Pathogenesis of steatohepatitis : insights from the study of animal models

Isabelle A. Leclercq

Laboratoire de gastro-entérologie, Université catholique de Louvain (UCL), Brussels, Belgium.

Abstract

Non-alcoholic steatohepatitis (NASH) is a disease of expanded clinical importance. Its pathogenesis remains poorly understood. Tools to identify patients at risk and targeted treatments are lacking.

The aim of this work was to analyse potential pathogenic mechanisms for inflammatory recruitment and fibrogenesis in NASH, using animal models.

We demonstrated that oxidative stress, invariably associated with NASH, is a primary and necessary event for disease progression. Inhibition of stress-activated transcription factor NF-κB prevents NASH. NF-κB therefore appears as a pathogenic link between oxidative stress and NASH.

Increased lipid β-oxidation in NASH could generate oxidative stress. We used a potent inducer of PPAR-alpha to stimulate β-oxidation in a model of steatohepatitis. Such treatment induced a complete clearance of steatosis together with a significant reduction of oxidative stress and oxidative injuries and prevention of inflammation and fibrosis. Thus in a situation of steatosis, stimulation of lipid combustion depletes the substrates for lipid peroxidation and thereby decreases oxidative stress. This effect is sufficiently powerful to prevent the development of steatohepatitis.

We demonstrated that leptin is a pro-fibrogenic adipocytokine and is implicated in the regulation of liver regeneration. Leptin plays this crucial physiological role in hepatic wound healing by controlling the production and the activation of cytokines.

The insulin sensitising drugs thiazolidinediones have anti-inflammatory and anti-fibrotic properties in rats. We demonstrated that such drugs are poorly effective in the treatment of pre-established hepatic fibrosis in rats and unable to prevent fibrogenesis *in vitro* as well as *in vivo* in mice. Direct anti-fibrotic effect of such substances remains to be demonstrated in humans.

In conclusion, our work demonstrates the importance of oxidative stress in the pathogenesis of NASH, the role of intrahepatic lipid overload and underlies the links between adipose tissue and the liver. (Acta gastroenterol. belg., **2007**, 70, **25-31**).

Introduction

Non alcoholic fatty liver disease (NAFLD) is a disease of our generation. Mostly unrecognised before 1980 (1) and seldom taken seriously until the past few years, NAFLD is now recognized as an entity of expanded clinical importance (2). In fact, NAFLD has emerged during the industrial revolution, which caused food to be processed differently and provided more abundantly, and made physical work less demanding. In the 1980's, information technology and virtual reality have enhanced sedentary lifestyle disorders. NAFLD shares these roots with obesity and type 2 diabetes mellitus and is now being considered as the hepatic complication of the metabolic syndrome (3).

The liver pathology that characterises non-alcoholic steatohepatitis (NASH) is similar to that observed in alcoholic liver disease (ALD). Because of this, patients with NASH were for many years thought to be alcoholics that denied alcohol abuse (1). NASH is part of the spectrum of non-alcoholic fatty liver diseases (NAFLD) that ranges from hepatic steatosis to cirrhosis. NASH is characterised by steatosis, lobular inflammation and progressive pericellular fibrosis (4) ; the latter may lead to cirrhosis, and it now seems likely that many cases of cirrhosis hitherto labelled as "cryptogenic" are actually the end stage of NASH. As expected, NASH-associated cirrhosis is also an important precursor for liver failure and hepatocellular carcinoma. To date, the treatment options for NASH are limited (5,6) and the identification of patients with NAFLD at risk for progression to NASH remains difficult.

The difficulties encountered in the diagnosis and treatment of this progressive disease are mainly related to the fact that the pathogenic mechanisms behind the development of NASH remain elusive. Steatosis results from altered lipid metabolism and lipid partitioning. Insulin resistance is strongly associated with NAFLD/NASH (3,7,8) which is, for this reason, now regarded as the hepatic complication of the metabolic syndrome (3,9). Insulin resistance encompasses impaired insulin signaling, alteration glucose homeostats and of the metabolism of the adipose tissue together with profound changes in the pattern and the balance of production of several adipocytokines.

One of the main challenges has been to identify the factors responsible for mediating the transition from benign steatosis to steatohepatitis. Insulin resistance is likely to be a significant determinant of NASH pathogenesis (3,7,8). An increase in oxidative stress on a background of steatosis is also thought to be important (10-13), but the molecular mechanisms linking oxidative stress and liver injury and inflammation are yet to be delineated in this setting. Also, the source(s) of reactive oxygen species (ROS) are still undecided. Various lines of evidence suggest that cytochrome P450 2E1 and 4A enzymes are important (10,14). Other options include mitochondria, peroxisomes, inflammatory cells and cytokines (reviewed in (15-17)). The role

Corresponding author : Isabelle Leclercq, M.D., Ph.D., Laboratoire de Gastro-entérologie, Université Catholique de Louvain, GAEN 53/79, Avenue Mounier, 53, B-1200 Brussels, Belgium. E-mail : isabelle.leclercq@gaen.ucl.ac.be.

Submission date : 12.12.2006
Acceptance date : 21.12.2006

of lipids and in particular lipid accumulation and altered lipid metabolism is still under-evaluated.

As NASH is associated with obesity, insulin resistance and type 2 diabetes mellitus, conditions that are increasingly common in the community, the prevalence of NASH is predicted to rise. It is clear that a better understanding of the pathogenic mechanisms involved in the development of NASH would assist in creating effective strategies to both prevent and treat this disease. My personal contribution to studies that explore the potential pathogenic mechanisms for steatohepatitis is developed here.

Pathogenesis of liver steatosis

Liver triglycerides content is a reflection of the balance of complex processes of input, output, synthesis and oxidation of fatty acids. Obesity and insulin resistance tip this balance towards triglycerides accumulation and fatty liver. Excess of dietary fat, saturation of capacity for lipid storage in adipocytes and increased activity of lipoprotein lipase due to insulin resistance in the adipose tissue increases the pool of serum free fatty acids. The liver readily takes those up. The hyperinsulinemia and the low-grade inflammatory state stimulate intrahepatic de novo lipogenesis. Together with decreased intrahepatic fat burning and impaired VLDL formation, all these mechanisms concur to fatty liver (reviewed in (3,18)).

While there has been explosion of interest about why and how hepatic steatosis develops in humans, little is known about the mechanistic basis for the clinically most important outcome : chronic inflammation and progressive hepatic fibrosis.

The source of oxidative stress and the role of lipid accumulation and/or altered lipid metabolism

Oxidative stress is constantly observed in NASH livers (11,12). Reactive oxygen species and reactive radicals modify key constituents of the cells such as lipids, proteins and nucleic acids and thereby alter their function. As a result, intracellular lesions develop and adaptative mechanisms are activated (13).

In previous work, we have shown that NASH livers are characterized by increased expression and activity of several cytochrome P450 isoforms capable to generate reactive radicals (10). The main siege for lipid β-oxidation is the mitochondria. Peroxisomes and endoplasmic reticulum participate to a lesser extent (oxidation of very long chain fatty acids or ω-oxidation). The process of oxidation of fatty acids is intrinsically associated to the production of reactive oxygen radicals (ROS, H_2O_2, $O_2.^-$, …) (17). We therefore asked whether enhancement of fatty acid β-oxidation, in the face of increased hepatic lipid load, could be implicated in the generation of deleterious oxidative stress and initiate the transition from fatty liver to NASH. To test this, we stimulated β-oxidation by the use of a potent inducer of PPAR-alpha (the "super fibrate" Wy-14,613) in mice with steatohepatitis induced by a diet deficient in methionine and choline. This results, in mice, in the up-regulation of key enzymes controlling both mitochondrial and peroxisomal pathways for β-oxidation of fatty acids (19). With PPAR-alpha treatment, we observed an almost complete clearance of fatty liver. But surprisingly, in these livers, the oxidative stress and the oxidative injury were significantly reduced, the development of inflammatory lesion was prevented as well as fibrogenesis.

In addition, PPAR alpha agonist appeared as a very effective treatment of pre-established fibrosing steatohepatitis, as the treatment reverted steatosis, inflammation as well as fibrosis (20). We suggested that, in a situation of fatty liver, stimulation of lipid combustion is associated with a decrease in intrahepatic lipid load. This consequently removes fatty acids as substrates for lipid peroxidation, an effect sufficiently powerful to prevent the development of steatohepatitis.

This represents a proof of principle that targeting steatosis is effective for the prevention and the treatment of NASH. Unfortunately, PPAR-alpha agonists do not significantly enhance hepatic fatty acid fat burning in human as they do in rodents. First, hepatic expression of PPAR-alpha is much lower in human than in mice. Second, in rodents, the rate-limiting enzymes of β-oxidation are exclusively controlled by PPAR-alpha. This is not the case in human. Indeed, fibrate treatment in humans lowers serum triglycerides but has no significant effect on steatosis nor on steatohepatitis (reviewed in (5)). To corroborate the prove of principle of a beneficial effect of hepatic fat clearance, the treatments leading to melioration of NASH today are those reducing hepatic steatosis such as life style adjustments (5,6) or glitazones (21).

NF-κB activation : a link between oxidative stress, chronic inflammation and fibrogenesis in fatty liver ?

In animal models, we showed that oxidative stress is linked to the development of inflammatory reaction and to fibrosis of the liver parenchyma. We analysed the temporal evolution of oxidative stress and oxidative injury in a dietary model of NASH (22). By comparative studies of several animal models of fatty liver, we determined that inflammatory lesions as well as fibrosis only develop when there is operation of oxidative stress and active peroxidation of lipids (23). We proposed the transcription factor NF-κB, which can be activated in response to oxidative stress, as a possible link between oxidative stress, oxidative injury, recruitment of inflammatory reaction and initiation of fibrogenesis. Indeed, when using the natural substance curcumin that has antioxidant properties and acts as an inhibitor of this transcription factor, we observed a significant improvement of the inflammatory reaction and fibrosis in mice fed a NASH-inducing diet (24) (Fig. 1). Similar results were obtained by specific blockade of NF-κB activation by

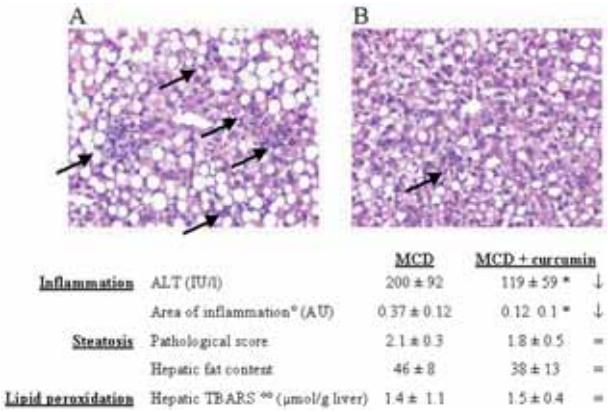

Fig. 1. — Prevention of NF-ϰB activation by curcumin decreases hepatic inflammation in the MCD-diet model of steatohepatitis.
Representative liver histology from mice receiving (A) the MCD diet or (B) the MCD diet together with curcumin, for 4 weeks. Arrows point to the inflammatory infiltrates. Note that curcumin decreases the severity of hepatic inflammation, but has no effect on steatosis and hepatic lipoperoxides.
°area of inflammation : relative area of the liver section occupied by inflammatory infiltrates. °°lipoperoxides are measured as Thiobarituric acid reactive substances (TBARS).
*statistically significant in Curcumin treated MCD-fed mice compared to MCD-fed mice.
Adapted from (24) with permission.

viral transfection of a non-degradable form of the inhibitory protein IϰB (25). In both sets of experiments, inhibition of NF-ϰB resulted in reduced production of pro-inflammatory and chemoattractant factors such as TNF or ICAM-1. Importantly, those manipulations had no effect on oxidative stress and hepatic steatosis. NF-ϰB can also be strongly induced in response to pro-inflammatory stimuli such as the cytokine TNF. However, abolition of TNF signalling such as in TNF-null mice or in mice lacking TNF receptor 1, does not prevent the activation of NF-ϰB, nor the development of steatohepatitis in our model (25). Therefore, we propose that oxidative stress and consequent peroxidation of lipids, are key mechanisms for activation of the transcription factor NF-ϰB, initiating inflammatory changes and disease progression in the context of hepatic steatosis.

Adipocytokines, hepatic fibrosis and altered wound healing in NASH

Non-alcoholic steatohepatitis belong to a cluster of metabolic abnormalities (Table 1) called the metabolic syndrome (MS). Resistance to the action of insulin is the corner stone of this syndrome which predisposes to increased risk of developing type II diabetes, cardio-vascular diseases or steatohepatitis (Table 1) (3,26). Two new paradigms may provide a framework for our understanding of the etiopathology of the MS : ectopic fat storage and endocrine dysfunction of the adipose tissue.

Table 1. — **Syndrome of insulin resistance : Abnormalities and related clinical outcomes**

Pathophysiological disturbances	Clinical manifestations
Glucose intolerance	Type II diabetes mellitus
Dyslipidemia	Increased risk of cardiovascular diseases
Endothelial dysfunction	Essential hypertension
Pro-inflammatory state	Polycystic Ovarian Syndrome
Pro-coagulant state	Non-alcoholic fatty liver diseases
Abnormal uric acid metabolism	Increased risk of cancer
Hemodynamic changes	Sleep apnea syndrome
Sleep disordered breathing.	

Adapted from (26).

Some evidence that the MS may represent a failure of lipo-homeostasis has emerged. Lipid overload is no longer confined to white adipocytes but also occurs in liver, skeletal muscle and pancreas, which results in lipotoxicity, insulin resistance and impaired insulin secretion (3,7,27). This view holds that the machinery to oxidize fat is not sufficient to match the dietary load or is not activated in a timely fashion by appropriate signals. Some of these are adipocyte-secreted hormones (collectively known as adipocytokines). These factors lay adipose tissue at the heart of a complex network influencing energy homeostasis, insulin sensitivity, glucose and lipid metabolism, vascular homeostasis, immune and inflammatory responses, cell cycle and cell differentiation (28). Any change in adipose tissue mass will cause altered adipocytokines production/action. Such a dysregulation triggers the development or worsens the evolution of the metabolic syndrome and its complications.

Several adipocyte-derived factors have been shown to modulate hepatic fibrosis : Adiponectin has anti-fibrotic properties (29), while angiotensinogen and PAI-1 are pro-fibrotic (30). Our work has provided conclusive evidence that leptin plays a central role in the control of hepatic wound healing (31-33). We have shown that leptin is an indispensable player in hepatic fibrogenesis. Leptin-deficient *ob/ob* mice are indeed protected against the development of hepatic fibrosis either induced by repeated toxic insult to the liver or in the context of steatohepatitis (31). We have shown that leptin-deficiency *per se*, and not the metabolic abnormalities resulting from the absence of leptin such as obesity, fatty liver or insulin resistance, is responsible for the fibro-protective effect (31). The literature provides contradictory data on the mechanisms implicated. For some authors, leptin has a direct effect on HSC : it stimulates the transcription of pro-fibrotic genes such as collagen or tissue inhibitor of metalloprotease-1 (TIMP-1) (34,35) and increases mitogenesis (36,37). But us and others support the proposition that leptin-dependent stimulation of TGFβ1 expression and released by Kupffer cells is the indirect mechanism by which leptin influences fibrogenesis (18,32,38-41) (Fig. 2).

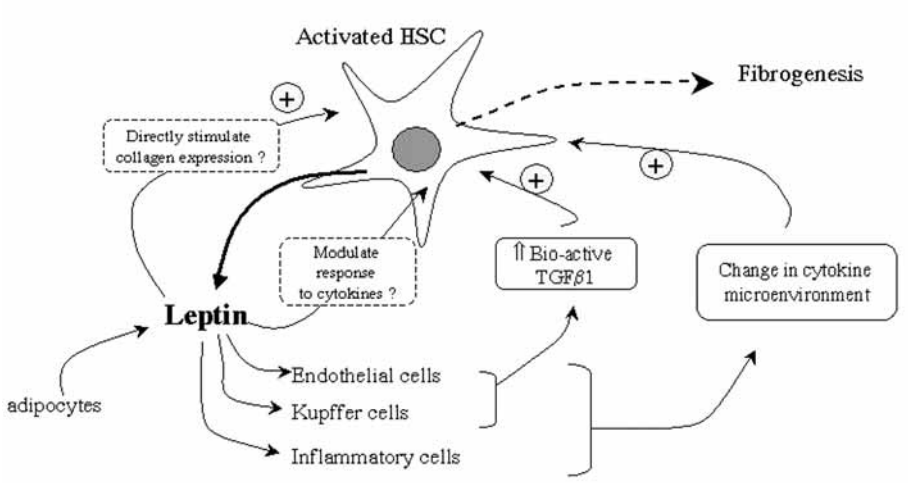

Fig. 2. — Pro-fibrogenic effect of leptin : possible mechanisms of action.
Leptin, derived from adipocytes or produced locally during fibrogenesis by activated HSC, participates in the paracrine/autocrine regulation of the cytokine microenvironment : Leptin increases the release and the bio-activation of the most potent fibrogenic cytokine, TGFβ1. Leptin is involved in the maturation and activation of immune cells and, thereby, modulates cytokine balance. Also, leptin regulates the expression of several cytokine receptors and responsiveness to cytokines. In addition, a direct effect of leptin on collagen I expression has been proposed.
Reproduced with permission from (41).

In addition to impaired fibrogenesis, leptin deficient *ob/ob* mice have also impaired liver regeneration. We demonstrated that, in *ob/ob* mice, impaired liver regeneration after acute toxic injury to the liver is rescued by the administration of exogenous leptin (32). In this context, the increased production of TNF, IL-6, and TGFβ1 seen in wild type animals in response to acute liver injury was blunted in *ob/ob* mice and restored after leptin repletion. Also, in a non-inflammatory model of liver regeneration that respects hepatocellular integrity, namely partial hepatectomy, hepatocyte proliferation is dampened in leptin deficient mice (33). However, by contrast to the toxic model, leptin repletion did not restore normal rate of liver cell regeneration, nor the normal pattern of hepatic cytokine expression. Thus, leptin does not directly signal to liver cells to promote hepatocyte proliferation and the obese phenotype is not solely responsible for impaired regeneration. Maturation of inflammatory cells and modulation of cytokine production by leptin is likely to play crucial physiological roles in hepatic wound healing.

Targeting PPARγ as a treatment for NASH and fibrosis ?

Thiazolidinediones or glitazones are agonist drugs of the transcription factor PPARγ, which is crucial for adipogenesis and for the control of adipocytokines production. Glitazones are used in the treatment of type 2 diabetes because they increase the sensitivity to insulin (42).

In the context of NASH, PPARγ agonist appears as a very attractive therapeutic option because it regulates the function of the adipose tissue, which is believed to be the primary dysfunctional site in the development of NASH. Animal and clinical studies have shown that PPARγ agonists effectively ameliorate insulin resistance and fatty liver associated with obesity or type 2 diabetes. They also ameliorate steatosis, inflammation and hepatic insulin resistance in animal models of alcoholic and non-alcoholic steatohepatitis (43-45). In pilot studies of NASH patients, glitazones (pioglitazone and rosiglitazone) ameliorate insulin resistance, decrease steatosis, inflammation and fibrosis (46,47). However, the effect on fibrosis has not been confirmed in more recent placebo-controlled trials (21,48).

In rat, the administration of PPARγ agonists prevents hepatic fibrosis induced by various means (49,50), possibly via direct control of the biology of hepatic stellate cells (51,52). Other mechanisms such as the control of intrahepatic inflammation or modulation of adipocytokines production have not been conclusively explored yet. Much to our surprise, although pioglitazone prevents fibrogenesis, it has a limited efficacy in the treatment of pre-established hepatic fibrosis : Pioglitazone reduces fibrosis only if administered early in the course of fibrotic disease, but is not effective in advanced or biliary diseases (45) (Fig. 3). Moreover, the anti-fibrotic effect appears to be limited to rats, since we failed to observe a similar fibro-preventive effect in mice *in vivo* as well as in primary murine hepatic stellate cells *in vitro* (53). The anti-fibrotic effect of such substances remains to be demonstrated in humans.

Agenda for future research

Many questions pertaining to the pathogenesis of NASH and the determinants of it progression remain unanswered. Among those open questions, we are

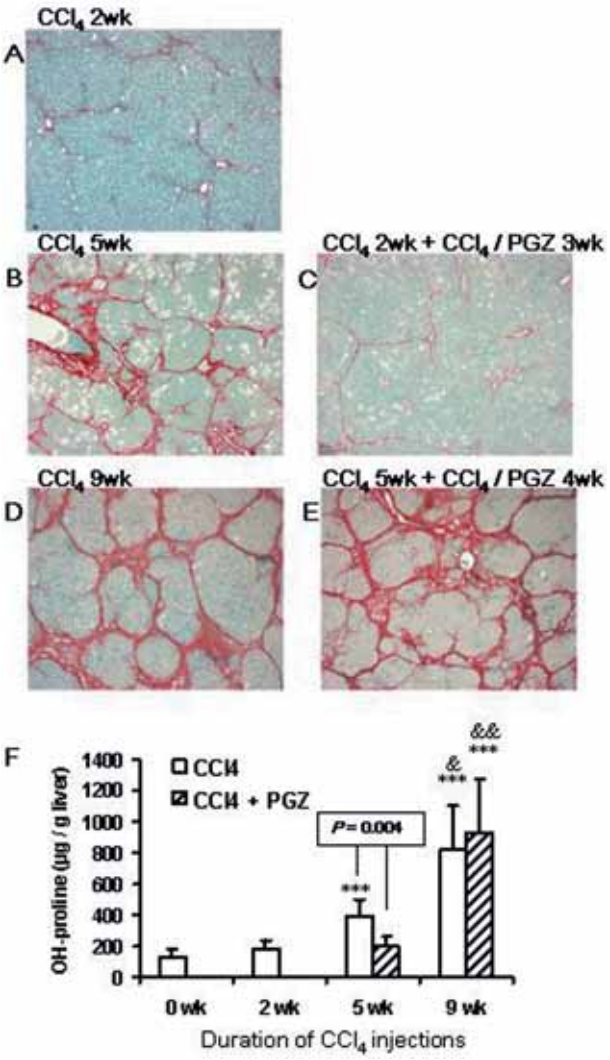

Fig. 3. — Effects of Pioglitazone (PGZ) treatment on the progression of CCl4-induced hepatic fibrosis.
Representative photomicrographs of Sirius red stained liver sections from rats received CCl4 for 2 weeks (A), for 5 wks without (B) or with PGZ treatment for the last 3 weeks (C), for 9 wks without (D) or together with PGZ treatment for the last 4 wks (E). Original magnification 5X. Quantification of hepatic hydroxy-proline content (F) in rats injected twice weekly with CCl4 for the indicated period of time (white bars) and in rats treated with PGZ for the last 3 wks of the 5 wks CCl4 regimen or the last 4 wks of the 9 wks CCl4 regimen (CCl4 + PGZ ; hatched bars). Data are expressed as mean ± SD for n=5/group. *** $p < 0.001$ compared to 2wk CCl4 livers, & $p < 0.05$ and && $p < 0.01$ compared to similar treatment at 5wks.
Reproduced with permission from (45).

particularly interested in the role of dysregulated adipose tissue function in the emergence of steatosis, inflammation and fibrosis. To resolve this, we will have to understand the intricated cross-talks between, adipose tissue (including altered adipocytokines balance, altered metabolic function and resulting chronic low-grade systemic inflammation) and the liver. This will have to be explored in terms of intrahepatic energy metabolism, but also in terms of consequences for the biology of intrahepatic inflammatory resident cells or hepatic effector cells of fibrosis.

Much remains to be learned about the consequences of insulin resistance for the liver and the links between insulin-resistance and the triggering of hepatic inflammation and fibrosis. Indeed, if insulin resistance is a hallmark of NASH, only a small proportion of patients with insulin resistance or type 2 diabetes will develop NASH. This implies that factors, others than impaired insulin signalling or severity of insulin resistance, participate to the pathogenesis of the disease. It is likely that complex additional factors such as diet, exercise and/or environment, as well as the efficiency of defence or protective mechanisms are implicated. In addition, the fact that the prevalence of NAFLD/NASH largely varies among different racial groups and that there are variable rates of disease progression within individuals with similar risk factors strongly suggests that genes also play a role in both prevalence and natural history of the disease. The interactions between environment and genetics, and the way they condition the hepatic phenotypic expression of insulin resistance remains largely unexplored.

Resolving this complex intricacy of metabolic, endocrine, environmental and genetic factors is the challenge that lies upon scientist interested in the field for the next decade. Indeed, the understanding of the pathogenesis of NASH is mandatory for identification of NAFLD or insulin resistant patients at risk of developing an aggressive fibrosing form of the disease. This understanding is also a prerequisite for the identification of potential therapeutic targets.

Acknowledgements

The author is thankful to all the people whom participated to the studies related in this review : Prof. Y. Horsmans, Dr. P. Starkel, Dr. I. Borbath, Mr. A. DaSilva, Ms. V. Lebrun, Mr. J. Abarca-Quinones, Ms. C. De Saeger and Ms. M. Petit from the laboratory of Gastroenterology ; Dr. C. Sempoux, department of pathology and Mr. A. Saliez for the department of experimental surgery, Université catholique de Louvain UCL, Brussels, Belgium. Prof. G. C. Farrell, Prof. J. George, Dr. N. Teoh, Dr. A. dela Pena, Dr. A. Ip, Dr. J. Wang, Ms. J. Field-Williams, Ms. R. Schreimer, Ms. N. Pera, Dr. G. Robertson from the Storr Liver Unit, University of Sydney, Sydney, Australia.

This work was funded by grants from the Australian NH&MRC (projet grant and programme grant), Belgian FNRS, Université Catholique of Louvain (FSR), action de recherche concertée de la direction de la recherche scientifique de la communauté française de Belgique (ARC 05/10-328). IL is research associate with the FNRS.

References

1. LUDWIG J., VIGGIANO T.R., MC GILL D.B., OH B.J. Nonalcoholic steatohepatitis : Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin. Proc.*, 1980, **55** : 434-8.
2. BACON B.R., FARAHVASH M.J., JANNEY C.G., NEUSCHWANDER-TETRI B.A. Nonalcoholic steatohepatitis : an expanded clinical entity. *Gastroenterology*, 1994, **107** : 1103-9.
3. BUGIANESI E., MC CULLOUGH A.J., MARCHESINI G. Insulin resistance : a metabolic pathway to chronic liver disease. *Hepatology*, 2005, **42** : 987-1000.
4. BRUNT E.M., JANNEY C.G., DI BISCEGLIE A.M., NEUSCHWANDER-TETRI B.A., BACON B.R. Nonalcoholic steatohepatitis : a proposal for grading and staging the histological lesions. *Am. J. Gastroenterol.*, 1999, **94** : 2467-74.
5. MENDES F.D., LINDOR K.D. Recent advances in the treatment of non-alcoholic fatty liver disease. *Expert Opin. Investig. Drugs*, 2005, **14** : 29-35.
6. JAMES O.F. NASH/NAFLD management. *Acta Gastroenterol. Belg.*, 2002 : **65**, 200-203.
7. SAMUEL V.T., SHULMAN G.I. Insulin resistance in NAFLD : potential mechanisms and therapies. *In* : FARRELL G.C., GEORGE J., DE LA M.HALL P., MC CULLOUGH A.J. (eds). Fatty Liver Disease -NASH and related disorders. Oxford : Blackwell Publishing Ltd, 2004, 38-54.
8. LARTER C.Z., FARRELL G.C. Insulin resistance, adiponectin, cytokines in NASH : Which is the best target to treat ? *J. Hepatol.*, 2006, **44** : 253-61.
9. MARCHESINI G., FORLANI G., BUGIANESI E. Is liver disease a threat to patients with metabolic disorders ? *Ann. Med.*, 2005, **37** : 333-46.
10. LECLERCQ I.A., FARRELL G.C., FIELD J., BELL D.R., GONZALEZ F.J., ROBERTSON G.R. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. *J. Clin. Invest.*, 2000, **105** : 1067-75.
11. IKURA Y., OHSAWA M., SUEKANE T., FUKUSHIMA H., ITABE H., JOMURA H., NISHIGUCHI S., INOUE T., NARUKO T., EHARA S., KAWADA N., ARAKAWA T., UEDA M. Localization of oxidized phosphatidylcholine in nonalcoholic fatty liver disease : impact on disease progression. *Hepatology*, 2006, **43** : 506-14.
12. SEKI S., KITADA T., YAMADA T., SAKAGUCHI H., NAKATANI K., WAKASA K. In situ detection of lipid peroxidation and oxidative DNA damage in non-alcoholic fatty liver diseases. *J. Hepatol.*, 2002, **37** : 56-62.
13. ALBANO E., MOTTARAN E., OCCHINO G., REALE E., VIDALI M. Review article : role of oxidative stress in the progression of non-alcoholic steatosis. *Aliment Pharmacol. Ther.*, 2005, **22** Suppl 2 : 71-3.
14. BASS N.M., MERRIMAN R.B. Fatty acid metabolism and lipotoxicity in th epathogenesis of NAFLD/NASH. *In* : FARRELL G.C., GEORGE J., DE LA M HALL P., MC CULLOUGH A.J. (eds). Fatty Liver Disease : NASH and related disorders. Oxford : Blackwell Publishing, Ltd, 2005, 109-22.
15. DIEHL A.M., LI Z.P., LIN H.Z., YANG S.Q. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. *Gut*, 2005, **54** : 303-6.
16. CORTEZ-PINTO H., DE MOURA M.C., DAY C.P. Non-alcoholic steatohepatitis : from cell biology to clinical practice. *J. Hepatol.*, 2006, **44** : 197-208.
17. PESSAYRE D., FROMENTY B. NASH : a mitochondrial disease. *J. Hepatol.*, 2005, **42** : 928-40.
18. LECLERCQ I.A., SCHROYEN B., DA SILVA M.A., VANHUL N.K., GEERTS A. Mechanisms and consequences of insulin resistance in liver cells. *J. Hepatol.*, 2007. In Press.
19. IP E., FARRELL G.C., ROBERTSON G., HALL P., KIRSCH R., LECLERCQ I. Central role of PPARalpha-dependent hepatic lipid turnover in dietary steatohepatitis in mice. *Hepatology*, 2003, **38** : 123-32.
20. IP E., FARRELL G., HALL P., ROBERTSON G., LECLERCQ I. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. *Hepatology*, 2004, **39** : 1286-96.
21. BELFORT R., HARRISON S.A., BROWN K., DARLAND C., FINCH J., HARDIES J., BALAS B., GASTALDELLI A., TIO F., PULCINI J., BERRIA R., MA J.Z., DWIVEDI S., HAVRANEK R., FINCKE C., DEFRONZO R., BANNAYAN G.A., SCHENKER S., CUSI K. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N. Engl. J. Med.*, 2006, **355** : 2297-307.
22. GEORGE J., PERA N., PHUNG N., LECLERCQ I., YUN HJ., FARRELL G. Lipid peroxidation, stellate cell activation and hepatic fibrogenesis in a rat model of chronic steatohepatitis. *J. Hepatol.*, 2003, **39** : 756-64.
23. STARKEL P., SEMPOUX C., LECLERCQ I., HERIN M., DEBY C., DESAGER J.P., HORSMANS Y. Oxidative stress, KLF6 and transforming growth factor-beta up-regulation differentiate non-alcoholic steatohepatitis progressing to fibrosis from uncomplicated steatosis in rats. *J. Hepatol.*, 2003, **39** : 538-46.
24. LECLERCQ I.A., FARRELL G.C., SEMPOUX C., DELA P.A., HORSMANS Y. Curcumin inhibits NF-kappaB activation and reduces the severity of experimental steatohepatitis in mice. *J. Hepatol.*, 2004, **41** : 926-34.
25. DELA P.A., LECLERCQ I., FIELD J., GEORGE J., JONES B., FARRELL G. NF-kappaB activation, rather than TNF, mediates hepatic inflammation in a murine dietary model of steatohepatitis. *Gastroenterology*, 2005, **129** : 1663-74.
26. REAVEN G. The metabolic syndrome or the insulin resistance syndrome ? Different names, different concepts, and different goals. *Endocrinol. Metab. Clin. North Am.*, 2004, **33** : 283-303.
27. YKI-JARVINEN H. Fat in the liver and insulin resistance. *Ann. Med.*, 2005, **37** : 347-56.
28. KERSHAW E.E., FLIER J.S. Adipose tissue as an endocrine organ. *J. Clin. Endocrinol. Metab.*, 2004, **89** : 2548-56.
29. KAMADA Y., TAMURA S., KISO S., MATSUMOTO H., SAJI Y., YOSHIDA Y., FUKUI K., MAEDA N., NISHIZAWA H., NAGARETANI H., OKAMOTO Y., KIHARA S., MIYAGAWA J., SHINOMURA Y., FUNAHASHI T., MATSUZAWA Y. Enhanced carbon tetrachloride-induced liver fibrosis in mice lacking adiponectin. *Gastroenterology*, 2003, **125** : 1796-807.
30. BERGHEIM I., GUO L., DAVIS M.A., DUVEAU I., ARTEEL G.E. Critical role of plasminogen activator inhibitor-1 in cholestatic liver injury and fibrosis. *J. Pharmacol. Exp. Ther.*, 2006, **316** : 592-600.
31. LECLERCQ I.A., FARRELL G.C., SCHRIEMER R., ROBERTSON G.R. Leptin is essential for the hepatic fibrogenic response to chronic liver injury. *J. Hepatol.*, 2002, **37** : 206-13.
32. LECLERCQ I.A., FIELD J., FARRELL G.C. Leptin-specific mechanisms for impaired liver regeneration in ob/ob mice after toxic injury. *Gastroenterology*, 2003, **124** : 1451-64.
33. LECLERCQ I.A., VANSTEENBERGHE M., LEBRUN V.B., VANHUL N.K., ABARCA-QUINONES J., SEMPOUX C.L., PICARD C., STARKEL P., HORSMANS Y.L. Defective hepatic regeneration after partial hepatectomy in leptin-deficient mice is not rescued by exogenous leptin. *Lab. Invest.*, 2006, **86** : 1161-71.
34. CAO Q., MAK K.M., REN C., LIEBER C.S. Leptin stimulates tissue inhibitor of metalloproteinase-1 in human hepatic stellate cells : respective roles of the JAK/STAT and JAK-mediated H2O2-dependant MAPK pathways. *J. Biol. Chem.*, 2004, **279** : 4292-304.
35. SAXENA N.K., IKEDA K., ROCKEY D.C., FRIEDMAN S.L., ANANIA F.A. Leptin in hepatic fibrosis : evidence for increased collagen production in stellate cells and lean littermates of ob/ob mice. *Hepatology*, 2002, **35** : 762-71.
36. ALEFFI S., PETRAI I., BERTOLANI C., PAROLA M., COLOMBATTO S., NOVO E., VIZZUTTI F., ANANIA F.A., MILANI S., ROMBOUTS K., LAFFI G., PINZANI M., MARRA F. Upregulation of proinflammatory and proangiogenic cytokines by leptin in human hepatic stellate cells. *Hepatology*, 2005, **42** : 1339-48.
37. SAXENA N.K., TITUS M.A., DING X., FLOYD J., SRINIVASAN S., SITARAMAN S.V., ANANIA F.A. Leptin as a novel profibrogenic cytokine in hepatic stellate cells : mitogenesis and inhibition of apoptosis mediated by extracellular regulated kinase (Erk) and Akt phosphorylation. *FASEB J.*, 2004, **18** : 1612-4.
38. IKEJIMA K., HONDA H., YOSHIKAWA M., HIROSE M., KITAMURA T., TAKEI Y., SATO N. Leptin augments inflammatory and profibrogenic responses in the murine liver induced by hepatotoxic chemicals. *Hepatology*, 2001, **34** : 288-97.
39. IKEJIMA K., TAKEI Y., HONDA H., HIROSE M., YOSHIKAWA M., ZHANG Y.J., LANG T., FUKUDA T., YAMASHINA S., KITAMURA T., SATO N. Leptin receptor-mediated signaling regulates hepatic fibrogenesis and remodeling of extracellular matrix in the rat. *Gastroenterology*, 2002, **122** : 1399-410.
40. GEORGE J., WANG J., RAMEZANI M., LONDON R., BRYMORA J., LECLERCQ I. Kupffer cells : the main player in leptin-induced liver fibrosis. *J. Gastroenterol. Hepatol.*, 2006 : **21** (Suppl.), A315.
41. LECLERCQ I.A., HORSMANS Y. Cell biology of NASH : fibrosis and cell proliferation. *In* : FARRELL G.C., GEORGE J., DE LA M HALL P., MC CULLOUGH A.J., (eds). Fatty Liver Disease : NASH and related disorders. Oxford : Blackwell Publishing, 2005,143-58.
42. YKI-JARVINEN H. Thiazolidinediones. *N. Engl. J. Med.*, 2004, **351** : 1106-18.
43. TOMITA K., AZUMA T., KITAMURA N., NISHIDA J., TAMIYA G., OKA A., INOKUCHI S., NISHIMURA T., SUEMATSU M., ISHII H. Pioglitazone prevents alcohol-induced fatty liver in rats through up-regulation of c-Met. *Gastroenterology*, 2004, **126** : 873-85.
44. ENOMOTO N., TAKEI Y., HIROSE M., KONNO A., SHIBUYA T., MATSUYAMA S., SUZUKI S., KITAMURA K.I., SATO N. Prevention of

ethanol-induced liver injury in rats by an agonist of peroxisome proliferator-activated receptor-gamma, pioglitazone. *J. Pharmacol. Exp. Ther.*, 2003, **306** : 846-54.

45. LECLERCQ I.A., SEMPOUX C., STARKEL P., HORSMANS Y. Limited therapeutic efficacy of pioglitazone on progression of hepatic fibrosis in rats. *Gut*, 2006, **55** : 1020-9.
46. PROMRAT K., LUTCHMAN G., UWAIFO G.I., FREEDMAN R.J., SOZA A., HELLER T., DOO E., GHANY M., PREMKUMAR A., PARK Y., LIANG T.J., YANOVSKI J.A., KLEINER D.E., HOOFNAGLE J.H. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. *Hepatology*, 2004, **39** : 188-96.
47. NEUSCHWANDER-TETRI B.A., BRUNT E.M., WEHMEIER K.R., OLIVER D., BACON B.R. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. *Hepatology*, 2003, **38** : 1008-17.
48. RATZIU V., CHARLOTTE F., JACQUEMINET S., PODEVIN P., SERFATY L., BRUCKERT E., GRIMALDI A., POYNARD T. A one year randomized, placebo-controlled, bouble-blind trial of rosiglitazone in non alcoholic steatohepatitis : results of the FLIRT pilot trial. *Hepatology*, 2006 : **44** (4, suppl.1), 201A.
49. MARRA F., EFSEN E., ROMANELLI R.G., CALIGIURI A., PASTACALDI S., BATIGNANI G., BONACCHI A., CAPORALE R., LAFFI G., PINZANI M., GENTILINI P. Ligands of peroxisome proliferator-activated receptor gamma modulate profibrogenic and proinflammatory actions in hepatic stellate cells. *Gastroenterology*, 2000, **119** : 466-78.
50. GALLI A., CRABB D.W., CENI E., SALZANO R., MELLO T., SVEGLIATI-BARONI G., RIDOLFI F., TROZZI L., SURRENTI C., CASINI A. Antidiabetic thiazolidinediones inhibit collagen synthesis and hepatic stellate cell activation in vivo and in vitro. *Gastroenterology*, 2002, **122** : 1924-40.
51. HAZRA S., XIONG S., WANG J., RIPPE R.A., KRISHNA V., CHATTERJEE K., TSUKAMOTO H. Peroxisome proliferator-activated receptor gamma induces a phenotypic switch from activated to quiescent hepatic stellate cells. *J. Biol. Chem.*, 2004, **279** : 11392-401.
52. SHE H., XIONG S., HAZRA S., TSUKAMOTO H. Adipogenic transcriptional regulation of hepatic stellate cells. *J. Biol. Chem.*, 2005, **280** : 4959-67.
53. DA SILVA M.A., ABARCA-QUINONES J., HORSMANS Y., STARKEL P., LECLERCQ I.A. Peroxisome proliferated-activated receptor gamma ligand, Pioglitazone, does not prevent hepatic fibrosis in mice. *Int. J. Mol. Med.*, 2007, **19** : 105-12.