PATHOGENIC MECHANISMS INVOLVED IN THE NON-ALCOHOLIC FATTY LIVER DISEASE

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Abstract

NAFLD pathogenesis is partially known. In determining NAFLD stages there are implied insulin resistance, magnitude and pattern of lipid deposits, lipid hepatic metabolism (synthesis, storage, β-oxydation use, elimination), oxydative stress, synthesis of cytokines and adipokines.

Key words*: steatosis, metabolic syndrome, insulin resistance, oxydative stress, cytokines, adipokines.*

I. Introduction. Definition.

Non-alcoholic steatosic hepatopathy represents a clinical entity frequently met in association with the elements of metabolic syndrome (type 2 diabetes mellitus, obesity, dyslipidaemia) and consists of fat accumulation within hepatocytes in the absence of a significant alcohol intake (< 20g alcohol/day).

Non-alcoholic fatty liver disease (NAFLD) consists of a hepatic damaging spectrum which presents as initial anomaly the hepatic steatosis, a benign condition, characterized through a hepatic fatty infiltration, followed by non-alcoholic steatohepatitis (NASH), consisting of steatosis, inflammation, necrosis and fibrosis. 25% of patients with NASH may progress to cirrhosis and its complications, including: portal hypertension, liver failure, hepatocellular carcinoma [1].

The first observations on NASH date since 1950s, the condition being described by using various names. In 1980, Dr. J. Ludwig uses the notion of non-alcoholic steatohepatitis (NASH) to designate a clinicopathological entity frequently met in obese female patients with diabetes mellitus who reject alcohol intake, but present a hepatic histological aspect similar to that of alcoholic hepatitis. For a long time, there has been considered that NASH is a completely harmless condition, the distinction between simple hepatic steatosis and non-alcoholic steatohepatitis being performed much later.

II. Epidemiology

The actual prevalence and incidence of NASH cannot be appropriately estimated due to the fact that a hepatic biopsy is required for diagnosis. The data derived from histological studies indicate a NASH prevalence, varying from 15 to 39%. NAFLD prevalence in diabetes mellitus is estimated between 34-74% [2], and in diabetes mellitus associated with obesity it is considered to be of 100% [3]. Steatosis was reported in 70% of the obese

patients and in 35% of patients with normal weight [4].

III. Pathogenesis

NAFLD pathogenesis is partially known. Strong correlation between conditions characterized by insulin resistance (diabetes mellitus, obesity, metabolic syndrome) to NASH, has determined some authors to support the idea that this is a hepatic manifestation of the metabolic syndrome. Insulin resistance plays a major part in determining hyperinsulinism and hepatic fat overload. [5,6].

In determining NAFLD stages there are implied insulin resistance, magnitude and pattern of lipid deposits, lipid hepatic metabolism (synthesis, storage, β-oxydation use, elimination), oxydative stress, synthesis of cytokines and adipokines.

Day [7] considers that NASH pathogenesis involves two progression stages. Initially, the normal liver becomes steatosic as a consequence of peripheral insulin resistance, which favourizes the AG transport from the fat tissue to the liver. The second stage involves oxydative stress and inflammatory cytokines, especially TNF α that exacerbates insulin resistance, oxydative stress, determines hepatic cellular dysfunction, leads to an inflammatory process, hepatocellular degeneration and fibrosis.

Hepatic steatosis reflects an imbalance between Fatty Acids (FA) intake and synthesis by the liver, between oxydation and elimination. Patients suffering from type 2 diabetes mellitus present dyslipidaemia characterized through high TG plasma levels, low HDL cholesterol level, small LDL predominance, a pattern also seen in patients with NAFLD [8]. High FFA levels may be the consequence of some multiple mechanisms, including novo synthesis, insulin resistance by interfering with the intracellular phosphorylation process, as well as from lipid peroxydation. The central anomaly in steatosis pathogenesis seems to be connected to insulin resistance resulting from major lipolysis, high levels of FFA which are afterwards assumed by the liver as an energy source. High FA levels increase hepatic mitochondrial β-oxydation, leading to FA accumulation in the liver. Lipid retention in hepatocytes, mainly TG, is a condition for NAFLD development [9]. Insulin resistance, generally present in NAFLD patients, is believed to play an important role in this process [10,11]. TG deposited in hepatocytes originates from increased FFA delivery from the plasma FFA pool reflecting the flux from the adipose tissue, from *de novo* lipogenesis, or from postprandial metabolism of chylomicrons. Balancing of FA metabolism is different in fasting and postprandial status. Recent data [12] from obese patients with hypertriglyceridemia and hyperinsulinemia have shown that 59% of hepatocytary TG derive from plasma pool in postprandial status, 26% of novo synthesis, 15% of diet. As both intestinal and hepatic sources contribute to the development of fatty liver, both B48 and B100 apoproteins from the chylomicron fraction and VLDL1 are high in subjects with hepatic fat overload.

A combination between genetic and environment factors, which determine insulin resistance, contribute to the development of hepatic steatosis. Severity of insulin resistance has proved to be paralel to the severity of liver

fatty overload, NASH being common in patients with type 2 diabetes mellitus [13].

The levels of unsaturated fats in the diet are correlated with a lower sensitivity to insulin, high levels of postprandial TG and to other aspects of the metabolic syndrome [14].

There are data indicating that the central and visceral fat tissue play a more important part than the peripheral fat tissue in FFA release and emergence of hepatic steatosis [15]. The reason seems to be the direct access to the liver through the portal system of fats derived from these tissues, as well as a lower secretion of leptin [16].

Under normal circumstances, the liver is ready to protect itself against the toxicity induced by FFA, through their esterification and TG formation, through oxydation or synthesis and VLDL release. PPAR α nuclear receptor plays an important role in this process [17]. In hyperinsulinism, the following process will take place: increase of FFA estretification and TG formation, increase of AG glycolysis and synthesis, oxydation inhibition (18), decrease of TG reelase within VLDL [19]. The B apolipoprotein secretion, necessary for VLDL formation, proved to be low in NASH [20]. These alterations of the lipidic metabolism contribute to the change of a healthy liver into a stetosic one.

Stetosic liver may become an insulin resistant organ. The mechanism is not completely understood, TNF α, NF k, FFA effects, PPAR α may also play a part [21]. Leptin may also regulate hepatic sensitivity to insulin [22].

In most patients, the process does not progress, although a minority of them pass to the next stage, characterized by inflammation and hepatocellular degeneration. In steatosis liver hepatocytes are vulnerable against external aggressive factors, and apoptosis becomes a frequent mechanism of cell death. [23]. Insulin resistance seems to be a major risk factor, independent from other factors involved in fibrosis progression [24]. The reason of the disease progression is not fully known, oxydative stress and cytokines being the main effectors of a vulnerable liver.

IV. 1 Oxydative Stress

Peripheral insulin reistance and high leptin levels allow the FFA that reached the liver to enter the mitochondria, as a consequence of a previous inhibition of oxydation (7). Besides that, oxydation of FA, with long and very long chains, partially takes place extramitochondrially (microsomes, peroxysomes), while the production of O_2 free radicals takes part mainly in the mitochondria [25]. Major hepatic intake of FFA, and particularly of acil-Co A, leads to an immediate PPAR α activation of the synthesis of enzymes responsible for oxydation, increasing the level of peroxydes [7]. Formation of O_2 free radicals in a rich fatty environment leads to lipidic peroxydation. Increase of oxydative stress and of lipidic peroxydation determines alterations of plasma membrane, of intracellular organite, of mitochondrial DNA and of respiratoty chain proteins. Moreover, resulting products from the oxydative stress activate NF k, mediate the synthesis of nitric oxyde, leading to the formation of peroxynitrites. AGL increase the expression of microsomal oxydase CYP 4 A and CYP 2 E1, responsible for the production of hydroxyl radicals [26]. Because CYP 2 E1 is inhibited by insulin, its levels are high in peripheral insulin resistance.

Mitochondrial dysfunction seems to be the critical event in NASH. Alterations caused by lipidic peroxydating products lead to alterations in the electron transport of the respiratory chain, this generating more O_2 free radicals [25]. Mitochondrial expression of Protein 2 decoupling is high. This protein reduces the synthesis of O_2 free radicals, but, in the same time, the ATP level decreases, this making the cell more vulnerable to aggressions [27] and facilitating hepatocellular apoptosis and necrosis. The decrease of ATP synthesis was reported in NASH after glucose perfusion. Electronic microscopy of hepatic biopsy in NASH patients showed the presence of paracrystalin inclusions in 10-30% of cases in hepatocellular mitochondria. The significance of this fact is unknown, these inclusions were noticed in patients with myopathies associated to mitochondrial DNA alterations that damage the respiratory enzyme chain and may represent a genetic basis used to determine the hepatic disease progression to NASH.

IV. 2 Cytokines, Insulin Resistance, Fatty Liver Disease

Present studies emphasize the main part played by cytokines in the NAFLD pathogenesis. Cytokines have a balancing role in the insulin sensibility, lipidic hepatic overload, hepatic injuries, inflammation, fibrosis and cirrhosis.

The main role is played by TNF α together with other proinflammatory cytokines in the development of obesity associated with insulin resistance and fatty overload of the liver. Literature data underline the major part played by TNF α in the development of insulin resistance. TNF α expression is high in the fatty tissue and liver in NASH patients whose TNF α seric levels are correlated with insulin resistance. Circulating, as well as liver and adipose tissue levels of TNF α are increased in obesity [28]. Administration of TNF α to culture cells in vivo or to animals impairs the insulin action, while the low TNF α levels or TNF receptors in animals were associated with the improvement of insulin sensibility. In humans, acute infusion of TNF α inhibits the distribution of insulin stimulated glucose [29], while TNF α polymorphism is connected to insulin resistance and NAFLD susceptability [30], supporting the importance of this cytokine in inflammation, insulin signal, fat accumulation.

The intracellular and molecular mechanisms responsible for TNF-α-induced insulin resistance and lipid overloading of liver cells have been increasingly elucidated and appear to involve both activation of stress-related protein kinases, such as Jun N-terminal kinase (JNK), as well as the inhibitor kappa beta kinase beta (IKKβ)/NF-κB pathway [31]. These mechanisms are induced by the increase of TNF α seric levels which stimulate the cells with surface receptors. It was demonstrated that JNK activation plays a role in insulin resistance associated with obesity [32]. JNK activation is high in the liver, the muscles, the fatty tissue, and loss of JNK1 activity prevents the development of insulin resistance in obesity. JNK activation may alter insulin action through IRS-1 phosphorylation at serine level [33]. The signal of insulinic receptor, normally occurring through tyrosine kinase, is inhibited by serine or treonine phosphorylation.

The connection between IKK β activity and insulin resistance is less known. IKK β

activation leads to the NF-k β translocation to the nucleus, resulting a process that promotes the synthesis of TNF α together with other mediators of inflammation which may cause insulin resistance [34]. IKK β heterozygous mice are protected against the development of insulin resistance. The selective activation degree of IKK β/NF-k β mechanism leads to a chronic inflammation process, with the increase of cyokine (TNF α, IL-6) and of hepatic and systemic insulin resistance [35].

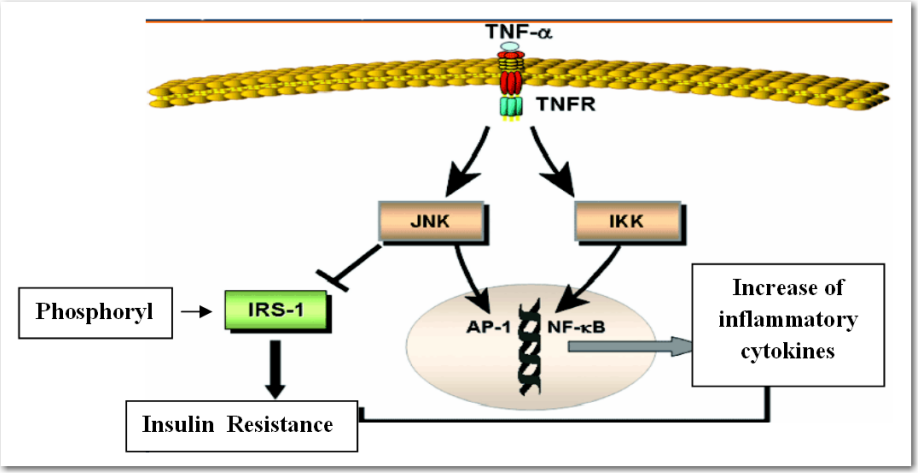

Fig. 1. Molecular Mechansim Through which TNF α Induces Insulin Resistance.
(from American Journal of Gastroenterology - Christine Carter-Kent, M.; Nizar N. Zein, MD; Ariel E. Feldstein, MD; May 2008 - modified)

There are at least two mechanisms contributing to the exacerbation of insulin resistance:

- IKK chronic activation (IB-kinase), which plays a part not only in producing the answer to proinflammatory cytokinescare such as TNF α, but also in the development of insulin resistance.
- Decreased clearance of insulin, that occurs in presence of a steatotic liver, thus creating a hyperinsulinemic medium that in turn enhances insulin resistance.

Other cytokines and adipokines like IL-6 and adiponectine were shown to play a significant role in the development of insulin resistance and fatty liver. Seric levels of IL-6 are high in animal models, in NAFLD patients and also in case of alcoholic liver [36]. IL-6 frequency was shown to be correlated in the liver on two NAFLD murine models.

In human NAFLD, IL-6 expression is high in hepatocytes and Kuffer cells, and its level is positively correlated with inflammatory activity and fibrosis degree [37]. Certain concepts suggest the fact that, in the liver, IL-6 mainly has a pro-survival and anti-apoptotic activity [37]. Still, there has been demonstrated that, despite all IL-6 functions of hepatic regeneration, excessive chronic exposure to IL-6 not only minimizes the protector effect, but it also favourizes liver injury and apoptosis [38]. The way in which hepatic chronic exposure to IL-6 excess promotes hepatic damage in NAFLD is under investigation on animal models. Immune cells, endothelial cells and, more recently, adipocytes are the main sources of IL-6, but it was also shown that hepatocytes may produce IL-6 and other proinflammatory cytokines such as TNF α [28]. Increasingly more often,

in vitro and in vivo studies showed that the liver represents the main target of IL-6 action, where the insulin signal is inhibited, thus resulting the increase of hepatic gluconeogenesis, consecutive hyperglycemia and compensatory hyperinsulinism. As omental fat may be an important source of IL-6 towards the liver, it is tempting the supposition that IL-6 hepatocytary production may play a part through a paracrine or autocrine action. In order to support this concept, studies on mice demonstrated that IL-6 sustained selective expression in hepatocytes leads to a paralel increase of IL-6 plasma level, sufficient enough for inducing insulin resistance [35]. More often in NAFLD patients, IL-6 hepatic expression is closely connected to IL-6 circulatory levels [37].

IV. 3 Cytokines and Progression to NASH

The main part in liver progression to NASH is played by TNF α together with other inflammatory cytokines. TNF α contribute to NASH pathogenesis, its increase of hepatocytes and Kupffer cells synthesis may be caused by:

- mediation of FFA oxydation by NF β, inducing oxydative stress [36]
- endogene toxemia, resulting from intestinal bacterial overgrowth

TNF has got several action models:

- induces insulin resistance by increased FFA level
- alteration of mitocondrial respiration, with emergence of O_2 free radicals [18]
- induces hepatocytary apoptosis and necrosis [37]

Genetic predisposal in NASH includes genes involved in:

- establishing insulin sensibility [38]
- hepatic fat storage, oxydation, release into blood flow [39]
- obesity and its distribution [40]
- balancing iron hepatic deposits and generating oxydative stress [41]
- cytokine synthesis [42]

Qualitative and quantitatve alterations of cytokines, and particularly an increase of TNF α/adiponectin ratio may play a part in the develpment of NASH.

Antibody treatment against TNF α in ob/ob mice with leptin defficiency improves NASH and hepatic insulin resistance [42]. Genetically deficient mice in the TNF Receptor 1 (TNFR 1) are resistant to steatosis and hepatic injury induced by a rich-carbohydrates and metionine-restrictive diet, namely the choline-deficient diet [28]. Additionally, recombined adiponectine injection in ob/ob mice decreases the TNF α level and determines steatohepatitis regression, while the metamorphin leads to regression of liver fat overload in these mice, at least partially, through inhibiting the TNF α hepatic expression [43]. Gene expression of TNF α and its receptor is high in the liver of patients with NASH, and it is higher in those patients with more severe NASH. Circulatory level of adiponectin is low, while TNF is high [44].

The molecular mechanism that leads to fat accumulation in hepatocytes or increase of TNF α production are on the way to be clarified. Exposure of human and murine hepatocyte cultures with long chain AGL results in the Bax translocation, a proapoptotic member of B lymphocytary cells family (Bcl-2), cells that form channels within the membrane towards lysosomes with secundary

lysosomal permeability and B cathepsin release, a cysteine protease. Once reaching the cytosol, B cathepsin starts a cascadel that peaks with the NFK β activation and its translocation within the nucleus where it produces the TNF α synthesis. TNF α may also produce lysosomal destabilisation, leading to the ongoing mechanism of hepatic injury [28]. Based on this concept, we may state that genetic or pharmacological inactivation of B cathepsin protects against hepatic steatosis, hepatic injury and insulin resistance [28]. B cathepsin causes mitochondrial dysfunction which leads to hepatocytary apoptosis.

Another mechanism through which TNF may contribute to steatosis and inflammation is by modulating of sterol regulatory element binding proteins (SREBP). This represents a family of membranary transcription factors which activate genes that codify enzymes of lipid biosynthesis. Out of the three identified members of this family, SREBP-1c is the major form expressed in the liver [45]. Its level is high in ob/ob mice and this increase is correlated with the increase of ARNm level of lipogenic enzymes and with hepatic fatty deposits [54]. Endo and colleagues reported that intraperitoneal injection of mice with TNF α or liposaccharides determine fatty overload of the liver, positively connected to the SREBP-1c level and to the ARNm level of FA synthesis [46].

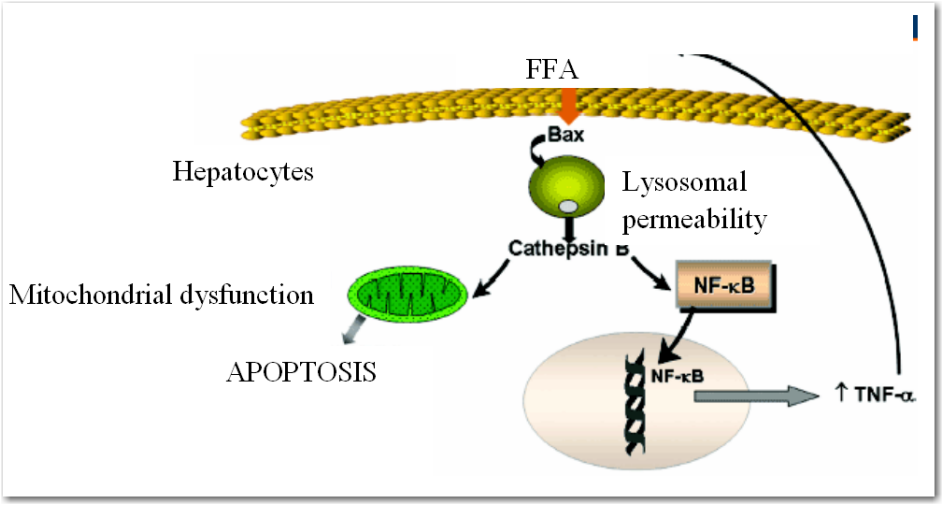

Fig. 2. Molecular Mechansim through which TNF α Induces Hepatic Damage in the Liver
(from *American Journal of Gastroenterology* - Christine Carter-Kent, M.; Nizar N. Zein, MD; Ariel E. Feldstein, MD; May 2008 - modified)

In contrast with TNF α, adiponectin presents antilipogenic, anti-inflammatory effects and numerous evidences suggest that hypoadiponectinemia may play an important role in NAFLD. In patients with biopsy-proven NAFLD, the decrease of seric adiponectin levels was positively correlated with severe stages of necroinflammatory activity. In these patients, hypoadiponectinemia and insulin resistance have been predicitve for the progression of steatosis to NASH. In morbidly obese patients diagnosed with either NASH or steatosis by liver biopsy, Kaser *et al.* showed that mRNA levels of both adiponectin and adiponectin receptor II were

significantly decreased in patients with NASH as compared with those with steatosis. [47].

Leptine is a peptidic hormone involved in the balancing of lipidic metabolism. It prevents fat accumulation in nonfat tissues by inhibiting lipogenesis and favouring lipolysis after excessive caloric intake. Present data reported the leptin involvement in NASH pathogenesis. Uygun [48] discovered high leptin seric levels in NASH and suggested that leptin may represent an independent, predicitve factor for steatosis severity in NASH, together with C peptide (reflecting insulin secretion) and age [22].

Resistin is an adipokine identified in mice, which induces insulin resistance in target tissues of insulin: liver, muscle, fatty tissue. The level of Resistin in humans was proven to be connected to necroinflammatory alterations in NASH [49].

IV. 4 Cytokines and Hepatic Fibrosis

Longitudinal studies on bioptically objectified NASH patients have shown that, after an evolution period of 3-4 years, the inflammation and fibrosis progress in approximately a third of the patients [50]. After 10 years, approximately 10% of NAFLD patients progress to cirrhosis with an increase in hepatic mortality in comparison to the general population. Cytokines seem to play an important role in fibrogenesis and fibrosis. Hepatic star cells are the main mediators of fibrosis. Upon activation, these cells release factors that determine the deposit of extracellular matrix (ECM). In chronic hepatic injury, sustained inflammation leads to the increase of star cells activation and reduction of their apoptosis with abnormal ECM production. TNF α has a direct role in Kupffer cells activation and stellate cells activation, leading to fibrosis.

Together with TNF α, leptine is another cytokine that plays an important part in fibrogenesis. Leptine works through its receptor OB-R leading to Janus kinase stimulation (JAK). The effects of leptine activation have been proposed to include increased release of TGF-β 1 from Kupffer cells.

V. Treatment Implications

There must be encouraged a change of lifestyle in NAFLD patients. Insulin resistance, known as main pathogenic mechanism, may be influenced by using a Metformin medication. In 2009, Loomba and colleagues have shown improvement of biochemical markers by using Metformin in NASH treatment. Taking into consideration the key role of cytokines in NAFLD pathogenesis, their treatment modulation seems to be a logical target.

Using TNF α inhibitors in animals may become promissing in the future. In 2007 Satapathy and colleagues used Pentoxyfyllinum in NASH patients, accompanied by the improvement of hepatic histology after a 12 months period [51]. Pentoxyfyllinum is a relatively poor and non-specific inhibitor of TNF, and the use of more selective blocants, like Infliximab and Adalimunab may represent promissing choices. IL-6 inhibition may be another treatment target. Tocilizumab inhibits IL-6 binding with its receptor. In theory, adiponectin administration may prevent the development of severe steatohepatitis. Pharmacological inactivation of cathepsin may theoretically protect against hepatic steatosis.

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