PLANT LONG-CHAIN ISOPRENOID ALCOHOLS (POLYPRENOOLS) PROTECT LIVER VIA STABILISATION OF CELL MEMBRANES AND ORGANELLE STRUCTURE IN A CARBON TETRACHLORIDE ANIMAL MODEL OF TOXIC LIVER DAMAGE

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The mechanism for the ability of pharmaceutical-grade plant long-chain isoprenoid alcohols (polyprenols) to improve liver function was investigated in a carbon tetrachloride animal model of liver damage. The pharmaceutical-grade polyprenol substance, Ropren®, was compared with a commonly used phospholipid substance, Essentiale Forte, for 21 days of treatment. The condition of hepatic cells and cellular membranes was investigated using electron and light microscopy. The study showed Ropren® restored liver function and morphology as soon as day 7–14 of treatment, an effect that was faster than improvement after treatment with Essentiale Forte. Similarly, levels of glycogen in the liver were restored faster after treatment with Ropren® than Essential Forte. Measurement of the activity of the membrane-bound enzymes, monoamine oxidase (MAO) and butyrylcholinesterase (BuChE) also showed Ropren® improved liver function by improving cellular membrane and mitochondrial membrane function. To our knowledge, these results show for the first time a mechanism of the stabilisation of cellular membranes after treatment with Ropren® along with improvements in liver enzymes. These functional improvements occur faster than Essentiale Forte, a commonly used hepatoprotector.

Keywords: long-chain isoprenoid alcohols, polyprenols, dolichol, Ropren, Essentiale Forte, carbon tetrachloride, hepatocytes, liver function

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Introduction

The carbon tetrachloride (CCL4) animal model of toxic liver damage leads to damage of plasma and intracellular membranes of hepatic cells and a loss of functional activity (Bebzborodkina et al. 2008). The CCL4 model offers an opportunity to study the ability of therapeutical substances to protect hepatic membranes and restore or partially restore the function of the liver. This includes ability of substances (or their endogenous analogues) to protect membranes, improve the architecture of disrupted membrane and/or regenerate damaged membranes.

In mammals, the endogenous long-chain isoprenoid alcohol is dolichol, a polyprenol present in almost all tissues as either a free alcohol or phosphorylated and/or esterified with fatty acid compounds. Dolichols are located inside the phospholipid bilayer of membranes. Their movement and distribution varies depending on the geometry of the membrane (Chojnicki et al 1988, Chojnicki et al. 2001), but they determine and modify fluidity, stability and permeability of membranes (Kurup and Kurrup, 2003). As a component of membranes, dolichols (free or bound with fatty acids) are present in high concentrations in the Golgi apparatus, where they can increase the fluidity and permeability of membranes, regulate transformation of glycoproteins and their secretion – the levels of dolichol in Golgi are markedly affected by CCL4 treatment (Pronzato et al. 1990). As the plant analogue of dolichol, long-chain isoprenoid alcohols (polyprenols) are also involved in membrane structure (Valtersson et al 1985; Khidrova and Sharhodoxov, 2002; Janas et al 2000; Surmacz and Swiezewska, 2011) and can change membrane fluidity and permeability (Ciepichal et al. 2011). Although these plant isoprenoid alcohols are known to improve liver function, the objective of this study was to directly look at the mechanism of action by examining the ability...
of a plant polyprene to restore hepatic cellular membranes, functional activity and mitochondria in the CCl₄ rat model of toxic liver damage.

To fulfil this objective, we used a pharmaceutical-grade form of long-chain isoprenoid alcohols (polyprenels) from conifers that is registered for clinical use in Russia as a hepatoprotector. The finished form of the substance, Ropren®, is used as a treatment in a range of clinical conditions including fatty degeneration of the liver of various aetiologies (including non-alcoholic steatohepatitis – NASH and non-alcoholic fatty liver disease – NAFLD), hepatitis, liver cirrhosis (in combination treatment), and in toxic liver damage (alcohol, drugs, and pharmaceutics) (Lapteva et al. 2006; Lapteva et al. 2007; Lapteva et al. 2015; Shabanov and Soultanov, 2011; Soultanov et al. 2010a; Soultanov et al. 2010b). This pharmaceutical-grade preparation has a wide spectrum of activity and can be used in clinical practice for treatment of a metabolic syndrome and has beneficial neurologic effects in both animal models and people (for review see Soultanov 2016).

Although Ropren® is widely used as a hepatoprotector in humans, the mechanism of its effect is not fully elucidated, although the pharmaceutical-grade polyprenels used in this study (Sviderskii et al. 2007) and other forms of polyprenels from *Ginkgo biloba* (Yang et al. 2011; Sun and Jia, 2015) have also been tested in CCl₄ animal models, including in comparison with commonly used hepatoprotectors such as Essentiale Forte. Plant polyprenels represent a newer class of hepatoprotector with the advantage that the pharmaceutical preparation Ropren® has a high level of safety, with no side effects encountered in the many preclinical and clinical studies conducted (Roschin and Soultanov 2003b; Lazarev et al. 2012; Soultanov 2016).

Other substances with a wide spectrum of activity used to treat hepatic conditions are also of plant origin. These include therapeutic substances from milk thistle (such as Carsil, Legalon, Silimarlin) and other drugs containing essential phospholipids, such as Essentiale Forte (phosphotidyl choline) that is extracted from soybeans (Okovity, 2002).

We have previously established in animal studies that Ropren® is well absorbed and tolerated by animals (even at a relatively very high dose of 2,000 mg/kg of weight) and does not disrupt the main neuromediating pathways of metabolism in the liver, kidneys, and brain in rats (Sviderskii et al. 2006). Moreover, we have demonstrated that in the case of membrane damage by CCl₄, Ropren®, as well as Gliatilin, protects cellular membranes in brain, possibly by participating in the redistribution of lipids in lipid bilayers (Sviderskii et al. 2007).

For this study, Essentiale Forte, a well-studied and commonly used phospholipid hepatoprotector, was selected as the comparator drug for Ropren®. Phospholipids embed in membrane layers to provide a cytoprotective effect (Ipatova et al. 1998; Gundermann, 2002), including in models of toxicity and hepatitis caused by CCl₄. Based on this property, Essentiale Forte is a useful comparator substance to study as its cytoprotective properties belong to a different group of compounds compared with Ropren®. This enables us to compare the rate of restoration of damaged membranes using enzymatic analysis of membrane-bound proteins and histological analysis using electron and light microscopy.

**Materials and methods**

**Animals**

Male Wistar male rats weighing 180–200 g were obtained from the Rappolovo Animal Farm (Leningrad region) and kept in the vivarium of the Sechenov Institute of Evolutionary Physiology and Biochemistry of the Russian Academy of Science. The animals were housed under standard conditions and their diet was balanced, with water and food available *ad libitum*.

**Substances**

Pharmaceutical-grade long-chain isoprenoid alcohols (polyprenels) are extracted from green vegetation of spruce trees (*Picea abies* (L) Karst), highly purified (Roschin and Soultanov 2003a) and produced at pharmaceutical concentrations of not less than 95% purity, as previously described (Fedotova, 2012). The finished form of this substance is registered in Russia as Ropren® (Frenolica Limited, Australia, formerly known as Solagran Limited) and contains 25% of the pharmaceutical-grade polyprenels and 75% oil. Its functional activity has been characterised and extensively studied (Fedotova et al. 2012; Fedotova et al. 2016; Soultanov et al. 2017; for review see Soultanov 2016). Based on extensive pre-clinical and clinical testing in Russia, in 2007 Ropren® was approved by the Russian Ministry of Health for entry into the Russian Pharmacopoeia as an effective treatment for human liver disease.

For this study, Ropren® was further diluted with natural vegetable oil to enable oral administration of correct doses (see below for doses) to rats. The comparator treatment was Essentiale Forte (Rhone-Poulenc Rorer Pharmaceuticals Inc., France), a mixture of essential phospholipids widely used in medical practice as a hepatoprotector. For this study, the Essentiale Forte was diluted with natural vegetable oil to enable oral administration.

CCl₄ was sourced from Merck Group, Germany.

**Treatment**

The CCl₄ model of toxic hepatitis involves subcutaneous injection of an oil solution of CCl₄ at a dose of 4 mg/kg (0.2 mL per 100 g of animal weight) for 4 days. For this study, four animal groups each contained 21 rats:
**Enzyme analysis**

Whole blood from sacrificed rats was allowed to clot and the clot was removed to produce serum by centrifugation at 1,500 x g for 15 minutes at 4 °C. The liver was removed and after preparation for the enzyme assays (see below), samples were immediately frozen and stored at -5 °C.

Butyrylcholinesterase (BuChE) activity in serum or homogenised liver was analysed as described in Ellman et al. (1961).

For MAO activity, liver mitochondria were partly separated from ballast protein and used as the source of MAO activity in the assay. MAO activity was analysed in liver using a spectrophotometric method (wavelength of 420 nm) measuring the amount of ammonia formed (over 60 minutes) as a result of the enzymatic oxidative deamination of monoamine serotonin-creatinine sulphate (Reanal, Hungary) (as described, Severina, 1979). MAO activity in the serum (Sigma) was analysed using benzylamine substrate and a spectrophotometric method (wavelength 241 nm) measuring the amount of benzaldehyde formed as a result of substrate deamination (as described, Severina, 1979). Protein content in enzyme preparations was measured as per Lowry et al. (1953).

Statistical analysis was performed using Student’s t test, with p < 0.05 considered significant.

**Histology**

Rat liver was analysed using both electron and light microscopy. For electron microscopy, the livers were fixed in a 2.5 % glutaraldehyde solution and then immersed in araldite. Ultra-thin cross-sections were prepared and examined using a Jem-100S electron microscope (Jeol, Japan) with accelerating potential of 80 k/volt and magnification of 12,000 times magnification.

For light microscopy livers were fixed in 10% formalin and immersed in paraffin. Paraffin cross-sections were prepared, stained with haematoxylin-eosin and examined using a Carl Zeiss light microscope at 120 times magnification.

Histochemical analysis of glycogen was conducted by Periodic acid-Schiff (PAS) staining. Fat was detected on semi-fine cross-sections impregnated with osmium and stained with methylene blue.

**Results and discussion**

**Ropren® improves the condition of intracellular organelles after hepatic damage induced by carbon tetrachloride**

To examine cell membrane structure, electron microscopic examination of the liver parenchyma was carried out on day 7, 14 and 21 (Figure 1).

Healthy cell structure was observed in the livers of the Control group 1 [no treatment] (Figure 1A). In comparison, CCl4 caused irreversible changes in hepatocytes of the animals untreated with either Ropren® or Essentiale Forte (Control group 2 [CCl4], Figure 1B and C). On Day 7 (Figure 1B), CCl4 caused lipid degeneration in the mitochondria, the rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER) and the Golgi apparatus. The cellular damage was still present on day 21 (Figure 1C). CCl4 has an active oxidising effect on cellular membranes, and the microscopy shows destruction of mitochondria and the tubules of the endoplasmic reticulum, and the accumulation of two types of lipid inclusions that persisted for up to 21 days (Figures 1B and C). Depending on their origins, these lipid inclusions have single or double-layered membranes, with a double-layered membrane indicating mitochondrial origins and a single-layered membrane indicating RER origins. Lipid dystrophy of the cells leads to their partial destruction and appeared as pyknosis of the nuclei and formation of micro-areas of necrosis. This damages cellular organelles responsible for protein synthesis: the RER, the smooth endoplasmic reticulum (SER), the Golgi apparatus and the mitochondria.

It has been established that the function of membranes in the endoplasmic reticulum (ER) varies, with the RER involved in protein synthesis and protein secretion and SER involved in synthesis of carbohydrates, metabolism of glycogen, steroids and various toxic substances that need to be neutralised. CCl4 treatment degranulates the RER, decreases the number of ribosomes associated with the ER and decreases the total number of ribosomes in hepatocytes (Castro et al. 1973; Vengerovski et al. 1987) causing a reduction in protein synthesis and a dysfunctional ER that leads to the accumulation of some viruses, in particular retroviruses, which reduce immunity.

As early as day 7, animals treated with Ropren® in addition to CCl4 (Experimental group 1 [CCl4 + Ropren], Figure 1D) had less lipid in their hepatic cells and membrane structure was restored in spite of the persisting toxic effect of CCl4. After 14 days of Ropren® treatment, an accumulation of glycogen in the cell was observed with subsequent restoration of cytoplasmic structures in the cytoplasm (Figure 1E). By day 21, Ropren® had restored membranes and the RER, with completed re-assembling of protein synthesizing complexes in the endoplasmic reticulum and mitochondria (Figure 1F).
In comparison, after 7 days of treatment with Essential Forte (Experimental group 2 [CCl₄ + Essenti- tiale Forte]), destruction of the tubules of the ER and destruction of mitochondria were observed (Figure 1G). On day 14, these destructive changes were less evident, although there was a process of developing lipid inclusion in place of mitochondria (Figure 1H). By day 21, the protein synthesising machinery of the liver was restored, as shown by an aggregation of mitochondria, bubbles forming from the endoplasmic reticulum and glycogen in the cytoplasm (Figure 1I).

**Ropren® improves liver structure and glycogen levels after hepatic damage induced by carbon tetrachloride**

Histological analysis with light microscopy and haematoxylin-eosin staining confirmed the data obtained in the experiment. Compared with healthy intact liver (Control group 1 [no treatment]; Figure 2A*), CCl₄ was strongly toxic and caused lipid dystrophy in hepatocytes, which was demonstrated by the degeneration of liver cells and pyknosis, followed by destruction of the nucleus and evidence of areas of leukocytic infiltration (Control group 2 [CCl₄]; Figures 2B*, C* and D*). Where significant damage was present on day 21, areas of cirrhosis were observed along with Councilman bodies in the cytoplasm of hepatocytes (Figure 2D*). On day 7, the exposure to CCl₄ resulted in vacuolisation of the liver cells – some cells had pyknotic nuclei with signs of cellular destruction (Figure 2B*). On day 14, pyknosis of nuclei was observed in the vacuolated cells from central sections (Figure 2C*). By day 21, death of cells was evident with Councilman bodies observed in the cytoplasm of individual hepatocytes along with areas of cirrhosis (Figure 2D*).

To summarise, although the hepatoprotective effect of Ropren® was comparable with that of Essenti- tiale Forte, the structural improvements occurred at a faster rate (as early as day 7) with Ropren® treatment. Ropren® promoted a faster decrease in the number of lipid-containing vacuoles and enabled formation of protein-synthesizing complexes of the RER and the mitochondria. These data provide a mechanism for the ability of Ropren® to restore disrupted functions of hepatocytes by restoring cellular membranes, especially of organelles important to protein synthesis.

*On color insert.*

**Figure 1:** Electron microscopy of liver from rats treated with carbon tetrachloride alone or with carbon tetrachloride plus Ropren® or Essentiiale Forte.

All panels show electron-diffrac- tion patterns (magnification x12,000). Panel A shows healthy untreated rat liver with endo- plasmic reticulum (1), nucleus (2), Golgi apparatus (3) and mitochondria (4). Panel B and C shows liver from rats treated with carbon tetrachloride alone at day 7 and day 21, respec- tively. Panel B shows two types of vacuoles: larger vacuoles without membranes derived from endoplasmic reticulum (1) and smaller vacuoles limited by two membranes (2). Panels D-F show liver from rats treated with carbon tetrachloride and Ropren® at days 7, 14 and 21, respectively. Panel D shows the nucleus (1), lipid inclusions (2) and mitochondria (3). Panel E shows the nucleus (1), lipid inclusions (2) and mitochondria (3). Panel E shows nucleus (1), mitochondria (2), Golgi apparatus (3) and glycogen (4). Panel F shows mitochondria (1), Golgi apparatus (2), granular endoplasmic reticulum (3) and glycogen (4). Panels G-I show liver from rats treated with carbon tetrachloride and Essentiiale Forte at days 7, 14 and 21, respectively. Panel G shows lipid (1), mitochondria (2), degenerating granular en- doplasmic reticulum (3) and the nucleus (4). Panel H shows lipid (1), mitochondria (2), Golgi ap- paratus (3) and the appearance of glycogen (4). Panel I shows mitochondria (1), endoplasmic reticulum (2), glycogen (3) and Golgi (4).
Ropren® restores levels of butyrylcholinesterase – an enzyme of the ER membrane – after treatment with carbon tetrachloride

One of the consequences of liver damage after exposure to CCl₄ is a significant reduction in the rate of protein synthesis. Using an enzymatic method of analysis we evaluated the effect of Ropren® and Essentiale Forte on the activity of the membrane-bound enzyme – butyrylcholinesterase (BuChE). CCl₄ disrupts the integrity of hepatocyte membranes and causes partial solubilisation of membrane-bound proteins, thereby disrupting activity and normal function. It is known that BuChE is produced in the liver and its activity significantly alters in cases of functional insufficiency of the liver (Moraev et al. 2007). BuChE is synthesised in ER of the parenchymal cells. The level of BuChE activity is an indicator of the functional condition of the liver that changes with various liver pathologies. According to the literature, a decrease of BuChE activity is found in cases of hepatitis and cirrhosis (Inage and Furuhama, 1997).

Furthermore, comparisons of blood biochemistry and data from liver biopsies have demonstrated that the reduction of cholinesterase activity correlates with exacerbation of hepatitis and development of liver fibrosis (Zou et al. 2001). In cases of hepatitis, BuChE activity decreases to 40–50 % of the minimal normal value (MNV) by day 3 to 6 of the disease and returns to the normal value by day 13 to 15 and, in severe cirrhosis, BuChE activity is only 10–20 % of the MNV (Zou et al. 2001). Onset of an increase in BuChE activity is a positive prognostic indicator (Shabanov et al. 2010).

In this study, treatment with Ropren® (Experimental group 1 [CCl₄ + Ropren®]) and Essentiale Forte (Experimental group 2 [CCl₄ + Essentiale Forte]) increased BuChE activity after CCl₄ treatment to a level not significantly different from healthy animals (Control group 1 [no treatment]). However, this increase in BuChE activity was at a faster rate after treatment with Ropren®, when BuChE activity in the serum was close to normal values as early as on day 7 in serum and day 14 in liver (Table 1).

The statistically significant reduction of BuChE activity in the liver and blood serum of rats treated with CCl₄ alone (Control group 2 [CCl₄]) shows the liver was damaged and is similar to data obtained by other researchers (Inage and Furuhama, 1997; Zou et al. 2001).

Table 1.
Changes in butyrylcholinesterase in the liver and blood serum after treatment with carbon tetrachloride alone or with carbon tetrachloride plus Ropren® or Essentiale Forte

<table>
<thead>
<tr>
<th>Animal groups (number of animals)</th>
<th>Butyrylcholinesterase activity (μmol ATCh/min/gram tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>Control group 1 [no treatment]</td>
<td>(n=7)</td>
</tr>
<tr>
<td>Control group 2 [CCl₄]</td>
<td>(n=7)</td>
</tr>
<tr>
<td>Experimental group 1 [CCl₄ + Ropren®]</td>
<td>(n=7)</td>
</tr>
<tr>
<td>Experimental group 2 [CCl₄ + Essentiale Forte]</td>
<td>(n=7)</td>
</tr>
</tbody>
</table>

* On color insert.

CCl₄ = carbon tetrachloride; ATCh = acetylthiocholine; * statistically significant difference (p < 0.05) from Control group 1 (Day 7); ** statistically significant difference (p < 0.05) from Control group 1 (Day 14); *** statistically significant difference (p < 0.05) from Control group 1 (Day 21).

In comparison, after 7 days of treatment with Essentiale Forte (Experimental group 2 [CCl₄ + Essentiale Forte]) levels of glycogen in the cells was as low as in animals treated with CCl₄ alone (Control group 2 [CCl₄]; data not shown). On day 14, hepatocytes showed some light staining for glycogen but still contained many vacuoles (data not shown). By day 21 the cells showed moderate and patchy staining for glycogen (Figure 3F*) at a similar level to that shown on day 7 of Ropren® treatment (Experimental group 1 [CCl₄ + Ropren®]; Figure 3C*) showing that Ropren® can restore the disrupted synthesis of glycogen more quickly than the phospholipid drug Essentiale Forte.

levels on days 14 and 21 (Experimental group 1 [CCl₄ + Ropren®]; Figures 3D* and E*).
Ropren® restores levels of monoamine oxidase – an enzyme of the mitochondrial membrane – after treatment with carbon tetrachloride

For additional information about the membrane-protective effect of the Ropren®, we also studied MAO, which is located predominantly on mitochondrial membranes of hepatocytes and other tissues. MAO is not only an enzyme required for metabolism of neuromediators and hormones, but it also has an important barrier function, including in the liver, for inactivation of biogenic amines and other toxic agents (Fowler and Saaf, 1985). MAO is contained in nearly all animal tissues with the highest concentration found in the liver, kidneys, spleen, and brain tissues. Within the cell, the enzyme is localised predominantly in the mitochondrial fraction, which is exactly where Ropren® shows its highest activity as a membrane-stabilising substance. Measuring changes in MAO activity in various conditions is of great importance to clinical medicine. The literature shows that some toxic agents radically affect MAO activity. In some cases MAO activity is inhibited, as with emotional stress or experimental atherosclerosis (Gorkin and Ovchinnikova, 1993), while in other cases, the enzyme’s activity is increased by thyroxin, drugs and alcohol, and at early stages of cirrhosis – as occurs in the CCl4 animal model. MAO not only defends the cellular membrane from the impact of poisons and toxins but also indirectly affects the immune system. For evaluation of the liver’s functional condition and its detoxifying properties, the level of MAO in the blood and the liver is a very indicative parameter. During non-alcoholic fatty degeneration of the liver with hepatitis, MAO activity in the human liver was increased by approximately 40% (Arima et al. 1977). Compared with untreated animals (Control group 1 [no treatment]), MAO activity in mitochondria of the rat liver increased with CCl4 treatment (Control group 2 [CCl4]) and remained almost unchanged until the end of the experiment at day 21. This indicates that the processes of deamination of monoamines in the body were disrupted and the membrane-bound MAO could not perform its main barrier function of inactivating biogenic amines and the toxic agent, CCl4. Dysfunctional MAO has been shown to lead to disruption of metabolism of neuromediators and hormones (Obata et al. 1988).

<table>
<thead>
<tr>
<th>Animal group</th>
<th>MAO activity in liver (nmol ammonia/min/mg protein)</th>
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<tbody>
<tr>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>Control group 1 [no treatment] (n=7)</td>
<td>2.26±0.16</td>
</tr>
<tr>
<td>Control group 2 [CCl4] (n=7)</td>
<td>3.57±0.14*</td>
</tr>
<tr>
<td>Experimental group 1 [CCl4 + Ropren®] (n=7)</td>
<td>3.15±0.12</td>
</tr>
<tr>
<td>Experimental group 2 [CCl4 + Essentiale Forte] (n=7)</td>
<td>3.50±0.20</td>
</tr>
<tr>
<td></td>
<td>Day 14</td>
</tr>
<tr>
<td>Control group 1 [no treatment] (n=7)</td>
<td>2.31±0.18</td>
</tr>
<tr>
<td>Control group 2 [CCl4] (n=7)</td>
<td>3.51±0.14***</td>
</tr>
<tr>
<td>Experimental group 1 [CCl4 + Ropren®] (n=7)</td>
<td>2.91±0.13***</td>
</tr>
<tr>
<td>Experimental group 2 [CCl4 + Essentiale Forte] (n=7)</td>
<td>3.39±0.27</td>
</tr>
<tr>
<td></td>
<td>Day 21</td>
</tr>
<tr>
<td>Control group 1 [no treatment] (n=7)</td>
<td>2.40±0.17#</td>
</tr>
<tr>
<td>Control group 2 [CCl4] (n=7)</td>
<td>3.53±0.17</td>
</tr>
<tr>
<td>Experimental group 1 [CCl4 + Ropren®] (n=7)</td>
<td>2.73±0.18#</td>
</tr>
<tr>
<td>Experimental group 2 [CCl4 + Essentiale Forte] (n=7)</td>
<td>2.33±0.16#</td>
</tr>
</tbody>
</table>

Here we show that CCl4 significantly increased MAO in the liver 7 days after the beginning of the experiment (p<0.05), and this level remained high until day 21 (Table 2).

Treating animals with Ropren® (Experimental group 1 [CCl4 + Ropren®]), decreased MAO activity by day 7 compared with CCl4 alone (Control group 2 [CCl4]) and was significantly decreased by day 14 compared with Control group 2 [CCl4] (p<0.05). Essentiale Forte also decreased MAO activity but these decreases were only statistically significant at day 21 compared with Control group 2 [CCl4] (p<0.05).

The ability of Ropren® to restore MAO activity in the liver and kidneys is related to participation of this therapeutic substance in processes of repair of damaged cellular membranes or changes in the viscosity of the hydrophobic components of mitochondrial membranes. The regenerative effect of Ropren® on MAO activity in the liver could thus be explained firstly by changes in the molecular properties of the enzyme itself, and secondly, by changes in the properties of mitochondrial membranes where this enzyme is localised.

The asymmetric structure of plasma membranes means that macromolecular receptors can be located on the surface of the membrane, can penetrate the entire membrane, or can be on the inside of the membrane (Grundahl et al, 2012). Based on this understanding, it can be assumed that Ropren® and Essentiale Forte have a membrane-protective effect on mitochondria in the liver.

The reason that improvements with Ropren® treatment are faster than Essential Forte is unclear, but it could be related to its ability to compensate for low levels of dolichol in the dolichyl-phosphate cycle. Data in the literature (Pronzato et al. 1990) shows that CCl4 affects the formation of glycoproteins and leads to changes in the level and structure of microsomes and Golgi apparatus in the rat liver. Pronzato and colleagues showed that 5–60 minutes after CCl4-induced liver damage, the level of total dolichol, free dolichol, and dolichyl phosphate sharply reduced in microsomes and Golgi apparatus, with the early decrease of total dolichol changing the secretory function of the Golgi...
as early as 15 minutes. Lipid peroxidation disturbed the structure of free dolichol and dolichyl-phosphate and leads to changes in the biosynthetic pathway of these important compounds (Pronzato et al. 1990). Therefore, it is possible that Ropren® prevents this process and protects dolichol from oxidative degradation and restores the secretory function of hepatocytes. This possibility requires further study.

Conclusion

1. CCl₄ disrupted liver cell function and structure, in particular the protein-synthesising machinery.
2. Treatment with pharmaceutical-grade polyproprenols for 21 days was hepatoprotective and restored liver function in the CCl₄ model of liver damage in rats. Ropren® restored the integrity of membranes and the RER, as well as protein-synthesising complexes in the ER and mitochondria.
3. Treatment with Ropren® restored liver morphology at a faster rate than Essentiale Forte – as early as day 7, with restoration peaking at day 21. Ropren® promoted accumulation of glycogen in the cytoplasm of damaged hepatocytes, decreased the number of lipid-containing vacuoles and enabled formation of protein-synthesising complexes in the endoplasmic reticulum and mitochondria, leading to restoration of membranes in damaged cells.
4. The metabolic protective effect of Ropren® included restoration of the activity of BuChE, as well as membrane-bound enzyme MAO – these improvements began on day 7 to day 14 of treatment and continued until the end of the experiment on day 21.
5. In comparison with a phospholipid drug, Essentiale Forte, the process of improved liver function was faster with Ropren® therapy, potentially because Ropren® more actively improves mitochondrial function than Essentiale Forte. This raises the possibility of using Ropren® to repair mitochondrial function in the liver, and in other tissues affected by mitochondrial disorders.

References


34. Soultanov VS, Agishev VG, Monakhova IA, Mokhovikova IA, Kulikov AP, Roschin VI, Nikitina TV. Ropren® improves liver and pancreatic function in patients with chronic alcoholism. Gastroenterology-St. Petersburg, 2010a;4:12–18.


К статье

Растительные длинноцепочные изопреноидные спирты предохраняют печень посредством стабилизации клеточных мембран и структуры органелл на модели токсического поражения печени углеродтетрахлоридом (Plant long-chain isoprenoid alcohols (polyprenols) protect liver via stabilisation of cell membranes and organelle structure in a carbon tetrachloride animal model of toxic liver damage), – стр. 94–101

Figure 2:
Light microscopy of liver from rats treated with carbon tetrachloride alone or with carbon tetrachloride plus Ropren®.

All panels show light microscopy of rat liver stained with haematoxylin and eosin (magnification х120). Panel A shows healthy untreated rat liver. Panels B-D shows liver from rats treated with carbon tetrachloride alone at day 7 and day 21, respectively. Panels E-F show liver from rats treated with carbon tetrachloride and Ropren® at days 7, 14 and 21, respectively.

Figure 3:
Light microscopy and staining for glycogen in liver from rats treated with carbon tetrachloride alone or with carbon tetrachloride plus Ropren® or Essential Forte.

All panels show periodic acid-Schiff (PAS) stain of rat liver (magnification х120), with more colour (magenta-purple) indicating a higher level of glycogen. Panel A shows glycogen in healthy untreated rat liver and panel B shows glycogen depleted 7 days after carbon tetrachloride treatment. In comparison, after carbon tetrachloride plus Ropren® treatment, a moderate level of glycogen is restored after 7 days (panel C), and a high level of glycogen after 14 days (panel D) and 21 days (panel E) of Ropren® treatment. After carbon tetrachloride treatment plus 21 days of Essential Forte treatment, a moderate level of glycogen was restored (panel F).