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NEW HEPATIC AND NEUROLOGICAL CLINICAL IMPLICATIONS OF LONG-CHAIN PLANT POLYPRENOLS ACTING ON THE MAMMALIAN ISOPRENOID PATHWAY

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НОВЫЕ ГЕПАТОЛОГИЧЕСКИЕ И НЕВРОЛОГИЧЕСКИЕ КЛИНИЧЕСКИЕ ЭФФЕКТЫ ДЛИННОЦЕПОЧЕЧНЫХ РАСТИТЕЛЬНЫХ ПОЛИПРЕНОЛОВ, ДЕЙСТВУЮЩИИХ НА ПУТЬ ИЗОПРЕНОИДНЫХ МЛЕКОПИТАЮЩИХ

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Summary

The mammalian isoprenoid synthesis pathway (also known as the mevalonate pathway) is fundamental to the metabolism and health of organisms, with products such as cholesterol (sterol isoprenoid), ubiquinone (coenzyme Q) and dolichol (non-sterol isoprenoids) having great importance to mammalian biology and physiology. Targeting the isoprenoid pathway results in novel therapeutic options for a diverse range of conditions. Plant polyprenols are biologically active molecules that affect the isoprenoid pathway — toxic side effects have never been observed during treatment with our pharmaceutical-grade polyprenols (Ropren®). Statins and bisphosphonates also act on this pathway but have the disadvantage of causing numerous side effects. Our unique ability to produce Ropren® containing not less than 95% pure polyprenols has enabled their clinical use in Russia for around eight years and has also enabled researchers to conduct trials into other therapeutic uses. Although polyprenols can treat conditions such as viral, bacterial and fungal infections, inflammation and other immune conditions, this paper focuses on the new pre-clinical and clinical effects of polyprenols in hepatic and neurological conditions. Recent pre-clinical studies have shown treatment with polyprenols from conifers had a range of neurological and cognitive effects, including improved cognitive performance in a rat model relevant to Alzheimer's disease and healthy levels of myelination in mice with an experimental model of multiple sclerosis. Early clinical data has shown Ropren® treatment improved antioxidant levels in people with diabetes and improved liver function in patients on chemotherapy treatment. Ropren® also had positive effects on electroencephalograms of people with alcohol-induced cirrhosis and Alzheimer's disease and significantly decreased symptoms in people with depression. These results pave the way for larger clinical trials and show how Ropren® is a valuable clinical tool to treat a wide range of liver and neurological conditions.

Keywords: Ropren, polyprenols, isoprenoid, hepatoprotector, neurological conditions, mevalonate pathway

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РЕЗЮМЕ

Путь синтеза изопреноидов у млекопитающих (также известный, как мевалонатный путь) является фундаментальным для метаболизма и здоровья организма, а такие соединения, как холестерин (стерольный изопреноид), убихинон (кофермент Q) и долихол (нестерольный изопреноид) имеют огромное значение для биологии и физиологии млекопитающих. Воздействие на путь обмена изопреноидов приводит к новым терапевтическим возможностям, применимым для широкого диапазона различных патологических состояний. Растительные полипренолы являются активными молекулами, которые влияют на путь обмена изопреноидов. При использовании наших полипренолов фармацевтического качества, каковым является Ропрен®, никогда не было зарегистрировано побочных эффектов. Статины и бисфосфонаты также влияют на этот путь обмена, но при этом проявляют множественные побочные эффекты. Наша уникальная возможность производить Ропрен®, содержащий не менее 95% чистых полипренолов, позволила использовать этот препарат в клинической практике в России около 10 лет, а также открыла возможность исследователям проводить клинические испытания для выявления других его терапевтических свойств. Не смотря на то, что полипренолы могут широко применяться для лечения заболеваний, связанных с вирусными, бактериальными и грибковыми инфекциями, воспалениями и другими иммунными состояниями, данная статья представляет новые доклинические и клинические эффекты полипренолов при заболеваниях печени и центральной нервной системы. Недавние доклинические испытания показали, что лечение полипренолами, полученными из хвои, имеет целый ряд неврологических и когнитивных эффектов, включая улучшение когнитивной функции у крыс с заболеванием Альцгеймера и восстановление здорового уровня миелинизации у мышей на модели рассеянного склероза. Более ранние клинические исследования продемонстрировали, что лечение Ропреном® улучшает уровни антиоксидантов у людей с сахарным диабетом, а также улучшает функцию печени у пациентов, проходящих химиотерапию. Ропрен® также оказывает положительное влияние на электроэнцефалограмму у людей с болезнью Альцгеймера и алкогольным циррозом печени и значительно снижает симптомы депрессии у пациентов.

Результаты дают основание для проведения более обширных клинических исследований и указывают на то, что Ропрен® является ценным клиническим «инструментом» в лечении целого ряда неврологических и гепатологических заболеваний.

Ключевые слова: Ропрен, полипренолы, изопреноид, гепатопротектор, неврологические заболевания, мевалонатный путь.

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Introduction

The mammalian isoprenoid synthesis pathway (also known as the mevalonate pathway) results in the production of a number of molecules of fundamental importance to the metabolism and health of the organism. Major products of the pathway such as cholesterol (sterol isoprenoid), ubiquinone (coenzyme Q) and dolichol (non-sterol isoprenoids) have great importance to mammalian biology, although it has become obvious that some of the other pathway intermediates have biological and potential clinical effects. The isoprenoid pathway is essential to many processes including inflammation (Tricarico et al. 2014), immunity (Akula et al. 2016), neuronal development (Li et al. 2016) and tumour progression (Thurnher et al. 2013; Gruenbacher and Thurnher, 2015).

There is growing evidence that targeting the isoprenoid pathway will result in novel therapeutic options for the future (Surmacz and Swiezewska, 2011). Interest in the targeting this pathway has grown from: (i) a body of work showing that plant isoprenoids are biologically active molecules; and (ii) evidence of the pleiotropic (diverse) effects of two classes of drugs (statins and bisphosphonates) that affect this pathway and are widely used and can have significant side effects. Both of these classes of drugs have pleiotropic effects beyond their clinical use to improve blood cholesterol levels (statins) and bone metabolism (bisphosphonates). The mechanisms of many of these additional effects are not fully understood and are, at times, controversial.

Given our unique ability to produce pharmaceutical-grade plant polyprenols, this paper will focus on the clinical effects of polyprenols for hepatic and neurological conditions. These substances can also treat other conditions such as viral, bacterial and fungal infections, inflammation and other immune conditions, which we will discuss in the near future. The therapeutic polyprenol substance produced by our researchers has pleiotropic therapeutic effects while having the advantage that after six years of extensive testing and clinical use, toxic side effects have never been observed.

Statins are the most prescribed drug in the world and as the number of people being treated with this class of drug increases, so does the number of patients who have not responded to therapy or who have suffered side effects. Given the high prescription rate of statins, adverse events are vigorously debated in the literature. Side effects include hepatotoxicity and muscle symptoms ranging from milder forms of myopathy to rhabdomyolisis, with intensive doses exacerbating the number of such adverse events and the risk for adverse events increasing with the concomitant use of lipid-lowering medications such as broad-spectrum fibrates, bile acid sequestrants, cholesterol absorption inhibitors or niacin. (Husband 2009; Silva et al. 2007). This situation has caused some to suggest the importance of research into non-statin alternatives (Rozman and Monostory, 2010). Plant polyprenols are such an alternative and one study has even showed that polyprenols from spruce can protect against statin-induced muscle weakness in rats (Jansone et al. 2016).

Polyprenols have been extracted from a number of species of plants but other clinical researchers have not been able to extensively study polyprenols in humans because of an inability to produce them in sufficient quantity and quality. Our researchers have solved this problem with the extraction of large quantities of concentrated pharmaceutical-grade polyprenols (Roschin and Soultanov, 2003a). The safety and efficacy of Ropren® (or Bioeffective R) has been shown and this substance is registered by the Russian Ministry of Health for use as a hepatoprotector in humans. Ropren® improves cholesterol levels in the blood and protects the liver and brain, especially in cases of encephalopathy (Sviderskii et al. 2006; Sviderskii et al. 2007). Unlike statins, adverse events have never been observed.

While the mechanisms governing the clinical effects of polyprenols are not fully characterised, given their influence on the isoprenoid biosynthetic pathway, it is not surprising that the clinical effects of polyprenols are as pleiotropic as those observed for statins and bisphosphonates.

Therapeutic drugs targeting the isoprenoid biosynthetic pathway can have toxic side effects

While it is not the purpose of this paper to fully discuss the statins and bisphosphonates, brief mention will be made of their pleiotropic effects. This will put into perspective the wide-ranging effects that are possible in manipulation of the isoprenoid biosynthesis pathway. Plant isoprenoids have similar wide-ranging biological activity but without the toxic side effects that can emerge with use of statins and bisphosphonates.

Statins

The potential pleiotropic effects of statins have led to suggestions that the iosprenoid pathway could provide new targets for novel drug design (Bedi et al. 2016). Statins can decrease blood low-density lipoprotein (LDL) by 20% to 55% (Jones et al. 1998; Jones et al. 2003) and have therefore become one of the most widely used drugs in the world. Statins block the active site of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (HMGR) (Istvan and Deisenhofer, 2001), which is the step in the pathway that converts HMG-CoA to mevalonic acid. This enzyme is common to the entire isoprenoid biosynthesis pathway and is highly regulated. Therefore, as well as lowering cholesterol levels, statins also reduce levels of ubiquinone and dolichol, thus affecting antioxidant activity and glycosylation in the body.

The side effects of statins are wide ranging, with good evidence for a causative relationship for diabetes mellitus but less clear evidence for neurological effects, muscle weakness, elevated creatine kinase (CK) levels and other statin-associated muscle symptoms such as myalgia, cramps, rhabdomyolysis (Thompson et al. 2016). While cognitive side effects remain controversial (Thompson et al. 2016), case studies continue to emerge in the literature (Okeahialam 2015; Samaras et al. 2016; Sureweera et al. 2016).

Bisphosphonates

Bisphosphonates inhibit osteoclast-mediated bone resorption and are therefore widely used for osteoporosis and other bone metabolism defects. There are two types of bisphosphonate: nitrogen-containing and non-nitrogen-containing. It is the nitrogen-containing class of this drug that affects the isoprenoid pathway by inhibiting farnesylpyrophosphate (FPP) synthase, thus decreasing levels of FPP and geranylgeranylpyrophosphate (GGPP) and subsequently, prenylation of proteins. This class of drugs has also exhibited marked anti-proliferative effects. Reduced protein prenylation affects the ability of small GTPases to insert into membranes. Many of these GTPases have a role in malignant transformation of cells by regulating multiple cellular processes, including cell signalling, growth motility and metastasis (Karlsson et al. 2009). *In vitro* and pre-clinical studies of the anti-proliferative effect of these drugs show anti-tumour activity in a number of cancers, including breast cancer, prostate cancer and pancreatic cancer via mechanisms such as inhibition of cell adhesion, migration and proliferation and modulation of the immune system (Buhaescu and Izzedine, 2007).

Unfortunately, bisphosphonates can have short-term (acute phase response, low calcium levels, ocular inflammation and severe musculoskeletal pain) and long-term side effects (increased risk of bone fracture including femoral fracture and (rarely) osteonecrosis of the jaw and kidney damage) (Hirschberg 2012; Kennel and Drake, 2009).

The role of plant isoprenoids as therapeutic substances

In the 30 years to 2012, approximately 50% of drugs approved were natural products or were derived from a natural product (Newman and Cragg, 2012). Although the pharmaceutical industry has turned to synthetic libraries as a focus of drug design, there is renewed interest in the active pursuit of natural products (including plants) using technologies such as genomic sequencing, genetic engineering and robotics (Li and Vederas, 2009).

It therefore comes as no surprise that substances based on isoprenoids from plants are of great interest. These substances have a long history of human use and have the potential to lead to novel therapies. There is currently widespread interest in microbial engineering of metabolites of the isoprenoid biosynthesis pathway for potential screening for commercial products for medicine and agriculture (Kirby and Keasling, 2009; Klein-Marcuschamer et al. 2007; Withers and Keasling, 2007).

Historically, the isoprenoids have also been referred to as terpenoids and some of the nomenclature for the metabolites still reflects this convention. As such, the terpenoids are classified based on the number of carbons and are known as hemiterpenes (five carbons), monoterpenes (10 carbons), sesquiterpenes (15 carbons), diterpenes (20 carbons) and triterpenes (30 carbons). The polyprenols are long chain isoprenoids where the number of five-carbon isoprene units can vary from 10 to 50 in number. One end of the molecule is hydroxylated (the α-residue). In polyprenols, this α-residue is unsaturated whereas in dolichol it is saturated.

The therapeutic potential of derivatives of polyprenols has been reviewed elsewhere (Pronin et al. 2014; Zhang et al. 2015) — this paper will focus on the clinical implications of the long-chain polyprenols.

Introduction to plant polyprenols

The polyprenols are essential membrane components conserved throughout evolution (Hartley and Imperiali, 2012), with polyprenols affecting the fluidity of membranes and changing the permeability of biological membranes (Ciepichal et al. 2011).

Like other plant isoprenoids, the use of plants that are rich in polyprenols for treating human conditions is historical. Pine, spruce and fir trees are a rich source of these substances and during WWII the Russian government used a paste of conifer needles for topical and internal use that was thought to have saved thousands of lives during the 900-day siege of Leningrad. This led to intense scientific research in Russia. Similarly, there has been strong interest from Japanese researchers. Like many areas of clinical research, the clinical effect is obvious before the mechanism of that effect is known. The wide-ranging effects of these substances have been viewed with scepticism by some, however, once an understanding of the basic biochemistry of isoprenoid metabolism is gained, it becomes obvious that substances that affect this pathway are fundamental to the metabolism of animals and plants.

Research of the closely related mammalian dolichol in the 1970s helped to suggest some possible mechanisms about how polyprenols are exerting their effect on human metabolism. Although the role of dolichol-phosphate in glycosylation is well characterised, interestingly the role of dolichol in many other biological processes is not yet fully elucidated.

The effects of polyprenols on mammalian cells

Modification of plant polyprenols to biologically active dolichol

Before we begin a discussion of dolichol, it is important to discuss absorption of polyprenols from the diet and the ability of those polyprenols to be converted into dolichol. As mentioned, polyprenols are unsaturated in the α-residue whereas dolichol is saturated. For dietary polyprenols to have a physiological effect by acting as a dolichol, mammalian tissues must be able to saturate the α-residue. It has been suggested that dietary dolichol and polyprenols have negligible contribution compared with *de novo* synthesis of dolichols (Adair and Keller, 1982; Keller et al. 1982). However, the α-residue of radiolabelled polyprenols containing 11 isoprene units were present in liver at higher

concentrations than those containing 19 isoprene units (Chojnacki and Dallner, 1983). Furthermore, a proportion of the saturated lipids was also phosphorylated and thus could potentially participate in glycosylation of proteins. When the levels of polyprenols in the average diet were taken into account, the ability to saturate polyprenols was not considered to be of physiological relevance (Chojnacki and Dallner, 1988; Keller et al. 1982). However in a breakthrough finding in the field, the polyprenol reductase (called SRD5A3) responsible for converting polyprenols to dolichols was identified (Cantagrel et al. 2010). The *SRD5A3* gene is highly expressed in human foetal brain tissue, especially in the cerebellum (Morava et al. 2010).

Dietary polyprenols can be metabolised by mammals into dolichol and participate in the dolichol cycle and so are related to other substances in the isoprenoid pathway such as cholesterol and dolichol (Muceniece et al. 2016).

As mentioned, our extraction of polyprenols at pharmaceutical concentrations (Roschin and Soultanov, 2003a) enables administration to patients at levels much higher than typical dietary levels. Given that there is now molecular evidence of the ability of mammalian cells to convert polyprenols to dolichol, the ability of these higher concentrations of polyprenols to exert a therapeutic effect is not surprising.

Potential direct effect of polyprenols on the isoprenoid pathway

As well as acting via the conversion into dolichol, it is also possible that plant polyprenols could exert a direct effect on the isoprenoid pathway. Dolichol was still present in human and mouse *SRD5A3* mutants suggesting that there might be a positive feedback mechanism on the isoprenoid biosynthetic pathway (Cantagrel et al. 2010). Whether this relates to a direct effect of polyprenols on the pathway remains to be investigated.

There is evidence that the smaller plant isoprenoids can directly exert a number of effects, especially on HMG-CoA at a post-transcriptional level (Mo and Elson, 2004; Crespo et al. 2013). It is thought that these molecules can have protective roles in cancer and cardiovascular disease by inhibiting the isoprenoid biosynthetic pathway (Mo and Elson, 2004). Of the longer chain isoprenoids, recent data shows that substances created by irradiation of ubiquinone have direct effects on enzymes of the isoprenoid biosynthesis pathway to increase synthesis of ubiquinone and decrease synthesis of cholesterol (Bentinger et al. 2008). The ability of polyprenols to directly affect isoprenoid metabolism requires thorough investigation and we hope that other researchers will be encouraged to enter this exciting field to study mechanisms.

Biological activity of dolichols

Studies of the efficacy of polyprenols were inhibited by an inability to extract them in sufficient qualities for treatment of humans. Nonetheless, a clinical interest in these substances to treat cardiovascular problems, cancer, infection and to modulate the immune system has resulted in an extensive number of patents (Grigor'eva and Moiseenkov, 1989).

With the extraction of pharmaceutical-grade substance Ropren®, it is now possible to administer these substances at pharmaceutical concentrations. It is assumed that the therapeutic effects observed after treatment with this higher concentration of polyprenols would in large part be due to its conversion into dolichol although, as mentioned, any direct effects cannot be ruled out at this stage. Nonetheless, a brief discussion of the biology of dolichols is warranted.

Dolichol is widely distributed in mammalian tissues. It exists in a phosphorylated form and a dephosphorylated free form. Dolichol-phoshate is the most intensively studied of the dolichols, as it is involved in N-linked glycosylation and thus is of fundamental importance to the cell. The role of dolichol-phosphate in N-linked glycosylation has been extensively studied (Burda and Aebi, 1999; Denecke and Kranz, 2009) and will not be covered in detail here. Although the function of the free form of dolichol is not fully characterised, it interacts with cellular membranes by intercalation into the lipid bilayer (Wood et al. 1989b; Ciepichal et al. 2011). It is also thought that dolichol regulates membrane fluidity, particularly in synaptic membranes (Wood et al. 1989b). Currently, there is no known mechanism of dolichol degradation. There is evidence that dolichol levels increase with age, in the brain (Söderberg et al. 1990) and in tissues, for which there is some understanding of mechanisms (Marino et al. 2002; Cavallini et al. 2004). Although the significance of these increases is not currently clear, dolichol has been proposed as a biomarker of aging (Parentini et al. 2005). A role for dolichol as an antioxidant and inhibitor of cell toxicity has also been proposed (Bergamini et al. 2004; Cavallini et al. 2016).

Interestingly, dolichol levels are decreased in brains of patients with AD while dolichol-phosphate and ubiquinone levels were increased; this is the opposite of what occurs in brains without AD (Söderberg et al. 1992). It was thought that dolichol-phosphate levels in AD brains might be higher due to increased glycosylation and ubiquinone levels and because of an increased need for protection from oxidative stress (Bergamini et al. 2004). Is it also possible that dolichol levels are lower in AD brains because it has been depleted during the mechanism of antioxidative protection. These questions need further study.

Recently, it has been proposed that upregulation of FFP and GGPP could result in prenylation of small GTPases that may be involved in the pathology of Alzheimer's disease (Hooff et al. 2010b). On the other hand, cholesterol, rather than prenylation, appears to affect beta-amyloid production in a neuroblastoma cell line (Hooff et al. 2010a).

In summary, the role of dolichol (and for that matter, ubiquinone) in Alzheimer's disease pathogenesis is unknown. In general, far more detailed investigation of the isoprenoid pathway in Alzheimer's disease is urgently required.

Chemical and radiation assault of liver cells also affect dolichol levels. It is possible that carbon tetrachloride treatment or irradiation of rat liver cells resulted in a reduction in the level of dolichol, possibly by the generation of free radicals (Parentini et al. 2003). In addition, it is well established that ethanol increases dolichol levels in the blood and urine of humans (Roine et al. 1992) and affects fluidity of membranes in mice (Wood et al. 1989a).

Pharmaceutical-grade plant polyprenols — chemistry and safety of Ropren®

For polyprenols to be clinically relevant, there is a need for them to be extracted and produced at high quality. Although there is widespread belief in the inability to extract sufficient quantities of polyprenols for industrial purposes, we have extracted high-quality polyprenols from confers for some time, with approximately 95% purity (Roschin and Soultanov, 2003a; for presentation of the structure see Fedotova et al. 2012) and in sufficient quantities for clinical use. This substance, Ropren® , is registered in Russia for use as a hepatoprotector — as a treatment for a variety of liver conditions such as alcoholic liver, cirrhosis, nonalcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD) and hepatitis.

Others have extracted polyprenols in smaller quantities for research purposes (Zhang et al. 2015).

During our extensive pre-clinical testing and clinical use we have not found Ropren® to be carcinogenic, teratogenic, irritating or toxic — we have not observed any side effects and this substance fulfills the human safety requirements of Russia and other countries. Testing of polyprenols extracted from *Ginkgo biloba* L. leaves has also found that polyprenols were safe (Wang et al. 2015).

Pre-clinical studies of polyprenols from conifers

The efficacy of Ropren® has been extensively shown in pre-clinical models of hepatic and neurological conditions. In the rat, long-term (30-day) administration of a high dose of Ropren® (2mg/kg) had no effect on cholinesterase (CE) or monoamine oxidase (MAO) in kidney and brain (striatum, hypothalamus, and medulla). This high dose did increase MAO and decrease CE in liver (Sviderskii et al. 2006). In a model of hepatic encephalopathy induced with carbon tetrachloride and affecting brain and blood levels of CE and MAO, treatment with Ropren® had some efficacy at improving levels of CE in medulla and, to a lesser extent, in striatum (Sviderskii et al. 2007).

In rat, Ropren® has been shown to protect the liver in experimental models of liver damage induced by treatment with dichloroethane and acetaminophen. Liver function was assessed using blood biochemistry in conjunction with histology and showed that Ropren® was able to significantly improve liver function as many parameters of liver function were restored to normal levels (Lapteva et al. 2006; Lapteva et al. 2007). Furthermore, Ropren® improved liver regeneration in rats and decreased total cholesterol, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Roschin and Soultanov, 2003b). Polyprenols extracted from fir also had a marked effect in a rat model of alcohol-induced hepatitis, normalising levels of total lipids, triglycerides and phospholipids, inhibiting lipid peroxidation and improving bile secretion (Vaĭs et al. 2012). Polyprenols extracted from *Ginkgo biloba* L. (Yang et al. 2011) and *Taxus chinesis* var. *mairei* (Yu et al. 2012) protected rat liver from carbon tetrachloride damage, with the polyprenols from *Ginkgo biloba* protecting to a similar extent as Essentiale (Yang et al. 2011).

Polyprenols such as Ropren® are also effective in animal models of neurological conditions potentially through a pleiotropic effect on metabolism and the immune system or through direct action on the brain.

Ropren® improves cognitive performance in a rat model with β-amyloid peptide-(25–35)-induced amnesia, which is relevant to Alzheimer's disease (Fedotova et al. 2012). In the same model of Alzheimer's disease in gonadectomised male rats, Ropren® enhanced memory (Fedotova et al. 2016) and had an antidepressant-like effect (Soultanov et al. 2016). Polyprenols have also improved cognitive performance in mice with D-galactose-induced cognitive impairment (Wang et al. 2014).

An exciting result in a mouse model of multiple sclerosis from Khodanovich and colleagues (2016) encourages future studies to determine if Ropren® is preventing demyelination or promoting re-myelination. Using a mouse model of multiple sclerosis where cuprizone induces demyelination in the corpus callosum, the researchers showed that mice treated with both cuprizone and Ropren® had a level of demyelination similar to healthy animals (Khodanovich et al. 2016).

Clinical studies of polyprenols from conifers

Other authors have pointed out the scarcity of clinical studies because of the difficulty of extracting high quality polyprenols in sufficiently high quantities. Fortunately Ropren®, which we have extracted at pharmaceutical grade and quantity for many years, is available to conduct a variety of clinical trials in humans.

In this paper, we will focus on newer clinical trials related to hepatic and neurological conditions, although it is worth mentioning that the pleiotropic action of Ropren® means its therapeutic action is also related to wide ranging effects on the immune system.

New clinical uses of polyprenols in conditions of human liver and metabolism

As the liver has a large metabolic influence on the body, we have long known that in addition to improving the function of the liver, Ropren® improves the blood biochemistry and cholesterol profile of patients.

In a study in patients with alcoholism, treatment with Ropren® for 12 weeks improved liver, renal and pancreatic function — blood chemistry for blood sugar, ALT, AST, alkaline phosphatase (AP), bilirubin and cholesterol returned to normal levels and this effect was more marked and occurred faster than in patients who received standard treatment including Essential Forte (Soultanov et al. 2010; Soultanov et al. 2012).

Parameter	Before Ropren® treatment	After Ropren® treatment (2 months)
Superoxide dismutase (units/l)	129.6 ± 6.36	155.0 ± 6.0 *
Catalase (mcmol/ml)	4.64 ± 0.32	5.2 ± 0.48 *
Glutathione peroxidise (units/l)	7645.7 ± 710.6	6575.2 ± 520.7 *

Table 1.
Changes in blood antioxidant enzyme levels in people with diabetes after treatment with Ropren® for two months
* significant difference ($p<0.05$) when levels after Ropren® treatment is compared with baseline levels before treatment

Parameter	Before Ropren® treatment	After Ropren® treatment (2 months)
Alanine transaminase (ALT)	54.9 ± 20.1	37.1 ± 16.6 *
Aspartate transaminase (AST)	75.4 ± 21.8	38.5 ± 17.4 *
Albumin	34.0 ± 4.1	35.0 ± 4.8
Alkaline phosphatase (AP)	251.5 ± 45.1	219.4 ± 38.8 *
Total bilirubin	26.9 ± 14.1	23.2 ± 10.7
Gamma-glutamate transpeptidase (GGTP)	168.2 ± 111.9	99.7 ± 78.3 *

Table 2.
Changes in blood biochemistry in people having chemotherapy before and after treatment with Ropren® for two months
* significant difference ($p<0.05$) when levels after Ropren® treatment is compared with baseline levels before treatment

Ropren® treatment has also been shown to have a marked affect on liver function for patients with NASH and, importantly, was able to decrease liver fibrosis, which is notoriously difficult to treat (Golovanova et al. 2010; Soultanov et al. 2012; Golovanova et al. 2016). Another study also showed normalisation of blood parameters and a decrease in body mass index for patients with metabolic syndrome and NASH and treated with Ropren® (Lapteva et al. 2015). These results are extremely important given the growing global epidemic of obesity and diabetes and an increase in the number of people with non-alcoholic fatty liver disease (NAFLD) and NASH, with the incidence of NAFLD even rising in children (Vajro et al. 2012).

Given the prevalence of diabetes, a small trial of 25 patients with diabetes (10 with type 1; 15 with type 2 diabetes) between the ages of 42 and 68 (and who were first diagnosed with diabetes at the ages of 4 to 12 years) was undertaken at St Petersburg Medical Academy of Postgraduate studies (MAPS), VG Baranov's Endocrinology Department. Ropren® treatment (four drops, twice per day, 48 mg per day) was given for two months, with patients having various clinical and laboratory tests at four visits including blood tests to establish baseline parameters during screening and at the completion of treatment at two months. While Ropren® did not improve patient-reported symptoms, glycaemic status (tested with HbA1c) or body mass index, there were statistically significant changes in antioxidant enzymes (*Table 1*).

Given the broad ranging metabolic damage caused by diabetes, the ability to provide antioxidant protection to cellular membranes could have long-term beneficial effects for people with diabetes. Larger clinical trials are needed to further study these findings but there is potential for Ropren® to be used for patients with type 1 and type 2 diabetes as antioxidant therapy in a combined treatment regimen that includes drugs for glycaemic control.

One of the most damaging assaults on the human body is chemotherapy or radiation therapy for cancer. Hepatic toxicity during treatment of malignant tumours can result in the need to change the dose of chemotherapy, prolong the interval between treatment courses, provide a detoxifying therapy, and in some cases, discontinue treatment for cancer.

Ropren®'s ability to protect the liver while not increasing side effects or toxic symptoms led to a small pilot trial (Lazarev et al. 2012) of 15 patients with first and second degree hepatic toxicity caused by chemotherapy. The age of the patients ranged from 18 to 62 years and included two men and 13 women. Ropren® treatment was six drops, three times per day (108 mg per day) for two months, with blood biochemistry analysed before and after treatment with Ropren® (*Table 2*).

After treatment with Ropren®, all patients reported a significant reduction in dyspeptic symptoms (heaviness in the right hypochondrium, flatulence, unstable stool) and asthenic symptoms (asthenia, fatigue, sleep disorders). The patients also noted better tolerance of the chemotherapy. Patients showed some improvement in white blood cell count suggesting an immunomodulating effect of Ropren® that has been found in other trials. Analysis of blood biochemistry showed improved ALT and AST levels ($p<0.05$) and a reduction ($p<0.05$) of activity of cholestasis enzymes — AP and gamma-glutamate transpeptidase (GGTP) (Table 1).

Larger clinical trials are needed but these results show the potential for Ropren® to reduce hepatic toxicity and improve tolerance of chemotherapy.

New clinical uses of polyprenols in conditions of the human neurological system

An independent pilot study examined the hepatoprotective and neuroprotective effects of Ropren® in 21 patients (aged 28–70) with alcohol-induced liver cirrhosis treated at a dose of eight drops, three times a day (144 mg/day) for four weeks (Minushkin et al. 2011). A reduction in the level of inflammation was indicated by a significant improvement of parameters including AST and AP, GGTP, bilirubin, similar to an earlier trial (Soultanov et al. 2010), and improved liver fibrosis. Ropren® treatment also decreased the symptoms of neuropathy and was associated with a statistically significant improvement in the neurological status of patients and the quality of life.

In the earlier trial of patients with alcoholism (Soultanov et al. 2010), we also took measurements of neurological and cognitive function in addition to the metabolic measurements discussed above. The study also recorded electroencephalograms (EEG) in a subset of patients to provide electrophysiological evidence of improved brain function. The investigation

Table 3.
Comparative analysis of experimental and control groups of patients according to electroencephalography data, before and after treatment

Changes	Experimental group of patients treated with Ropren® (%)	Control group of patients that received standard therapy for alcoholism (%)
Negative changes	2	23.5
No significant changes	14	35
Neutral changes	18	18
Total:	34	76.5
Small positive changes	24	23.5
Positive changes	11	0
Marked positive changes	31	0
Total:	66	23.5

included 56 patients in the experimental group, with EEG recorded before and after treatment with Ropren® (*Table 3*).

EEG data showed that Ropren® intensified the alpha-rhythm, and decreased readings showing irritation and stimulation (especially in the frontal areas), while also diminishing or removing readings showing vascular instability. By the end of treatment with Ropren®, 66% of patients had improved EEG readings, with 31% showing marked improvement. Only 23.5% of the patients receiving standard therapy showed small improvements in their EEG readings and none of the patients had marked improvement (Table 3).

Clinical observational studies have also shown Ropren® improved neurological symptoms in patients with Alzheimer's disease (Soultanov et al. 2010; Monakhova et al. 2010). In this pilot study of 25 patients, 40% showed marked improvement of cognitive function, while 80% of patients had improved EEG readings, 25% had improved neurological status and Ropren® normalised blood levels of BuChE and MAO.

Neurological improvements have also been found in healthy patients in a study performed by Swinburne University in Australia (Stough and Soultanov, 2007). In a randomised placebo-controlled trial, Ropren® (100 mg per day) was administered for 12 weeks to healthy humans aged between 65 and 80 years. Even in this smaller trial of 79 participants, measures of working and long-term memory consolidation were significantly improved. In a smaller subset of the group tested with EEG, there was an increase in alpha frequencies and a decrease in delta and theta frequencies suggesting that Ropren® might aid in relaxation and focus attention. Furthermore the HDL/LDL ratio was significantly improved. LDL was lower, although not significantly lower in this smaller group of 79. These data in humans supports a large body of work already performed by researchers in Russia showing the ability of Ropren® to protect both liver and brain.

A small pilot study into the use of Ropren® for mental health conditions in humans was conducted at the Department of Affective States of the Research Institute of Occupational Diseases of the Siberian Branch of the Russian Academy of Medical Sciences. Ropren® was used to treat six outpatients (two male and four female), one with a recurrent depressive disorder and five with mild/moderate depressive disorder. The average duration of the current depressive episode was 4.17 ± 2.32 months.

All the patients received Ropren® for the first two weeks at a dose of three drops, three times a day (54 mg per day), increasing to four drops, three times a day (72 mg per day) on day 14 and six drops, three times a day on day 28 (108 mg per day). In patients treated with Ropren®, the average severity of depression (using the Montgomery–Åsberg Depression Rating Scale) decreased from 18.33 ± 3.20 points to 2.67 ± 2.50 ($p < 0.001$), with 100% of cases showing a significant improvement after 14 days of treatment.

Conclusion

The global literature for the therapeutic effect of polyprenols through the isoprenoid pathway is an exciting field that is about to enter a new phase of growth, with an increasing emphasis of the diverse therapeutic potential of non-statin substances. Although, polyprenols represent a safe and clinically relevant substance, the ability of most researchers to produce only small amounts has hampered clinical trials.

The large-scale production of polyprenols as Ropren® has enabled clinical use in Russia for the last eight years and has also enabled researchers to conduct further trials into other clinical uses and applications for this substance. Multiple observational studies suggest Ropren® is a valuable clinical tool to treat people with a wide range of liver diseases as well as a range of neurological conditions such as memory decline, depressive disorders and other cognitive conditions.

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