MODULATION OF KUPFFER CELL ACTIVITY : PHYSIO-PATHOLOGICAL CONSEQUENCES ON HEPATIC METABOLISM

par

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I. Involvement of Kupffer cells in the regulation of hepatic metabolism

The liver is one of the most generous organs, involved in whole body homeostasis. It plays a crucial role in xenobiotic and intermediary metabolism. Although hepatocytes are considered as the major cells involved in the control of hepatic metabolism, recent evidences highlight some important and complex interactions between hepatocytes and sinusoidal nonparenchymal cells to serve these hepatic functions. Such a viewpoint is supported by the findings that nonparenchymal sinusoidal cells have the capacity to synthesize various molecules, which can have profound influences on hepatocytes.

Kupffer cells -the resident macrophages of the liver- are able to release a tremendous array of mediators upon inflammatory conditions, such as infection, and their role in innate immunity is well described in the literature. However, the impact of these Kupffer cell-derived mediators on liver homeostasis is unknown. In the present study, the physiological involvement of Kupffer cells was investigated in the regulation of hepatic metabolism.

I.1. $GdCl_3$ administration and Precision-Cut Liver Slices (PCLS) in culture : valuable tools to investigate Kupffer cell-hepatocyte interactions

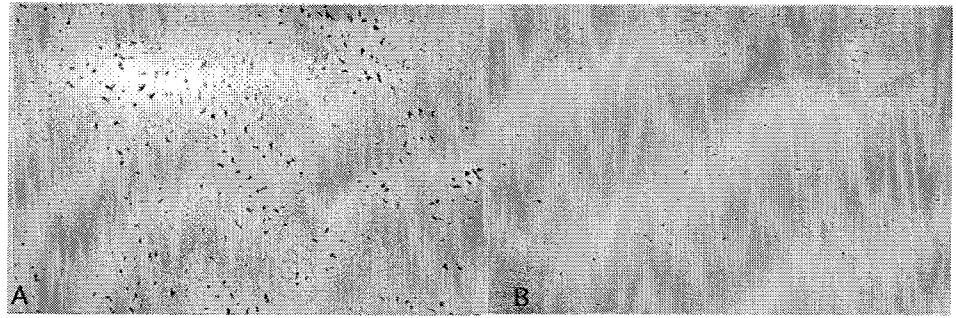

Fig. 1
Distribution of colloidal carbon in liver 20 min after injection in the portal vein. NaCl (0.9% i.v.) (A) or $GdCl_3$ (10 mg/kg i.v.) (B) were injected two days before the experiment. Colloidal carbon is mainly taken up by Kupffer cells in the liver. Original magnification x 200

and physiological responses during septic shock; 2) prostaglandin E_2 (PGE_2), a lipid mediator, is also mainly produced by Kupffer cells within the liver and is a potent inhibitor of macrophage activation in assays of cytokine release (immuno-suppressive action) and endotoxin-induced hepatic injury (role of cytoprotection); 3) nitric oxide ($NO^{\cdot}$) (indirectly determined by NOx concentration) is known to be a crucial factor in acute inflammation and sepsis, which may have protective effects in endotoxin-induced hepatic injury; $NO^{\cdot}$ is produced by Kupffer cells in response to endotoxin as well as by hepatocytes in response to Kupffer cell-derived cytokines (TNF-α). The consequences of $GdCl_3$ treatment on parenchymal cell integrity has also been analysed.

PCLS in culture were able to produce TNF-α, NOx and PGE_2 [1]. In addition, we have checked PCLS response to inflammatory stimulus *in vitro* induced by lipopolysaccharide (LPS). We have shown that the presence of LPS in the incubation medium increased

I.2. Involvement of Kupffer cells in the regulation of paracetamol metabolism

In the first experimental investigation, we have analysed the metabolism of paracetamol *in vitro* by using PCLS model without any LPS challenge.

Our results suggest that the presence of Kupffer cells inside PCLS could affect phase II metabolism in the adjacent hepatocytes, since a higher proportion of paracetamol glucuronide metabolites was observed in the incubation medium of PCLS obtained from $GdCl_3$-treated rats[2]. The secretion of inflammatory mediators derived from Kupffer cells, such as TNF-α, PGE_2 and NO·, may be involved in the lower glucuronidation capacity observed in control PCLS.

I.3. Involvement of Kupffer cells in the regulation of lipid metabolism

In this work, we analysed also lipid metabolism occurring in PCLS after Kupffer cell inhibition. We found a higher incorporation of both [^{14}C]-acetate and/or [^{14}C]-palmitate into lipids (triglycerides, phospholipids and cholesterol) of PCLS obtained from $GdCl_3$-treated rats, suggesting that Kupffer cells play a role in the regulation of lipid synthesis[3]. *In vivo*, $GdCl_3$ treatment is able to promote fatty acid release by adipose tissue and favours their esterification in liver tissue, leading to hepatic triglyceride accumulation in the fasting state (table 1).

In fed rats, Kupffer cell inhibition leads to a higher hepatic content of both triglyceride and cholesterol although activity of several key enzymes involved in lipid synthesis, measured in liver tissue, was not affected by $GdCl_3$ treatment. These results suggest that other pathways than cholesterogenesis, lipogenesis and fatty acid esterification could be affected by $GdCl_3$ treatment in the fed state. Could intra-hepatic lipid accumulation be due to a shift in lipoprotein metabolism such as a decreased VLDL secretion by the liver? To answer this question, a complete qualitative and quantitative analysis of lipoprotein contents in the serum should be performed.

	fasting state		port-absorbtive state	
	Gd-	**Gd+**	**Gd-**	**Gd+**
Hepatic lipids (nmol/mg protein)				
TG	42.7 ± 5.3	77.9 ± 13.9*	30.0 ± 1.8	37.9 ± 3.0*
PL	109.4 ± 2.8	116.2 ± 7.0	91.1 ± 3.0	97.0 ± 1.0
CHO	37.3 ± 1.0	43.2 ± 2.9	43.9 ± 1.4	49.3 ± 0.5*
Enzyme activities in the liver				
- lipogenesis (mIU/mg protein)				
ACC	n.d.	n.d.	0.17 ± 0.03	0.19 ± 0.03
FAS	2.9 ± 0.2	3.5 ± 0.3	5.5 ± 0.5	5.5 ± 0.5
ATPCL	9.9 ± 0.5	9.3 ± 1.6	16.5 ± 1.9	15.6 ± 1.9
ME	15.6 ± 0.9	13.9 ± 2.5	27.7 ± 1.7	24.2 ± 1.7
- esterification (mIU/mg prot.)				
PAP	10.9 ± 0.9	14.7 ± 0.5*	12.1 ± 0.5	12.8 ± 0.4
GPAT	n.d.	n.d.	1.69 ± 0.11	1.90 ± 0.10
- cholesterogenesis(pmol/min/mg/prot.)				
HMG-CoA reductase	n.d.	n.d.	12.1 ± 4.6	8.0 ± 2.4
Serum lipids (mM)				
TG	0.48 ± 0.06	0.43 ± 0.09	0.82 ± 0.08	0.93 ± 0.18
PL	1.25 ± 0.07	1.74 ± 0.10*	1.18 ± 0.03	1.47 ± 0.08*
CHO	1.56 ± 0.12	2.30 ± 0.23*	1.39 ± 0.05	1.79 ± 0.12*

Table 1

$GdCl_3$ (10 mg/kg i.v., Gd+) or NaCl (0.9% i.v., Gd-) was injected two days before liver and serum sampling. TG, triglycerides; PL, phospholipids; CHO, cholesterol; ACC, acetyl-CoA carboxylase; FAS, fatty acid

dietary components (and their digestion products) and the wide intestinal immune system. We have selected three types of nutrient, which could offer possibilities to interact with immune cells present in the liver. We analysed their capacity to modulate Kupffer cell activity or Kupffer cell response towards septic stimuli. Such a study might be useful to develop alternative and non-toxic strategy for modulating Kupffer cell activity via dietary advices.

II.1. Influence of nutritional transition: high carbohydrate-fat free diet given after fasting

We failed to demonstrate involvement of Kupffer cells in fasting-refeeding nutritional transition leading to lipid metabolism alteration in the liver similar to the one developed in the obese Zucker rat[5]. However, their role in the pathogenesis of nonalcoholic fatty liver disease remains an attractive hypothesis to investigate.

II.2. Influence of an amino acid: glycine

We investigated the effects of diet enriched with glycine on lipid metabolism. Glycine is structurally the simplest of amino acids and has been recently described as an anti-inflammatory immunonutrient capable to blunt LPS-induced Kupffer cell activation. In the fasting state, hepatic triglyceride content increased three days after dietary glycine, a phenomenon related to a higher hepatic phosphatidate phosphohydrolase activity and fatty acid released from adipose tissue (table 2).

The higher incorporation of palmitate into triglycerides by PCLS obtained from rats treated with glycine[4] confirmed the metabolic shift of fatty acids towards their

	control diet	glycine diet

esterification into triglycerides and accumulation in liver tissue compared to rats fed with a control diet. However, by contrast to $GdCl_3$ administration, we failed to demonstrate any influence of the glycine diet on mediator secretion by PCLS. These results do not exclude a putative effect of glycine *in vivo* during the feeding period on hepatic macrophage activity.

Furthermore, we determined whether glycine added to the incubation medium of PCLS affects both parameters known to assess PCLS viability upon incubation and secretion of key mediators released by Kupffer cells under inflammatory stimuli (LPS). We show that glycine, added *in vitro*, decreases TNF-α secretion, as well as NOx production, by PCLS prepared from LPS-treated rats, supporting the idea that the prevention of both ATP and glycogen depletion in PCLS by glycine is, in part, mediated through inhibition of cytotoxic mediator (TNF-α) and reactive intermediates ($NO^{\cdot}$) release[6]. The effect of glycine *in vitro* on mediator secretion was less pronounced if rats did not receive an injection of LPS prior to PCLS preparation : only TNF-α secretion was reduced independently of the presence of LPS *in vitro*[6]. The different pattern in mediator secretion could be related to the accumulation and activation of leukocytes inside the liver tissue after LPS administration, such as neutrophils or monocytes (as suggested by the enhanced peroxidase activity in the liver[6]). Before clarifying the involvement of Kupffer cells in the protective effect of glycine upon inflammation *in vivo*, kinetic studies, by determining inflammatory mediator concentrations in the serum after LPS challenge, would be necessary. Furthermore, analysis of Kupffer cell markers assessing their presence or activity (ED2 staining, peroxidase activity, colloidal carbon uptake) should be performed upon treatment with a glycine diet. If glycinemia is able to influence the hepatic immune system, an interesting question could be addressed: are inflammatory parameters or immune response modified in patients with nonketotic hyperglycinemia?

II.3. Influence of a non-digestible carbohydrate: oligofructose

products, through gut-derived immuno-modulatory mediators or immune cells or through gut-derived short chain fatty acids). We have tested the hypothesis that dietary oligofructose was able to modulate toxicity associated with LPS challenge in rats. Our study demonstrated that, following LPS injection (10 mg/kg), TNF-α – a proinflammatory cytokine – and PGE_2 – an immunosupressive mediator – were higher in rats treated with 10% oligofructose in the diet for three weeks than in controls receiving the standard diet[7]. Interestingly, oligofructose treatment allowed to avoid the increase in ALT activity occurring 24 h after LPS administration, suggesting a lower hepatic injury confirmed through histological analysis[7].

This would mean that higher Kupffer cell phagocytic activity and secretion capacity through oligofructose supplementation improves LPS clearance in liver tissue, and would help to avoid hepatocyte alterations. This study supports the hypothesis that oligofructose might decrease liver tissue injury following endotoxic shock and sepsis. Those preliminary findings indicate that fructan-type prebiotics may indeed modulate intra-hepatic immune cell activity and might constitute a novel nutritional strategy to improve resistance against systemic infection.

We conclude that the diet has a profound influence on hepatic immunity.

RÉSUMÉ

Le maintien et le contrôle de l'homéostasie hépatique sont classiquement attribués à l'activité métabolique des cellules parenchymateuses du foie. Cependant, de récentes découvertes mettent en évidence des interactions complexes et contrôlées entre les hépatocytes et les autres cellules hépatiques.

Les cellules de Kupffer sont les macrophages résidents du foie capables de sécréter un éventail impressionnant de médiateurs dans des conditions inflammatoires, telle que l'infection. Bien que leur rôle en tant que macrophage soit bien décrit dans la littérature, l'impact des médiateurs issus des cellules de Kupffer sur l'homéostasie du foie n'est pas connu jusqu'à ce jour.

L'objectif principal de cette étude était d'explorer le rôle physiologique des cellules de Kupffer dans la régulation du métabolisme hépatique. Il a fallu tout d'abord valider l'utilisation d'un agent capable

glycine, un acide aminé simple, dans la diète des rats influençait le métabolisme hépatique des lipides. Cependant, une relation directe entre ces effets métaboliques et l'activité des cellules de Kupffer n'a pu être démontrée. En revanche, nos résultats suggèrent que l'utilisation de la glycine *in vitro* permettrait d'élucider les interactions cellule de Kupffer-hépatocyte grâce au modèle des PCLS, et pourrait constituer une méthode alternative pour inhiber *in vitro* la production des médiateurs inflammatoires issus des cellules de Kupffer. En outre, un régime enrichi en oligofructose – un glucide non-digestible mais fermentescible connu pour son effet prébiotique – favorise l'activité phagocytaire du foie et la sécrétion des médiateurs produits par les PCLS en incubation. Nos résultats indiquent que l'augmentation de l'activité des cellules de Kupffer serait responsable de l'hépato-protection observée après un apport alimentaire en oligofructose dans le cadre d'un choc septique.

SUMMARY

Classically, the maintenance and control of liver homeostasis are assigned to the metabolic activity of parenchymal cells. However, recent evidence highlights complex and tightly regulated interactions between hepatocytes and other intra-hepatic cells.

Kupffer cells - the resident macrophages of the liver – are able to release a tremendous array of mediators upon inflammatory conditions, such as infection, and their role in innate immunity is well described in the literature. However, the impact of these Kupffer cell-derived mediators on liver homeostasis is unknown.

In this study, we investigated the physiological involvement of Kupffer cells in the regulation of hepatic metabolism. It was first necessary to validate the use of a compound able to selectively deplete Kupffer cells. We confirmed that gadolinium chloride ($GdCl_3$) injection to rats eliminated ED2-positive Kupffer cells and strongly decreased both their phagocytic and peroxidase activities. Moreover, we demonstrated that precision-cut liver slices (PCLS) – an original *in vitro* model allowing to maintain intact hepatic architecture and cellular heterogeneity – obtained from $GdCl_3$-treated rats released lower amounts of inflammatory mediators. Therefore, we proposed to use $GdCl_3$ prior to PCLS preparation in order to investigate the role of Kupffer cells in the control of hepatic metabolism.

Among various metabolic functions of the liver, we focused, in particular, on paracetamol and lipid metabolism as example of drug and intermediary metabolism, respectively. Our results suggest that the presence of Kupffer cells in liver tissue can affect the viability of PCLS in culture and are involved in the regulation of paracetamol metabolism, in particular the glucuronidation pathway. Furthermore, inhibition of Kupffer cells leads to a metabolic shift of fatty acids towards their esterification (at least, in fasted rats) and accumulation in the liver tissue, supporting a key role of Kupffer cells in the regulation of intra-hepatic lipid metabolism. Results obtained from *in vitro* studies suggest that Kupffer cell-derived prostaglandin

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