ГЕПАТОПРОТЕКТОР РОПРЕН ДЛЯ ЛЕЧЕНИЯ БОЛЬНЫХ С НЕАЛКОГОЛЬНЫМ СТЕАТОГЕПАТИТОМ: ОБСЕРВАЦИОННОЕ ПРОГРЕССИВНОЕ ИССЛЕДОВАНИЕ†

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THE HEPATOPROTECTOR ROPREN FOR THE TREATMENT OF PATIENTS WITH NONALCOHOLIC STEATOHEPATITIS: A PROSPECTIVE OBSERVATIONAL STUDY†

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Summary

Objectives. New and effective drugs to improve liver function and fibrosis are urgently needed for patients with nonalcoholic steatohepatitis (NASH). This study examines the effectiveness of treatment of patients with NASH with the registered hepatoprotector, Ropren®. **Methods**. This observational study used Ropren® in a post-registration setting to treat 20 females (38–56 years) with chronic NASH unresponsive to standard treatment. Ropren® is a biopolymer made up of polyprenols (analogue of dolichol) isolated from the green verdure of spruce (Picea abies (L) Karst). Ropren® was given orally, three times a day (54 mg/day). Measurements before and after treatment included symptoms and blood biochemistry (triglycerides, high-density lipoproteins, low-density lipoproteins, alanine transaminase, aspartate transaminase, alkaline phosphatase and gamma-glutamyl-transpeptidase). Liver fibrosis was measured with indirect ultrasound elastometry. **Results**. After 12 weeks of Ropren® treatment, improvements were found for blood lipids and clinical and biochemical signs, including reductions in total cholesterol and triglycerides ($p < 0.05$). Ropren® also significantly decreased the liver fibrosis index ($p < 0.05$). No side effects were observed. **Conclusions**. Ropren® treatment increased the elasticity of the liver and might be useful to reduce the risk of cirrhosis. Although the sample size in this study was small, the results demonstrated that a randomised-controlled trial of Ropren® for NASH is warranted.

Keywords: non-alcoholic steatohepatitis, NASH, lipid, polyprenols, dolichol, hepatoprotectors, Bioeffective, Ropren®.

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Worldwide epidemics of type 2 diabetes and obesity have resulted in an increase in the incidence of non-alcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). Of particular concern is that NAFLD is the most common cause of liver disease in children because of the increase in childhood diabetes and obesity [1]. Given the ageing population and the increase in obesity, NAFLD and NASH are public health problems that are expected to worsen in the future.

NAFLD has the potential to progress to non-alcoholic steatohepatitis (NASH), with and without liver cirrhosis, and liver hepatocellular carcinoma [2]. While risk factors for this condition include obesity and type 2 diabetes, the presence of NAFLD and NASH also increase the risk of patients developing metabolic syndrome and fibrosis/cirrhosis of the liver [3, 4] and NAFLD is considered both a consequence and cause of insulin resistance [5].

NAFLD also covers a spectrum of liver conditions where fatty liver is benign, especially if fibrosis of the liver and inflammation are not present. The progression of NAFLD is in the following stages: steatosis, steatohepatitis, fibrosis and cirrhosis of the liver. Liver

steatosis is characterised by the accumulation of fat, mainly triglycerides, in hepatocytes and stellate cells. There is also an increased release of free fatty acids (FFA) into the liver due to: 1) the high content of fatty acids in food; 2) the release of fatty acids during lipolysis of the adipose tissue; and 3) the transformation of excess carbohydrates into fatty acids by the liver. The accumulation of fat droplets in the hepatocytes leads to the compression of cell organelles, along with damage, stretching and rupture of liver cell membranes.

The next phase of disease progression is the formation of steatohepatitis. Continued insulin resistance and oxidative stress can cause NALFD to progress into NASH, with increased fibrosis of the liver and the potential to develop cirrhosis (and complications such as portal hypertension and liver failure) and hepatocellular carcinoma. Liver fibrosis is considered a predictor of mortality and more severe disease [6].

The incidence of NAFLD varies depending on ethnicity, but a long-term study of over 11,000 people in the USA found that 34% had NAFLD, with 28.3% of those having fibrosis scores indicating an intermediate level of fibrosis and 3.2% having a score indicating severe fibrosis [7].

An important part of the treatment of NASH is the management of metabolic syndrome with lifestyle management. This begins with improving the patient's diet and is followed by the gradual reduction of body mass and an increase in exercise to adequate levels. Beyond that, the use of medications to increase insulin sensitivity and decrease oxidative stress can also be considered. Metformin (a biguanide) and second-generation insulin sensitisers such as pioglitazone are also in wide use. Treatment with metformin or simvastatin for three months was associated with fewer deaths [6].

A cautious approach is necessary when choosing lipid-lowering medications, as combining the arsenal of medications available to practitioners (statins, fibrates) may potentially provoke the development of drug-induced hepatitis in NASH patients [8, 9]. Furthermore, treatment of paediatric NAFLD also requires careful consideration [1], with a preference for less toxic approaches.

At this stage, treatment for NAFLD and especially NASH is not standardised and clinical trials of treatments are needed. Treatment is even more critical for NASH and the need for medications that might effectively decrease liver fibrosis is especially urgent because there has been a specific lack of translational studies that take pre-clinical results into humans [10].

The aim of hepatoprotective therapy in NASH is the reduction of free fatty acid accumulation in the liver (including omega-3 and omega-6 fatty acids), repair of hepatocyte membrane damage, protection of cell organelles, reduction of oxidative stress, and the reduction of fibrosis. Therefore, pharmaceuticals for treating NASH require direct cytoprotective activity to normalise the structure and function of liver cell membranes and intracellular organelles and to promote the normalisation of lipid metabolism.

At the present time, various hepatoprotectors are used for treatment of NASH: essential phospholipids, S-adenosyl-L-methionine, Saint-Mary-Thistle (Silybum marianum) flavonoids, Liv-52 and ursodeoxycholic acid. The efficacy of these preparations does not always meet the expectations of both doctors and patients, making the search for a new, highly efficient and safe hepatoprotector an urgent task.

Scientists worldwide have demonstrated a key role for polyprenols in the biosynthesis of a number of the most important compounds necessary for vital functions in the body and in various organs. Prenols are the plant analogues of the endogenous transport lipid known as dolichol [11–14]. Dolichols play an extremely important role in basic biological processes of the human body. Found in the phospholipid bilayer, dolichols modify the fluidity and permeability of membranes and participate in the regeneration, differentiation and proliferation of cells. Moreover, dolichols are essential for the synthesis of glycoproteins, such as membrane glycoconjugates, receptors, hormones and immunoglobulins. The dolichol-phosphate cycle is a necessary metabolic mediator in the processes of regeneration, differentiation and proliferation of cells [15–18]. In acute and chronic inflammatory/degenerative liver disease of various etiologies (characterised by progressive damage to cell membranes caused by dolichol deficiency and insufficiency of dolichol-phosphate cycle), exogenous substitution with prenols improves the immune status of the cell and restores and stabilises protein molecules in the membrane [19–21]. The discovery of the steroid 5 reductase type 3 gene for a polyprenol reductase that can convert polyprenols to dolichols [22] has created a resurgence of interest in polyprenols worldwide.

As a hepatoprotector, Ropren® is an antioxidant that protects cell membranes [23], modulates the immune system [24]. Ropren® also has significant neurological effects [25–28]. The hepatoprotective activity of the polyprenol preparation Ropren® was studied in a pre-clinical animal model of toxic liver damage induced by dichloroethane or acetaminophen [29,30]. Therapy with Ropren® significantly improved the functional and morphological state of the liver in laboratory rats exposed to hepatotoxic agents and liver resection.

Clinical trials of the efficacy and safety of Ropren® were conducted on patients with liver disease of various etiologies such as hepatitis B and C and alcohol- and drug-induced damage. A comparative study of Ropren® with Essentiale Forte as a comparator drug was conducted in four clinical centers in St Petersburg [31]. In that study, treatment with Ropren® monotherapy improved cell damage (as measured by a reduction in the level of alanine transaminase and aspartate transaminase in the blood) and was faster acting then Essentiale Forte. Administration of Ropren® led to improvements in the blood lipid spectrum (an increase in high-density lipoproteins and a reduction in low-density lipoproteins) and a significant increase of general antioxidants in the blood serum. Ropren® also improved liver function in patients with chronic alcoholism [32].

With the increasing incidence of NAFLP and NASH, especially in children, hepatoprotectors with very low toxicity would be extremely useful. Given the success of Ropren® in other types of liver disease, the purpose of this prospective observational study was to examine Ropren® as a therapy for patients with non-alcoholic steatohepatitis.

MATERIALS AND METHODS

ETHICS

The study was approved by the Ethics Committee of the Central Scientific Research Institute of Moscow. The research followed the guidelines of the Declaration of Helsinki and Tokyo for humans. Each patient gave informed consent for participation in the trial.

ROPREN® FINISHED PRODUCT

Ropren® is a biopolymer made up of long-chain polyisoprenoid alcohols (polyprenols) extracted from green verdure of spruce trees (*Picea abies* (L) Karst). Ropren® is a highly purified polyprenol [25, 33, 34] that is produced at pharmaceutical concentrations (not less than 95% purity). Based on extensive pre-clinical and clinical testing in Russia, Ropren® was approved by the Russian Ministry of Health for entry into the Russian Pharmacopoeia as an effective treatment for liver disease in 2007 [35].

Ropren® is manufactured by the St Petersburg Pharmaceutical Factory Pty Ltd and packed in flasks with a dosimeter, containing four millilitres (mL) of the finished product. The finished product is a transparent, oily, yellow- to orange-coloured liquid with a specific coniferous odour.

SUBJECTS

Twenty patients with NASH and disorders of lipid metabolism were included in the trial, which was conducted between 2008 and 2009. Outpatients enrolled in the study satisfied the inclusion criteria for the presence of NASH as determined by blood biochemistry and measurement of the liver fibrosis using ultrasound (see below). Patients with liver damage caused by alcohol, drugs or infectious agents and patients who required treatment with any medication were excluded from the study. The low number of patients was due to the rarity of suitable subjects. Males screened had liver damage caused by alcohol, drugs or infectious agents and so all patients were females (38–56 years of age) who had a chronic condition that had not improved with other treatments. All were outpatients who had not taken any medications in the three weeks before the commencement of the trial. Patient commitment to the treatment was high.

This was a post-registration study and so all patients were treated with Ropren®. During the registration process, the Ministry of Health (Russian Federation) required Ropren® to be compared with Essential Forte [35]. Essentiale Forte is a longstanding treatment for liver conditions and, in some conditions, is the standard of care.

The study design controlled for confounders such as genetics, socio-economic status and severity of underlying disease [36]. As this was a post-registration study, all patients were treated.

Side effects of the treatment were monitored throughout the trial using questionnaires administered by clinicians during patient interviews.

TREATMENT AND CLINICAL TESTS

Patients received Ropren® orally, 3 drops, 3 times a day (54 mg/day) for a period of 12 weeks. Standard clinical diagnostic protocols were used assess the treatment. We evaluated the dynamics of the patient's symptoms (dyspepsia and asthenia) and the blood levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (AP), gamma-glutamyl-transpeptidase (GGTP) and lipids, including total cholesterol, triglcyerides , low-density lipoproteins (LDL) and high-density lipoproteins (HDL). The liver fibrosis index (FI) was measured with indirect ultrasound elastometry using a Fibroscan device before and after the treatment.

STATISTICAL ANALYSIS

Statistical analysis was conducted using Student's t-test.

RESULTS AND DISCUSSION

Over 12 weeks of Ropren® monotherapy, a reduction in the intensity of dyspeptic symptoms (feeling of heaviness in the right hypochondrium, meteorism, and unstable stool) and asthenic syndromes (weakness, fatigue, sleep disorders) was observed in all patients. There was not a single case of reported side effects of the treatment.

Ropren® significantly improved the lipid spectrum in the blood by normalizing the dyslipidemia that was initially present. There was a reliable decrease

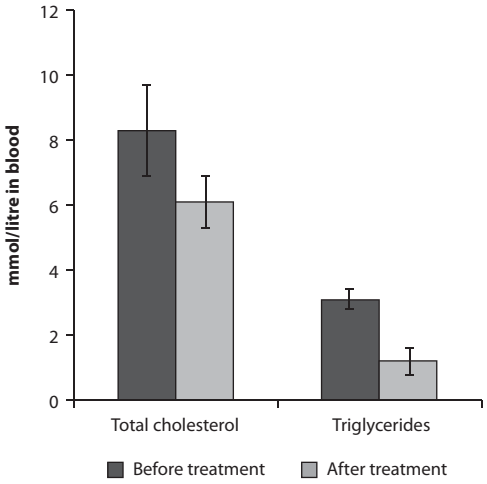

Fig. 1 (left)
Mean total cholesterol and trigyclerides in the blood of patients with nonalcoholic steatohepatitis (NASH) before and after treatment with Ropren (54 mg/day) for 12 weeks. The decreases in mean blood levels after treatment compared with before treatment were statistically different (*p < 0.05)

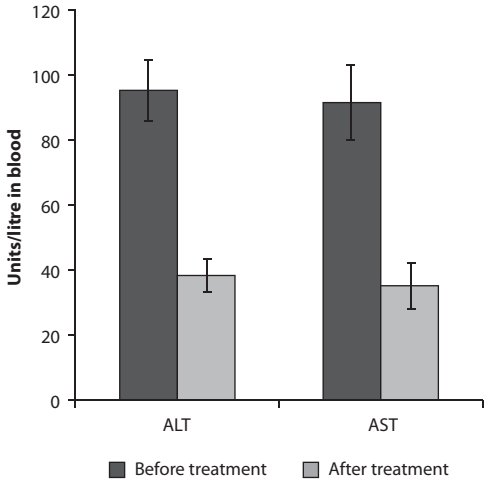

Fig. 2 (right)
Mean blood alanine transaminase (ALT) and aspartate transaminase (AST) levels in the blood of patients with nonalcoholic steatohepatitis (NASH) before and after treatment with Ropren® (54mg/day) for 12 weeks

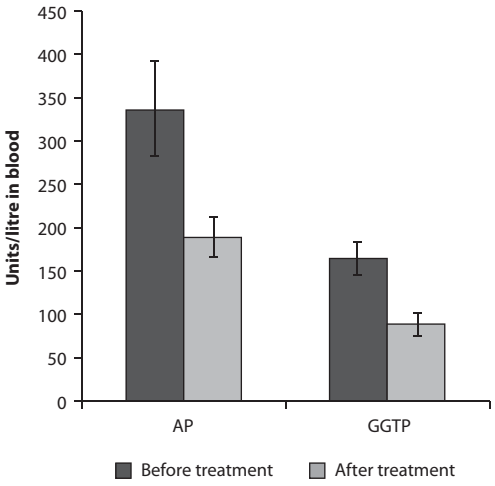

Fig. 3 (left)
Evaluation of cholestasis using mean levels of alkaline phosphatase (AP) and gamma-glutamyl transpeptidase (GGTP) in the blood of patients with nonalcoholic steatohepatitis (NASH) before and after treatment with Ropren® (54 mg/day) for 12 weeks

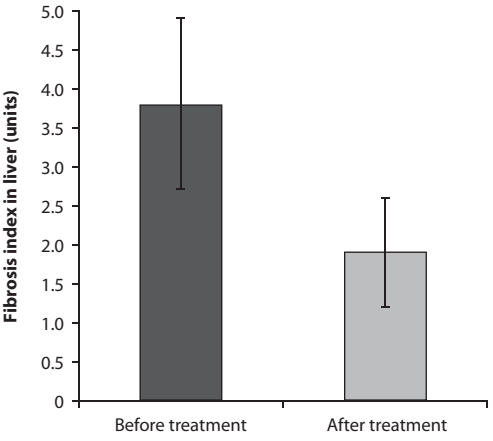

Fig. 4 (right)
Measurement of the elasticity of the liver using the fibrosis index (FI, units) in patients with nonalcoholic steatohepatitis (NASH) before and after treatment with Ropren® (54 mg/day) for 12 weeks. The decrease in mean FI units after treatment compared with before treatment was statistically different (*p < 0.05)

in total cholesterol levels (from 8.3 ± 1.4 to 6.1 ± 0.8 mmol/l, p < 0.05) and triglycerides (from 3.1 ± 0.3 to 1.2 ± 0.4 mmol/l, p < 0.05) (Figure 1). Levels of LDL decreased from 4.3 ± 1.1 to 2.1 ±1.0 mmol/l (p > 0.05), whereas HDL increased from 0.75 ± 0.2 to 1.1 ± 0.4 mmol/l (p > 0.05).

In studying the blood biochemistry to measure inflammation of the liver, we found a trend towards reduced cell damage as indicated by decreased levels of alanine transaminase (ALT) and aspartate transaminase (AST) after Ropren® therapy (Figure 2). ALT decreased from 95.2 ± 9.3 Units/L of blood before treatment to 38.2± 5.3 Units/L of blood after treatment (p > 0.05). AST decreased from 91.5 ± 11.6 Units/L of blood before treatment to 35.1 ± 7.2 Units/L of blood after treatment (p > 0.05). Although the decreases are large, the low number of patients in this pilot study does not allow us to call the decreases statistically significant. Nonetheless, the trends were clear and indicate that a study with more patients and greater statistical power is essential.

Similarly, there was also a decrease (p > 0.05) in the activity of alkaline phosphatase (AP) and gamma-glutamyl transpeptidase (GGTP) (Figure 3) suggesting improved cholestasis. AP decreased from 338.2 ± 55.4 Units/L of blood before treatment to 189.9 ± 23.1 Units/L of blood after treatment (p > 0.05). GGTP decreased from 165.3 ± 18.6 Units/L of blood before treatment to 88.4 ± 12.5 Units/L of blood after treatment (p > 0.05). Again, the low patient numbers do not allow us to say that these results are statistically significant but the results do indicate that a study with larger statistical power is warranted.

Prior to treatment, all patients had very high levels of fibrosis as measured by indirect elastometry (3.8 ± 1.1 FI units). This can be explained by the significant reduction in the elasticity of the liver parenchyma caused by marked infiltration of the hepatocytes with fat. None of the patients in this group with NAFLD had cirrhosis. During the 12 weeks of Ropren® monotherapy, there was a statistically significant reduction in the FI (Figure 4) to 1.9 ± 0.7 units (p < 0.05). Fibrosis is a significant indicator of mortality [6] and so this is a noteworthy result given the low statistical power of this study.

Ropren® improved the clinical and biochemical signs of NASH and facilitated the normalisation of the lipid spectrum. In combination, the results suggest that recovery of the elasticity of the liver, the improved lipid profile of the blood, the reduction in inflammation of the liver and the improved general condition of the patients could contribute to a reduced risk of cirrhosis.

CONCLUSION

Ropren® is an effective and safe hepatoprotector and is currently being used as both a monotherapy and combined therapy for chronic hepatitis and cirrhosis of various etiologies. Given that Ropren® has low or no side effects, this study suggests that Ropren® also has great potential as a treatment for patients with NASH, especially in populations such as children [1], where treatments with low side effects are important. A larger multi-centre trial with greater statistical power is recommended.

Conflict of Interest

This study was performed at the Central Scientific Institute of Gastroenterology, funded by the Russian Government's Ministry of Healthcare and Social Development. Prenolica Limited supplied the Ropren®.

V. Soultanov is an academic scientist involved in decades of research into substances from conifer needles in Russia. He is a Director and shareholder of Prenolica Limited, which is the company that is commercialising the technology.

The remaining authors have no conflicts to disclose for this research.

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