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Molecular Targets for Therapeutic Intervention in Patients with Nonalcoholic Fatty Liver Disease (NAFLD)

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Abstract

Pathogenesis of nonalcoholic fatty liver disease (NAFLD) is multi factorial; hence there is no single treatment for this disease entity. Multiple molecules involved in the pathogenesis thus become important targets for therapeutic intervention in NAFLD. Insulin resistance plays most important role in the pathogenesis of NAFLD. Interplay of adipokines like tumor necrosis factor-alpha (TNF-á), adiponectin and leptin is involved causing insulin resistance. Life style interventions, drugs and bariatric surgery which are used for weight reduction in NAFLD have been shown to be beneficial by improving the insulin sensitivity by changing the adipokine profile. Similarly various anti oxidant drugs like vitamin C, vitamin E, betaine, probucol and metadoxine used in NAFLD have been shown to be beneficial by acting at molecular level. Unchecked, some of the patients with NAFLD can progress on to develop hepatic fibrosis and cirrhosis. Renin angiotensin system (RAS) and leptin have been thought to be involved in liver fibrosis. Treatment by angiotensin II receptor antagonist (losartan) has been shown to be effective in reducing hepatic fibrosis in patients with NAFLD. In future, anti apoptotic drugs and manipulation at genetic level may be beneficial in patients with NAFLD because of role of apoptosis and various genes in the pathogenesis of NAFLD.

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Introduction

Nonalcoholic fatty liver disease (NAFLD) presents a spectrum ranging from steatosis only to nonalcoholic steatohepatitis (NASH) which can in some cases progress to cirrhosis and hepatocellular carcinoma (1, 2). Pathogenesis of NAFLD involves deposition of fat in the liver; increased fatty acid oxidation, oxidative stress and altered levels of cytokines, finally leading to steatohepatitis and fibrosis in some of these patients who require appropriate treatment (3). Pharmacological treatment for NAFLD is still evolving and multiple factors in the pathogenesis may be important targets for therapeutic intervention.

Pathogenesis and molecular targets

The pathogenesis of the NASH is not completely understood. Pathogenesis involves deposition of fat in the liver and later development of steatohepatitis and fibrosis in some of these patients. Various pathogenetic mechanisms include insulin resistance, with or without metabolic syndrome, oxidative stress, cellular apoptosis and finally hepatic fibrosis. Various molecules responsible for these alterations can be attractive therapeutic targets.

Insulin resistance

Insulin resistance is thought to be the key factor, which leads to an increase in lipolysis and increased uptake of fatty acids by hepatocytes. Hyperinsulinemia occurring as a result of insulin resistance also increases the intrahepatocytic fatty acids by increasing glycolysis and decreasing apolipoprotein B-100 resulting in decreased export as VLDL (4).

Insulin resistance is almost a universal phenomenon in patients with NAFLD (5-8). Insulin resistance is related to an imbalance between pro-insulin and anti-insulin cytokines particularly those secreted from adipose tissue (adipokines). Tumor necrosis factor-alpha (TNF-á) is an anti insulin cytokine and its levels are elevated in patients with NAFLD (9). TNF – á activates stress related protein kinases including inhibitor kappa beta kinase beta (IKK beta) and Jun N-terminal kinase (JNK). These kinases promote serine phosphorylation of insulin receptor substrate-2 (IRS-2) molecules, inhibiting the propagation of signals from the insulin receptor. IKK beta also induces nuclear translocation of nuclear factor kappa beta (NF-kB) which further increases TNF production and also inhibits proliferator activator protein gamma (PPAR-ã) (10, 11). Source of TNF-á could either be the immature adipocytes, or the increased macrophages in the mature adipose tissue (especially the visceral adipose tissue) or the gut flora contributing to the release of the cytokines (12, 13).

Adiponectin is an adipokine which enhances some of the metabolic effects of

insulin and prevents insulin resistance by antagonizing the actions of anti-insulin cytokines like TNF-á. NF-kB inhibits PPAR-ã which prevents transcription of adiponectin gene thus leading to the insulin resistance by uninhibited actions of TNF-á.

The pharmacological treatment of patients with NAFLD is still evolving. No single therapy for NAFLD has clearly been proven effective. Dietary restriction, exercise and weight reduction, which improve insulin sensitivity, are usually the first line of treatment. Weight reduction in obese has been found to improve the serum ALT levels and liver steatosis (14). The weight reduction has to be slow and sustained because rapid weight loss may worsen necro-inflammation. A weight loss of no more than 1.6 kg in a week with a maximum of 10% of the baseline over a period of six months is recommended (15). Such a weight reduction is achieved by dietary fat restriction along with moderate sustained exercise like swimming, jogging, cycling, running etc. Weight reduction has also been attempted through use of drugs like orlistat and sibutramine but the results are generally not encouraging (16). Use of bariatric surgery in patients with NAFLD and morbid obesity has shown a biochemical and histological improvement (17).

Molecular basis of the benefits of life style interventions is improvement in insulin sensitivity by improving the cytokine profile. In a study, 60 women were randomly assigned to weight reduction of 10% or more through diet and increased physical activity (18). The control group (n = 60) were given general information about healthy food choices and exercise. After 2 years benefits seen in intervention group included reduction in BMI, decrease in serum concentrations of interleukin- 6 (IL-6), interleukin-18 (IL-18) and C reactive protein (CRP) and significant increase in adiponectin levels. The cytokine improvement was independent of changes in insulin sensitivity (18). In another study of 106 participants, 51 were treated with orlistat 360 mg/day for one year and compared with 55 age and sex and BMI matched controls. The orlistat group achieved reduction in leptin and increase in adiponectin, independent of changes in % body fat and waist circumference (19). Effect of bariatric surgery on cytokines was studied in 40 obese patients who achieved weight loss with either diet control (n=14) for 6 months or vertical banded gastroplasty (VBG) (n=13) or biliopancreatic diversion with duodenal switch (BPD-DS) (n=13). BMI significantly decreased in all 3 groups. Serum fasting ghrelin level increased after diet and VBG but decreased after BPD-DS. Leptin concentration decreased and adiponectin increased in all groups (20). In a mice model, treatment with anti-TNF antibodies improved liver histology, reduced hepatic

total fatty acid content, and decreased serum alanine aminotransferase (ALT) levels. These benefits were associated with decreased hepatic expression of TNF-alpha mRNA (21). Similarly treatment with anti TNF agent pentoxyphylline in patients with NAFLD has been shown to improve insulin resistance and serum ALT levels. Twenty patients were given pentoxifylline (400 mg q.i.d.) for 12 months. ALT and AST levels were significantly lower at 12 months compared to baseline (p= 0.002, p= 0.003). Nine patients (45%) withdrew from the study because of nausea (22). In an Indian study eighteen patients were given pentoxifylline 400 mg t.i.d. for 6 months. Mean serum levels of AST and ALT reduced significantly ($p < 0.0001$) and normalized in 60% of subjects at 6 months. HOMA (IR)) improved ($p = 0.046$) and the serum TNF-alpha reduced ($p = 0.011$) with none experiencing any side effects (23). Metformin by improving the insulin sensitivity and by its anti TNF action has been shown to be useful in humans and animal models of NAFLD. Likewise, we have also shown it to be useful in those not responding to lifestyle modifications and administration of UDCA (6,8). In an open-label study of pioglitazone in 18 NASH patients, serum levels for adiponectin, leptin, IL-1a, IL-6, and TNF-alpha, and paired liver biopsy specimens were studied. Adiponectin levels increased from 3.7 to 10.3 mug/mL at week 48 ($P < 0.01$). The levels of other cytokines were unchanged. There was an improvement in steatosis, inflammation and fibrosis. The change in adiponectin level was associated with improvement in steatosis ($P = 0.03$) as well as NASH activity index score ($P = 0.01$) (24).

Oxidative stress

The increased load of fatty acids in the hepatocytes increases the mitochondrial β-oxidation, and an increase in cytochrome P-450 4A and cytochrome P450 2E1 levels, leading to an increase in reactive oxygen species. The increased mitochondrial oxidative stress leads to the second hit from steatosis to steatohepatitis and fibrosis by three main mechanisms: lipid peroxidation, cytokine induction, and Fas ligand induction. A high ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) protects individuals against oxidative stress. Decreased hepatic GSH levels have been demonstrated and an association has been shown between the depleted hepatic GSH levels and lipid peroxidation in patients with NAFLD.

Various antioxidants used in NAFLD include vitamin C, vitamin E, betaine, probucol and metadoxine. In a recent experimental study, glutathione enhancing agents S-adenosylmethionine (SAMe) and propylthiazolidine carboxylic acid (PTCA) have been shown to improve the liver biochemistry and histology (25).

Hepatic Cellular Apoptosis

Emerging data suggests that hepatic apoptosis may play an important role in

liver injury and disease progression in patients with NAFLD. *In vivo* liver cell apoptosis has also been used as a biomarker of disease severity in these patients. Since apoptosis has been shown to play a major role in the pathogenesis of NAFLD, anti-apoptotic agents hold promise in the treatment of such patients (26).

Hepatic fibrosis

Leptin is an adipokine released from adipose tissue sharing with insulin several metabolic effects. Higher levels of leptin are found in patients with NAFLD due to leptin resistance. Increased leptin levels are also involved in causing fibrosis in these patients. In patients with lipodystrophies, recombinant human leptin treatment has shown to improve hepatic steatosis without any change in fibrosis. Ten patients comprising of generalized lipodystrophy (8 patients) or Dunnigan's partial lipodystrophy (2 patients) underwent paired liver biopsies at baseline and after treatment with recombinant methionyl human leptin (r-metHuLeptin) of mean duration 6.6 months. Significant improvements in steatosis ($P = 0.006$) and ballooning injury ($P = 0.005$), with a reduction of mean NASH activity by 60% ($P = 0.002$) was noticed but fibrosis remained unchanged (27).

Renin angiotensin system (RAS) has now been recognized as a major contributor towards hepatic fibrosis. Studies have shown higher positivity for RAS receptors on activated hepatic stellate cells (HSC) than on quiescent HSCs. In patients with NAFLD, treatment with losartan an angiotensin II antagonist has shown improvement in hepatic necro-inflammation and fibrosis as well as reduction in the activated HSCs (28,29). Seven patients with both NASH and hypertension were treated with angiotensin II receptor antagonist losartan (50 mg/d) for 48 weeks. Decrease in blood markers of hepatic fibrosis i.e. plasma TGF-beta1 and serum ferritin were assessed. There was improvement in serum aminotransferase levels, hepatic necro-inflammation (5 patients), reduction of hepatic fibrosis (4 patients) and disappearance of iron deposition (2 patients). No side effect of treatment was noted at any time during the study (28). In another study by the same authors quiescent and activated HSCs were identified by double immunostaining using anti-p75 and -smooth muscle actin antibodies. Activated HSCs were dominantly distributed in NASH vs simple steatosis which acted as controls. The 48-week losartan treatment induced a remarkable decrease in activated HSCs (29).

Genes

Although most patients with obesity and Type 2 diabetes have steatosis, only a minority will ever develop NASH, fibrosis and cirrhosis. Family studies suggest that genetic factors are important in the disease progression. Preliminary studies have

shown associations between severity of steatosis, NASH, fibrosis with genes whose products are involved in lipid metabolism, oxidative stress, and endotoxin-cytokine interactions. Identification of many other unknown genes involved in the pathogenesis of NAFLD can help in designing future therapies (30).

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