ELSEVIER

Journal of Hepatology 36 (2002) 466–473

Journal of Hepatology

www.elsevier.com/locate/jhep

Inhibition of Kupffer cell activity induces hepatic triglyceride synthesis in fasted rats, independent of lipopolysaccharide challenge

Audrey M. Neyrinck, Henryk S. Taper, Valérie Gevers, Barbara Declerck, Nathalie M. Delzenne*

Unité de Pharmacocinétique, Métabolisme, Nutrition et Toxicologie, Département des Sciences Pharmaceutiques, Université Catholique de Louvain, 73 Avenue Mounier, B-1200 Brussels, Belgium

***Background*: Lipopolysaccharides (LPS), cleared from the blood by Kupffer cells, induce hypertriglyceridemia.**

***Aims*: To test the hypothesis that $GdCl_3$, through inhibition of large Kupffer cell activity, modulates LPS-induced hyperlipidemia in rats.**

***Methods*: Male Wistar rats received a single intravenous injection of $GdCl_3$ (10 mg/kg) or saline, 24 h before intraperitoneal LPS (1.5 mg/kg) administration. Serum and hepatic lipids as well as activity of key enzymes controlling fatty acid synthesis and esterification in liver tissue were measured. The incorporation of labeled precursors into lipids was assessed in cultured precision-cut liver slices.**

***Results*: $GdCl_3$ does not prevent hypertriglyceridemia occurring in LPS-treated rats. Surprisingly, $GdCl_3$ per se is able to promote triglycerides accumulation in the liver tissue, an effect related to an increase in hepatic fatty acid esterification. Such an effect also occurs in rats receiving a dietary supplementation with glycine (5%) known to inhibit Kupffer cell secretory capacity.**

***Conclusions*: Large Kupffer cell inhibition does not prevent LPS-induced hypertriglyceridemia and even leads to a metabolic shift of fatty acids towards their esterification and accumulation in the liver tissue, suggesting that Kupffer cells play a role in the regulation of lipid metabolism of the adjacent hepatocytes, independent of any inflammatory stimulus.**

***Keywords*: Kupffer cell; Triglyceride; Gadolinium chloride; Lipopolysaccharide; Liver; Precision-cut liver slice; Glycine**

1. Introduction

Kupffer cells are resident macrophages distributed along the hepatic sinusoids, which play a major role in clearing circulating LPS from the blood [1]. Several lines of evidence suggest that Kupffer cells may be the predominant cells responsible for initiating the inflammatory cascade resulting in septic shock [2]. Bioactive molecules, including cytokines (tumor necrosis factor-alpha (TNF-α), interleukin (IL-1, IL-6), interferon-alpha (IFN-α)), reactive intermediates ($NO^{\cdot}$), lysosomal enzymes or prostaglandins, generated by Kupffer cells in response to LPS stimulation could act as paracrine factors to modulate hepatocyte metabolism.

Gadolinium chloride ($GdCl_3$), when injected intravenously, is a specific inhibitor that completely eliminates large Kupffer cells in the liver tissue; it blocks Kupffer cell phagocytosis without exerting any toxic effect on hepatocytes, endothelial cells, circulating monocytes or other macrophages pools [3–5]. Moreover $GdCl_3$ is able, at least in vitro, to suppress mediator release (IL-6, TNF-α and $NO^{\cdot}$) by Kupffer cells upon LPS activation [6].

The host response to infection and inflammation mimicked by LPS injection in animals, often leads to hypertriglyceridemia due to an increase in very low density lipoprotein (VLDL) secretion by the liver [7–9]. Much evidence suggests that liver is the key organ in total body lipid metabolism regulation when challenged either with LPS or cyto-

Received 6 June 2001; received in revised form 29 November 2001; accepted 8 December 2001

* Corresponding author. Tel.: +32-2-764-7367; fax: +32-2-764-7359.
E-mail address: delzenne@pmnt.ucl.ac.be (N.M. Delzenne).

Abbreviations: LPS, lipopolysaccharide; PCLS, precision-cut liver slices; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon; VLDL, very low-density lipoprotein; TG, triglycerides; PL, phospholipids; NEFA, non-esterified fatty acids; CHO, cholesterol; HDL, high-density lipoprotein; FAS, fatty acid synthase; ATPCL, ATP citrate lyase; ME, malic enzyme; PAP, phosphatidate phosphohydrolase; LDL, low-density lipoprotein.

0168-8278/02/$20.00
PII: S0168-8278(02)00009-0

kines. In vivo administration of LPS or IL-6 produces a rapid increase in hepatic de novo fatty acid synthesis [9,10]; IL-6 directly induces hepatocyte lipogenic capacity in primary-culture rat hepatocytes [11]. Similarly, TNF-α, TNF-β, IL-1 and IFN-α induce a rapid increase, but sustained for 17 h, in hepatic de novo fatty acid synthesis [11–14]. Those data suggest that the activity of Kupffer cells, through the release of mediators, could play a role in the modulation of hepatic lipid metabolism after LPS challenge. In the present study, we examined the hypothesis that the inhibition of Kupffer cell activity, namely by $GdCl_3$, could modulate LPS-induced hyperlipidemia in rats.

2. Materials and methods

2.1. Chemicals

Most chemicals of purest grade available were purchased from Sigma (Filter Service, Belgium) or Boehringer (Mannheim, Germany). [1-^{14}C]acetic acid (specific activity 60 mCi/mmol) and [1-^{14}C]palmitic acid (specific activity 8.4 mCi/mmol) were obtained from Amersham Pharmacia Biotech Europe (Buckinghamshire, UK) and NEN Products (Boston, MA), respectively. Thin-layer chromatography silica gel 60 F_{254} plates were purchased from Merck (Darmstadt, Germany), Williams' medium E, L-glutamine, penicillin and streptomycin from Gibco BRL (Middlesex, UK).

2.2. In vivo studies

2.2.1. Experiment 1: effect of $GdCl_3$ and/or LPS treatments (Fig. 1)

Male Wistar rats (240–260 g, Iffa Credo, les Oncins, France) received AO4 standard diet (UAR, Villemoisson-sur-Orge, France) and tap-water ad libitum. $GdCl_3$ (10 mg/kg body weight) or NaCl 9‰ were injected intravenously to rats 24 h before intraperitoneal LPS administration (1.5 mg/kg body weight) or NaCl 9‰. Food was withdrawn after the injection of LPS. Two hours after LPS or saline injection, serum was collected from the tail vein. Animals were killed under pentobarbital (60 mg/kg) anesthesia 18 h after LPS or saline injection; blood was collected from the descending vena cava and liver pieces were either clamped in liquid nitrogen and stored at −80 °C before analysis or kept in formalin. All the animals received care in compliance with institution's guidelines from the National Academy of Sciences (NIH publication 86-23).

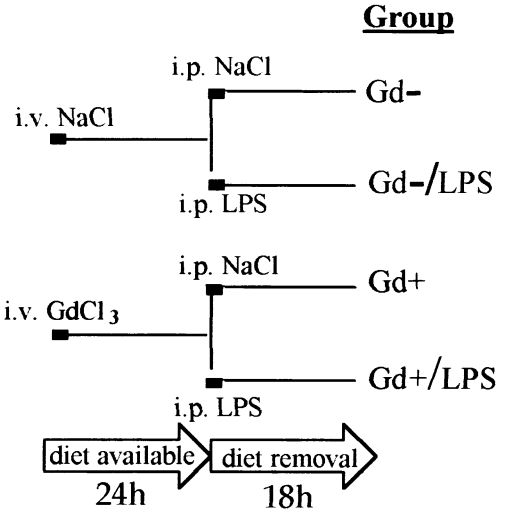

Fig. 1. Schematic representation of the experimental design of the study. Gd, gadolinium ($GdCl_3$); LPS, lipopolysaccharide; i.v., intravenous; i.p., intraperitoneal.

2.2.2. Experiment 2: effect of a diet enriched with glycine

Rats were fed a powdered AO4 standard diet containing 5% glycine (glycine diet) or 5% casein (control diet) ad libitum for a period of 3 days. Rats were then fasted for 18 h before blood and liver removal.

2.2.3. Colloidal carbon uptake by the liver and spleen macrophages

Phagocytic activity of Kupffer cells and of spleen macrophages towards colloidal carbon has been assessed on tissue slices as previously described [15].

2.2.4. TNF-α assay

TNF-α level was determined in frozen aliquots of serum (Quantikine M Rat TNF-α immunoassay, R&D Systems). Limit of detection was 5 pg/ml.

2.2.5. Biochemical parameters

Triglycerides (TG), phospholipids (PL), non-esterified fatty acids (NEFA), total cholesterol (CHO) and high-density lipoprotein-cholesterol (HDL-CHO) were measured in the plasma or serum by using kits coupling enzymatic reaction and spectrophotometric detection of reaction end-products (EliTech Diagnostics, Brussels, Belgium); liver lipid analysis was performed after Folch chloroform–methanol extraction [16]. Fatty acid synthase (FAS), ATP citrate lyase (ATPCL) and malic enzyme (ME) activities were measured in the cytosolic fractions [17–19]. Phosphatidate phosphohydrolase (PAP) activity was measured in microsomal fractions [20]. Protein was assayed by the method of Lowry et al.[21].

2.2.6. Fat histochemical detection

Liver pieces were frozen in liquid nitrogen for cryostat sections and fat detection by staining with Sudan Red 7b (Janssen Chimica, Belgium).

2.3. In vitro studies

2.3.1. Study on precision-cut liver slices (PCLS) in culture

PCLS were prepared from rats pretreated with $GdCl_3$ (10 mg/kg body weight) (Gd+) or NaCl 9‰ (Gd−) 48 h after injection as previously described [22,23]. PCLS were incubated in Williams' E medium (supplemented with penicillin (100 IU/ml), streptomycin (100 μg/ml), glutamine (2 mM) and insulin (100 nM), 0.3% bovine serum albumin) containing 0.2 mM [^{14}C]palmitate (0.2 mCi/mmol) or 2 mM [^{14}C]acetate (0.2 mCi/mmol) and incubated for 1 h 30 min and 3 h, respectively. PCLS were sonicated in 0.5 ml NaCl (0.05 M) before lipid extraction and separation by thin-layer chromatography with hexane/ether/acetic acid (80:20:1) [16,24]. Spots corresponding to TG, PL and CHO were scrapped from the plate and counted in 10 ml scintillation fluid (Ultima Gold) in a beta Wallac-1410 counter.

ATP content of PCLS was greater than 8 nmol/mg protein in all experiments.

2.3.2. Study on hepatocytes in primary culture

Four experiments were performed, using distinct hepatocyte preparations from fed rats [25]. Cell viability assessed by Erythrosine B exclusion test was greater than 80%. Hepatocytes were incubated in a medium supplemented with penicillin (100 IU/ml), streptomycin (100 μg/ml), bovine serum albumin (0.1%), insulin (100 nM) and glutamine (2 mM) in the presence of 10 μg $GdCl_3$/ml or the same volume of NaCl 9‰. After 24 and 48 h of incubation, the medium was replaced by fresh medium containing 0.2 mM [^{14}C]palmitate (0.2 mCi/mmol) or 2 mM [^{14}C]acetate (0.2 mCi/

mmol). After 3 h of incubation, the medium was removed and hepatocytes were sonicated in 0.5 ml NaCl (0.05 M) before lipid extraction and separation as described above.

2.4. Statistical analysis

In experiment 1, statistical significance of observed variations (effect of $GdCl_3$-treatment or LPS-treatment alone and the $GdCl_3 \times$ LPS interaction) was assessed by applying the two-factor analysis of variance (ANOVA) test, using SPSS statistical software.

For in vitro studies, all the parameters were measured at least in triplicate (three slices or three hepatocyte culture plates obtained from the same rat). A mean value was calculated for each experiment. Results were analyzed by Student's *t*-test, $P < 0.05$ being considered as the significant level.

3. Results

3.1. Analysis of colloidal carbon uptake by liver and spleen macrophages

Kupffer cells having taken up colloidal carbon (colored in black) appeared in the Gd− rats as large and irregular cells mainly localized in the periportal zone (Fig. 2). Twenty-four hours after $GdCl_3$ treatment, colloidal carbon uptake by Kupffer cells was strongly depressed whereas it increased in the spleen. This phenomenon persisted 48 h after $GdCl_3$ injection (Fig. 3). Colloidal carbon uptake was not modified 18 h after LPS administration.

3.2. Analysis of TNF-α concentration in the serum

TNF-α was only detectable in the serum after LPS administration. 2 h after the LPS injection, serum TNF-α level reached 3.9 ± 1.2 and 4.6 ± 1.9 ng/ml in Gd−/LPS and Gd+/LPS rats, respectively (Student *t*-test: $P > 0.05$). Serum TNF-α level measured 18 h after LPS administration remained detectable in two Gd−/LPS animals out of seven; the residual concentration of TNF-α (17 ± 1 pg/ml) was detectable in all Gd+/LPS rats.

3.3. Effect of $GdCl_3$ and/or LPS treatment on lipid metabolism

TG concentration was significantly increased by 66% 2 h after LPS administration (0.78 ± 0.02 and 1.30 ± 0.10 mM for Gd− and Gd−/LPS rats, respectively; $P < 0.05$,

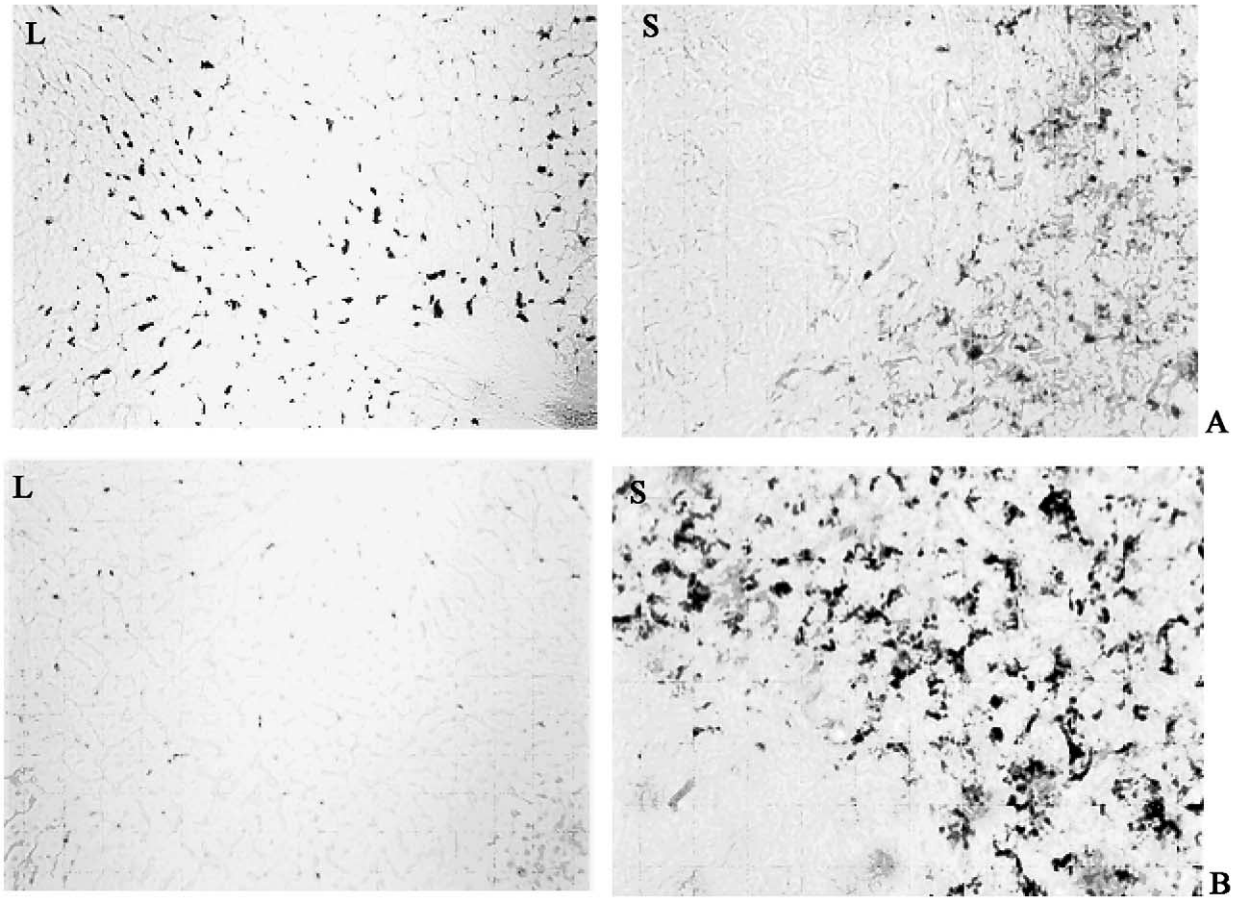

Fig. 2. Distribution of colloidal carbon in the liver (L) and spleen (S) 20 min after injection in the portal vein, 24 h after $GdCl_3$ injection (B) or saline injection (A) (original magnification ×300). In the liver, colloidal carbon is mainly taken up in Kupffer cells (dark spots). In the spleen, colloidal carbon is taken up by macrophages of the red pulp.

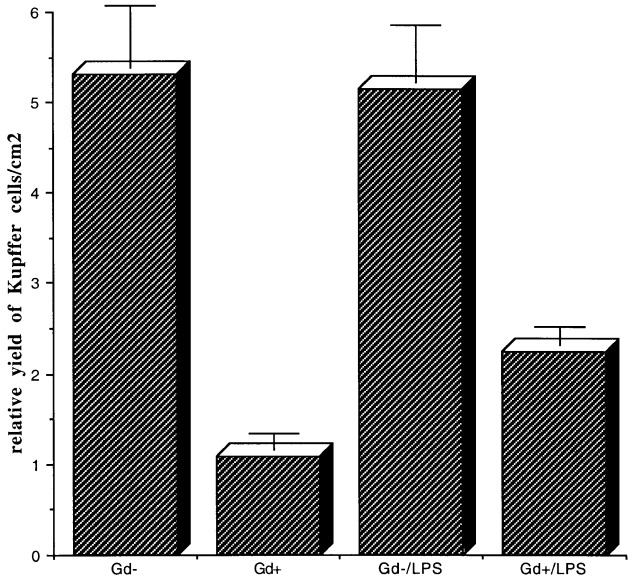

Fig. 3. Semi-quantification of colloidal carbon uptake by Kupffer cells, 20 min after intravenous injection of colloidal carbon in the portal vein, by in situ densitometry on liver section. Relative yield of black cells per cm^2 corresponding to Kupffer cells containing colloidal carbon was calculated after scanning and computerizing the image of liver slices (three fields per rat; all taken in the medium lobe of the liver). Values are mean ± SEM for four rats in each group. #Significant effect of $GdCl_3$ treatment ($P < 0.05$, ANOVA).

ANOVA) and by 44% in $GdCl_3$-pretreated rats (0.80 ± 0.07 mM and 1.15 ± 0.09 mM for Gd+ and Gd+/LPS rats, respectively; $P < 0.05$, ANOVA). Eighteen hours after LPS injection, TG concentration was 33% higher in Gd−/LPS group than in Gd− group and 85% in Gd+/LPS group

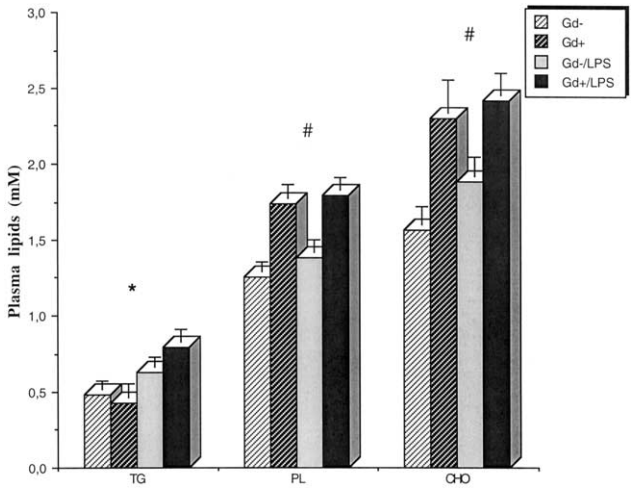

Fig. 4. Plasma lipids after $GdCl_3$ and/or LPS treatment. $GdCl_3$ (10 mg/kg, intravenously) or NaCl 9‰ was given 1 day before LPS (1.5 mg/kg, intraperitoneally) and the 18 h-fasted rats were killed 18 h after LPS treatment. Values are mean ± SEM of at least four rats. *Significant effect of LPS-treatment; #significant effect of $GdCl_3$-treatment ($P < 0.05$, ANOVA). TG, triglycerides; PL, phospholipids; CHO, cholesterol.

than in Gd+ group (Fig. 4). LPS injection changed neither PL, nor CHO levels 18 h after the treatment. Surprisingly, plasma PL and CHO concentration significantly increased 2 days after $GdCl_3$ injection as compared to controls, independent of LPS challenge. This increase in total CHO observed in the plasma of Gd+ rats was only due to an increase in HDL-CHO (0.75 ± 0.12 and 1.46 ± 0.16 mM for Gd− and Gd+ rats, respectively; $P < 0.05$, ANOVA); low density lipoprotein-cholesterol (LDL-CHO) concentration, estimated following the Friedewald equation [26], was not modified by the $GdCl_3$-pretreatment (0.68 ± 0.15 and 0.65 ± 0.11 mM for Gd− and Gd+ rats, respectively). Moreover, NEFA level increased 24 h after $GdCl_3$ treatment (0.74 ± 0.03 and 0.98 ± 0.05 mM for Gd− and Gd+ rats, respectively; $P < 0.05$, ANOVA).

LPS treatment lead to an increase in liver weight without hepatic TG content modification as compared to controls (Table 1). A twofold increase of TG content in the liver tissue occurred in Gd+ and Gd+/LPS rats: numerous fat positive hepatocytes were localized mainly in periportal area (Fig. 5). Hepatic PL content was not modified after LPS injection or by $GdCl_3$ pretreatment. However, hepatic CHO concentration slightly decreased 18 h after LPS injection whereas it increased 2 days after $GdCl_3$ administration ($P = 0.058$, ANOVA); those opposite effects allowed a 'normalization' of hepatic CHO content in rats treated with both $GdCl_3$ and LPS.

Among enzymes controlling fatty acid synthesis (FAS, ATPCL and ME) and esterification (PAP), only ATPCL activity slightly raised after LPS administration (Table 1). Pretreatment of rats with $GdCl_3$ promoted significantly the effect of LPS on ATPCL. Surprisingly, PAP activity was increased by 35% in Gd+ rats compared to Gd− rats.

Since $GdCl_3$ is able to promote per se the accumulation of TG in the liver tissue, the 'intrinsic' capacity of the liver tissue to synthesize lipids after $GdCl_3$ treatment was assessed in PCLS: PCLS obtained from Gd+ rats allowed a higher incorporation of [^{14}C]acetate or [^{14}C]palmitate into lipids (CHO, TG, PL) as compared to the Gd− rats (Fig. 6).

3.4. Does $GdCl_3$ act on lipid metabolism through inhibition of Kupffer cell activity?

Pretreatment of hepatocytes in culture with $GdCl_3$ failed to increase acetate or palmitate incorporation into the different lipid fractions after 24 or 48 h of incubation (Table 2). Moreover, the metabolic effects of $GdCl_3$ were compared to dietary supplementation with glycine, another treatment known to decrease Kupffer cell activity. Hepatic TG level and PAP activity in the liver tissue were significantly increased in rats receiving 5% glycine diet as compared to control rats (Fig. 7). Serum NEFA level was increased (1.5 fold) in glycine-fed rats as compared to controls (vena cava: $P = 0.07$; portal vein: $P < 0.05$).

Table 1
Modification of lipid metabolism in the liver tissue after $GdCl_3$ and/or LPS treatment[a]

	Gd−	Gd+	Gd−/LPS	Gd+/LPS
Liver weight (g)*	8.22 ± 0.33	7.65 ± 0.11	9.26 ± 0.26	9.54 ± 0.08
Hepatic content (nmol/mg protein)				
Triglycerides[#]	42.7 ± 5.3	77.9 ± 13.9	38.9 ± 5.7	70.2 ± 9.8
Phospholipids	109.4 ± 2.8	116.2 ± 7.0	115.3 ± 1.5	106.2 ± 4.2
Cholesterol*	37.3 ± 1.0	43.2 ± 2.9	35.1 ± 0.8	36.2 ± 1.0
Enzyme activities (mIU/mg protein)				
Fatty acid synthase	2.9 ± 0.2	3.5 ± 0.3	3.8 ± 0.5	3.5 ± 0.4
ATP citrate lyase*[◇]	9.9 ± 0.5	9.3 ± 1.6	10.8 ± 0.8	15.1 ± 1.4
Malic enzyme	15.6 ± 0.9	13.9 ± 2.5	18.1 ± 1.5	16.3 ± 1.4
Phosphatidate phosphohydrolase[#]	10.9 ± 0.9	14.7 ± 0.5	10.6 ± 0.4	14.5 ± 0.7

[a] $GdCl_3$ (10 mg/kg, intravenously) or NaCl 9‰ was given 1 day before LPS (1.5 mg/kg, intraperitoneally) and the 18 h-fasted rats were killed 18 h after LPS treatment. Values are mean ± SEM of at least four rats. *Significant effect of LPS-treatment; [#]significant effect of $GdCl_3$-treatment; [◇]significant interaction between LPS and $GdCl_3$ treatments ($P < 0.05$, ANOVA).

4. Discussion

4.1. Effect of $GdCl_3$ and/or LPS treatment on Kupffer cells activity: which relationship with serum TNF-α?

$GdCl_3$ i.v. injection had previously been shown to inhibit large Kupffer cells activity 6 h after the treatment [3]. We show here that colloidal carbon uptake by liver macrophages is also reduced 24 h (before LPS challenge) or 48 h after $GdCl_3$ administration, whereas splenic phagocytic activity is increased, thus suggesting a 'compensatory' role of this organ [27]. Even if a liver TNF-α mRNA induction may occur a few hours after $GdCl_3$ injection [28,29], no TNF-α was detectable in the serum of rats 1, 2, 4, 6, 30 or 48 h after $GdCl_3$ injection (data not shown) as previously shown in endotoxin-sensitive mice 2 days after $GdCl_3$ administration [30]. Moreover, we have previously shown, in a similar protocol to the one used in the present study, that rat PCLS prepared 24 h after $GdCl_3$ administration exhibited a lower release of TNF-α in the medium [31]. A higher serum TNF-α level occurred after LPS challenge in $GdCl_3$-treated animals, despite a depression of Kupffer cell phagocytic activity. Putative explanations could be that (1) the inhibitory effect of $GdCl_3$ primarily occurs on ED2-positive mature Kupffer cells whereas other hepatic macrophage/monocyte populations may remain present in the tissue which are even more sensitive to LPS in term of TNF-α production [32]; (2) peritoneal and splenic cells rather than Kupffer cells become the primary source of circulating TNF-α after sepsis [5,33,34]. Would the promotion of spleen macrophage activity by $GdCl_3$ lead to a release of TNF-α, after LPS treatment? This hypothesis is denied by Kohno et al., showing that splenectomy does not affect plasma TNF (TNF-α and TNF-β) in $GdCl_3$-endotoxemic rats [30].

4.2. Effect of LPS on lipid metabolism

A single intraperitoneal injection of LPS induces hypertriglyceridemia [7–9]. The rapid increase in serum triglycerides, shown here 2 h after LPS administration, could be due to an inhibition of lipoprotein lipase activity in the adipose tissue and/or a stimulation of hepatic lipogenesis [7,9,10]. When LPS is given in a high dose (50 μg/100 g body weight

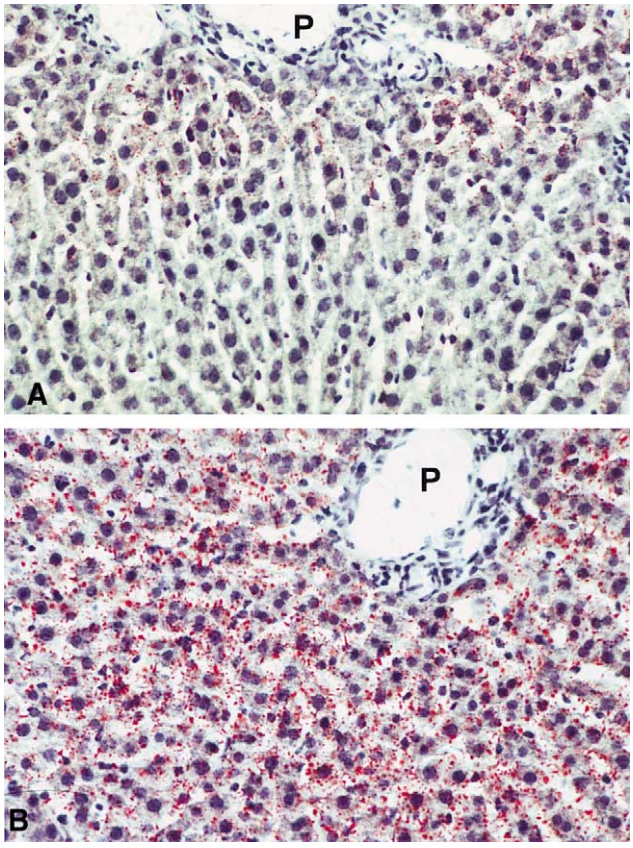

Fig. 5. Histological analysis of liver cryostat sections from Gd− rats (A) and Gd+ rats (B) stained with Sudan Red 7b for lipid detection and counterstained with Mayer's Haemalum (original magnification ×750). Rare hepatocytes with cytoplasmic Sudan 7b slightly positive microvacuoles are dispersed in the periportal area of liver lobule from Gd− rats (A). Almost all hepatocytes in the periportal area demonstrate cytoplasmic Sudan 7b positive micro- and macrovacuoles in the liver of Gd+ rats (B). P, portal space.

like in our study), the major metabolic effect results from a decrease in TG-rich lipoprotein clearance, through inhibition of lipoprotein lipase activity [9]. The hypertriglyceridemia persists 18 h after LPS injection in fasted rats. At that time, despite a higher liver weight probably due to hepatotrophic effect of endotoxin, no modification of liver TG content was observed after LPS treatment. Only a slight increase in hepatic ATP-citrate lyase activity (ATPCL), a lipogenic enzyme controlling fatty acid and cholesterol synthesis by providing acetyl-CoA in the cytoplasm, is observed in LPS-treated rats, this increase being potentiated by $GdCl_3$ pretreatment. ATPCL is mainly induced in the fed state through the activation of gene transcription by glucose and insulin, a regulation involving sterol regulatory element-binding protein (SREBP-1) [35,36]. Surprisingly, in our study, the increase in ATPCL activity due to LPS treatment occurs in fasted rats and is independent of any induction of other lipogenic enzymes. TNF-α, released after LPS treatment, might be involved in the regulation of hepatic ATPCL activity since it induces SREBP-1 maturation in human hepatocytes in a dose-dependant manner [37].

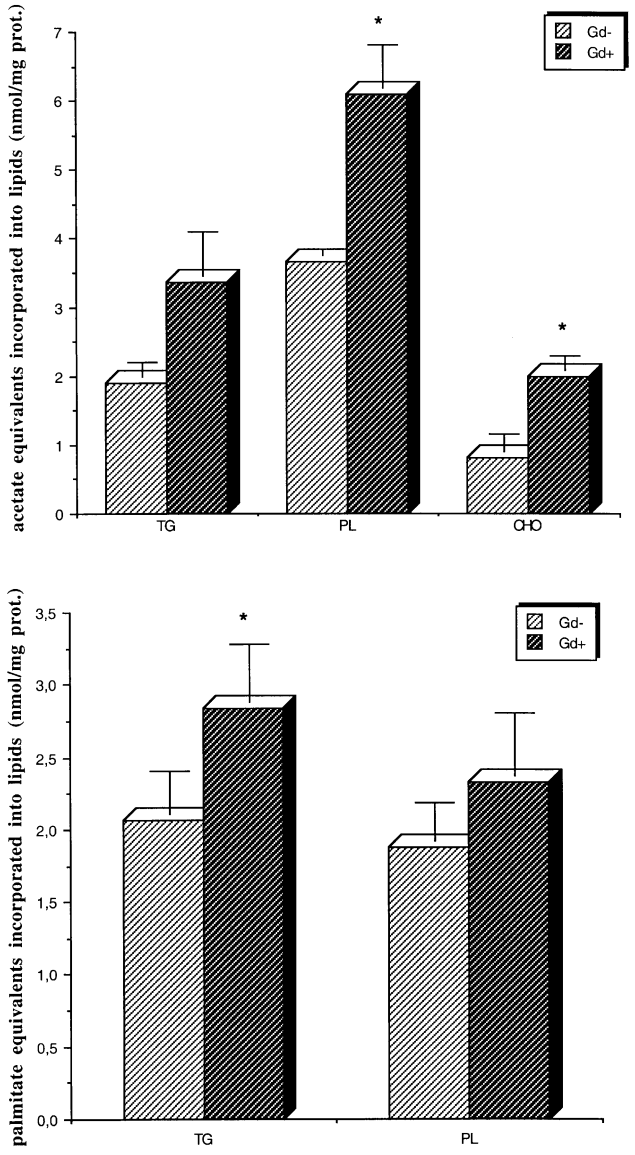

Fig. 6. Incorporation of [^{14}C]acetate or [^{14}C]palmitate into liver lipids (TG, triglycerides; PL, phospholipids; CHO, cholesterol) by PCLS. PCLS from rats pretreated with $GdCl_3$ (Gd+) or NaCl (Gd−) were incubated in medium containing 2 mM [^{14}C]acetate for 3 h (A) or 0.2 mM [^{14}C]palmitate for 1 h 30 min (B). Values are mean ± SEM of four independent experiments. *$P < 0.05$, Student's unpaired *t*-test.

Table 2
Effect of $GdCl_3$ on [^{14}C]acetate and [^{14}C]palmitate incorporation into liver lipids in hepatocytes in primary culture[a]

	24 h	48 h
Rate of [^{14}C]acetate incorporation (% of control)		
Triglycerides	97 ± 7	102 ± 18
Phospholipids	100 ± 6	90 ± 13
Cholesterol	87 ± 5	96 ± 9
Rate of [^{14}C]palmitate incorporation (% of control)		
Triglycerides	104 ± 6	109 ± 4
Phospholipids	105 ± 8	102 ± 4

[a] Rat hepatocytes were treated with $GdCl_3$ 10 μg/ml for 24 or 48 h and further incubated with [^{14}C]acetate or [^{14}C]palmitate for 3 h. Cells were harvested for quantification of incorporation rates into different lipid fractions as described in Section 2. Rates normalized to control = 100 permit assessment of $GdCl_3$ effects despite variability between preparations in the absolute control rates of incorporation into lipids. Values are mean ± SEM of four independent experiments.

4.3. Effect of $GdCl_3$ on lipid metabolism

In contrast to LPS, $GdCl_3$ treatment alone does not

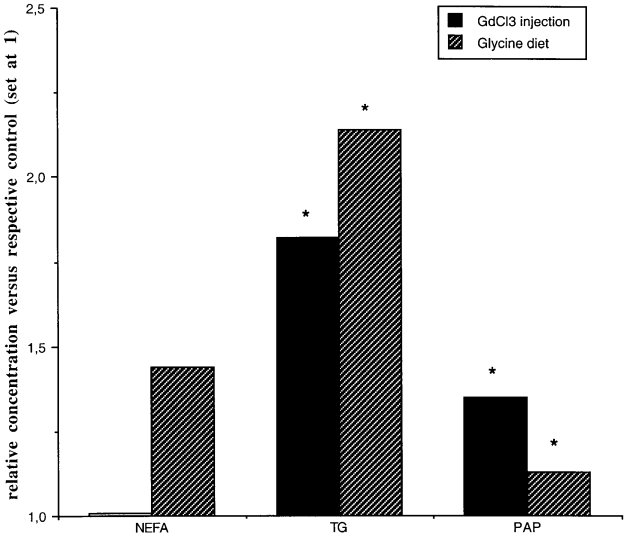

Fig. 7. Effect of dietary glycine (glycine 5% during 3 days, then 18 h fasting) on serum non-esterified fatty acids (NEFA), hepatic triglycerides (TG) and phosphatidate phosphohydrolase (PAP) activity in the liver, as compared to the effect of $GdCl_3$ treatment. Values represent the relative concentration of parameters in glycine diet-fed rats or $GdCl_3$-treated rats as compared to respective control rats (*$P < 0.05$, rats receiving glycine diet versus control diet or Gd+ rats versus Gd− rats; Student's unpaired *t*-test).

provoke an increase in serum TG, but leads to a strong and unexpected accumulation of liver TG 48 h after the treatment. This may result from the higher PAP activity, a key enzyme controlling the synthesis of TG and PL from diacylglyceride phosphate [38]. In accordance with the in vivo results, palmitate and acetate incorporation into PL and TG was higher in precision-cut liver slices obtained from $GdCl_3$-treated than control rats. In addition, $GdCl_3$ promotes the availability of fatty acids by increasing NEFA concentration in the serum one day after injection, a phenomenon which could also contribute to the liver steatosis observed later on. Cascales et al. proposed that an increased availability of fatty acids promotes the activation and translocation of PAP from the cytosol to the membranes, thus increasing synthesis of TG in the liver [38]. As PAP activity is also affected by glucocorticoids [39], insulin [40], noradrenaline [40], cAMP [41] or TGF-β [42], the influence of $GdCl_3$ on those parameters merits further study.

Phospholipids, the main lipid component of HDL, as well as cholesterol-HDL levels, were increased in the plasma of rats two days after $GdCl_3$ treatment. These results, together with the higher cholesterol content in the liver tissue, suggest a higher cholesterogenesis and cholesterol-HDL secretion in $GdCl_3$ treated animals. In fact, PCLS obtained from $GdCl_3$-treated rats exhibited a higher cholesterogenesis, as shown by the extent of incorporation of acetate into cholesterol. PAP and HMG-CoA reductase, a key enzyme of cholesterogenesis, have previously been shown to change in parallel under various conditions [43].

In order to investigate the possibility that $GdCl_3$ directly acts on hepatocytes to increase lipid synthesis, hepatocytes in primary culture have been incubated with $GdCl_3$ (at 10 μg/ml, a concentration known to inhibit the LPS-induced release of IL-6 or TNF-α by Kupffer cells in culture, without affecting LDH release by hepatocytes in primary culture [6]) for 24 and 48 h before assessing lipid synthesis. No increase of [^{14}C]acetate or [^{14}C]palmitate incorporation into TG, PL or CHO occurred in hepatocytes incubated with $GdCl_3$ versus control. We thus propose that the increase in cholesterogenesis and fatty acid esterification occurring in $GdCl_3$-treated animals is rather a consequence of inhibition of Kupffer cell activity than a direct effect on hepatocytes, suggesting a role of Kupffer cells in the regulation of liver lipid metabolism. Moreover, the major $GdCl_3$ effects (hepatic steatosis, increased serum NEFA and hepatic PAP activity) were reproduced in rats previously treated with a dietary supplementation with glycine. Although phagocytic activity by Kupffer cells (colloidal carbon uptake) was not modified by glycine supplementation in the diet (data not shown), such a treatment inhibits the production of eicosanoids and cytokines by Kupffer cells [44]. Taken together, these results support the hypothesis that the inhibition of Kupffer cell phagocytic and/or secretory capacity leads to a metabolic shift of fatty acids towards their esterification and accumulation in the liver tissue even in fasting state.

5. Conclusions

In this study, we have shown that $GdCl_3$ treatment, although it reduces Kupffer cell activity in the liver, does not prevent either TNF-α release in the serum, or hypertriglyceridemia in LPS-treated rats, and even promotes certain metabolic alterations due to LPS treatment. $GdCl_3$ is largely used to study the role of Kupffer cells in several models of liver injury such as CCl_4- or Pb-induced toxicity [45,46], partial hepatectomy [28], reperfusion injury after liver transplantation [47] or ethanol-induced steatosis in the enteral ethanol feeding model [48]. Surprisingly, $GdCl_3$ per se is able, in 18 h-fasted rats, and without any further stimulus, not only to promote fatty acid release by the adipose tissue but also to favor their esterification in the liver tissue, leading to steatosis. This observation could be physiologically relevant since an inhibition of Kupffer cell activity in the liver correlates with the development of steatosis as shown recently in obese Zucker fa/fa rat [49]. The contribution of bioactive compounds released by Kupffer cells in the regulation of lipid metabolism in adjacent hepatocytes should be clarified.

References

[1] Mathison JC, Ulevitch RJ. The clearance, tissue distribution and cellular localisation of intravenously injected lipopolysaccharide in rabbits. J Immunol 1979:1233–2143.
[2] Laskin DL, Pendino KJ. Macrophages and inflammatory mediators in tissue injury. Annu Rev Pharmacol Toxicol 1995;135:655–677.
[3] Hardonk MJ, Dijkhuis FWJ, Hulstaert CE, Koudstaal J. Heterogeneity of rat liver and spleen macrophages in gadolinium chloride-induced elimination and repopulation. J Leukoc Biol 1992;52:296–302.
[4] Gloor B, Todd KE, Lane JS, Lewis MP, Reber HA. Hepatic Kupffer cell blockade reduces mortality of acute hemmorrhagic pancreatitis in mice. J Gastrointest Surg 1998;2(5):430–435.
[5] Ayala A, O'Neill PJ, Uebele SA, Herdon CD, Chaudry IH. Mechanism of splenic immunosuppression during sepsis: key role of Kupffer cell mediators. J Trauma 1997;42(5):882–888.
[6] Saad B, Frei K, Scholl FA, Fontana A, Maier P. Hepatocyte-derived IL-1 and TNF-α mediate the LPS-induced acute-phase response and NO release by cultured rat hepatocytes. Eur J Biochem 1995;229: 349–355.
[7] Kaufmann RL, Matson CF, Beidel WR. Hypertriglyceridemia produced by endotoxin: role of impaired triglyceride disposal mechanism. J Infect Dis 1976;133:548–555.
[8] Hardardottir I, Grunfeld C, Feingold KR. Effects of endotoxin and cytokines on lipid metabolism. Curr Opin Lipidol 1994;5:207–215.
[9] Feingold KR, Staprans I, Memon R, Moser AH, Shigenaga JK, Doerrler WT, et al. Endotoxin rapidly induces changes in lipid metabolism that produce hypertriglyceridemia: low doses stimulate hepatic triglyceride production while high doses inhibit clearance. J Lipid Res 1992;33:1765–1769.
[10] Adi S, Pollock AS, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C. Role for monokines in the metabolic effects of endotoxin. J Clin Invest 1992;89:1603–1609.
[11] Brass EP, Vetter WH. IL-6, but not TNF-α, increases lipogenesis in rat primary cultures. Biochem J 1994;301:193–197.
[12] Feingold KR, Grunfeld C. Role of cytokines in inducing hyperlipidemia. Diabetes 1992;41(Suppl 2):97–101.
[13] Feingold KR, Soued M, Serio MK, Moser AH, Dinarello CA, Grun-

feld C. Multiple cytokines stimulate hepatic lipid synthesis in vivo. Endocrinology 1989;125:267–274.
[14] Grunfeld C, Soued M, Adi S, Moser AH, Fiers W, Dinarello CA, Feingold KR. Interleukin 4 inhibits stimulation of hepatic lipogenesis by tumor necrosis factor, interleukin 1, interleukin 6 but not interferon-α. Cancer Res 1991;51:2803–2807.
[15] Neyrinck AM, De Wispelaere LD, Vanhulle VP, Taper HS, Delzenne NM. Are Kupffer cells involved in the metabolic adaptation of the liver to dietary carbohydrates given after fasting? Biochim Biophys Acta 2000;1475:238–244.
[16] Folch J, Lees M, Sloane-Standley G. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957;226:497–509.
[17] Linn T. Purification and cristallization of rat fatty acid synthetase. Arch Biochem Biophys 1981;209:613–619.
[18] Takeda Y, Suzyki F, Inoue H. ATP citrate lyase (citrate cleavage enzyme). Methods Enzymol 1969;13:236–238.
[19] Hsu R, Lardy H. Malic enzyme. Methods Enzymol 1969;13:230–235.
[20] Cheng C, Saggerson E. Rapid antagonistic actions of noradrenaline and insulin on rat adipocyte phosphatidate phospho-hydrolase activity. FEBS Lett 1978;93:120–124.
[21] Lowry O, Rosebrough N, Farr A, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951;193:265–273.
[22] Goethals F, Deboyser D, Lefebvre V, Decoster I, Roberfroid M. Adult rat liver slices as a model for studying the hepatotoxicity of vincaalkaloids. Toxicol in Vitro 1990;4:435–438.
[23] Vanhulle VP, Martiat GA, Verbeeck RK, Horsmans Y, Calderon PB, Eeckhoudt SL, et al. Cryopreservation of rat PCLS by ultrarapid freezing; influence on phase I and II metabolism and on cell viability upon incubation for 24 h. Life Sci 2001;68:2391–2403.
[24] Liao W, Florèn CH. Upregulation of low density lipoprotein receptor activity by TNF, a process independent of TNF-induced lipid synthesis and secretion. Lipids 1994;29:679–684.
[25] Slegen PO. Preparation of isolated rat liver cells. Methods Cell Biol 1976;13:29–83.
[26] Friedewald WT. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin Chem 1972;18:499–502.
[27] Rüttinger D, Vollmar B, Wanner GA, Messmer K. In vivo assessment of hepatic alterations following gadolinium chloride-induced Kupffer cell blockade. J Hepatol 1996;25:960–967.
[28] Rai RM, Yang SQ, McClain C, Karp CL, Klein AS, Diehl AM. Kupffer cell depletion by gadolinium chloride enhances liver regeneration after partial hepatectomy in rats. Am J Physiol 1996;270:G909–G918.
[29] Rose ML, Bradford BU, Germolec DR, Lin M, Tsukamoto H, Thurman RG. Gadolinium chloride-induced hepatocyte proliferation is prevented by antibodies to TNF-α. Toxicol Appl Pharmacol 2001;170:39–45.
[30] Kohno H, Yamamoto M, Iimuro Y, Fujii H, Matsumoto Y. The role of splenic macrophages in plasma TNF levels in endotoxemia. Eur Surg Res 1997;29:176–186.
[31] Neyrinck AM, Eeckhoudt SL, Meunier CJ, Pampfer S, Taper HS, Verbeeck RK, Delzenne NM. Modulation of paracetamol metabolism by Kupffer cells: a study on rat liver slices. Life Sci 1999;65:2851–2859.
[32] Wake K. Cells of the hepatic sinusoids, anno 1998 – concluding remarks at the IXth international symposium, 1999. In: Wiss E, Knook DL, de Zanger R, Fraser R, editors. Cells of the hepatic sinusoid, vol. 7. Leiden: Kupffer Cell Foundation, 1999. pp. 308–310.
[33] Wu JZ, Ogle CK, Mao JX, Szczur K, Fischer JE, Ogle JD. The increased potential for the production of inflammatory cytokines by Kupffer cells and splenic macrophages eight days after thermal injury. Inflammation 1995;19:529–541.
[34] Ayala A, Perrin MM, Kisala JM, Ertel W, Chaudry IH. Polymicrobial sepsis selectively activates peritoneal but not alveolar macrophage to release inflammatory mediators (IL-1, IL-6 and TNF). Circ Shock 1992;36:191–1999.
[35] Fukuda H, Katsurada A, Iritani N. Effects of nutrients and hormones on gene expression of ATP citrate-lyase in rat liver. Eur J Biochem 1992;209:217–222.
[36] Saton R, Okamotot A, Inoues J, Miyamoto W, Sakai Y, Emoto N, et al. Transcriptional regulation of the ATP- citrate-lyase gene by sterol regulatory element-binding proteins. J Biol Chem 2000;275:12497–12502.
[37] Lawler JF, Yin M, Roberts E, Diehl AM, Chatterjee S. TNF-α stimulates the maturation of sterol regulatory element binding protein-1in human hepatocytes through the action of neutral sphingomyelinase. J Biol Chem 1998;273:5053–5059.
[38] Cascales C, Mangiapane EH, Brindley DN. Oleic acid promotes the activation and translocation of phosphatidate phosphohydrolase from the cytosol to particulate fractions of isolated rat hepatocytes. Biochem J 1984;219:911–916.
[39] Brehier A, Benson BJ, Williams MC, Mason RJ, Ballard PL. Corticosteroid induction of phosphatidic acid phosphatase in fetal rabbit lung. Biochem Biophys Res Commun 1977;77:883–890.
[40] Cheng CHK, Saggerson ED. Rapids antagonistic actions of noradrenaline and insulin or rat adipocyte phosphatidate phosphohydrolase activity. FEBS Lett 1978;93:120–124.
[41] Moller E, Wong KH, Green P. Control of fat cell phosphohydrolase by lipolytic agents. Can J Biochem 1981;59:9–15.
[42] Dixon MC, Yeaman SJ, Agius L, Day CP. Transforming growth factor beta increases the activity of phosphatidate phosphohydrolase-1 in rat hepatocytes. Biochem Biophys Res Commun 1997;230:365–369.
[43] Björkhem I, Berglund L. Stimulatory effect of mevinolin on rat liver phosphatidic acid phosphatase. Biochim Biophys Acta 1987;920:20–25.
[44] Ikejima K, Iimuro Y, Forman DT, Thurman RG. Diet containing glycine improves survival endotoxin shock in the rat. Am J Physiol 1996;271:G97–G103.
[45] Edwards MJ, Keller BJ, Kauffman FC, Thurman RG. The involvement of Kupffer cells in carbon tetrachloride toxicity. Toxicol Appl Pharmacol 1993;119:275–279.
[46] Carla EC, Ruzittu MT, De Luca M, Dini L. Soluble factors released from activated Kupffer cells promote hepatocyte apoptosis. In: Wiss E, Knook DL, de Zanger R, Fraser R, et al., editors. Cells of the hepatic sinusoid, vol. 7. Leiden: Kupffer Cell Foundation, 1999. pp. 70–72.
[47] Schemmer P, Schoonhoven PR, Swenberg JA, Bunzendahl H, Thurman RG. Gentle in situ liver manipulation during organ harvest decreases survival after liver transplantation: role of Kupffer cells. Transplantation 1998;65:1015–1020.
[48] Enomoto N, Ikejima K, Yamashina S, Enomoto A, Nishiura T, Brenner DA, et al. Kupffer cell-derived prostaglandin E2 is involved in alcohol-induced fat accumulation in rat liver. Am J Physiol Gastrointest Liver Physiol 2000;279:G100–G106.
[49] Yang SQ, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. Proc Natl Acad Sci USA 1997;94:2557–2562.