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NOVEL THERAPEUTIC APPROACH IN NONALCOHOLIC FATTY LIVER DISEASE

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Rezumat

Boala ficatului gras nealcoolic: noi abordări terapeutice

Boala ficatului gras nealcoolic (BFGNA) este cea mai răspândită maladie hepatică în lumea occidentală, cu o prevalenţă de 20-33% în populaţia generală. Epidemiologia globală a BFGNA devine tot mai cunoscută, însă o farmacoterapie precisă nu a fost încă stabilită. Tratamentul cu statine este adeseori evitat la bolnavii cu o BFGNA, iar medicii sunt îngrijoraţi de prescrierea statinelor la pacienţii cu valoarea crescută inexplicabilă a enzimelor hepatice sau cu o maladie hepatică activă. Monocolinele acţionează ca inhibitori reversibili ai reductazei 3-hidroxi-3-metil-glutaril-coenzimă A, reduc concentraţiile de colesterol şi sunt capabile să scadă nivelul de lipoproteine cu densitate mică. Noi abordări în terapia BFGNA ce vizează metabolismul colesterolului pot deveni utile pentru a reduce grăsimea hepatică, astfel diminuând leziunile hepatice în aceste maladii.

Cuvinte-cheie: *boala ficatului gras nealcoolic, dislipidemie, statine, monocoline*

Резюме

Новый терапевтический подход при неалкогольной жирной болезни печени

Безалкогольная жирная болезнь печени (БАЖБП) является наиболее распространенным заболеванием печени в западных странах (до 20-33% из общей популяции). Несмотря на растущее понимание глобальной эпидемии БАЖБП, до сих пор нет определенной фармакотерапии для этого заболевания. Очень часто статины не используются у пациентов с БАЖБП и многие врачи обеспокоены назначением статинов пациентам с необъяснимым постоянным повышением ферментов печени или активным заболеванием печени. Моноколины действуют как обратимые ингибиторы 3-гидрокси-3-метил-глутарил-кофермент-редуктазы, снижают концентрацию холестерина и способны уменьшать уровень липопротеинов низкой плотности. Новые подходы в терапии БАЖБП, влияющие на метаболизм холестерина, могут стать полезными для снижения уровня жировой инфильтрации в печени, что уменьшает повреждение печени при этой патологии.

Ключевые слова: *неалкогольная жирная болезнь печени, дислипидемия, статины, моноколин*

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in the western world with prevalence of 20–33% in general population, encompasses a spectrum of hepatic histopathological changes ranging from non-inflammatory intracellular fat deposition to non-alcoholic steatohepatitis (NASH), which may progress into hepatic fibrosis, cirrhosis, or hepatocellular carcinoma. NAFLD is associated with metabolic syndrome, type 2 diabetes mellitus and increased cardiovascular disease (CVD) which contribute to the morbidity and mortality besides that due to hepatic disease.

Despite growing understanding of the global epidemic of NAFLD, there is no definite pharmacotherapy available. Most available data on pharmacologic interventions consists of small nonrandomized studies, which lack a control group and histological evaluation. This review article will evaluate the current evidence on pharmacological and nonpharmacological therapeutic options of NAFLD, and highlight administration of statins and natural statins (monocoline) effect in patient with NAFLD.

I. *Non pharmacological treatment*

I.1. Lifestyle Intervention. Obesity and metabolic syndrome are the most important risk factors for NAFLD [6]. Excessive food intake,

particularly western diet that includes complex carbohydrates and saturated fats along with sedentary lifestyle, has increased the prevalence of obesity and metabolic syndrome leading to NAFLD. Therefore, lifestyle intervention targeting weight loss has been of great interest in terms of being a therapeutic option for NAFLD. Nevertheless, adjusting such changes to one's lifestyle and achieving the target weight reduction are difficult and many factors may play a role. Furthermore, weight loss of at least 7% is required for histological improvement, and greatest improvement achieved with >10% weight loss [10]; however, only 50% of patients are able to achieve that goal.

I.2. Dietary Modification. It is important to recognize the role of nutritional imbalance between saturated and unsaturated fatty acids, as well as carbohydrates and proteins, in development and progression of NAFLD. Studies show that dietary deficiency of polyunsaturated fatty acids (PUFA) can lead to NAFLD and patients with NASH seem to have overall decreased intake of PUFA and increased intake of saturated fat, and trans fatty acids. Furthermore, fructose is another common ingredient used as sweetener in many food products and soft drinks, which is found to have a strong association with development of metabolic risk factors and 2-3-fold increased risk of developing liver steatosis.

I.3. Dietary Modification Plus Physical Activity. Dietary modifications such as calorie restriction as well as carbohydrate and fat restriction in combination with exercise have been assessed in multiple studies and combination strategy has shown better results. In a prospective study involving 261 patients with biopsy proven NASH, lifestyle modification with hypocaloric diet and moderate intensity exercise show that weight loss of >5% was associated with reduction of NASH and 90% of the participants with >10% weight loss had resolution of NASH [10].

II. Bariatric surgery

Weight loss through bariatric surgery has been proposed as a possible treatment option for nonalcoholic fatty liver disease because of its positive effect on liver histology. Nevertheless, rapid sudden weight loss such as a result of bariatric surgery may lead to progression of liver failure in some NAFLD patients. A French study looked at the metabolic markers and histology before bariatric surgery and 1 year and 5 years after the surgery in 381 patients with severe obesity [7]. This study showed an improvement in steatosis, ballooning, and overall NASH with significant reduction in percentage of NASH patients at 5 years compared to before surgery (27,4% to 14,2%).

III. Pharmacological treatment

III.1. Weight Loss Medication. Orlistat and Sibutramine have been studied as a treatment option for nonalcoholic fatty liver disease, though without much convincing results. Orlistat is a pancreatic lipase inhibitor that reduces absorption of free fatty acids. Sibutramine is a centrally acting serotonin-norepinephrine reuptake inhibitor, which enhances satiety. In a randomized prospective trial, 50 overweight patients with biopsy proven NASH were assigned to either 1400 kcal/day diet plus vitamin E 800 IU alone or with combination of orlistat for 9 months [4]. Both groups showed reduction in steatosis, necroinflammation, ballooning, and NAS in addition to reduction of weight and improvement in aminotransferases.

III.2. Antidiabetic Medications

III.2.1. Thiazolidinediones (TZDs) are peroxisomal proliferator activated receptor (PPAR-) gamma agonists that promote hepatic fatty acid oxidation, which increases hepatic lipogenesis and insulin sensitivity. Most studies have shown improved biochemical efficacy with TZDs in NAFLD patients and some of them resulted in histological improvements such as steatosis and inflammation.

III.2.2. Metformin improves insulin resistance by reducing hepatic gluconeogenesis and fatty acid oxidation, increasing peripheral and hepatic insulin sensitivity, decreasing intestinal glucose absorption, and lowering serum lipid concentration. Recent animal studies have also suggested a possible role of metformin in prevention of hepatocellular carcinoma. Although, some of the studies showed improvement in metabolic markers, aminotransferases, and liver histology, most of the benefits may be due to metformin's known effect of causing improvement in insulin sensitivity, weight loss, and other markers of metabolic syndrome [5].

III.2.3. Other Antidiabetic Agents Studies have been done with meglitinides and incretin mimetics (GLP-1 analogs) to assess their effect on fatty liver. Meglitinide stimulates pancreatic insulin release and pancreatic beta cell growth. The data on use of meglitinide as treatment for NAFLD/NASH is scarce. Although some of the

pilot studies involving these agents have shown improvement in metabolic biomarkers and histology, these studies involved very small sample sizes.

III.3. Antioxidants

III.3.1. Vitamin E has been shown to improve aminotransferases as well as histological markers in subjects with NASH. Some studies have also shown complete resolution of steatohepatitis, but there are mixed results on its effect on liver fibrosis. As described above in the pioglitazone section, the PIVENS trial, a randomized, multicenter, double blind, placebo-controlled trial [9]. The effect of vitamin E on NAFLD has also been evaluated in many combination therapy studies. In a prospective, double-blind, randomized, placebo-controlled trial, 45 patients with biopsy proven NASH were enrolled to receive either vitamin E 1000 IU plus vitamin C 1000mg or placebo for 6 months [3]. At the end of treatment, the vitamin E + C group had statistically significant improvement in fibrosis but no improvement in necroinflammation. Similar results were seen in a small study evaluating combination of vitamin E and pioglitazone compared to vitamin E alone.

III.3.2. Other Antioxidants. Changes in methionine/folate metabolism may contribute to the development of steatosis. S-adenosyl methionine (SAM) and betaine are nutritional supplements that have anti-TNF alpha, cytoprotective, antiapoptotic, and antisteatogenic activity and can cause reversal of insulin resistance. N-acetyl cysteine (NAC) is a glutathione precursor, which increases glutathione in hepatocytes and limits the reactive oxygen species that causes hepatocellular injury.

III.4. Anti-Inflammatory Agents. Pentoxifylline is a xanthine derivative that inhibits TNF alpha, which is a pro inflammatory cytokine that has been shown to activate reactive oxygen species by lipid peroxidation and promote necroinflammation, fibrogenesis, hepatic insulin resistance, and apoptosis. In a randomized placebo-controlled trial, 49 patients with biopsy proven NASH were randomized to pentoxifylline versus placebo for 1 year. All patients in the treatment group had either improved NASH or no change. Compared to placebo, pentoxifylline group had significant improvement in Alanine aminotransferase (ALT), steatosis, inflammation, and fibrosis, but no change in ballooning. A decrease in NASH of ≥2 was seen in 50% of patients in pentoxifylline group versus 15,4% in placebo group. Additionally, 25% of NASH patients in the pentoxifylline group had resolution of NASH at the end of treatment.

III.5. Probiotics. Bacterial overgrowth in the bowel is present in 50% of patients with NASH and changes in the intestinal bacterial content may be related to the pathogenesis of NASH due to enhanced intestinal permeability, activation of inflammatory cytokines, and absorption of endotoxins. Therefore, probiotics have been suggested as a treatment option in NASH patients. However, some studies have shown worsening of steatosis with the use of probiotics.

III.6. Cytoprotective and Antiapoptotic Agents. Ursodeoxycholic acid (UDCA) has a beneficial effect on hepatobiliary diseases by its cytoprotective, immunomodulatory, and antiapoptotic effects. The efficacy results of UDCA on NAFLD/NASH have been mixed, with some trials showing improvement in ALT and other histological markers such as steatosis, inflammation, and fibrosis.

III.7. Other Therapeutic Agents

III.7.1. Inhibitors of RAAS (Renin-Angiotensin-Aldosterone System). Emerging evidence, mainly in animal studies, has shown that inhibiting RAAS pathway decreases hepatic stellate cell activity, which in turn prevents fibrosis and that ACE inhibitors and angiotensin receptor blockers may be useful in treating NAFL/NASH as they may lead to decreased fibrosis.

III.7.2. Coffee is rich in sources of bioactive phytochemicals including methylxanthines (caffeine), amino acids, phenolic acids, and polyphenols, which may protect against liver disease. A study involved 195 patients participating in a questionnaire about coffee and espresso as well as other caffeinated drinks and chocolate. All patients underwent liver biopsies and compared to espresso, regular coffee consumption was associated with decreased odds ratio of liver fibrosis.

III.7.3. Vitamin D. There is not enough data assessing the effect of vitamin D supplementation on NAFLD, even though studies have shown vitamin D deficiency is associated with development of NAFLD. A study of 60 patients with biopsy proven NAFLD and 60 healthy controls shows that, compared to controls, NAFLD patients had a significant decrease in vitamin D levels and that levels of vitamin D also negatively correlated with histological severity of steatosis, necroinflammation, and fibrosis independent of other variables or presence of metabolic syndrome.

III.7.4. Phlebotomy. Although there has been a significant recent interest in the role of iron in

NAFLD, the results have been conflicting. A recent study showed that patients who were suspected to have NAFLD had higher body iron and a greater hemoglobin level.

III.8. Antilipidemic Drugs

III.8.1 Statins. Statins is a class of drugs that lowers the level of cholesterol in the blood by reducing the production of cholesterol by the liver. (The other source of cholesterol in the blood is dietary cholesterol.) Statins block the hydroxymethylglutaryl-coenzyme A reductase (HMG-CoA reductase), enzyme in the liver that is responsible for making cholesterol.

Statins are often underused in patients with non-alcoholic fatty liver disease and many physicians are concerned about the prescription of statins to patients with unexplained persistent elevation of liver enzymes or active liver disease. Based on currently available data, statin therapy, at low-to-moderate doses, seems to be safe and has low liver toxicity. Treatment of dyslipidaemia in patients with NAFLD is recommended and may also improve liver function tests. In these patients, the risks of not taking statins could outweigh the risks of taking the drug. Conversely, the usefulness of statins for the treatment of NAFLD /non-alcoholic steatohepatitis is still a matter of debate and randomized clinical trials of adequate size and duration are required [8].

Recently, the 2013 guidelines by the American College of Cardiology (ACC) and the American Heart Association (AHA) for the treatment of cholesterol expanded the indications for statin therapy for the prevention of cardiovascular disease in patient with NAFLD. Long-term use of statins results in important reductions in the risk of experiencing major coronary and vascular events in patients with a wide range of lipid levels, both in primary and secondary prevention.

Long-term statin treatment and liver toxicity

A. Liver toxicity of statins. Relevant statin-related liver toxicity is a rare but important adverse event occurring during statin treatment In fact, while asymptomatic elevations in serum ALT are relatively common in patients treated with statins, severe hepatic toxicity has been rarely described. During statin treatment, an asymptomatic elevation in ALT should not be considered a sign of ongoing liver disease or injury.

Nevertheless, previous studies reported that statin use might induce an autoimmune hepatitis. In particular, some of a research described three cases of autoimmune hepatitis after treatment with fluvastatin – in two cases and atorvastatin – in one.

B. Safety and efficacy of long-term statin treatment in patients with abnormal liver tests. The issue of possible liver-related adverse effects of statin treatment in patients with coronary heart disease and liver enzyme elevation was addressed in a post hoc analysis of the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) Study. The frequency of liver-related adverse effects was low (1.1%) and did not differ from rates reported in patients not treated with statins (0.4%; $p = 0.2$). Additionally, 227 patients with abnormal rises in Aspartate aminotransferase (AST) or ALT concentrations of up to three times the upper limit of normal at entry in the trial, who were given a statin had a substantial improvement in liver tests during 3-year follow-up, whereas 210 not treated had a further increase of transaminases.

In conclusion, statins seem to be effective and safe for the treatment of hypercholesterolemia and/or atherogenic dyslipidaemiain patients with elevated serum liver enzymes, without inducing a further elevation of liver enzymes in treated patients.

C. Safety and efficacy of statins for the treatment of dyslipidaemia in patients with non-alcoholic fatty liver disease. Hyperlipidaemia is frequently associated with NAFLD. Most patients with moderately elevated ALT levels have "atherogenic dyslipidaemia", which is characterized by increased serum triglycerides, low high-density lipoprotein (HDL) cholesterol and the presence of small, dense Low-density lipoprotein (LDL) particles, a common finding also in insulin resistance and metabolic syndrome. "Atherogenic dyslipidaemia" is frequently associated with other features of metabolic syndrome such as obesity, diabetes mellitus, and hypertension. Aggressive treatment of dyslipidaemia plays a critical role in the overall management of patients with NAFLD.

However, there is concern that patients with NAFLD or NASH and hyperlipidaemia who are treated with statins could develop serum ALT elevation or a further increase of already elevated enzymes. Therefore, in clinical practice, management of dyslipidaemia in patients with NAFLD has been often a matter of concern and under-treatment with statin therapy because of potential liver damage. Safety of statin treatment of dyslipidaemia in patients with NAFLD has been addressed in numerous studies [1].

D. Safety and efficacy of statins for the treatment of NAFLD and NASH. Statins have anti-

inflammatory, anti-oxidant and anti-thrombotic effects that are independent of their lipid-low ring activity. Therefore, they have been proposed for the treatment of NAFLD and NASH, since in these conditions both inflammation and oxidative stress play an important pathogenetic role.

III.8.2 Dietary supplemens which reduce cholesterolemia

A relatively large number of dietary supplements and nutraceuticals have been studied for their supposed or demonstrated ability to reduce cholesterolemia in humans. These supplements include soluble fibers, phytosterols, soy proteins, ω-3 polyunsaturated fatty acids, monacolins, policosanols, berberine and garlic extracts.

Monocolins act as reversible inhibitors of the 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme in cholesterol biosynthesis. The main source of monocolins are Chinese red yeast rice (dietary supplement), made by fermenting the yeast, *Monascus purpureus*, over rice. In addition to the inhibition of HMG-CoA reductase, red yeast rice has been found to contain sterols (β-sitosterol, campesterol, stigmasterol, and sapogenin), isoflavones and isoflavone glycosides, and monounsaturated fatty acids [2], all capable of lowering low-density lipoprotein cholesterol (LDL-C).

Several trials conducted in the People's Republic of China showed that consumption of red yeast rice reduced cholesterol concentrations by 11–32% and triacylglycerol concentrations by 12–19%, both in animal and human models. These positive effects were also confirmed in an American population after 12 weeks of treatment and in a Norwegian population after 16 weeks of treatment, in two randomized, double-blind, placebo-controlled trials .

Summary, conclusion and perspective

Due to the complex multi-factorial nature of the disease, combined treatment may be needed to achieve better results. Targeting cholesterol accumulation represents a potentially useful therapeutic approach in NAFLD. In patients with NAFLD, inhibition of cholesterol synthesis by statins alone or in combination with antioxidants was shown beneficial.

Unloading the liver from an excess of cholesterol in the clinical setting would likely require a combination of dietary and pharmacological interventions that need to be designed and validated. Currently used drugs to treat hyperlipidemia may be of benefit in NAFLD/NASH but this specific effect warrants evaluation in large clinical trials. Also, new approaches targeting nuclear receptors or cholesterol metabolism pathways may become useful to reduce hepatic fatty thus reducing liver injury in NAFLD/NASH.

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