Prediction of Cytochrome 450 Mediated Drug-Drug Interactions by Three-Dimensional Cultured Hepatocytes

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Abstract: Cytochrome P450 (CYP) inhibition and induction are the key mechanisms in drug-drug interactions which should be avoided in clinic for the uncertain influence on the efficacy and safety of drug co-administration. The CYP-mediated drug-drug interactions urgently need to be predicted by *in vitro* models before animal and clinical trials, while the primary hepatocytes may represent the most appropriate experimental system by now. However, traditional two-dimensional (2D) culture of hepatocyte monolayer, regardless of the good facilitation and repeatability, rapidly loses its CYP-inducibility during short-term culture due to the deviated microenvironments from *in vivo*. Also, 2D culture did not reproduce the CYP-inhibition mediated hepatotoxicity in drug co-administration. Recently, three-dimensional (3D) cultured hepatocytes have been recognized as the promising models for predicting *in vivo* drug-drug interactions. The 3D cultures such as sandwich and gel entrapped hepatocytes showed the comparable response to CYP inhibitors and inducers as *in vivo* and well reflected the inhibitor/inducer mediated hepatotoxicity. In this regard, this review, for the first time, compares the effect of 2D and 3D hepatocyte cultures on reflecting CYP-mediated drug-drug interactions *in vivo*. And the advantage of 3D cultured hepatocytes on predicting *in vivo* CYP-induction/inhibition will be particularly discussed.

Keywords: Drug-drug interactions, 3D culture, CYP 450, induction, inhibition, prediction.

1. INTRODUCTION

Multiple drug therapy, particularly in patients with several diseases, increases the possibility of drug-drug interactions which is broadly defined as the effects of one drug on the metabolic clearance of another [1]. The highly important drug-drug interactions in clinic are mostly mediated by cytochrome P450 (CYP) inhibition/induction in the metabolism of drugs [2-4]. The CYP-mediated drug-drug interactions consist of two procedures: the CYP-induction/inhibition by one drug and subsequent alteration on therapeutic effect and toxicity of another drug [5].

Currently, pharmaceutical industry routinely focuses on the CYP-mediated drug-drug interactions to reduce the failure of drug development [4, 6]. Since CYP 450 mainly distributes in hepatic parenchymal cells (hepatocytes) of liver, the hepatocyte or its fragment named microsomes have been long utilized as *in vitro* tools in screening CYP-inductor/inhibitor at early stage of drug development [3, 7, 8]. However, the consequence of CYP-induction/inhibition on the therapeutic efficacy or toxicity has to be detected late in the phase II and III clinical trails [3]. Only a few cases of CYP-induction/inhibition mediated hepatotoxicity are possible to be reflected by hepatocytes *in vitro* [9, 10] since liver is the sole involved organ.

Nowadays, several *in vitro* liver models have been optimized and standardized for drug screening, namely microsomes, liver slices, hepatic cell lines and primary hepatocytes [11], which provide the facilitated tools for quick or high-throughput screening of drug candidates. CYP-inhibition, essentially the immediate chemical reaction with enzymes, is mostly investigated by hepatic microsomes that retain the CYP activities [2]. But the available studies showed the poor accuracy by hepatic microsomes whereas about 50% cases (sum of false negatives and false positives) were falsely predicted [8] and only 37% of drugs had the prediction within two-fold of the clinical value [12]. By contrast, CYP-induction should be studied in cultured hepatocytes because most of the induction is a slow regulatory process lasting several days [3]. Since freshly isolated hepatocytes can only shortly survive for a few hours in suspension [13], the two-dimensional (2D) monolayer culture [14,15] and three-dimensional (3D) tissue-like culture [16,17] have offered the improvement in hepatocyte survival. But 2D monolayer culture rapidly loses many liver-specific functions including the activity of CYP 450 [18-20] within 2~3 days [21]. By contrast, 3D cultures are developed associating with liver tissue engineering which is dedicated to rationally manipulate biophysical regulatory mechanisms in the design of hepatocellular constructs [22]. The typical 3D cultures include hepatocytes in sandwich, gel entrapment and spheroid. Hepatocytes in sandwich culture usually use the technique that cells on a single surface are overlaid with a second layer of ECM to create a "sandwich" configuration [20, 23] or it is simplified by coating the dish with a single layer of Matrigel before cell seeding [24, 25]. Gel entrapment is that hepatocytes encapsulated or entrapped within hydrogel such as alginate [26] and collagen [27]. The hepatocytes inside the gel form small clusters which mimic the *in vivo* structure of cells surrounding by ECM. Spheroid,

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the most approached 3D culture to liver, promotes hepatocytes to self-assemble into global aggregates with bile duct structure inside [28-30]. Notably, hepatocytes in these 3D cultures show spherical morphology and express elevated liver-specific functions indicative of a highly differentiated state when compared to 2D cultures with elongated morphology [31, 32]. In this regard, 3D cultured hepatocytes may display the potential value in predicting CYP-mediated drug-drug interactions *in vivo* [28, 33, 34].

Therefore, this paper reviews the predictive effect on *in vivo* CYP-inhibition/induction by 2D&3D cultured hepatocytes since little attention has been paid on this issue in previous literatures. Moreover, inhibitor/inducer mediated hepatotoxicity represented for CYP-inhibition/induction induced adverse effect predicted by 2D&3D cultured hepatocytes is also discussed. It should be mentioned that this review largely summarizes the results from rat hepatocytes and includes limited data on human hepatocytes due to the rarely published reports with human hepatocytes.

2. MECHANISM ON CYP INHIBITION AND INDUCTION

The CYP 450 constitutes a superfamily of isoforms that dominate the drug metabolism including numerous oxidative reactions of carbon hydroxylation, heteroatom oxygenation, dealkylation and epoxidation in human and animals [3, 35]. The major CYP 450 isoforms of human are CYP 1A2, 1B1, 2A6, 2A13, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5 [36], while those of rat are 1A1, 1A2, 2A1, 2B1, 2C6, 2C11, 2D1, 2D2, 2E1, 3A1, 3A2 [7].

The mechanism of CYP inhibition and induction is quite different. CYP inhibition can be grossly divided into 3 categories: reversible inhibition, quasi-irreversible inhibition and irreversible inhibition [2]. Reversible inhibition is the most common mechanism which arises as a result of competition at the CYP active site [2]. Many of reversible CYP inhibitors are nitrogen-containing drugs such as ketoconazole, which can bind to the heme iron or lipophilic region of CYP 450 [2]. The quasi-irreversible inhibitors form quasi-irreversible complexes of CYP-metabolites which functionally inactivate the CYP [37]. Methylenedioxybenzenes, alkylamines and hydrazines have been known as quasi-irreversible inhibitors [2]. The irreversible inhibitors, however, cause the irreversible inactivation of CYP prior to the reactive intermediates release from the active site, which is also called mechanism-based inactivators or suicide substrates. Allylisopropylacetamide, ethinylestradiol and diallyl sulphide are typical irreversible inhibitors [2]. For the direct inactivation on the active site of enzymes, CYP inhibition is almost immediate response [38].

CYP induction, unlike inhibition, is a slow regulatory process in a time-dependent manner [3]. The mechanisms of CYP 3A4, 2B6 and 1A1 induction in human involve the activation of the transcription factors pregnane X receptor (PXR), constitutive androstane receptor (CAR) and aryl hydrocarbon receptor (AhR), but CYP 2E1 induction is nonreceptor mediated [39]. In most cases, induction of CYP is a consequence of increase in gene transcription *via* ligand activation of key receptor transcription factors [40]. But the CYP 2E1 can be induced *via* either nontranscriptional mechanisms by ethanol, acetone and isoniazid [2] or *via* transcriptional mechanisms by interleukin-4 [41]. From a biological view, CYP induction is an adaptive response that protects the cells from toxins by increasing the detoxification activity [3].

CYP-induction *in vivo* is affected by physiological factors (e.g. hormones, vitamins, bile acids, cytokines, growth factors), exogenous factors (e.g. diet, drugs, smoke, environmental pollutants) and pathological factors (e.g. inflammation and infection) [42]. The *in vitro* induction is also largely determined by cell density, cell shape, cell polarity, medium composition and contacted extracellular matrix (ECM), *etc* [43]. For example, the round hepatocytes on Matrigel gel showed higher CYP induction than elongated hepatocytes on type I collagen [44]. And microtubules disruption severely impaired the cell polarity which decreased the inducible expression of human CYP 1A1, 2B6, 2C8, 2C9, 2C19, 3A4, and rat CYP 1A2, 2B1, 2B2, 3A23 *via* inhibition on AhR and glucocorticoid receptor (GR) [42]. Moreover, Table **1** displays the typical inducers and inhibitors on human CYP.

3. PREDICTION OF *IN VIVO* CYP INHIBITION

In vivo, the degree of CYP inhibition is expressed as the increase in the area under the plasma concentration-time curve (AUC) of substrate by determining the drug plasma

Table 1. Major Cytochrome P450 (CYP) Inducers and Inhibitors in Human

CYP	Inducer	Inhibitor	Reference
1A2	3-methylcholanthrene, β-naphthoflavone, omeprazole	fluvoxamine, furafylline	[2, 7]
2B6	phenobarbital, efavirenz, nevirapine, rifampicin	2-phenyl-2-(1-piperdinyl)propane	[89]
2C9	barbiturates, rifampicin	sulfaphenazole	[2]
2C19	phenobarbital	Ticlopidine	[7, 90]
2D6	none identified	quinidine, ajmaline	[2]
2E1	ethanol, isoniazid, acetone	4-methylpyrazole, disulfiram (or its metabolite diethyldithiocarbamate)	[2, 7]
3A4	barbiturates, rifampicin, troglitazone, dexamethasone, carbamazepine, nevirapine, sulfinpyrazone	gestodene, troleandomycin, ketoconazole, itraconazole, ritonavir	[2, 6]

concentration profiles in the presence and absence of inhibitors [8]. When the metabolism of a substrate is inhibited by an inhibitor *in vivo*, the AUC of substrate is decreased by a factor related to the inhibitor concentration to the enzyme [I] and the inhibition constant (K_i) (equation 1) [8].

$$AUC\ ratio = 1 + [I] / K_i \quad (1)$$

As Ki values can be readily obtained from *in vitro* studies, the predicted *in vivo* AUC ratio can be calculated using the *in vitro* data of Ki. In fact, the *in vitro* CYP inhibition on hepatocytes has been less frequently studied. Usually, according to a few *in vitro* reports, CYP inhibition was simply represented by the inhibitory percentage of specific CYP activity. In our own results, the inhibitory percentage on CYP 2E1, 3A and 1A2 of diethyldithiocarbamate at 5 μM, ketoconazole at 3 μM and fluvoxamine at 5 μM was detected to be 35%, 51% and 45% in 2D monolayer vs. 35%, 85% and 63% in 3D gel entrapment of rat hepatocytes [45-47]. Based on the reduced percentage of CYP 450, the value of Ki (inhibitor concentration at 50% inhibition) can be roughly calculated. Table **2** lists the Ki value of three inhibitors above in 2D&3D cultures of rat hepatocytes. Therefore, the predicted AUC ratio calculated by equation 1 was respectively 1.7, 2.0, 1.9 in 2D monolayer and was 1.7, 2.7, 2.3 in 3D gel entrapment culture for diethyldithiocarbamate, ketoconazole and fluvoxamine, while the corresponding AUC ratio *in vivo* was 6~9 [48], 1.77~24.4 [8] and 1.41~3.63 [8] for the corresponding inhibitors.

In this regard, *in vivo* inhibition of ketoconazole and fluvoxamine could be predicted by both 2D&3D cultures in some extent although 2D culture showed the less significant inhibition. Interestingly, among the three inhibitors above, diethyldithiocarbamate is an irreversible mechanism-based inhibitor for CYP 2E1, which showed much lower inhibition ratio of 1.7 in 2D&3D hepatocyte cultures compared to the inhibition value of 6~9 in rats [48]. Similarly, cimetidine [49, 50], another mechanism-based inhibitor, required typically 100~600 times higher concentrations (0.5~1.0 mM) in hepatocytes [51] than the plasma concentration (0.4~0.8 μg/ml, equivalent to 1.6~3.2 μM) associated with the same inhibition of drug metabolism in patients [52]. Thus, the 2D&3D cultured hepatocytes seem to well predict the reversible CYP-inhibition *in vivo* but insensitive to mechanism-based inhibitors.

4. PREDICTION OF *IN VIVO* CYP-INDUCTION

Due to the slow regulatory process (usually 1~3 days) in CYP induction, the cultured hepatocytes, not microsomes, have been widely applied in studies of induction. In cultured hepatocytes, absolute CYP activity can not be comparable to that *in vivo* because of the unavoidable decrease on CYP baseline during cell culture [53]. Thus, the induction fold has been normally used as a comparable index to extrapolate the *in vitro* data to *in vivo* [53]. In fact, more than 2-fold of the *in vitro* induction has been accepted as a good reflection on induction of *in vivo* despite that the *in vivo* data did not exactly match the *in vivo* fold [20, 54]. Table **3** compares the CYP induction in 2D&3D rat hepatocytes as well as in rats.

Compared with the 3D cultured hepatocytes, 2D hepatocyte monolayer has been realized to poor reflect the *in vivo* CYP induction. The quick loss on CYP activity in 2D culture results in its disappeared CYP-inducibility [3, 7, 20, 53]. For example, rat hepatocyte monolayer lost CYP 2B/2C/3A inducibility within 1~2 days of culture and did not sustain CYP 1A inducibility at 6 days of culture [20]. Rifampicin, a CYP 3A inducer in rats [55], did not replicate such induction in rat hepatocyte monolayer [56]. Although human hepatocytes seem to better preserve CYP-inducibility than rat hepatocytes [57], CYP 3A4 in human hepatocyte monolayer was not induced by dexamethasone [58] as in clinic [59].

For the shortcoming of 2D hepatocyte monolayer, 3D cultured hepatocytes have been enthusiastically proposed to predict *in vivo* CYP induction. The following content will introduce the effects of typical 3D cultures (sandwich, gel entrapment and spheroid) on predicting *in vivo* CYP induction.

In recent decades, sandwich culture has replaced the 2D monolayer to be the routine model in investigation of CYP induction *in vitro*. Despite that hepatocytes in sandwich culture appear as a more flattened morphology away from those in liver tissue sections [60], they can reestablish the

Table 2. Summary of Inhibitors on CYP Activity and Drug Hepatotoxicity Using Rat Hepatocytes in 3D Gel Entrapment and 2D Monolayer

Hepatotoxic drug	Involved CYP 450	Inhibitor	Gel Entrapment		Monolayer		Reference
			Ki (μM)[b]	Hepatotoxicity	Ki (μM)[b]	Hepatotoxicity	
Acetaminophen	2E1	Diethyldithiocarbamate	7.1	Reduced	7.1	No effect	[45]
Isoniazid				Reduced		No effect	[76]
Amiodarone	3A	Ketoconazole	1.8 [a]	Reduced	2.9 [a]	No effect	[77]
Clozapine				Reduced		No effect	[46]
Tacrine	1A2	Fluvoxamine	4.0	Reduced	5.6	No effect	[47]

Note: a. unpublished data in our lab.
b. Ki is the concentration of 50% inhibition on CYP isoforms by inhibitors calculated from the inhibitory percentage in Ref. [45, 47]. Used concentration of diethyldithiocarbamate, ketoconazole and fluvoxamine was 5, 3 and 5 μM.

Table 3. Comparison of CYP 450 Inducibility on 2D and 3D Cultured Rat Hepatocytes

CYP isoenzymes	Induction Fold				
	2D monolayer	Sandwich	Gel entrapment	Spheroids	Liver *in vivo*
1A[a]	7~15 [20], 2.7 [91]	40~55 [20], 21 [54]	2.4 [91]	2.3 [91]	22 [54]
1A[b]	36.9 [92], 1.8~5.6 [93]	1.9~9.2 [93]	150 [53]	~50 [63]	57 [53]
2B[c]	No induction [20]	3~7 [20], 273 [54]	127.5 [53]	5 [62]	61.3 [54]
3A[d]	1.0~2.2 [20]	2.5~5 [20], 13 [54]	2.2 [g]	11 [62]	5 [54]
3A[e]	No induction [94]	No induction [56]	2.0 [g]	Not reported	5~14 [55]
2E1[f]	No induction [95]	1.3~2.5 [96]	1.5 [97]	Not reported	6~16 [98]

Note. CYP induction fold by [a]β-naphthoflavone (BNF), [b]3-methylcholanthrene (3-MC), [c]phenobarbital, [d]dexamethasone, [e]rifampicin, [f]ethanol, [g]unpublished data in our lab.

cell polarity [60] which plays the private role in CYP-induction [43]. As shown in Table **3**, rat hepatocytes in sandwich exhibited the over 2-fold induction to β-naphthoflavone (BNF), 3-methylcholanthrene (3-MC), phenobarbital and dexamethasone as in rats, indicative of a well prediction of the *in vivo* CYP induction. Besides, sandwich cultured hepatocytes in flat membrane bioreactor preserved long-term CYP-induction for 14-21 days [61]. Unfortunately, there is an unpredicted case that rifampicin did not induce CYP 3A in sandwich cultured rat hepatocytes (Table **3**).

Gel entrapment culture of rat hepatocytes in alginate microsphere, as shown in Table **3**, presented extremely high induction factors (150-fold and 127.5-fold) on CYP 1A and 2B at an individual exposure to 3-MC and phenobarbital which is even higher than those in rats (57-fold and 61.3-fold) [53, 54]. And the authors suggested that the sensitive response to inducers *in vitro* would be preferred in predicting *in vivo* induction [53]. Moreover, our studies on rat and human hepatocytes by collagen gel entrapment showed 2.2 and 2.0 fold induction on CYP 3A after treatment of dexamethasone and rifampicin, indicating its more inducibility than that in 2D monolayer (Table **3**). Like sandwich culture, CYP in hepatocytes of gel entrapment are also capable to be induced for a long-term of 2 weeks' culture [14].

Hepatocyte spheroid has been less applied in drug studies than other 3D cultures due to the difficulty on fabrication. Table **3** lists the available data of CYP-induction in rat hepatocyte spheroid, which shows well prediction of inducibility in rats. Similarly, hepatocyte spheroids also have the advantage of long-term preservation of CYP-inducibility over 10-day culture [62-64]. Recently, hepatocyte spheroid arrays within micro-wells have provided a high effective and standard tool by development of microfabrication technology [63, 64], which may promote the application of hepatocyte spheroid on CYP induction.

Nevertheless, all the 3D cultures showed low induction on CYP 2E1 (Table **3**). This might be due to the different mechanism of CYP 2E1 induction from other CYPs [40] and need further clarification.

5. PREDICTION OF CYP INHIBITOR/INDUCER MEDIATED HEPATOTOXICITY *IN VIVO*

Drug hepatotoxicity *in vivo*, elicited by parent drug or its metabolites, can be significantly changed by inducers or inhibitors of CYP. When the hepatotoxicity is caused by the reactive metabolites, CYP inhibitors attenuate the toxicity *via* reducing the drug metabolism [65-67] while CYP inducers exacerbate the toxicity *via* increasing the drug metabolism. On the contrary, if hepatotoxicity is elicited by parent drug, CYP inhibitors exacerbates the hepatotoxicity *via* prolonging drug clearance [68] while CYP inducers are on vice versa. In this regard, the hepatotoxicity mediated by CYP inducers and inhibitors might elicit four situations: (1) inducer exacerbates toxicity; (2) inducer attenuates toxicity; (3) inhibitor exacerbates toxicity; (4) inhibitor attenuates toxicity. The detail will be describes as following.

As the hepatotoxicity induced by parent drug occurs much less than by reactive metabolites, only a few *in vitro* regulation of parent-drug hepatotoxicity by CYP 450 inhibitor/inducer have been reported. In 2D cultured rat hepatocytes, hepatotoxicity of leflunomide was enhanced by six CYP inhibitors but decreased by CYP inducer of dexamethasone [69], illustrating the toxicity elicited by parent drug. Similarly, sanguinarine, a toxin inhibiting the Na^+-K^+-ATPase on cell membrane was reduced hepatotoxicity by two CYP inducers, dioxin and phenobarbital in rat hepatocytes monolayer [70], indicating that sanguinarine, rather than its metabolites, served as a toxin. The limited cases have showed that the toxicity induced by parent drug can be regulated by CYP inhibitor/inducer in 2D cultured hepatocytes, but the effect of 3D cultures has not been reported in this issue.

Relatively, the drug hepatotoxicity induced by reactive-metabolites has been more frequently focused and, therefore, its regulation by induction of CYP has been enthusiastically investigated *in vitro*. Commonly, CYP inducer enhanced the drug hepatotoxicity in both 2D and 3D cultured hepatocytes. As a support, our previous study found that the drug hepatotoxicity was exacerbated by CYP inducer in both 2D and 3D cultured human hepatocytes, regardless the more sensitive response to hepatotoxicity in 3D culture [9]. This *in vitro* study corresponded well with the situation *in vivo* [71]. Similar comparison on 2D&3D cultures is that sandwich

cultured hepatocytes [10] sensitively reflected the acetaminophen-ethanol hepatotoxicity *in vivo* [72] but 2D monolayer showed exacerbation only at the highest concentrations of ethanol and acetaminophen [73]. By the *in vitro* studies, enhanced acetaminophen hepatotoxicity has been known to be caused by the ethanol induction on CYP 3A and 2E1 activities *via* producing more reactive metabolite of N-acetyl-p-benzoquinone imine (NAPQI) [74]. The feasibility of 3D culture has again been proved that toxicity of diclofenac was enhanced by phenobarbital induction on CYP 3A in sandwich cultured rat hepatocytes [75]. Taken together, the studies above indicate that CYP inducers mediated hepatotoxicity can be predicted by 2D&3D cultured hepatocytes, but 3D cultures show more sensitive response to the regulation of CYP inducers.

Considering the attenuated drug hepatotoxicity by CYP inhibitors, the systematic comparison on 2D&3D cultures seems to be only conducted in our lab [46, 47, 76, 77]. As shown in Table **2**, five drugs forming reactive metabolites *via* CYP biotransformation have been investigated whose hepatotoxicity should be reduced by the corresponding CYP inhibitor. However, 2D cultured rat hepatocytes, though showed the decreased CYP activity after treatment of inhibitors, can not demonstrate the protective effects on drug toxicity (Table **2**). By contrast, 3D cultured hepatocytes reflected not only CYP-inhibition but also the attenuated hepatotoxicity by the inhibitors (Table **2**). The non-inhibited hepatotoxicity in 2D culture might be due to the low CYP activity which was insufficient to produce reactive metabolites as *in vivo*. It might be indirectly supported by the result from the other two groups who have ever used hepatocyte monolayer culture for investigation of the mediation of CYP 450 inhibitor on drug hepatotoxicity [70, 78]. In their studies, hepatocyte monolayer needed an extra pre-incubation with CYP inducer before subsequent inhibitor treatment for reducing drug hepatotoxicity [70], or extremely high dose of toxic drug with a very short exposure of 2 h were used immediately after formation of hepatocyte monolayer to obtain a significant protective effect by inhibitor [78]. It seems that drug hepatotoxicity detection routinely needs exposure of hepatocytes to drugs for at least 24 h, only 3D cultures can reflect the attenuation on drug toxicity by CYP inhibitors.

Except for predicting drug-drug interactions *in vivo*, the CYP inducers/inhibitors also facilitate the mechanistic studies by identifying the involved CYP isoforms in drug hepatotoxicity. Here we would like to introduce our findings with the effect of CYP inducer on isoniazid hepatotoxicity. It has been well known that rifampicin could exacerbate the isoniazid hepatotoxicity in clinic [71] while lack such synergistic effect in rats [79, 80]. The involved mechanism was unclear. By using gel entrapped rat/human hepatocytes, it was shown that rifampicin significantly induced CYP 2E1 activity in human hepatocytes while performed no induction in rat hepatocytes. As CYP 2E1 has been found to involve isoniazid hepatotoxicity [76], we suggested that the differential induction of CYP 2E1 activity between rat and human hepatocytes might account for the differential exacerbation of isoniazid hepatotoxicity by rifampicin [9]. Meanwhile, as rifampicin can induce several CYP isoforms, among which CYP 3A is the most typical one, Yue *et al.* claimed that the CYP 3A induction by rifampicin could enhance isoniazid hepatotoxicity in their comments [81]. To identify this argument, dexamethasone as another CYP 3A inducer was employed, replacing rifampicin, to co-treat gel entrapped rat/human hepatocytes with isoniazid. As expected, dexamethasone however, failed to enhance the isoniazid hepatotoxicity in either rat or human hepatocytes (Fig. **1**). Moreover, the equivalently same gene expression level of CYP 3A post to rifampicin induction in either rat or human hepatocytes (Fig. **2**) solidify the non-involvement of CYP 3A. And the differentially induced CYP 2E1 gene expression between rifampicin and dexamethasone (Fig. **2**) could further demonstrate its involvement in exacerbating isoniazid hepatotoxicity.

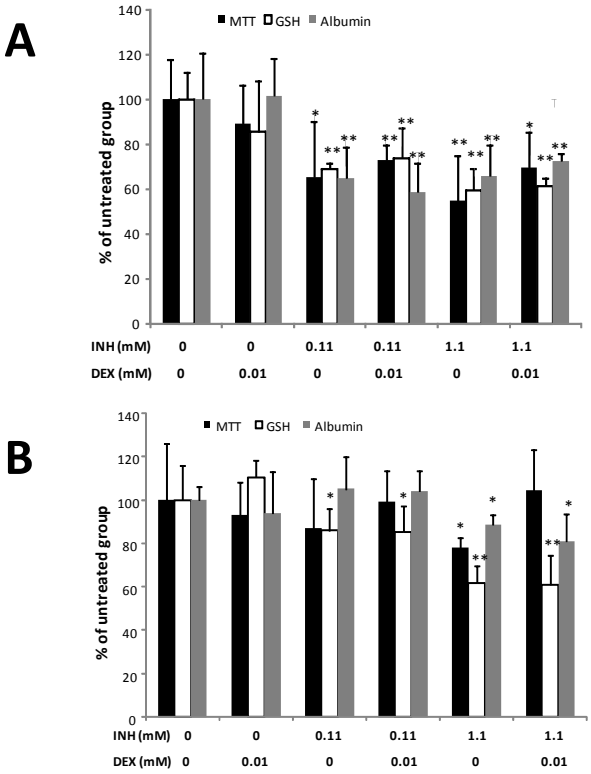

Fig. (1). Effect of dexamethasone on isoniazid hepatotoxicity in gel entrapped rat (**A**) and human (**B**) hepatocytes. The hepatocytes were co-treated with dexamethasone (DEX, 0.01 mM) and isoniazid (INH, 0.11 or 1.1 mM), or treated with dexamethasone and isoniazid alone for 48 h. The drug hepatotoxicity was evaluated by methyl thiazolyl tetrazolium (MTT) reduction, glutathione (GSH) depletion and albumin secretion. Data are given as means±s.d. for each point of three separate rats/donors. *$p < 0.05$, **$p < 0.01$ compared to control without drug treatment.

Nevertheless, the CYP inducer/inhibitor mediated hepatotoxicity has not been widely investigated on hepatocytes *in vitro*, possibly due to the insignificant response in 2D cultured hepatocytes. The current researches are more likely to feed the animals with CYP

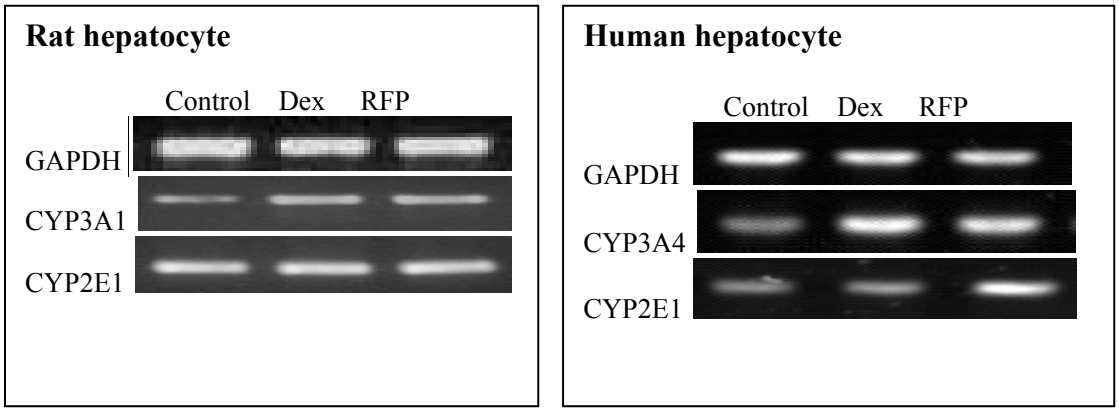

Fig. (2). Dexamethasone (Dex) and rifampicin (RFP) treatment on gene expression of CYP 3A and 2E1 in gel entrapped rat and human hepatocytes. The hepatocytes were incubated with Dex at 10 μM or RFP at 12 μM for 48 h before gene analysis. GAPDH means the housekeeping gene of glyceraldehyde-3-phosphate dehydrogenase.

inducer/inhibitor *in vivo* and then harvest hepatocytes from animal liver for subsequent studies on drug hepatotoxicity *in vitro* [82, 83]. This process is complicated and expensive. We strongly recommend to conduct such researches on 3D cultured hepatocytes which can serve as a convenient platform combining inhibition/induction with hepatotoxicity.

CONCLUSION AND PROSPECT

CYP-mediated drug-drug interactions are urgently needed for prediction by *in vitro* models before animal and clinical trials. Unfortunately, 2D cultured hepatocytes, though own the advantages on easy handle and high efficacy, rapidly lose CYP inducibility and can not reflect the CYP inhibitor attenuated drug hepatotoxicity. However, the hepatocytes in 3D cultures have showed the promising future on predicting CYP-induction/inhibition as well as inhibitor/inducer mediated hepatotoxicity. Compared with the animals and human being, 3D cultured hepatocytes also facilitate the mechanism studies on drug metabolism and hepatotoxicity utilizing the specific CYP inducers/inhibitors which can selectively alter the pathway of drug biotransformation. Besides the positive effects on CYP-mediated drug-drug interaction, the hepatocytes in 3D cultures have also been proved to obtain the better prediction than 2D cultures on *in vivo* drug hepatotoxicity [84], transporter-mediated drug interactions [85], drug clearance [86, 87], *etc*.

Nevertheless, the *in vivo* CYP-induction/inhibition can only predict the trend without a quantification. The alternated therapeutic efficacy or toxicity caused by CYP-induction/inhibition is even more difficult to be reproduced *in vitro* since multi-organs are usually involved in the process. Because of the instinct limitation such as lack of multi-organ interaction and blood circulation, the *in vitro* models, even 3D cultured hepatocytes, can only partly replace the animal and clinical trails. Nowadays, the cell culture and microreactor techniques have promoted the development of integrated multi-organ models with two or more types of organ cells cocultured in one bioreactor, which shows the possibility of *in vitro* studies on drug-drug interactions between multi-organs [88]. In this regard, the techniques of 3D cell cultures and multi-organ bioreactors will show great value to approach the 3R (reduction, refinement, and replacement) principles of animal testing and save the cost in pharmaceutical industry.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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PATIENT CONSENT

Declared none.

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