepG2 cells. Cells were treated with amiodarone or desethylamiodarone at different concentrations and time periods. Fig. 2 shows the relative viability of HepG2 cells after treatment with these compounds at concentrations of 6.25–100 μ M for 6 h or 24 h. Desethylamiodarone treatment resulted in significantly less cell viability at all tested concentrations for both the 6 and 24 h-incubation periods compared to that with amiodarone treatment. This demonstrated the higher cytotoxic potential of desethylamiodarone to HepG2 cells compared to its parent amiodarone.

3.3. Detection of desethylamiodarone and amiodarone

To investigate the formation of desethylamiodarone after amiodarone treatment, an HPLC mass spectrometry method was established based on the SIR monitoring of amiodarone (m/z 646), amiodarone-d4 (m/z 650), desethylamiodarone (m/z 618), and desethylamiodarone-d4 (m/z 622). Fig. 3 shows representative HPLC–MS chromatograms of the amiodarone, desethylamiodarone, and their deuterated internal standards detected in human microsomal incubations with 12.5 μ M amiodarone. The retention times of amiodarone and amiodarone-d4 were 10.69 min while desethylamiodarone and desethylamiodarone-d4 eluted at 10.24 min. Linear quantification ranges from 1 ng/ml up to 10 μ g/ml were established with R^2 0.999 for both amiodarone and desethylamiodarone.

3.4. Desethylamiodarone formation in HepG2 cells overexpressing various CYPs

The amount of the metabolite desethylamiodarone formed in HepG2 cells overexpressing 14 individual CYPs was measured after amiodarone treatment. As shown in Fig. 4, significantly increased levels of desethylamiodarone were found in CYP1A1 and CYP3A4 overexpressing HepG2 cells when compared with that of control cells. Considering the higher cytotoxic potency of desethylamiodarone to HepG2 cells (Fig. 2), this would explain why amiodarone treatment leads to lower cell viability in CYP1A1- and CYP3A4-overexpressing HepG2 cells.

3.5. Effect of CYP1A1 and CYP3A4 inhibition on the desethylamiodarone formation

To elucidate further the role of desethylamiodarone formation in cytotoxicity of amiodarone, CYP1A1 and 3A4 activities were inhibited with α -naphthoflavone and ketoconazole, respectively, in human microsomal incubations (Fig. 5A) or CYPs overexpressing HepG2 cells (Fig. 5B). Using P450-Glo™ Assays (1A1 and 3A4), and at the concentrations used in the CYP450 inhibition studies, α -naphthoflavone and ketoconazole were confirmed to abolish enzymatic activity of CYP1A1 or 3A4 (data not shown). Fig. 5A shows the amounts of parent amiodarone remaining and desethylamiodarone formed in human microsomes incubated with 12.5 μ M amiodarone for 60 min. The CYP1A1 inhibitor α -naphthoflavone decreased desethylamiodarone levels from 1.960 ± 0.020 μ g/ml to 0.453 ± 0.057 μ g/ml ($p < 0.05$). Similarly, the CYP3A4 inhibitor ketoconazole significantly inhibited

desethylamiodarone formation to 0.020 ± 0.000 $\mu\text{g/ml}$ ($p < 0.05$) compared with amiodarone treatment alone. In addition, the remaining levels of amiodarone were significantly higher in the microsomal incubations containing the inhibitors. Fig. 5B shows the effects of CYP inhibitors on amiodarone metabolism in intact HepG2 cells. Treatment with α -naphthoflavone decreased desethylamiodarone formation from 4.647 ± 0.630 $\mu\text{g/mg protein}$ to 0.115 ± 0.021 $\mu\text{g/mg protein}$ in CYP1A1 overexpressed HepG2 cells. Co-incubation with 10 μM ketoconazole also inhibited the formation of desethylamiodarone to 0.165 ± 0.041 $\mu\text{g/mg protein}$ in CYP3A4 overexpressed HepG2 cells. Higher levels of parent amiodarone remained in both cell lines after inhibition of CYP1A1 or CYP3A4.

3.6. Effect of CYP1A1 and CYP3A4 inhibition on the cytotoxicity of amiodarone in HepG2 cells

The above experiments showed CYP1A1 and CYP3A4 inhibition can significantly reduce formation of the potent cytotoxic metabolite desethylamiodarone in both human microsomal incubations and intact HepG2 cells. On the basis of this observation, we tested whether inhibition of these two CYPs has an effect on the cytotoxicity of amiodarone in HepG2 cells. Cells were pre-incubated with inhibitor for 60 min before amiodarone treatment. Fig. 6 shows amiodarone treatment alone exhibited significantly greater cytotoxicity in CYP1A1 overexpressed HepG2 cells compared with the control cells. Co-incubation of α -naphthoflavone attenuated toxicity in CYP1A1 overexpressing HepG2 cells, although the cytotoxicity was still higher than that observed in control cells. Ketoconazole treatment completely eliminated the cytotoxicity of amiodarone in CYP3A4 overexpressed HepG2 cells.

4. Discussion

Recent *in vitro* work has demonstrated that the metabolism of amiodarone contributes to its toxicity and that a number of CYPs, notably CYP3A4, are directly involved with the metabolism-mediated toxicity caused by amiodarone (Zahno et al., 2011). We aimed to identify and study the contribution of various human CYP isoforms in amiodarone's hepatic toxicity. Taking advantage of our previously established cell lines, which can be utilized to study the involvement of 14 individual CYPs, we treated each of these CYP-overexpressed HepG2 cell lines with 12.5 μM amiodarone and then investigated the metabolic profiles, compared the resulting cytotoxicity, and quantified the formation of the major metabolite desethylamiodarone.

With enzymatic analysis using different recombinant human CYPs and inhibitors, Elsherbiny et al. have reported that multiple forms of CYPs participate in the biotransformation of amiodarone, with human CYP1A1 and 3A4 showing the highest efficiency in converting amiodarone to desethylamiodarone (Elsherbiny et al., 2008). In line with these findings, our study also indicated that CYP enzymes 1A1 and 3A4 are the two major enzymes that metabolize amiodarone, as the formation of desethylamiodarone was significantly increased in CYP1A1 or 3A4 overexpressed cells, compared to the other forms of CYPs or control cells (Fig. 4). Further, cell viability was significantly decreased by 12.5 μM amiodarone exposure in CYP1A1- or CYP3A4-expressing cells compared to its

counterpart, HepG2/empty vector (Fig. 1). Other studies have reported that CYP 2C8 is involved in the metabolism of amiodarone and formation of desethylamiodarone (Ohya et al., 2000a). In our study, we also observed an increase in desethylamiodarone formation in CYP2C8-expressing cells (Fig. 4); however, the extent was much less than observed with CYP1A1- or CYP3A4-expressing cells and there was not an increase in toxicity (Fig. 1). These results suggest that the measured cytotoxic effects of amiodarone are mainly attributed to metabolism by CYP3A4 and 1A1. These data are supported by the experiment where amiodarone-induced toxicity was attenuated (Fig. 6) when desethylamiodarone formation was blocked by CYP 3A4 and 1A1 inhibitors (Fig. 5B).

CYP3A4 is the one of the most important drug metabolizing enzymes, both in terms of the abundance (accounting for approximately 30% of the total cytochrome P450 protein in human liver) and the number of drugs metabolized (more than 50% of therapeutics are metabolized by CYP3A4) (Guengrich, 1999). Remarkable high inter-individual variability of CYP3A4, with 50-fold differences in CYP3A4 activity and 641-fold differences in gene expression among human population, has been documented (Rodriguez-Antona et al., 2001; Yang et al., 2013).

Multifactorial components contribute to inter-individual variability of CYP3A4, including genetic variations, environmental and dietary exposure, pathological and hormonal modulation. Genetic variants have been found in CYP3A4 (www.cypalleles.ki.se) and some of the genetic variants may influence the level of expression or function (catalytic activity) of CYP3A4 protein. Whether or not genetic variants modulate inter-individual variability of CYP3A4 protein production among human populations is not fully understood, clinic data suggested that there are associations between CYP3A4 polymorphisms and drug metabolisms; thus could impact drug efficiency and toxicity. For example, the calcineurin inhibitor tacrolimus is metabolized by CYP3A4 and 3A5, a phenotype-genotype association study suggested that the CYP3A4 wild-type carriers need higher doses of tacrolimus to have pharmacological effects owing to a faster clearance rate in these patients, compared to the mutant (CYP3A4*1B) homozygotes (Hesselink et al., 2003). In another example with anti-cancer drug docetaxel, inter-individual variability in CYP3A4 activity played a key role in drug clearance and toxicity. Docetaxel exhibited a much higher risk of drug toxicity among patients with lower CYP3A4 activity, due to a decreased clearance rate of docetaxel (Hirth et al., 2000). Since amiodarone is metabolized by CYP3A4 and amiodarone-induced hepatocellular toxicity is metabolite-dependent, the inter-individual variability of CYP3A4 may have clinic implications. When the drug is prescribed, health service providers should consider the possibility that high dose amiodarone may have a potential to cause liver toxicity in patients with high CYP3A4 activity.

Xenobiotics can modulate CYP3A4 enzymatic activity by inhibiting or inducing protein production. CYP3A4 inhibition is usually achieved through competitive binding of an inhibitor to the enzyme active sites, in such a way the enzymatic activity of CYP3A4 towards metabolizing designated drugs is decreased (Lynch and Price, 2007). A variety of drugs, such as clarithromycin, diltiazem, erythromycin, itraconazole, and ketoconazole, are potent inhibitors for CYP3A4 (Lynch and Price, 2007). On the other hand, the induction of CYP3A4 is commonly mediated by nuclear receptors, constitutive androstane receptor

(CAR), and pregnane X receptor (PXR) (Pascussi et al., 2000). The inhibition and induction of CYP3A4 could result in clinic significance by drug-drug interactions that can cause adverse drug reactions or therapeutic failure (Lynch and Price, 2007). Drug interactions with amiodarone have been reported to have dramatic effects on the kinetics and metabolisms of cardiovascular therapeutics, and thus cause adverse reactions of a number of drugs, including procainamide, aprindine, warfarin, digoxin, quinidine, and beta-blocking agents (Marcus, 1983). On the other hand, other drugs can induce CYP3A4 and in turn affect the therapeutic effects of amiodarone, and potentially cause toxicity (Vrobel et al., 1989). Therefore, when amiodarone and CYP3A4 inducers (such as carbamazepine, phenobarbital, phenytoin, and rifampin) are prescribed together to patients, close monitoring the side effects may be important.

CYP3A4 has been found to be the major enzyme involved in the metabolism of drugs. By contrast, CYP1A1 has a more limited role, most likely due to the low basal expression level in human liver cells. However, CYP1A1 can be induced at both the mRNA and enzyme levels by a variety of chemicals *via* regulation of the aryl hydrocarbon receptor signaling pathway. As such, large variations in the induction of CYP1A1 have been reported in human populations (Ma and Lu, 2007). This CYP1A1 induction has been recognized to have impact on the toxicity and carcinogenicity of many environmental chemicals and drugs (Ma and Lu, 2007). Besides classes of compounds, such as polycyclic aromatic hydrocarbons, a wide array of substances has been found to induce CYP1A1, including prescription drugs, cigarette smoke, and dietary supplements (Guo et al., 2010b; Li et al., 2011; Ma and Lu, 2007). For instance, we have reported that herbal dietary supplements, such as kava and *Ginkgo biloba*, dramatically induce CYP1A1 (Guo et al., 2009; Guo et al., 2010a; Guo et al., 2010c; Li et al., 2011). It may be helpful for the patient administered amiodarone to take precautions to avoid the activation of CYP 1A1 as a result of their use of such dietary supplements or other CYP 1A1 inducers, as this could potentially affect the metabolism of amiodarone and its cytotoxicity.

Although amiodarone is metabolized primarily to mono-N-desethylamiodarone, other minor metabolites have been identified in rat liver microsomal incubation (Myung et al., 2000), rats feces, urine and plasma (Varkhede et al., 2014), human HepG2 cell lines (Zahno et al., 2011), human plasma (Ha et al., 2005), and human bile (Deng et al., 2011; Myung et al., 2000; Varkhede et al., 2014; Zahno et al., 2011). In the current study, we also observed several other amiodarone-derived metabolites, although the concentrations of these minor metabolites are much lower than desethylamiodarone (data not shown). Considering the predominance of desethylamiodarone in the metabolism of amiodarone treatment in our studies, we focused on the quantification of desethylamiodarone to demonstrate the overall metabolism of amiodarone. In future investigations, we plan to identify and study the potential contributions of the minor metabolites to the overall systemic toxicity of amiodarone.

In summary, our results support the hypothesis that amiodarone is metabolized by CYP3A4, 1A1, and to a lesser degree, 2C8. We also showed that metabolism by CYP3A4 and 1A1 is linked to the hepatotoxicity of amiodarone.

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HIGHLIGHTS

- Toxic effect of amiodarone was compared in 14 HepG2 cells expressing individual CYP.
- Higher cytotoxicity of amiodarone was observed in HepG2 cells overexpressing CYP3A4 or CYP1A1.
- Higher levels of the more potent hepatotoxic metabolite were detected in cells expressing CYP3A4 or CYP1A1.
- Metabolism by CYP 3A4 and 1A1 is linked to the hepatotoxicity of amiodarone.

Fig. 1.

Amiodarone-induced cytotoxicity in HepG2 cells expressing various human CYPs. Empty vector (EV)-transduced or HepG2 overexpressing human CYP cells were treated with 12.5 μM of amiodarone for 24 h (A) (data were collected and re-analyzed from previous publication (Xuan et al., 2015)). In a separate study, cell viability after short-term exposure (6 h) was compared with that of 24-h exposure (B). Cell viability was measured by the CellTiter-Glo assay at the end of the treatment. Data were presented as mean±SD of three independent experiments. * $P < 0.05$ compared with that of EV at the same time point.

Fig. 2.

Cytotoxicity of amiodarone (AM) and its major metabolite desethylamiodarone (DEA) in HepG2 cells. HepG2 cells were treated with 6.25–100 μM of amiodarone or desethylamiodarone for 6 or 24 h. The cell viability was measured by a CellTiter-Glo assay at the end of treatments. Data were presented as mean±SD of three independent experiments. * $P < 0.05$ compared with the cell viability of amiodarone at the same concentration.

Fig. 3.

HPLC-QDa mass spectrometry chromatograms for amiodarone (AM) and its metabolite desethylamiodarone (DEA) in an extract from a human microsomal incubation of 12.5 μ M amiodarone for 60 min. The analytes were separated using a Waters e2695 HPLC System and monitored with an ACQUITY QDa detector in a positive-electrospray ionization mode. The chromatogram shows selected ion recording (SIR) channels for (a) amiodarone (m/z 646), its internal standard (b) amiodarone-d4 (m/z 650), (c) desethylamiodarone (m/z 618), and its internal standard (d) desethylamiodarone-d4 (m/z 622).

Fig. 4.

Desethylamiodarone formation in HepG2Cells overexpressing human CYPs. Different human CYPs overexpressed or empty vector-transduced HepG2 cells were treated with 12.5 μ M of amiodarone for 6 h. At the end of treatment, cell pellets were harvested and extracted with ice-cold acetonitrile containing 100 ng/ml of deuterated internal standard desethylamiodarone-d4. Desethylamiodarone was quantified with LC-MS as described in Section 2. Data were presented as mean $\pm$ SD of three independent experiments. * $P < 0.05$ compared with that of HepG2/empty vector.

Fig. 5.

(A) Effects of CYP1A1 or CYP3A4 inhibition on the formation of desethylamiodarone in human microsomal incubation of amiodarone. Human microsomes were incubated with 12.5 μM of amiodarone or co-incubated with α-naphthoflavone (50 μM) or ketoconazole (50 μM) in a total volume of 200 μl for 60 min. The incubations were quenched by adding 1 ml of ice-cold acetonitrile containing deuterated internal standards. The amount of amiodarone and desethylamiodarone in extractions was measured with LC-MS as described in Materials and Methods. Data are presented as mean±SD of three independent experiments. * $P < 0.05$ amiodarone amount compared to that of cells without CYP inhibitor addition; # $P < 0.05$ desethylamiodarone formation compared to that of cells without CYP inhibitor addition. (B) Effects of CYP inhibition on the formation of desethylamiodarone in HepG2 cells overexpressing CYP1A1 or CYP3A4. HepG2 cells overexpressing CYP1A1 were treated with 12.5 μM of amiodarone (AM) or co-incubated with α-naphthoflavone (10 μM) for 6 h; and HepG2 cells overexpressing CYP3A4 were treated with 12.5 μM of amiodarone or co-incubated with ketoconazole (10 μM) for 6 h. Cell pellets were harvested and extracted with

ice-cold acetonitrile containing deuterated internal standards. The extractions were measured by LC–MS for the amounts of amiodarone and desethylamiodarone amounts. The bar graphs show as mean±SD of three independent experiments. * $P < 0.05$ versus without inhibition.

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Fig. 6.

Effects of CYP1A1 or CYP3A4 inhibition on amiodarone's cellular toxicity. HepG2 cells overexpressing CYP1A1 were treated with 12.5 μM of amiodarone or co-incubated with α-naphthoflavone (10 μM) for 24 h; and HepG2 cells overexpressing CYP3A4 were treated with 12.5 μM of amiodarone or co-incubated with ketoconazole (10 μM). HepG2/empty vector cells were treated with 12.5 μM of amiodarone only. Cell viability was measured by a CellTiter-Glo assay at the end of treatments. Data were re-analyzed from supplementary table of (Xuan et al., 2015). Data are presented as mean±SD of three independent experiments. * $P < 0.05$ compared with the cell viability of HepG2/empty vector cells, # $P < 0.05$ cell viability of CYP1A1 or CYP3A4 overexpressing HepG2 cells compared to that without CYP inhibition.