P.O.Box 2345, Beijing 100023,China
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com www.wjgnet.com

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• BASIC RESEARCH •

Influence of Kupffer cells on hepatic signal transduction as demonstrated by second messengers and nuclear transcription factors

Hong Ding, Jie-An Huang, Jing Tong, Xin Yu, Jie-Ping Yu

Hong Ding, Jing Tong, Xin Yu, Department of Pharmacology, College of Pharmacy, Wuhan University, Wuhan 430072, Hubei Province, China
Jie-An Huang, Department of Internal Medicine, the First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China
Jie-Ping Yu, Department of Internal Medicine, the First Affiliated Hospital of School of Medicine, Wuhan University, Wuhan 430064, Hubei Province, China
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Correspondence to: Dr. Hong Ding, Wuhan University College of Pharmacy, Wuhan 430072, Hubei Province, China. dinghong2000@263.net.cn
Telephone: +86-27-87682339 **Fax:** +86-27-87682339
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Abstract

AIM: To understand the influence of Kupffer cell (KC) on signal transduction pathways in the liver.

METHODS: To decrease selectively the number and function of KC, Kunming mice were ip injected with a single dose of gadolinium chloride ($GdCl_3$, 20 mg·kg^{-1}), the time-effect relationship assessment was performed after 1 d, 3 d and 6 d. sALT, sGST, liver glycogen content, phagocytic index, and expression of CD68 were assessed as the indexes of hepatotoxicity and functions of KC respectively, and morphology of KC was observed with transmission electron microscopy. Furthermore, cAMP, PGE_2 level, nitric oxide(NO) content, and mRNA expression of NFkappaBp65, Erk1, STAT1 were examined.

RESULTS: $GdCl_3$ could selectively cause apoptosis of KC and obvious reduction of KC's activity, but no hepatotoxicity was observed. One day after KC blockade, NO, PGE_2, cAMP contents in the liver were reduced 21.0 %, 6.94-fold, 8.3 %, respectively, and mRNA expression of NFkappaBp65 was decreased 3.0-fold. The change tendency of NO, PGE_2, and cAMP contents and mRNA expression of NFkappaBp65 were concomitant with recovery of the functions of KC. The contents of NO, PEG2, cAMP were increased when the functions of KC was recovered. However, all of the changes could not return to the normal level except NO content after 6 d $Gdcl_3$ treatment. No obvious changes were found in STAT1 and Erk1 mRNA expression in the present study.

CONCLUSION: Hepatic NO, PGE2, cAMP level and mRNA expression of NFkappaBp65 are closely related with the status of KC. It suggests that KC may play an important role in the cell to cell signal transduction in the liver.

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INTRODUCTION

Kupffer cells (KCs) account for a major portion of the tissue macrophages and play an important role in the defense mechanisms of the body[1]. KCs are involved in the pathogenesis of chemically mediated liver injury through release of biologically active mediators that promote the pathogenic process[2]. KCs can synthesize and release a variety of immunomodulating and inflammatory mediators such as oxygen-derived free radicals, nitric oxide, lipid mediators, and cytokines, etc. There are certain points to be elucidated that KCs involve in the pathophysiologic response of liver injury[3]. And now, many new functions have been found. KCs can reverse liver fibrosis and are critical for the progression of alcoholic injury[3,4]. Abolishment of KCs sensitization could prevent alcoholic liver injury[5]. KCs are major contributors to cytokine production in hepatic ischemia/reperfusion[6] and play a stimulatory role in liver regeneration[7]. Up to now, few studies about the influence of KCs on signal transduction in the liver have been reported. NO, PGE_2, cAMP are important second messengers transmitting and magnifying messages to modulate gene expression. NFkappaB, STAT, Erk are important nuclear transcription factors, which are involved in the regulation of cell proliferation and differentiation[8,9]. To understand the effect of KCs on the second messengers and nuclear transcription factors is of great importance in studying the mechanism of liver diseases. $Gdcl_3$, as an inhibitor of KCs, is often used as a tool for studying the role of KC[10]. Kupffer cell toxicant $GdCl_3$ prevents stellate cell activation and the development of fibrosis[11]. The present study was designed to clarify the effect of KC on signal transduction pathway in the liver following $GdCl_3$-induced KC blockade.

MATERIALS AND METHODS

Reagents

Gadolinium chloride ($GdCl_3$), collagenase IV, Indian ink were purchased from Sigma, USA. NO, PGE_2 detection kits were obtained from Bangding Biotechnology Co., Ltd. cAMP detection kit was obtained from Shanghai College of Chinese Traditional Medicine. CD68 immunohistochemical kit and NFkappaBp65, STAT1, Erk1 *in situ* hybridization kit were purchased from Wuhan Boster Biological Technology Co., Ltd. Other reagents were all of A.R.

Animal treatment

Kunming ♂ mice (aged 4-6 wk), weighing 22±3 g were obtained from the Experimental Animal Center of School of Medicine, Wuhan University. The animals were fed on a standard diet in pellets, and allowed free access to water. The mice were randomly distributed to control group, $GdCl_3$-1d

Table 1 Influence of $GdCl_3$ on hepatic function and activity of KCs in mice (n=8, $\bar{x}\pm s$)

Group	sALT ($mmol\cdot min^{-1}\cdot L^{-1}$)	sGST ($\mu mol\cdot min^{-1}\cdot L^{-1}$)	Liver glycogen ($\mu mol\cdot mg^{-1}\cdot pro^{-1}$)	Phagocytic activity(α)	Expression of CD68 (relative O.D.)
Control	2.4±0.3	15.2±2.2	4.9±0.9	9.7±0.7	0.131±0.018
$GdCl_3$-1d	2.6±0.9	14.9±1.9	5.4±1.4	5.2±0.4[b]	0.065±0.010[b]
$GdCl_3$-3d	2.6±1.0	16.5±3.1	5.8±1.1	6.0±1.1[a]	0.084±0.015[b]
$GdCl_3$-6d	2.6±1.4	15.7±2.3	5.0±0.2	6.8±1.3[a]	0.108±0.014[b]

[a]P<0.05, [b]P<0.01 *vs* control.

group, $GdCl_3$-3d group, $GdCl_3$-6d group, in which the mice received ip injection of a single dose of 20 $mg\cdot kg^{-1}$ of $GdCl_3$, and were sacrificed after administration of $GdCl_3$ for 1 d, 3 d, 6 d, respectively.

Test for phagocytic function

14 % Indian ink (10 $ml\cdot kg^{-1}$) was injected into the mice tail vein. After 1 min and 5 min, 20 μl blood was obtained from the orbital vein of the mice and added into 2 ml of 0.1 % Na_2CO_3 solution. Absorbance (OD) at 600 nm was read, and the phagocytic activity (α) of KCs was calculated as described[12].

Biochemical assay

Twenty-five percent liver homogenate was prepared, the glycogen content was quantified by an enzymatic reaction as previously described[13]. NO content was measured by Griess reaction[14]. PGE_2 and cAMP concentration were determined by radioimmunoassay, and the radioactivity of the samples was measured with a P△CK△RD CA-2000 liquid scintillation spectrometer[15]. The protein content of liver homogenate was determined by Lowry[16].

In situ hybridization and immunohistochemistry methods

The livers were briefly washed in cold 0.1 M phosphate buffer containing 0.1 % DEPC and then fixed in cold 4 % formaldehyde (in 0.1 M phosphate buffer, pH7.4), paraffin-embedded sections in 5-6 μm thickness were cut and placed onto aminopropyltriethoxysilane-coated glass slides. The expression of CD68 was determined by i*n situ* hybridization with DIG detection system kit. The anti-sense sequence of the probe was 5' -AAGCT TGGCC CAAGC CACCT TGGTT TTAGA-3' for Erk1 (extracellular signal-regulated kinase), 5' -CAGGT TGTCT GTGGT CTGAA GTCTA GAAGG-3' for STAT1 (signal transducers and activators of transcription), 5' -AGTTG ATGTC CGCAA TGGAG GTCTT-3' for NF-kBp65 (nuclear factor kappa B p65).

Image analysis of immunohistochemistry and in situ hybridization

Microscopic images through an interference filter (Nikon, Tokyo, Japan) were transferred to the processor (HPIAS image analysis system, Wuhan Tongji Medical University). Average absorbances in defined areas of the sections were measured, relative optical density(OD) was used to evaluate expression level.

Liver cells isolation and transmission electron-microscopic study

Liver cells were isolated as described[17]. In brief, after washed in D-Hanks, liver tissue was digested with 0.075 % collagenase for 30 min. The resulting suspension was passed through 70 μm gaze and then 1 g of sediment was generated after 10 min. Hepatocytes were fixed in 2.5 % glutaraldehyde in 0.1 $mol\cdot L^{-1}$ phosphate buffer, transmission electron- microscope was used to observe the morphology of KCs.

Statistical analysis

The data were presented as $\bar{x}\pm s$, and statistical analysis was performed with Student's t-test.

RESULTS

Effect of $GdCl_3$ on sALT, sGST, liver glycogen and activity of KCs

After administration of $GdCl_3$, no changes in sALT, sGST level and liver glycogen content were observed. Liver expression of CD68 (specific surface antigen of macrophage) and phagocytic activity (α) obviously reduced 102 %, 86 % respectively after 1 d of $GdCl_3$ treatment, then the function of KCs was gradually recovered. However they could not return to the normal level after 6 d of $GdCl_3$ treatment.

Electron microscopic study

The characteristics of apoptosis (the membrane of KCs was integrate, chromatin in the nucleus presented uneven distribution and was close to nuclear measure) were observed after treatment of $GdCl_3$ (Figure 1).

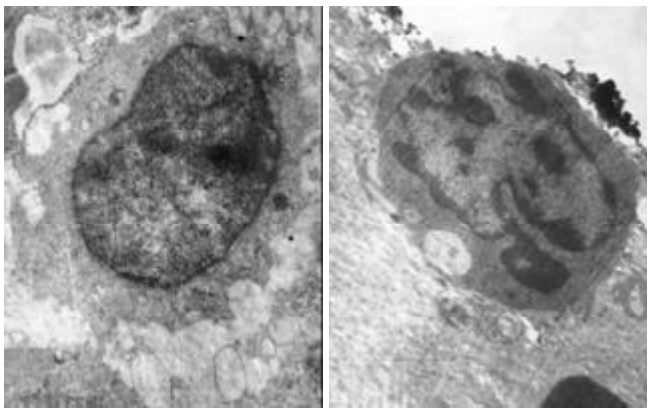

Figure 1 Influence of $GdCl_3$ on morphology of KCs under EM.

Effect on NO, PGE_2, cAMP content

After 1 d of $GdCl_3$ treatment, NO, PGE_2, cAMP contents were reduced 21.0 %, 6.94-fold, 8.3 %, respectively, and then they were gradually recovered. However, PGE_2 and cAMP contents could not return to the normal level after 6 d of treatment.

Table 2 Influence of $GdCl_3$ on NO, PGE_2, cAMP contents in liver (n=8 mice, $\bar{x}\pm s$)

Group	NO content ($pmol\cdot mg^{-1}\cdot pro^{-1}$.)	PGE_2 content ($pg\cdot mg^{-1}\cdot pro^{-1}$.)	cAMP content ($pmol\cdot mg^{-1}\cdot pro^{-1}$.)
Control	2.5±0.4	6.8±1.8	0.157±0.031
$GdCl_3$-1d	2.1±0.3[a]	0.9±0.2[b]	0.145±0.027[a]
$GdCl_3$-3d	2.0±0.3[a]	2.5±1.3[b]	0.131±0.010[a]
$GdCl_3$-6d	2.2±0.3	5.0±2.6[a]	0.133±0.010[a]

[a]P<0.05, [b]P<0.01 *vs* control.

Effect on NFkappaB, STAT1 and Erk1 mRNA expression

The time course of alteration of NFkappaB, STAT1 and Erk1 mRNA expressions after administration of 20 mg· kg^{-1} $GdCl_3$ showed that NFkappaB mRNA expression was decreased (3-fold) after administration of $GdCl_3$ for 1 d, then it was gradually recovered, but did not return to the normal level after 6 d of treament. No obvious influence on STAT1, Erk1 mRNA expressions was observed (Figure 2).

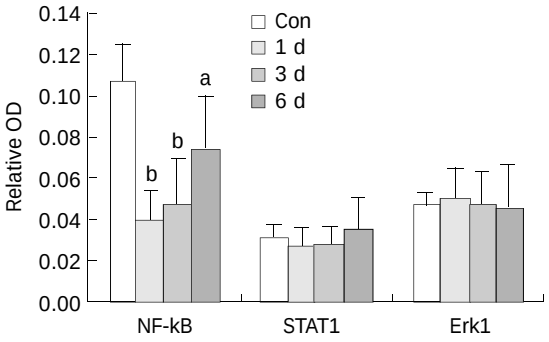

Figure 2 Time course of alteration of NFkappaB, STAT1 and Erk1 mRNA expressions after administration of 20 mg· kg^{-1} $GdCl_3$ (n=6, $\bar{x}\pm s$). $^aP<0.05$, $^bP<0.01$ *vs* control.

DISCUSSION

In the present study, ip injection of a single dose of $GdCl_3$ could selectively cause apoptosis of KCs, but did not induce hepatotoxicity. Therefore, it can be used as a tool for studying the role of KCs.

KCs are the phagocytic macrophages in the liver. NO, PGE_2, and cAMP could transmit and magnify extracellular messages to cells through a cascade system to regulate gene expression and cell proliferation and differentiation[18]. NO, PGE_2, and cAMP had different functions in hepatoprotection and hepatic injury. NO from KCs could induce membrane barrier dysfunction in liver sinusoid[19]. But according to Abou-Elella *et al*, the exacerbation of hepatocyte death by KCs was not related to NO[20]. Hsu *et al*, sproposed that KCs be the major source of induction of inducible NO synthase(iNOS) activity and NO production have a beneficial role in hepatic IR injury and the constitutive isoforms of NOS play a hepatoprotective role in hepatic injury[21-23]. The protective function of NO against hepatic injury might lie in that it could reduce tissue oxygenation[24]. PGE_2 derived from KCs increased cAMP, which caused triglyceride accumulation in the liver and fatty liver[3]. Increase of cAMP levels in KCs occurred during the late stage of polymicrobial sepsis, and might contribute to the depression of macrophage phagocytic function[25]. The current study showed that NO, PGE_2, and cAMP contents in the liver were reduced following KC blockade, and recovered following the functional recovery of KCs. It suggests that Kupffer cells may mediate signaling of second messengers in the liver. Moreover, in the present experiment, the hepatic function did not change following the alterations of NO, PGE_2, and cAMP content, the reasons remain to be researched.

It has widely been accepted that NFkappaB activation plays an important role in the pathophysiology of inflammatory disorders[26]. NFkappaB is an essential component of TNF proliferative pathway and TNF-induced changes in IL-6 mRNA, STAT3, and c-myc mRNA are dependent on NFkappaB activation[27]. NFkappaB activation may be important in "switching off" the cytokine cascade during acute pancreatitis[28]. The current study showed that mRNA expression of NFkappaB in the liver was down-regulated after KC blockade, suggesting that KCs may play an important role in mediating liver diseases and inflammatory disorders via changes of the signal transduction pathway in the liver.

In this experiment, STAT1 and Erk1 mRNA expressions were not affected by $GdCl_3$. STAT1 played a harmful role in Con A-mediated hepatitis, whereas STAT3 protected against liver injury[29]. Inhibition of STAT1 activation without reduction of STAT1 protein level might be one of the factors that are involved in the cAMP-dependent stellate cell growth arrest[30]. In this study, we only examined the expression of STAT1 mRNA, the relationship between STAT1 and hepatic injury needs to be further studied.

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