Therapeutic targets and the management of NASH

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Contents

• Overview of pathophysiology of NASH
• Therapeutic targets for NASH
• Management of NAFLD/NASH
  ➢ Short review of current therapy
• New emerging targets and therapeutics
• Summary
NAFLD: Overview

Obesity
Metabolic Syndrome
Type 2 diabetes
Dyslipidemia

Insulin resistance
Gut dysbiosis
Lipotoxicity
Oxidative stress
Mitochondrial injury
Inflammatory signals
Apoptosis cell death
Immune dysregulation
Stellate cell activation

Cardiovascular risk
Cancer risk and Death

Liver-related death

Hepatocellular carcinoma (HCC)

Non-alcoholic fatty liver disease (NAFLD)

Steatosis (without fibrosis)
Steatohepatitis (non-fibrotic/fibrotic)
Cirrhosis

NAFLD: Who to Treat

Features associated with Progression:

- Multiple features of M.S.
- Weight gain
- Persistent elevation of ALT
- Hepatocytes Ballooning
- Mallory bodies

Need for Therapy: General and Liver-specific
Pathophysiology of NAFLD/NASH: Multiple complex pathways

- Patatin-like phospholipase 3 (PNPLA3)
- Fasting-induced adipocyte factor (Fiaf)

Int. J. Mol. Sci. 2013, 14, 20704-20728
Potential Therapeutic Targets for NASH

Steatosis

Insulin resistance + Metabolic dysregulation
Wt gain + Gut Dysbiosis

NASH

FFA + insulin + cytokines

ER stress

Oxidative stress

Mitochondrial injury

Inflammatory signaling

Apoptosis Cell death

Stellate cell activation

Fibrosis
Potential Targets and Therapeutics for NASH according to the pathogenesis

**Metabolic syndrome**
- Insulin resistance
- Dyslipidemia

**DM**
- Pioglitazone
- Metformin
- Pentoxifylline
- Incretin mimetics
- FXR agonist
- Pre/Probiotics
- AdipoRon
- PPAR-α/δ agonist

**Obesity**
- Statins
- Clofibrate
- Gemfibrozil
- Probucol
- Omega-3-FA
- Rimonabant
- Orlistat
- Ezetimibe
- FXR agonist
- PPAR-α/δ agonist

**NAFL**
- Pentoxifylline
- Pioglitazone
- UDCA
- Pre/Probiotics
- FXR agonists
- AdipoRon
- PPAR-α/δ agonist
- ARBs
- Anti-apoptotic agents
- pan-caspase inhibitors
- Anti-fibrotic agents

**Inflammation**
- Oxidative stress
- Apoptosis
- ER stress
- Fibrosis
- Mito dysfunction

**NASH**
- Vitamin E, C
- Pentoxifylline
- UDCA
- 4-phenylbutyric acid
- AdipoRon
- Betaine
- Sylimarin
- L-carnitine
Management of NAFLD/NASH

• No standard treatment specific for NAFLD
• Treating risk factors & complications
• Lifestyle modification, Pharmacological therapy and Bariatric surgery

Schwenger K et al. World J gastroenterol 2014;20:1712-1723.
Current Treatment for NAFLD

• NAFLD/NASH is a lifestyle-associated disease:
  - lifestyle interventions comprising dietary modifications and exercise is a mainstay of treatment.
• Treatment options for non-diabetic patients with NASH:
  - Vitamin E Supplementation (anti-oxidant)
• 2nd-line treatment options for patients with NASH:
  - PPAR-γ agonist pioglitazone (insulin sensitizer)
• Lipid-lowering drugs for NASH patients with dyslipidemia:
  - Statins, Omega-3 PUFA, Ezetimibe
• Others: Pentoxiphylline, UDCA, Anti-obesity drugs
• Bariatric surgery for NASH patients with severe obesity
Lifestyle interventions for the treatment of NAFLD in Adults: A systematic review

- 23 studies included
- Lifestyle modifications leading to weight reduction and/or increased physical activity consistently reduced liver fat and improved glucose control/insulin sensitivity.
- The magnitude of body weight change was reflected in liver fat.
- Exercise only interventions produce a modest but significant effect upon liver lipid, without weight loss.
- Limited data also suggest that lifestyle interventions may hold benefits for histopathology.

# Anti-oxidant: Efficacy of Vitamin E

<table>
<thead>
<tr>
<th>Authors</th>
<th>N</th>
<th>Dose</th>
<th>Comparators</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arendt</td>
<td>80</td>
<td>1000 IU/d</td>
<td>Placebo</td>
<td>Improved*</td>
</tr>
<tr>
<td>Sanyal</td>
<td>247</td>
<td>800 IU/d</td>
<td>Pioglitazone, placebo</td>
<td>Improved†</td>
</tr>
<tr>
<td>Lavine</td>
<td>173</td>
<td>800 IU/d</td>
<td>Metformin, placebo</td>
<td>Improved‡</td>
</tr>
<tr>
<td>Harrison</td>
<td>45</td>
<td>1000 IU/d</td>
<td>Placebo</td>
<td>Improved§</td>
</tr>
<tr>
<td>Sanyal</td>
<td>20</td>
<td>400 IU/d</td>
<td>Vitamin E + pioglitazone</td>
<td>Improved†</td>
</tr>
<tr>
<td>Dufour</td>
<td>48</td>
<td>800 IU/d</td>
<td>UDCA + placebo, placebo</td>
<td>Improved†</td>
</tr>
</tbody>
</table>

*CT scan assessment of steatosis only. †Steatohepatitis and ballooning. ‡All histologic parameters excluding fibrosis. §Fibrosis improvement.

Long-term safety concern: all-cause mortality and prostate cancer

Insulin sensitizer: Role of PPAR-γ Agonists (thiazolidinediones, Glitazones) in NASH

- Systematic review and meta-analysis of thiazolidinedione effects on histologic improvement in NASH
  - 7 randomized trials (n = 489) with histologic outcomes and 4 placebo-controlled trials (n = 355)
- It showed improvements in
  - Fibrosis: RR 1.38, 95% CI 1.01 to 1.89
  - Steatosis: RR 2.03, 95% CI 1.57 to 2.62
  - Inflammation: RR 1.71, 95% CI 1.32 to 2.21
  - Ballooning: RR 1.62, 95% CI 1.15 to 2.28.
- Treatment increased weight by an average of 4.4 kg (CI 2.6–5.2 kg)
- Concerns: fracture, bladder tumor, CV risk (CHF)

Lipid lowering agents

- Statins
- n-3 PUFAs
- Ezetimibe
- Fibrates
- Niacin
Ezetimibe

- Niemann-Pick C1-like protein (NPC1L1) inhibitor
- A sterol transporter: important for the absorption of cholesterol in the enterocytes and hepatocytes.
- Reduce hepatic cholesterol accumulation and decrease lipotoxicity

Clin Liver Dis 18 (2014) 73–89
Efficacy of long-term ezetimibe therapy in patients with NAFLD

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Histological changes in 33 patients with NAFLD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis grade</td>
<td>Baseline</td>
</tr>
<tr>
<td>0</td>
<td>2.3 ± 0.7</td>
</tr>
<tr>
<td>1</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2</td>
<td>5 (16)</td>
</tr>
<tr>
<td>3</td>
<td>14 (44)</td>
</tr>
<tr>
<td>4</td>
<td>14 (41)</td>
</tr>
<tr>
<td>Necroinflammatory grade</td>
<td>Baseline</td>
</tr>
<tr>
<td>0</td>
<td>1.9 ± 0.7</td>
</tr>
<tr>
<td>1</td>
<td>10 (30)</td>
</tr>
<tr>
<td>2</td>
<td>16 (48)</td>
</tr>
<tr>
<td>3</td>
<td>7 (21)</td>
</tr>
<tr>
<td>Fibrosis stage</td>
<td>Baseline</td>
</tr>
<tr>
<td>0</td>
<td>2.0 ± 0.8</td>
</tr>
<tr>
<td>1</td>
<td>1 (3)</td>
</tr>
<tr>
<td>2</td>
<td>6 (18)</td>
</tr>
<tr>
<td>3</td>
<td>17 (52)</td>
</tr>
<tr>
<td>4</td>
<td>9 (27)</td>
</tr>
<tr>
<td>5</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ballooning score</td>
<td>Baseline</td>
</tr>
<tr>
<td>0</td>
<td>1.4 ± 0.5</td>
</tr>
<tr>
<td>1</td>
<td>0 (0)</td>
</tr>
<tr>
<td>2</td>
<td>19 (64)</td>
</tr>
<tr>
<td>3</td>
<td>14 (36)</td>
</tr>
<tr>
<td>NAS score</td>
<td>Baseline</td>
</tr>
<tr>
<td>4</td>
<td>5.5 ± 1.6</td>
</tr>
<tr>
<td>5</td>
<td>0 (0)</td>
</tr>
<tr>
<td>6</td>
<td>19 (64)</td>
</tr>
<tr>
<td>7</td>
<td>14 (36)</td>
</tr>
</tbody>
</table>
New Emerging Candidates for NASH Treatment

- Prebiotics & probiotics
- Incretin mimetics: new anti-diabetic drugs
- PPAR alpha/delta dual agonist (GFT-505)
- Adiponectin receptor agonist
- Anti-apoptotic agents: Caspase inhibitors
- Anti-fibrotic therapy: ARBs and Anti-lysyl oxidase monoclonal antibody
- Farnesoid X receptor agonist: Obeticholic acid
Gut Dysbiosis in NAFLD/NASH

Gut microbiota and Pathophysiology of NAFLD

Nat. Rev. Gastroenterol. Hepatol. 7, 691–701 (2010);
Hepatology 2014;59:328-339
Gut dysbiosis: Potential therapeutic strategies to prevent or treat liver disease (including NASH)
Prebiotics: non-digestible fiber compounds that stimulate the growth and/or activity of advantageous bacteria
Prebiotics: animal model

Prebiotic approach alleviates hepatic steatosis: Implication of fatty acid oxidative and cholesterol synthesis pathways

Scope: Recent data suggest that gut microbiota contributes to the regulation of host lipid metabolism. We report how fermentable dietary fructo-oligosaccharides (FOS) control hepatic steatosis induced by n-3 PUFA depletion, which leads to hepatic alterations similar to those observed in non-alcoholic fatty liver disease patients.

Methods and results: C57Bl/6J mice fed an n-3 PUFA-depleted diet for 3 months were supplemented with FOS during the last 10 days of treatment. FOS-treated mice exhibited higher caecal Bifidobacterium spp. and lower Roseburia spp. content. Microarray analysis of hepatic mRNA revealed that FOS supplementation reduced hepatic triglyceride accumulation through a proliferator-activated receptor α-stimulation of fatty acid oxidation and lessened cholesterol accumulation by inhibiting sterol regulatory element binding protein 2-dependent cholesterol synthesis. Cultured precision-cut liver slices confirmed the inhibition of fatty acid oxidation. FOS effects were related to a decreased hepatic micro-RNA 33 expression and to an increased colonic glucagon-like peptide 1 production.

Conclusions: The changes in gut microbiota composition by n-3 PUFA-depletion and prebiotics modulate hepatic steatosis by changing gene expression in the liver, a phenomenon that could implicate micro-RNA and gut-derived hormones. Our data underline the advantage of targeting the gut microbiota by colonic nutrients in the management of liver disease.

Prebiotics: clinical trial in NASH

Effects of oligofructose on glucose and lipid metabolism in patients with nonalcoholic steatohepatitis: results of a pilot study

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¹Unit of Pharmacokinetics, Metabolism, Nutrition and Toxicology, Université Catholique de Louvain, Brussels, Belgium; ²Unit of Gastroenterology, Cliniques Universitaires St Luc, Université Catholique de Louvain, Brussels, Belgium; and ³Institute of statistics, Université Catholique de Louvain, Louvain-la-Neuve, Belgium

Objective: In experimental animals, recent results suggest that the addition of inulin-type fructans such as oligofructose (OFS) in the diet decreases triacylglycerol accumulation in the liver tissue. Therefore, we have investigated the effect of daily ingestion of OFS in seven patients with nonalcoholic steatohepatitis (NASH), confirmed by liver biopsies.

Design: They received 16 g/day OFS or maltodextrine (placebo) for 8 weeks in a randomized double-blind crossover design. Energy intake, body composition, liver steatosis and blood parameters were analysed after 4 and 8 weeks of dietary supplementation.

Results: Compared to placebo, OFS decreased significantly serum aminotransferases, aspartate aminotransferase after 8 weeks, and insulin level after 4 weeks, but this could not be related to significant effect on plasma lipids.

Conclusion: This pilot study supports the putative interest of OFS in the management of liver diseases associated with abnormal lipid accumulation in humans.

Published online 16 March 2005
Probiotics: “live micro-organisms which confer a health benefit on the host, when administered in adequate amounts”

• **Metabolic effects**
  - Reduce availability of calories from indigestible carbohydrates
  - Enhance insulin sensitivity
  - Modulate intraluminal bile salt metabolism
  - Lower cholesterol
  - Produce conjugated linoleic acid
  - Reduce hepatic fatty acid oxidation

• **Anti-inflammatory effects**
  - Compete with/displace pathogenic strains in intestinal bacterial overgrowth
  - Antibacterial effects mediated by bacteriocins
  - Modify inflammatory pathways induced by overgrown bacteria
  - Ameliorate intestinal barrier function
  - Enhance integrity of the intestinal epithelium
  - Directly inhibit production of proinflammatory mediators
  - Stimulate release of immunoglobulin A

### Probiotics: Studies on NAFLD

<table>
<thead>
<tr>
<th>Studies</th>
<th>Participants/Duration</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal models</td>
<td>Ob/ob mice fed HFD / 4 w</td>
<td>VSL # 3</td>
<td>hepatic FA content, ALT level, activity of Jun N-terminal kinase, NF-κB and fatty acid β-oxidation, improved hepatic IR, and NAFLD histology</td>
</tr>
<tr>
<td>Animal models</td>
<td>mice fed HFD / 4 w</td>
<td>VSL # 3</td>
<td>Ameliorate hepatic NK cell depletion, steatosis, IR and inflammation, cholesterol and TG in the liver and plasma</td>
</tr>
<tr>
<td>Animal models</td>
<td>mice fed HFD / 8 w</td>
<td>Lactobacillus rhamnosus PL60</td>
<td>liver steatosis, improved histological steatosis manifestation</td>
</tr>
<tr>
<td>Animal models</td>
<td>Rats fed HFD &amp; HCD / 6 w</td>
<td>Bacillus polyfermenticus SCD</td>
<td>LDL, cholesterol and triglycerides</td>
</tr>
<tr>
<td>Animal models</td>
<td>Rats fed high-fructose diet / 8 w</td>
<td>Lactobacillus acidophilus and Lactobacillus casei</td>
<td>oxidative stress and ameliorate IR in liver</td>
</tr>
<tr>
<td>Animal models</td>
<td>Rats fed HCD / 5w</td>
<td>Lactobacillus plantarum MA2</td>
<td>cholesterol and triglycerides</td>
</tr>
<tr>
<td>Animal models</td>
<td>Rats fed HFD / 4w</td>
<td>VSL # 3</td>
<td>Improved the hepatic inflammatory, steatotic, peroxidative factors, serum aminotransferase levels</td>
</tr>
<tr>
<td>Animal models</td>
<td>mice fed MCD / 9w</td>
<td>VSL # 3</td>
<td>only improved liver fibrosis without effect on steatosis and inflammation</td>
</tr>
<tr>
<td>Animal models</td>
<td>Rats / 8w</td>
<td>Lactobacillus paracasei F19</td>
<td>hepatic inflammation, steatosis and fibrosis, innate inflammatory cytokines</td>
</tr>
<tr>
<td>Pilot study</td>
<td>10 NASH patients / 2 m</td>
<td>L. acidophilus, L. bulgaricus, B. lactis, B. bifidus, L. plantarum, L. breve, L. casei, L. salivarius, L. rhamnosus vsFOS and vitamin</td>
<td>Improved liver damage and liver function test ALT, AST, and GGT activity Ameliorate MDA and 4-HN plasma level</td>
</tr>
<tr>
<td>Open pilot</td>
<td>22 patients / 3 m</td>
<td>VSL # 3</td>
<td>improved liver damage and liver function test ALT, AST, and GGT activity Ameliorate MDA, and 4-HN plasma level</td>
</tr>
<tr>
<td>OL, pilot trial</td>
<td>4 patients / 4 m</td>
<td>VSL # 3</td>
<td>liver fat After washout time, no effects in blood or clinical parameters</td>
</tr>
<tr>
<td>R, DB, PC</td>
<td>28 patients / 3 m</td>
<td>L. bulgaricus, S. thermophilus</td>
<td>ALT, AST, and GGT activity</td>
</tr>
</tbody>
</table>

Hepat Mon 2012;12(11)
Incretin Mimetics

- GLP-1 (glucagon like peptide 1) agonists: SQ
- DPP-IV (dipeptidyl peptidase 4) inhibitors: oral

- Improve hepatic steatosis by direct effect on liver signaling pathways
- Lead to weight loss and reversal of lipotoxicity & hyperglycemia
Incretin-based therapy in animal models

<table>
<thead>
<tr>
<th>Species</th>
<th>Diet to induce NAFLD</th>
<th>Treatment</th>
<th>Outcomes</th>
<th>Associated with Weight loss?</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLP-1R agonists</td>
<td>Obese Ob/ob mice (leptin deficient)</td>
<td>Ex4 injection</td>
<td>↓ Hepatic lipid</td>
<td>Yes</td>
<td>Ding, 2006</td>
</tr>
<tr>
<td></td>
<td>C57BL/6 mice and Db/Db (leptin receptor deficient)</td>
<td>Helper dependent Adenovirus expressing Ex4 Osmotic pump administration of Ex4</td>
<td>↓ Hepatic TG&lt;br&gt;↓ Hepatic TG content</td>
<td>Yes</td>
<td>Samson, 2008; Samson, 2011</td>
</tr>
<tr>
<td></td>
<td>C57BL/6 mice</td>
<td>Liraglutide injection</td>
<td>↓ Hepatic lipid staining (Oil Red O)</td>
<td>No</td>
<td>Sharma, 2011; Melis, 2012; Tomas, 2011</td>
</tr>
<tr>
<td></td>
<td>C57BL/6 mice</td>
<td>ALIOS</td>
<td>↓ Hepatic lipid</td>
<td>No</td>
<td>Sharma, 2011; Melis, 2012; Tomas, 2011</td>
</tr>
<tr>
<td></td>
<td>Ob/ob (leptin deficient) and C57BL/6 mice</td>
<td>ALIOS with cholesterol (2 wt%) and HTF or HLF (40 kcal% fat)</td>
<td>↓ Hepatic Lipid: ↓ Collagen-I; ↓ ALT, not AST</td>
<td>Yes</td>
<td>Trevaskis, 2012</td>
</tr>
<tr>
<td></td>
<td>C57BL/6</td>
<td>HFD (45 kcal% fat)</td>
<td>↓ Hepatic TG</td>
<td>No</td>
<td>Lee, 2012</td>
</tr>
<tr>
<td>DPP4</td>
<td>DPP4-deficient rats</td>
<td>Chow and HFD (40 kcal% fat) High sucrose (36%) with linoleic or oleic acid</td>
<td>DPP4-deficient rats</td>
<td>No</td>
<td>Ben-Shlomo, 2011</td>
</tr>
<tr>
<td></td>
<td>C57BL/6 mice</td>
<td>N.A. Des-fluoro-sitagliptin</td>
<td>↓ Hepatic TG; ↓ ALT, AST&lt;br&gt;↓ Hepatic TG; ↓ Histologic grade of steatosis (Masson-Goldner staining)</td>
<td>No</td>
<td>Shirakawa, 2011</td>
</tr>
</tbody>
</table>

## Incretin mimetics:
The clinical study on NAFLD/NASH

<table>
<thead>
<tr>
<th>Studies</th>
<th>Anti-diabetic agents (dose/duration)</th>
<th>ALT (U/L)</th>
<th>Histology</th>
<th>BMI or weight (If BMI was not reported)</th>
<th>HbA1c (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yilmaz et al (^{19})</td>
<td>Sitagliptin 100 mg daily for a year</td>
<td>65→40</td>
<td>Improved</td>
<td>Decreased</td>
<td>Change is not statistically significant</td>
</tr>
<tr>
<td>Iwasaki et al (^{20})</td>
<td>Sitagliptin 50 mg daily for 4 months</td>
<td>55.6→35.9</td>
<td>Not evaluated</td>
<td>Change is not statistically significant</td>
<td>8.1→6.8</td>
</tr>
<tr>
<td>Ohki et al (^{21})</td>
<td>Liraglutide 0.3-0.9 mg daily for about 8 months</td>
<td>65→48</td>
<td>Improved(^*)</td>
<td>Decreased</td>
<td>7.7→6.9</td>
</tr>
<tr>
<td></td>
<td>Sitagliptin 50-100 mg daily for about a year</td>
<td>75→61</td>
<td>Change is not statistically significant</td>
<td>8.4→7.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pioglitazone 15 mg daily for about 41 months</td>
<td>87→53</td>
<td>Improved(^#)</td>
<td>Increased</td>
<td>7.7→6.9</td>
</tr>
<tr>
<td>Kenny et al (^{22})</td>
<td>Exenatide 5-10 μg twice a day for 7 months</td>
<td>69→45</td>
<td>Improved in 50% of series</td>
<td>Decreased</td>
<td>7.1→6.1</td>
</tr>
</tbody>
</table>

\(^*\): NAFLD activity score is a tool to measure changes in NAFLD during therapeutic trials. The scoring system comprises 14 histological features, 4 of which were measured semi-quantitatively: steatosis (0-3), lobular inflammation (0-2), hepatocyte ballooning (0-2), and fibrosis (0-4). Another nine features were recorded as present or absent. NAFLD score and NASH score are synonyms that were used interchangeably in this review based on how it was listed in the original papers. 

\(^#\): AST to platelet counts ratio index (APRI) was used in this study as fibrosis/cirrhosis assessment tool instead of biopsy.
The efficacy of dual PPAR-α/δ agonist GFT505 was assessed in animal models of NAFLD/NASH and liver fibrosis (Western diet [WD]-fed human apolipoprotein E2 [hApoE2] transgenic mice, methionine- and choline-deficient diet-fed db/db mice, and CCl4-induced fibrosis in rats. GFT505 demonstrated hepatoprotective effects on steatosis, inflammation, and fibrosis. In addition, GFT505 improved liver dysfunction markers, decreased hepatic lipid accumulation, and inhibited proinflammatory and profibrotic gene expression. The dual PPAR-α/δ agonist, GFT505, is a promising liver-targeted drug for treatment of NAFLD/NASH.
Adiponectin: Synthetic adiponectin receptor agonist

- Insulin-sensitizing, anti-lipotoxic, and anti-inflammatory adipocytokine
- Antidiabetic effects via activation of AMPK and PPAR-α pathways

*Adiponectin Receptor*

Low adiponectin → Inflammation

AdipoRon: Orally active small molecule AdipoR agonists

Impact of pan-caspase inhibition in animal models of established steatosis and non-alcoholic steatohepatitis

Background & Aims: Non-alcoholic fatty liver disease is a progressive condition comprising steatosis, steatohepatitis, and cirrhosis. Caspase activation mediates apoptosis and the inflammatory response. Studies demonstrate increased apoptotic activity in NASH although its pathophysiological importance is uncertain. We sought to determine the effects of irreversible pan-caspase inhibition in murine models of established steatosis (high fat diet, HFD) and steatohepatitis (methionine-choline deficient diet, MCD).

Methods: In one study arm, male C3H/HeN mice were fed HFD; in the other, Db/Db mice were fed MCD. Once disease was established, animals were randomised to receive caspase inhibitor (VX-166), TPGS/PEG vehicle or no additional therapy until the end of the study. Biochemical and histological indices were examined to determine NASH activity and tissue oxidative stress. Apoptotic activity and cell turnover were assessed immunohistochemically by staining for caspase-cleaved CK-18 and PCNA.

Results: MCD and HFD significantly increased apoptosis, which was reduced by VX-166 treatment. VX-166 did not reduce steatosis but reduced histological inflammation, serum ALT levels, and oxidative stress, particularly in the MCD model. TPGS/PEG vehicle also exhibited some anti-inflammatory activity.

Conclusions: In both models, VX-166 inhibited apoptosis and reduced histological inflammatory infiltrate although there was a more modest impact on other indices of liver injury. In addition, TPGS/PEG vehicle also exhibited some anti-inflammatory activity, likely through the antioxidant effects of vitamin E and changes in gut flora/mucosal interactions. These data suggest that caspase inhibition may represent a valid therapeutic approach; however, further studies to assess the long-term value of more selective caspase inhibition are merited.

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Liver fibrogenesis is attenuated under pan-caspase inhibitor (Emricasan, IDN-6556) treatment in HFD-fed mice

A Phase 2, Randomized, Double-Blind, Placebo-Controlled Study of GS-9450 in Subjects With Nonalcoholic Steatohepatitis

In nonalcoholic steatohepatitis (NASH), the extent of hepatocyte apoptosis correlates with disease severity. Reducing hepatocyte apoptosis with the selective caspase inhibitor GS-9450 has a potential for altering the course of the liver disease. In this phase 2, double-blind study, 124 subjects with biopsy-proven NASH were randomized to once-daily placebo or 1, 5, 10, or 40 mg GS-9450 for 4 weeks. Absolute and percent changes from baseline in ALT levels, AST levels, and caspase-3–cleaved cytokeratin (CK)-18 fragments at week 4 were assessed by an analysis of covariance model with adjustment for baseline values. In the 40-mg group, mean (SD) ALT decreased by 47 (43) U/L from baseline to week 4 (P < 0.0001 versus placebo), and the proportion of subjects with normal ALT increased from 0% to 35% at week 4. In the 40-mg group, mean AST decreased by 13 U/L from baseline (not significant), and the proportion with normal AST increased from 20% at baseline to 48% at week 4. By week 4, mean CK-18 fragment levels had decreased to 393 (723) U/L in the GS-9450 10-mg group and 125 (212) U/L in the 40-mg group, but these reductions were not statistically significant. No serious adverse events were reported during treatment, and the percentage of subjects with at least one treatment-emergent grade 3 or 4 laboratory abnormality ranged from 11.5% to 17% across the GS-9450 treatment groups versus 35% in the placebo group. Conclusion: GS-9450 treatment induced significant reductions in ALT levels in NASH patients. Reductions in CK-18 fragment levels also occurred, although they were not statistically significant. At appropriate therapeutic indices, selective caspase inhibitors may be a promising treatment option in patients with NASH. (Hepatology 2012;55:419-428)
Angiotensin-receptor blockers (ARBs) in hypertension-associated NASH

- Angiotensin-II stimulates contraction and proliferation of the activated hepatic stellate cells and increases TGF-b expression through angiotensin type-I receptors.
- Telmisartan increases adiponectin level through PPAR-γ activation.
  (Current Vascular Pharmacology, 2011, 9, 158-161)

- Clinical trial
- 54 pts with NASH and HTN; Telmisartan vs Valsartan for 2 years
- Significant improvement in HOMA-IR & NAS, fibrosis in telmisartan group

Direct inhibition or reversal of fibrosis by Lysyl oxidase homolog 2 inhibitory monoclonal antibody

Lysyl oxidase is an extracellular copper enzyme that catalyzes formation of aldehydes from lysine residues in collagen and elastin precursors. It is essential for cross-linking collagen and elastin.

*Expert Opin. Drug Discov.* (2014) 9(6)
FXR: a nuclear receptor that suppresses cholesterol 7 alpha-hydroxylase (CYP7A1), the rate-limiting enzyme in bile acid synthesis from cholesterol.
Major roles of FXR in regulating lipid and carbohydrate metabolism and inflammatory responses

G-protein-coupled BA receptor 1 (GPBAR1, or TGR5)

Biochemical Pharmacology 86 (2013) 1517–1524
FXR & NASH in mice

FXR Agonist: Obeticholic Acid

- A semisynthetic derivative of CDCA
- A potent and selective FXR agonist endowed with anti-cholestatic activity

**Obeticholic Acid (INT-747)**
6a-ethyl chenodeoxycholic acid

**CDCA**
Chenodeoxycholic acid

\[
\text{FXR EC}_{50} (\text{agonist}) \quad 0.099 \text{ mM} \\
8.66 \text{ mM}
\]

\(~2 \log \uparrow \text{FXR agonism}\)

J Med Chem. 2002;45:3569-3572. Copyright 2013, American Chemical Society
a double-blind, placebo-controlled, proof-of-concept study to evaluate the effects of OCA on insulin sensitivity in patients with NAFLD and type 2 DM. Patients were randomly assigned to groups given placebo (n ¼ 23), 25 mg OCA (n ¼ 20), or 50 mg OCA (n ¼ 21) once daily for 6 weeks.

Table 5. Enhanced Liver Fibrosis Markers

<table>
<thead>
<tr>
<th>ELF Component/Treatment Group</th>
<th>Mean (± SD)</th>
<th>Mean Change (± SD)</th>
<th>P valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 43</td>
<td>(Day 43 - Day 0)</td>
</tr>
<tr>
<td><strong>ELF score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 23)</td>
<td>8.2 ± 1.2</td>
<td>8.5 ± 1.2</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td>OCA 25 mg (n = 20)</td>
<td>8.4 ± 0.9</td>
<td>8.2 ± 0.9</td>
<td>-0.2 ± 0.4</td>
</tr>
<tr>
<td>OCA 50 mg (n = 20)</td>
<td>8.0 ± 1.0</td>
<td>8.1 ± 1.0</td>
<td>0.03 ± 0.8</td>
</tr>
<tr>
<td><strong>Hyaluronic acid (ng/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 23)</td>
<td>47.5 ± 94.2</td>
<td>54.1 ± 92.9</td>
<td>6.7 ± 15.4</td>
</tr>
<tr>
<td>OCA 25 mg (n = 20)</td>
<td>33.6 ± 40.8</td>
<td>30.8 ± 35.9</td>
<td>-2.9 ± 14.5</td>
</tr>
<tr>
<td>OCA 50 mg (n = 20)</td>
<td>31.0 ± 38.0</td>
<td>25.2 ± 23.7</td>
<td>-5.8 ± 31.4</td>
</tr>
<tr>
<td><strong>Procolagen 3 amino-terminal peptide (ng/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 23)</td>
<td>5.5 ± 2.7</td>
<td>6.0 ± 2.3</td>
<td>0.5 ± 1.3</td>
</tr>
<tr>
<td>OCA 25 mg (n = 20)</td>
<td>6.6 ± 2.5</td>
<td>6.1 ± 2.2</td>
<td>-0.5 ± 1.2</td>
</tr>
<tr>
<td>OCA 50 mg (n = 20)</td>
<td>5.6 ± 3.1</td>
<td>6.2 ± 3.6</td>
<td>0.6 ± 3.5</td>
</tr>
<tr>
<td><strong>Tissue inhibitor of metalloproteinase 1 (ng/mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 23)</td>
<td>609.1 ± 151.2</td>
<td>655.4 ± 156.6</td>
<td>46.3 ± 105.8</td>
</tr>
<tr>
<td>OCA 25 mg (n = 20)</td>
<td>649.9 ± 127.0</td>
<td>638.5 ± 101.2</td>
<td>-11.4 ± 57.5</td>
</tr>
<tr>
<td>OCA 50 mg (n = 20)</td>
<td>591.5 ± 101.5</td>
<td>627.3 ± 187.1</td>
<td>35.8 ± 158.9</td>
</tr>
</tbody>
</table>

aMean differences were tested using the t test for independent samples.
FXR is a molecular target for the effects of vertical sleeve gastrectomy

Karen K. Ryan¹, Valentina Tremaroli², Christoffer Clemmensen¹³, Petia Kovatcheva-Datchary², Andriy Myronovych⁴, Rebekah Karns⁵, Hilary E. Wilson-Pérez¹, Darleen A. Sandoval¹, Rohit Kohli⁴, Fredrik Bäckhed²⁶ & Randy J. Seeley¹

Bariatric surgical procedures, such as vertical sleeve gastrectomy (VSG), are at present the most effective therapy for the treatment of obesity, and are associated with considerable improvements in co-morbidities, including type-2 diabetes mellitus. The underlying molecular mechanisms contributing to these benefits remain largely undetermined, despite offering the potential to reveal new targets for therapeutic intervention. Substantial changes in circulating total bile acids are known to occur after VSG. Moreover, bile acids are known to regulate metabolism by binding to the nuclear receptor FXR (farnesoid-X receptor, also known as NR1H4). We therefore examined the results of VSG surgery applied to mice with diet-induced obesity and targeted genetic disruption of FXR. Here we demonstrate that the therapeutic value of VSG does not result from mechanical restriction imposed by a smaller stomach. Rather, VSG is associated with increased circulating bile acids, and associated changes to gut microbial communities. Moreover, in the absence of FXR, the ability of VSG to reduce body weight and improve glucose tolerance is substantially reduced. These results point to bile acids and FXR signalling as an important molecular underpinning for the beneficial effects of this weight-loss surgery.
Summary

• **Lifestyle modification** remains the mainstay of therapy for NASH.
• Drug therapy should be considered in those with NASH with activity and some fibrosis, especially in those with multiple features of metabolic syndrome and persistently elevated ALT.
• **Vitamin E** supplement in non-diabetics improves NASH with no serious adverse events in clinical trials; its long-term safety issue remains unsettled.
• **Pioglitazone** has beneficial effects on NASH but adverse effects limit its wide use.
• **Lipid lowering agents and ARBs** should be considered in NASH patients with dyslipidemia and hypertension, respectively.
• Several new **emerging agents** under study appear to be effective.
• **Combination therapy with different mode of actions** can be tried in the future.
Thank you for your attention.