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## Precision-cut liver slices in culture as a tool to assess the physiological involvement of Kupffer cells in hepatic metabolism

Audrey M Neyrinck\*, Cristina Gomez and Nathalie M Delzenne

Address: Unité de Pharmacocinétique, Métabolisme, Nutrition et Toxicologie, Département des Sciences Pharmaceutiques, Université Catholique de Louvain, PMNT-UCL 73 avenue Mounier, B-1200 Brussels, Belgium

Email: Audrey M Neyrinck\* - Audrey.Neyrinck@pmnt.ucl.ac.be; Cristina Gomez - Audrey.Neyrinck@pmnt.ucl.ac.be; Nathalie M Delzenne - Delzenne@pmnt.ucl.ac.be

\* Corresponding author

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### Introduction

Hepatic macrophages have the capacity to secrete a tremendous array of molecules, which can be divided into 3 categories – cytokines (TNF- $\alpha$ ), lipid mediators (prostaglandins PGE<sub>2</sub>) and reactive intermediates (NO $\cdot$ ) – in response to stimulus, such as lipopolysaccharides (LPS) [1,2]. Such mediators are capable to modulate both the metabolism and the integrity of hepatocytes *in vitro* [2]. The physiological role of Kupffer cell in hepatic metabolism regulation has been approached in the present study by using the original *in vitro* model of precision-cut liver slices (PCLS) in culture; this model allows preserving the liver lobule architecture, by maintaining namely cell diversity in physiological proportion and cell-cell interactions [3]. First, we established whether non-parenchymal cells are still viable in rat PCLS and are able to respond to LPS *in vitro*; TNF- $\alpha$ , PGE<sub>2</sub>, NOx (reflecting NO $\cdot$  release) were measured in the incubation medium of PCLS from rats previously treated with GdCl<sub>3</sub> – a specific inhibitor of Kupffer cell phagocytosis [4] – or NaCl as a control- in order to evaluate the contribution of Kupffer cell in mediator release. Moreover, by using the same model, we have investigated the role of Kupffer cell in the regulation of lipid synthesis in PCLS, in order to approach the biochemical mechanism explaining our last results, which indicate that the inhibition of Kupffer cell by GdCl<sub>3</sub> leads to triglycerides accumulation in liver tissue [5].

### Methods

#### Materials

Male Wistar rats weighing 240–280 g were used for the preparation of PCLS or for isolation of hepatocytes. Most

chemicals of purest grade available were purchased from Sigma (Filter Service, Belgium), Roche Diagnostics Belgium or Invitrogen™ (Belgium). [1-<sup>14</sup>C]-acetic acid (specific activity 60 mCi/mmol) was obtained from Amersham Pharmacia Biotech Europe (Buckinghamshire, United Kingdom).

#### Study of mediator secretion by PCLS in culture

PCLS were prepared from treated with GdCl<sub>3</sub> (10 mg/kg *i.v.*) (Gd+) or NaCl 0.9% (Gd-) 24 h before liver removal according to a procedure previously described [6] and were incubated in William's E medium, supplemented with penicillin (100 IU/ml), streptomycin (100 micrograms/ml), glutamine (2 mM), insulin (100 nM) and bovine serum albumin 0.1 % containing LPS at 0 – 0.1 – 10 micrograms/ml. Medium was frozen after 2 h and 20 h of incubation for further analysis. PGE<sub>2</sub> and TNF- $\alpha$  concentration were measured in frozen aliquots incubation medium with immunoassay kits (PGE<sub>2</sub> Immunoassay, DE0100 and Quantikine rat TNF- $\alpha$  immunoassay, RTA00 from R&D Systems) whereas NOx (NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup>) concentration was measured by the Griess reaction [7]. ATP content of PCLS was greater than 8 nmol/mg protein in all experiments.

#### Study of lipid synthesis by PCLS

PCLS were prepared from treated with GdCl<sub>3</sub> (10 mg/kg *i.v.*) (Gd+) or NaCl 0.9% (Gd-) 48 h before liver removal according to a procedure previously described [6]. PCLS were incubated as described above. Blood was collected from vena cava for serum PGE<sub>2</sub> measurement. After 2 h of preincubation, medium was frozen for further analysis

**Table 1: Effect of Kupffer cell inhibition by GdCl<sub>3</sub> on lipid synthesis by PCLS**

Acetate equivalent incorporated into lipids (nmol/mg prot.)	Gd-	Gd+
- phospholipids	3.7 ± 0.1	6.1 ± 0.6*
- triglycerides	2.4 ± 0.6	4.0 ± 0.8*
- cholesterol	0.8 ± 0.2	2.0 ± 0.2*

PCLS, prepared from 24 h-fasted rats pretreated with GdCl<sub>3</sub> (Gd+) or NaCl (Gd-) 2 days before the experiment, were incubated in medium containing 2 mM [<sup>14</sup>C]-acetate for 3 h. Values are means ± S.E.M (n ≥ 4) (\*p < 0.05, Student t-test).

and PCLS were transferred into fresh medium containing 2 mM [<sup>14</sup>C]-acetate (0.2 mCi/mmol); after 3 h of incubation, 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) [8,9]. Spots corresponding to triglycerides, phospholipids and cholesterol were scrapped from the plate and counted in 10 ml scintillation fluid (Ultima Gold) in a beta counter.

#### **Study of lipid synthesis by isolated hepatocytes in suspension**

The hepatocytes were isolated from fed animals [10], through perfusion with a buffer containing Liberase™ (35 micrograms/ml). Cells were incubated in the presence PGE<sub>2</sub> (Cayman Chemicals) dissolved in DMSO at 10 micromolar and 2 mM [<sup>14</sup>C]-acetate (0.2 mCi/mmol) in the same medium described above. After 15 min of incubation, hepatocytes were pelleted and sonicated in 0.5 ml NaCl 0.05 M before lipid extraction according to Folch [8]. The chloroform phase was counted in 10 ml scintillation fluid (Ultima Gold) in a beta counter.

#### **Statistical analysis**

Values are presented as means ± S.E.M. In the study of mediator secretion by PCLS, statistical analysis was performed by two-way ANOVA with the Tukey's post hoc test (with SPSS™ statistical software). Other data were analysed by Student-*t* test. Statistical significance was set at p < 0.05.

## **Results**

### **Validation of the PCLS model to assess the functionality of Kupffer cells**

Unstimulated-PCLS in culture release in the medium significant amount of TNF-alpha, PGE<sub>2</sub> and NOx (Figure 1) which level increases with the time of incubation. TNF-alpha concentration increases significantly in the presence of LPS; the effect is dependent on the dose of LPS and is already present after 2 h of incubation. The extent of both PGE<sub>2</sub> and NOx production, is also dependent on LPS concentration; the increase in both parameters appears significantly only after 20 h of incubation. The administration of GdCl<sub>3</sub> 1 day before PCLS preparation strongly reduces

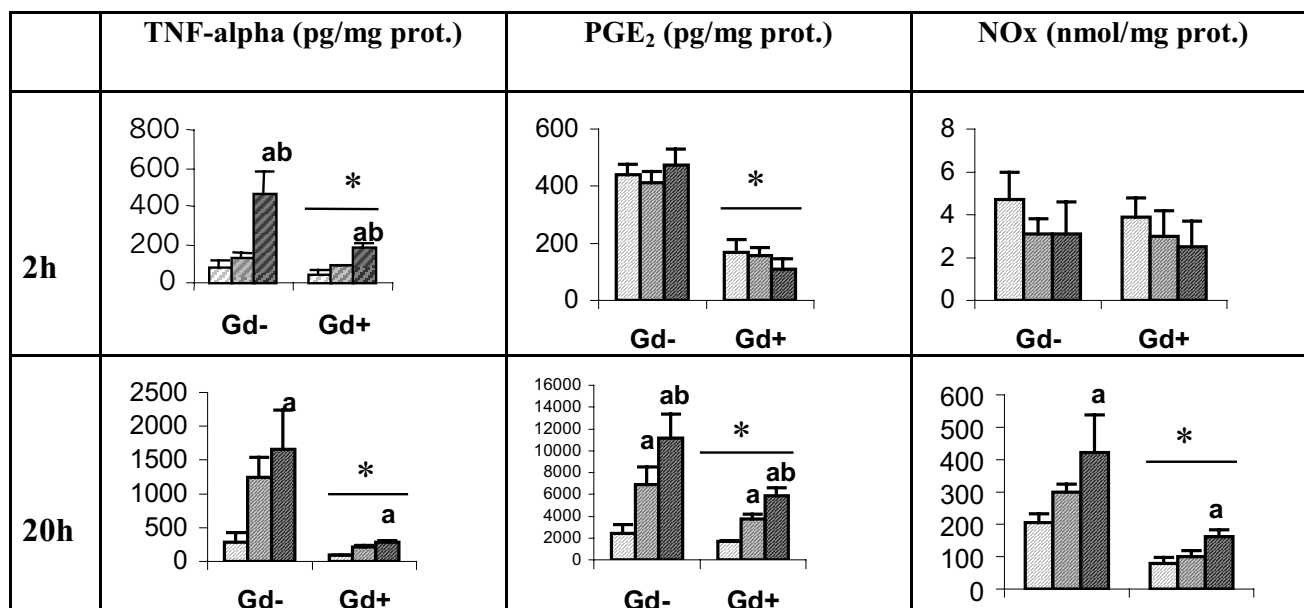
the basal production of the 3 mediators measured as well as upon stimulus by LPS.

### **Role of Kupffer cell in the physiological regulation of lipid synthesis in the liver tissue**

PCLS obtained from rats treated with GdCl<sub>3</sub>, 2 days before experiment, are characterised by a higher incorporation of [<sup>14</sup>C]-acetate into lipids (table 1), on one hand, and by a lower release of PGE<sub>2</sub> in the preincubation medium, on the other hand (PGE<sub>2</sub> concentration in the medium after 2 h: 5.4 ± 0.8 nM and 0.9 ± 0.1 nM for Gd- and Gd+ rats respectively; \*p < 0.05, Student t-test). Moreover, PGE<sub>2</sub>, measured in the serum obtained from vena cava, was much lower in animals treated with GdCl<sub>3</sub> 2 days before blood sampling (< 1 nM) as compared to control rats (26.7 ± 12.1 nM). The role of PGE<sub>2</sub> in the short-term control of lipid synthesis was assessed by incubation of isolated hepatocytes in suspension: the addition of PGE<sub>2</sub> (10 micromolar) to the culture medium rapidly decreases the incorporation of [<sup>14</sup>C]-acetate into total lipids in isolated hepatocytes after 15 minutes of incubation, total lipids – expressed in nmol acetate equivalent/mg protein – reached 1.3 ± 0.2 and only 0.87 ± 0.1 in control conditions (DMSO) and after addition of PGE<sub>2</sub> 10 micromolar, respectively (\*p < 0.05, Student t-test).

## **Discussion**

Our study demonstrates that GdCl<sub>3</sub>, a specific inhibitor of Kupffer cell phagocytosis [4], decreases the rate of LPS-induced TNF-alpha, PGE<sub>2</sub> and NOx production by PCLS; those 3 molecules are known as typical mediators produced by macrophages (and namely by Kupffer cells in culture) upon stimulation by LPS (cytokines, lipid mediators and reactive intermediates) [10]. Moreover, we have shown that PCLS are able, independently of any stimulus (absence of LPS or any toxic agents) to produce TNF-alpha, NOx and PGE<sub>2</sub>. In this case also, the release of mediators is decreased by previous GdCl<sub>3</sub> treatment. Therefore, we conclude that PCLS from GdCl<sub>3</sub>-treated rats – compared to PCLS obtained from control rats – is a convenient in vitro system to study the complex Kupffer cell-hepatocyte interactions retained inside the liver tissue, in basal conditions or after an inflammatory stimulus. PGE<sub>2</sub>

**Figure 1**

Effect of Kupffer cell inhibition on mediator secretion (TNF-alpha, PGE<sub>2</sub> and NOx) by PCLS, obtained from rats pretreated with GdCl<sub>3</sub> (Gd+) or NaCl (Gd-) 24 h before experiment; PCLS were incubated during 2 h and 20 h in the presence of LPS (from left to right, each group of three bars correspond to: 0 micrograms/ml, 0.1 micrograms/ml, 10 micrograms/ml). Values are means ± S.E.M (n ≥ 3; \*p < 0.05 Gd+ vs Gd-, two-way ANOVA; <sup>a</sup>p < 0.05 LPS 10 micrograms/ml vs LPS 0 micrograms/ml and <sup>b</sup>p < 0.05 LPS 10 micrograms/ml vs LPS 0.1 micrograms/ml, two-way ANOVA followed by Tukey's post hoc test).

merits special attention: when PCLS are prepared from Gd+ animals, PGE<sub>2</sub> release is strongly depressed (mainly during short time incubation) suggesting that Kupffer cells are important producers of PGE<sub>2</sub> by the liver. Since GdCl<sub>3</sub> treatment leads to a strong depression of PGE<sub>2</sub> concentration in the serum, we may propose that Kupffer cells inside the liver constitute an important source of circulating PGE<sub>2</sub>, known to exert pleiotropic effects outside the liver (control of lipolysis, platelet aggregation, gastric acid secretion, immunoregulation, neurotransmitter release, contraction-relaxation of smooth muscle) [11]. On the other hand, we show here that PGE<sub>2</sub> release by Kupffer cells, not only plays a role in the systemic availability of this prostaglandin, but is also able to exert paracrine effect on hepatocyte, with relevant metabolic consequences in the liver tissue: we had previously shown that, *in vivo*, the accumulation of lipids in the liver tissue after GdCl<sub>3</sub> administration, modifies the histological image of the liver, revealing steatosis [6]. We have demonstrated in the results presented here that the inhibition of Kupffer cells by GdCl<sub>3</sub> leads to a higher cholesterol, triglycerides and phospholipids synthesis by PCLS, a phenomenon related to a lower PGE<sub>2</sub> release. Since PGE<sub>2</sub> alone added in the culture medium of isolated hepatocytes decreases lipid synthesis, we may postulate that the lower PGE<sub>2</sub> secretion

after GdCl<sub>3</sub> treatment is, at least partly, responsible for a higher lipid synthesis inside the liver tissue. This is in favour of a role of Kupffer cell-released PGE<sub>2</sub> in the physiological control of lipid synthesis in hepatocytes. By which mechanism PGE<sub>2</sub> could control lipid synthesis in the liver tissue? The phosphorylation rate of hepatic enzymes involved in the lipogenesis or cholesterologenesis pathways (acetyl-CoA synthetase, HMG-CoA-reductase) could be modulated by PGE<sub>2</sub> which is released at concentration sufficient to alter the phosphorylation of specific proteins inside the hepatocytes [12]. The use of indomethacin, known to inhibit prostaglandin production, in the incubation medium of PCLS could help to elucidate the role of PGE<sub>2</sub> released by Kupffer cell in the regulation of hepatic metabolism.

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