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• BASIC RESEARCH •

Modulation of Kupffer cells on hepatic drug metabolism

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Abstract

AIM: To observe the effects of Kupffer cells on hepatic drug metabolic enzymes.

METHODS: Kunming mice were ip injected with $GdCl_3$10, 20, 40 mg/kg to decrease the number and block the function of kupffer cells selectively. The contents of drug metabolic enzymes, cytochrome P450, NADPH-cytochrom C redutase (NADPH-C), aniline hydroxylase (ANH), aminopyrine N-demethylase (AMD), erythromycin N-demethylase (EMD), and glutathione s-transferase (mGST) in hepatic microsome and S9-GSTpi, S9-GST in supernatant of 9 000 g were accessed 1 d after the injection. The time course of alteration of drug metabolic enzymes was observed on d 1, 3, and 6 treated with a single dose $GdCl_3$. Mice were treated with *Angelica sinensis* polysaccharides (ASP) of 30, 60, 120 mg/kg, ig, qd ×6 d, respectively and the same assays were performed.

RESULTS: P450 content and NADPH-C, ANH, AMD, and EMD activities were obviously reduced 1 d after Kupffer cell blockade. However, mGST and S9-GST activities were significantly increased. But no relationship was observed between $GdCl_3$ dosage and enzyme activities. With single dose $GdCl_3$ treatment, P450 content, NADPH-C, and ANH activities were further decreased following Kupffer cell blockade lasted for 6 d, by 35.7%, 50.3%, 36.5% after 3 d, and 57.9%, 57.9%, 63.2% after 6 d, respectively. On the contrary, AMD, EMD, mGST, and S9-GST activities were raised by 36.5%, 71.9%, 23.1%, 35.7% after 3 d, and 155%, 182%, 21.5%, 33.7% after 6 d, respectively. Furthermore, the activities of drug metabolic enzymes were markedly increased after 30 mg/kg ASP treatment, and decreased significantly after 120 mg/kg ASP treatment. No change in activity of S9-GSTpi was observed in the present study.

CONCLUSION: Kupffer cells play an important role in the modulation of drug metabolic enzymes. The changes of drug metabolic enzyme activities depend on the time of kupffer cell blockade and on the degree of Kupffer cells activated. A low concentration of ASP increases the activities of drug metabolic enzymes, but a high concentration of ASP decreases the activities of drug metabolic enzymes.

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INTRODUCTION

Drug metabolic enzymes can detoxify endogenous and exogenous compounds and also generate potentially carcinogenic or toxic compounds in the process of catalyzing the metabolism of xenobiotics, and inhibition and induction of their activities are also the key mechanisms in drug-drug interactions[1,2]. The induction or inhibition of metabolizing enzyme activities by a great deal of substances (including drugs, foods, inflammatory factors, *etc*.) influences their toxicological or pharmacological outcomes as well as those of other xenobiotics or drugs[3-5].

The role of sinusoidal cells in hepatic metabolism has been greatly underestimated until now. However, Kupffer cells, despite their size represents 80% to 90% of all fixed macrophages in the body and approximately 14% of the hepatic cellular mass, the function in hepatic metabolism of Kupffer cells is unknown. Some reports suggested that Kupffer cells might play an important role in xenobiotic-induced hepatotocixity, which is often dependent on their metabolism. Although hepatotocytes are the major site of xenobiotic metabolism, several enzymatic activities such as glutathione s-transferase, UDP-glucuronosyltransferase and cytochrome P450-dependent oxidase have been found in nonparenchymal cells (mainly Kupffer cells) and may play an important role in the metabolism and cellular effects of paracetamol[6,7]. Only a small number of reports demonstrated the relationship between drug metabolic enzymes and activities of Kupffer cells. The aim of this work was to investigate the effects of kupffer cell mediated metabolism as demonstrated by hepatic drug metabolic enzymes.

MATERIALS AND METHODS

Reagents

Gadolinium chloride ($GdCl_3$), glutathione (GSH), erythromycin, aniline, NADPH, aminophenazone, isocitric acid, isocitric acid dehydrogenase, 1-chloro-2,4-dinitrobenzene (CDNB), ethacrynic acid (EA) were purchased from Sigma, USA. All other reagents used in this study were of AR grade.

Animals and treatment

Kunming strain male mice (aged 4-6 wk) weighing 18-24 g were obtained from the Experimental Animal Center, Wuhan University School of Medicine. The animals were fed with a standard diet in pellets, and allowed free access to water. The mice were randomly divided into 9 groups, 10 per group: control group; $GdCl_3$10-1d group; $GdCl_3$20-1d group; $GdCl_3$40-1d group: mice received ip injection of $GdCl_3$10, 20, 40 mg/kg, respectively, and killed after 1 d; $GdCl_3$20-3 d group; $GdCl_3$20-6 d group: mice were treated with $GdCl_3$ at a single dose of 20 mg/kg, and killed after 3 d, and 6 d, respectively; ASP1 group; ASP2 group; ASP3 group: mice were given ASP 30, 60, and 120 mg/kg, respectively, i.g, qd×7 d, and were then killed on the 7th d. The livers were collected for the assay of drug metabolic enzyme activities.

Preparation of liver sample S9 and microsomal fractions

Microsomal fractions were preparated as previously described[8]. Total protein concentration in mice liver microsomes was determined by the method of Lowry *et al*.[9] (1951) using BSA as the standard. All operations were performed at 4 ℃.

Assays for metabolic enzyme activities

Total cytochrome P450 (P450) content was based on the use of the extinction coefficient of 105 mmol/L· cm for reduced cytochrome P450 minus oxidized P450 with a UV-1601 spectrophotometer[10].

NADPH-cytochrome C reductase (NADPH-C) activity was determined with a spectrophotometer at 550 nm by monitoring the reduction of cytochrome C (0.5 mg /mL) at 37 ℃ in an incubation mixture containing potassium phosphate (300 mmol/L, pH7.7) EDTA (0.1 mmol/L), liver microsomes (20 μg/mL) and NADPH (2 mg/L)[11].

Aminopytine N-demethylase (AMD) and erythomycin N-demethylase (EMD) were detected by measuring the production of formaldehyde. Its reaction system contained Tris-HCl 50 mmol/L, $MgCl_2$ 10 mmol/L, KCl 50 mmol/L and an NADPH-generating system (including $NADP^+$ 0.4 mmol/L, isocitric acid 10 mmol/L, and isocitric acid dehydrogenase 0.6 units). Erythromycin 0.4 mmol/L or aminopyrine 8 mmol/L was added, the reaction was initiated by the NADPH-generating system, the supernatant was incubated with the Nash reagent at 60 ℃ for 20 min and the color absorbance was measured at 415 nm with a UV-1601 spectrophotometer[12].

Aniline hydroxylase (ANH) was assessed with aniline as substrate as previously described[13].

Microsome glutathione s-transferase (mGST) and S9 glutathione s-transferase (S9-GST) activities were measured with CDNB as substrate (ε=9.6 mmol/L· cm)[14]. The assay mixture contained 850 μL of 0.1 mol/L sodium phosphate-1 mmol/L EDTA (pH6.5), 50 μL of 20 mmol/L GSH, 50 μL of 50 mmol/L CDNB, and 50 μL of sample. The absorbance at 340 nm was continuously recorded for 1 min[15]. S9-GSTpi was determined with EA and GSH as substrates (ε=5.0 mmol/L· cm)[14]. The assay mixture contained 850 μL of 0.1 mol/L sodium phosphate, 1 mmol/L EDTA (pH6.8), 50 μL of 50 mmol/L GSH, 50 μL of 50 mmol/L EA, and 50 μL of sample. The absorbance at 340 nm was continuously recorded for 1 minute.

Statistical analysis

The data were presented as mean±SD. Comparisons were performed using one-way analysis of variance (ANOVA) followed by the *posteriori* Student-Newman-Keuls' *t*-test. $P<0.05$ was considered statistically significant.

RESULTS

Influence of different dose of $GdCl_3$ on P450 content and P450 isoform activities

The content of P450 and the activities of NADPH-C, ANH, AMD, and EMD were obviously reduced after 1 d after $Gdcl_3$ treatment, and no relationship was found between the enzyme activities and the dose of $Gdcl_3$ (Table 1).

Time course of alterations of P450 content, activities of P450 isoforms, S9-GSTpi, S9-GST and mGST by Kupffer cell blockade

The content of P450 and activities of NADPH-C and ANH were reduced by 35.7%, 50.3%, 36.8% after Kupffer cell blockade for 3 d, and 57.9%, 57.9%, 63.2% for 6 d, respectively. However, the activities of AMD, EMD were raised by 36.5%, 71.9% after 3 d, and 155%, 181% after 6 d, respectively. S9-GST, mGST were markedly increased by Kupffer cell blockade. However, the changes were not related with the time of Kupffer cell blockade. No changes in S9-GST were observed (Table 2).

Influence of ASP on P450, activities of P450 isoforms, S9-GSTpi, S9-GST and mGST

The content of P450 and the activities of NADPH-C, ANH,

Table 1 Influence of different dose of $GdCl_3$ on P450 content and P450 isoform activities

Group	P-450 (nmol/mg·pro)	NADPH-C (nmol/min·mg·pro)	ANH (nmol/min·mg·pro)	AMD (nmol/min·mg·pro)	EMD (nmol/min·mg·pro)
Control	1.4±0.4	14.5±2.8	0.038±0.009	0.96±0.07	0.89±0.06
$GdCl_3$10-1 d	1.13±0.25	10.9±1.0[b]	0.032±0.016	0.76±0.07[b]	0.78±0.12
$GdCl_3$20-1 d	0.87±0.28[b]	10.5±1.8[b]	0.033±0.006	0.82±0.03[a]	0.74±0.13[a]
$GdCl_3$40-1 d	1.01±0.29[a]	6.0±1.0[b]	0.036±0.010	0.77±0.17[a]	0.74±0.18[a]

$GdCl_3$10-1 d, $GdCl_3$20-1 d, $GdCl_3$40-1 d: Mice were intraperitoneally injected with $GdCl_3$ 10, 20, 40 mg/kg, respectively, and sacrificed after 1 d. The hepatic microsome was prepared to assess the content of P450 and the activities of P450 isoforms. P450: cytochrome P450; NADPH-C: NADPH-cytochrome c reductase; ANH: aniline hydroxylase; AMD: aminopytine N-demethylase; EMD: erythomycin N-demethylase (n=8, mean±SD, [a]$P<0.05$, [b]$P<0.01$ compared with control group).

Table 2 Time course of alterations of P450 content, activities of P450 isoforms, S9-GSTpi, S9-GST and mGST by Kupffer cell blockade

Group	Control	$GdCl_3$20-1 d	$GdCl_3$20-3 d	$GdCl_3$20-6 d
P-450 (nmol/mg·pro)	1.4±0.4	0.87±0.28[b]	0.90±0.21[b]	0.59±0.24[b]
NADPH-C (nmol/min·mg·pro)	14.5±2.8	10.5±1.8[b]	7.2±1.0[b]	6.1±0.8[b]
ANH (nmol/min·mg·pro)	0.038±0.009	0.033±0.006	0.024±0.006[b]	0.024±0.006[b]
AMD (nmol/min·mg·pro)	0.96±0.07	0.82±0.03[a]	1.31±0.24[b]	1.31±0.24[b]
EMD (nmol/min·mg·pro)	0.89±0.06	0.74±0.13[a]	1.53±0.26[b]	2.5±0.3[b]
S9-GSTpi (nmol/min·mg·pro)	1.07±0.28	0.93±0.17	1.1±0.4	0.98±0.23
S9-GST (nmol/min·mg·pro)	8.3±0.91	0.2±1.5[a]	11.4±0.9[b]	11.1±1.3[b]
mGST (nmol/min·mg·pro)	0.25±0.03	0.28±0.06	0.31±0.04[b]	0.305±0.015[b]

$GdCl_3$20-1 d, $GdCl_3$20-3 d, $GdCl_3$20-6 d: Mice were intraperitoneally injected with a signal dose of 20 mg/kg of $GdCl_3$, and sacrificed after 1 d, 3 d, and 6 d of $GdCl_3$ treatment. The hepatic microsome was prepared to assay the P450 content, and activities of P450 isoforms, S9-GSTpi, S9-GST and mGST. (n=8, mean±SD, [a]$P<0.05$, [b]$P<0.01$ compared with control group).

Table 3 Influence of ASP on P450 content, P450 isoform activities and activities of S9-GSTpi, S9-GST and mGST

Group	Control	ASP1	ASP2	ASP3
P-450 (nmol/mg·pro)	1.4±0.4	2.1±0.4[b]	1.2±0.4	0.60±0.13[b]
NADPH-C (nmol/min·mg·pro)	14.5±2.8	19.7±2.2[b]	19.1±4.3[a]	7.6±1.1[b]
ANH (nmol/min·mg·pro)	0.038±0.009	0.045±0.009[b]	0.018±0.006[b]	0.010±0.003[b]
AMD (nmol/min·mg·pro)	0.96±0.07	1.29±0.11[b]	1.11±0.19	0.61±0.16[b]
EMD (nmol/min·mg·pro)	0.89±0.06	1.30±0.21	1.14±0.33	0.41±0.09[b]
S9-GSTpi (nmol/min·mg·pro)	1.07±0.28	1.2±0.3	1.15±0.26	0.78±0.19[a]
S9-GST (nmol/min·mg·pro)	8.3±0.9	0.5±1.9[a]	11.8±1.5[a]	7.9±0.7[a]
mGST (nmol/min·mg·pro)	0.25±0.03	0.29±0.03[a]	0.35±0.08[b]	0.155±0.016[b]

ASP1, ASP2, ASP3: Mice were given 30, 60, and 120 mg/kg ASP, ig, qd×7 d, respectively and sacrificed on day 7. The hepatic microsome was prepared to assay the content of P450, and activities of P450 isoforms, S9-GSTpi, S9-GST and mGST. (n=8, mean± SD, [a]P<0.05, [b]P<0.01 compared with control group).

AMD, EMD were obviously increased by 50.0%, 35.9%, 18.4%, 34.4%, and 46.1% after 30 mg/kg ASP treatment, and obviously decreased by 57.1%, 47.6%, 73.7%, 36.5%, and 53.9% after 120 mg/kg ASP treatment, respectively. Thirty and 60 mg/kg of ASP could increase the activity of S9-GST by 26.5%, 42.3%, respectively. The alterations of mGST activity were similar to those of S9-GST. The activities of S9-GST and mGST were reduced by 4.8%, 38.0% after administration of 120 mg/kg ASP, respectively. No changes in S9-GSTpi were observed (Table 3).

Influence of different dose of $GdCl_3$ on activities of S9-GSTpi, S9-GST and mGST

S9-GST activity was increased by 18.1%, 22.9% after 10 mg and 20 mg/kg of $GdCl_3$ treatment, respectively, and the alterations in mGST activity were similar to those of S9-GST. No changes in S9-GSTpi were observed (Table 4).

Table 4 Influence of different dose of $GdCl_3$ on activities of S9-GSTpi, S9-GST and mGST

Group	S9-GSTpi	S9-GST	mGST
	(nmol/min·mg·pro)		
Control	1.07±0.28	8.3±0.9	0.25±0.03
$GdCl_3$10-1d	1.16±0.0.19	9.8±1.5[a]	0.289±0.026[a]
$GdCl_3$20-1d	0.93±0.17	10.2±1.5[a]	0.28±0.06
$GdCl_3$40-1d	0.91±0.12	7.9±0.8	0.264±0.012

The administration of $GdCl_3$ and the treatment of animals were the same as described in Table 1. S9-GST: glutathione s-transferase in S9; S9-GSTpi: glutathione s-transferase pi in S9; mGST: glutathione s-transferase in microsome. (n=8, mean±SD, [a]P<0.05 compared with control group).

DISCUSSION

Neyrinck *et al*.[16,17] reported that Kupffer cells might play a role of xenobiotic metabolism in hepatocytes *in vitro*, and treatment of $GdCl_3$ could decrease the total hepatic content of cytochrome P450. Kupffer cells were involved in some liver diseases in which the activities of drug metabolic enzymes changed[18,19]. Few studies until now have reported an effect of $GdCl_3$ injection on the activities of specific CYP isoforms and GST, and on the time course of developmental changes of their activities after Kupffer cells blocked by $GdCl_3$ *in vivo*. The present study showed that Kupffer cell blockade indicated a tendency to decrease cytochrome P450 content and its isoform activities, and to increase GST (including S9-GST, and mGST) activities. However, CYP isoforms and GST activities showed different changes following different time of Kupffer cell blockade. The total cytochrome P450 content, NADPH-C and ANH (as marker of CYP2E1) activities had a sustained decrease following prolonged Kupffer cell blockade, but AMD, EMD (as marker of CYP3A) activities obviously raised 3 d after Kupffer cell blockade, and GST activities kept a high level 6d after Kupffer cell blockade. Some authors have already reported that the treatment of $GdCl_3$ causing inactivation of Kupffer cells could protect liver against damage induced by some toxic chemicals (such as ethanol, CCl_4, *etc*.) through inhibiting CYP2E1[20,21]. The present study offered further support for a relevant role of Kupffer cells through the control of hepatocyte metabolism mediated liver injury. CYP3A contributed significantly to the biotransformation of xenobiotic chemicals such as drugs, and toxic chemicals[22]. GSTs constitute a multigene family of phase II conjugating enzymes broadly distributed phylogenetically. Detoxification of electrophilic compounds by GSTs may occur via catalytic conjugation of electrophilic intermediates with GSH, via GSH-dependent reduction of organic peroxides, or via direct binding to lipophilic compounds. GST might have an important cytoprotective function[23]. The current study showed CYP3A and GST activities were rapidly recovered, which might be a self-regulated and self-protected mechanism of the body.

The changes in CYP and GST activities were related with the state of Kupffer cells activated by Angelica sinensis polysaccharides (ASP). Now, some new functions of ASP have been reported. ASP could promote ulcer healing, protect hepatic injury and might have antitumor effects[24-26]. Immunoactivity is the most important function of ASP. It could enhance the proliferative response of lymphocytes *in vitro* and differential expression of genes in the liver of immunological injury mice[27,28]. In the present study, 30 mg/kg ASP could increase cytochrome P450 content , P450 isoform activities and GST activities. However, 120 mg/kg ASP could obviously decrease the activities of these enzymes. This phenomenon might be concerned with the immunoactivity of ASP. Its mechanism remains to be further studied.

GSTpi, a glutathion s-transferase of placenta type, often presented high expression in tumor tissues related with drug resistance[29]. The present study indicated that GSTpi activity was not influenced by Kupffer cell blockade or activated, suggesting that GSTpi activity is not easily induced in normal tissues.

In conclusion, Kupffer cells and their adjacent hepatocytes capable of regulating interactions could be demonstrated[30], suggesting that Kupffer cells may mediate hepatic functions including drug metabolism.

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