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Conclusions:

1. In mild immune-mediated hepatitis caspase-mediated apoptosis intensifies.
4. In the experimental model of Con-A-induced mild hepatitis, Plaferon LB speeds up recovery of liver to its normal structure.
5. In the experimental model of Con-A-induced mild hepatitis, Plaferon LB modulates blood NO levels: in the most destructive phase it reduces NO levels while in the regenerative phase the opposite occurs in a statistically significant manner.
6. In a mild immune-mediated liver injury, Plaferon LB prevents development of oxidative stress.
7. Under the influence of Plaferon LB, increase in hepatocyte apoptosis and their simultaneous enhanced proliferative activity facilitates acceleration of regeneration processes in liver.

Practical Recommendations:

1. In weak immune-mediated infections (mild forms of Hepatitis B and Hepatitis C), Plaferon LB and other pro-apoptotic immunomodulators are recommended in the treatment of immune-mediated liver diseases.
2. To investigate the mechanism of Plaferon LB effects thoroughly, it is reasonable to study its influence on the balance of pro-inflammatory and anti-inflammatory cytokines via Con-A induced hepatitis and other experimental models of autoimmune diseases.
3. Identification of Plaferon LB fraction with immunomodulatory potential is reasonable to conduct using Con-A induced hepatitis model.
The work has been done at the Institute of Medical Biotechnology

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or because of inherited deficiencies in GSH synthesis. Thus, low GSH levels are observed during sepsis, acetaminophen intoxication, chronic alcohol consumption, and in acute Wilson’s disease. Moreover, hepatic GSH is subject to pronounced circadian alterations. Pharmacological enhancement of hepatic GSH renders the liver less vulnerable and protects against many direct hepatotoxins (Prescott et al., 1982). Increasing evidence argues for a dichotomal role of GSH with respect to cellular damage.

In some paradigms of cell death where the primary event is apoptosis, a protective (i.e., anti-apoptotic), and not an aggravating, effect of GSH depletion was reported. To date, NO-induced apoptosis of macrophages, CD95-mediated apoptosis of T cells, and cytokine-mediated hepatocyte apoptosis in vivo (Hentze et al, 1999) were found to depend on a sufficient intracellular GSH level of the respective cells. In these studies, the redox sensitivity of apoptosis-executing caspases, i.e. aspartate-specific cysteine proteases, was hypothesized to be responsible for the observed protection because of decreased GSH levels. However, elevated intracellular GSH levels can also abrogate apoptosis in various cell lines (Uhlig et al., 1992).

At the next stage of the research, we studied changes of glutathione reductase (GR) in animal serum. Analysis of the results indicated that 8hr after the Con-A injection, the GR level was reduced in a statistically significant manner (p<0.01). Throughout the experiment, Plaferon LB maintained GR level at a certain level but did not reduce it to the normal one (Diag. 5). Assumably, Plaferon LB keeps GR at a level necessary for the proper operation of mitochondria.

Similar to the activity of Plaferon LB, dexamethasone kept GR levels high over the experiment. However, at 8hr Plaferon weakened the GR activity significantly (p<0.01).

The results of our study fully correspond to the data of other studies that indicated an increase in GR activity in cirrhosis and hepatocellular carcinoma compared to the control tissue (Czeczot et al., 2006). At the cellular level, GSH homeostasis is ensured by the balance of biosynthesis, consumption, oxidation and export. The homeostasis disorder can affect the liver capacity to protect itself from oxidative stress (Fernandez-Checa et al., 1997).

At the last stage of our experiment, we studied mitochondrial activities of hepatocytes by means of MTT test. Spontaneous stimulation index (SI) of intact mice hepatocytes was equal to 1.14±0.2. In Con-A-induced mild hepatitis, at 24hr this parameter was 1.5±0.3 (compared to the control figure, p<0.05), while at 48hr it amounted to 1.16±0.11.

Plaferon LB influence 24hr after the Con-A-injection did not differ from I group. However, at 48hr the figure increased (2.06±0.12) so that it became statistically significant to exceed the analogous figure for the intact mice (p<0.01). It is noteworthy that in case of dexamethasone mitochondrial function was inhibited at 48hr (Diag. 6).

Thus, given the results, it can be concluded that Plaferon LB strengthens mitochondrial function and boosts hepatocyte proliferation stimulating regenerative processes.
Hampton and Orrenius demonstrated that prolonged or excessive oxidative stress inhibits caspase activities and accordingly blocks apoptosis (Hampton et al., 1998). In order to find out possible relationship between hepatocyte apoptosis and changes of redox potential, as well as to elucidate pro-caspase activity of Plaferon LB potentially arising from its anti-oxidant nature, we studied NO and glutathione reductase levels in the blood, as well as the hepatocyte mitochondrial activity.

Metabolic changes occurring in various organs eventually come down to changes in the blood. This is why the blood is the most suitable object to study metabolic processes taking place in the organism. Our study conducted on the animal blood through the Con-A induced hepatitis gave the picture that is characteristic of oxidative stress.

As seen from Diag. 3, NO levels are elevated at 8 hours after Con-A treatment without any further significant change till the end of the experiment. Administration of dexamathasone does not affect NO levels in Con-A-induced mild hepatitis. Thus, it lack an antioxidant capacity and hence the power to reduce lipid super-oxidation.

Treatment by Plaferon LB significantly decreases NO level at 24h compared to I and III groups. However, under influence of Plaferon LB the synthesis of nitric oxide was significantly intensified at 48h (Diag. 4), the stage that coincides with regenerative phase.

It is known from the previous studies that nitric oxide participates in the regulation of the apoptotic process. It exhibits both anti- and pro-apoptotic qualities. The anti-apoptotic activity proceeds via two main mechanisms: cGMP-dependent and cGMP-independent. By stimulating production of cGMP, NO induces activation of soluble guanylyl cyclic which protects cells against apoptosis (Kim et al., 1997). This type of mechanism is not fully studied yet. The second one – cGMP-independent mechanism – involves inhibition of lipid super-oxidation, peroxyl radical bonding, Bcl-2 cleavage, cytochrome c release (Kim et al., 1998) and induction of proteins such as Hsp70 and Bcl-2 (Moss et al., 1997; Genaro et al., 1995). Besides, NO expresses its protective nature by S-nitrosilating proteins. By S-nitrosilating 62nd cistern of p50 and p65 proteins, it can inhibit activation of NF-xB. NO donors inhibit activation of NF-xB by TNFα by stabilization of IκB-α and increasing expression of its gene. Due to high content of cisterns, caspases represent ideal targets of s-nitrosilation.

It is also interesting to examine influence of NO on regulation of NF-xB and inflammation. Its mechanism is not quite known yet. However, both positive and negative effects have been described. These activities depend on NO levels, redox status, stimulus and cell type (Kim et al., 1997). Endogenously produced by eNOS and/or nNOS, small quantities of NO, can play a crucial role in modulation of NF-xB activation in the presence of various stimuli.

It has been shown that in vitro and in vivo iNOS prevented TNF or Fas induced apoptosis (Bogdan et al., 2001). Even more, in iNOS deficient mice in vivo liver regeneration was inhibited.

Besides, Sass et al. (2001) could not find 3-nitrotyrosine - the marker of tissue injury by NO - in cytoprotective effects. Even more, it can be deduced that this enzyme at least partially protects the cells.

According to our results, Plaferon LB modulates NO levels. At 24hr, it reduces NO levels (p<0.01) to stop expansion of destructive activities, while at 48hr it raises inductible NO levels (up to the threshold concentrations) to accelerate proliferative processes.

The intracellular concentration of reactive oxygen species (ROS) is tightly regulated by multiple defense mechanisms involving ROS scavenging enzymes and small antioxidant molecules. Among these antioxidant systems acting as antioxidants or scavengers are glutathione and GSH dependent enzymes, which are one of the protective mechanisms vs. oxidative damage, both in the circulation and in various tissues, including liver.

The total intracellular glutathione (GSH) concentration varies considerably, especially in the liver, and hepatic GSH can dramatically decrease as a result of drug metabolism after oxidative stress,
Apart from caspase-3, activity of caspase-8 was assessed too. Changes in its levels were similar to those of caspase-8. Namely, caspase-8 levels soared (p<0.01) 8 hr after the Con-A injection but decreased at 24 hr and 48 hr without reaching the normal value. (Diag. 3).

Next we elucidated that Plaferon LB was raising caspase-8 levels at all levels: at I and II time points these increases were not statistically significant; at III time point, however, the change was prominent compared to the control group (p<0.01). The results of our analysis showed that the treatment with dexamethasone tends to suppress caspases without statistically significant effect (Diag. 3). In contrast, Plaferon LB was clearly pro-apoptotic in the regenerative phase of mild hepatitis as it boosted caspase-3 and -8 activities significantly.

As an additional instrument, TUNEL method was employed to study apoptotic processes in liver. The results of the study revealed that administration of Plaferon in experimental mild hepatitis model increases the number of apoptotic hepatocytes at 48 hr but not in previous time points. One more noteworthy fact was that dexamethasone did not have similar effects (Table 1).

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Healthy</th>
<th>Concanavalin-A</th>
<th>Concanavalin-A+PLB</th>
<th>Concanavalin-A+Dex</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 hr</td>
<td>54.23 ± 9.09</td>
<td>154.39±15.22</td>
<td>180.39±17.2</td>
<td>164.39±15.02</td>
</tr>
<tr>
<td>24 hr</td>
<td>“-“</td>
<td>76.41±12.12</td>
<td>82.34±11.15</td>
<td>86.14±9.11</td>
</tr>
<tr>
<td>48 hr</td>
<td>“-“</td>
<td>75.41±10.11</td>
<td>280.23±15.21</td>
<td>84.12±9.10</td>
</tr>
</tbody>
</table>

The excessive apoptosis is identified in acute and chronic viral hepatitis, alcoholic and non-alcoholic hepatitis, cholestatic liver disease, Wilson’s disease, and graft versus host disease (GVHD). Sustained apoptosis is linked to the development of hepatic fibrosis. In contrast, insufficient apoptosis has been associated with development and progression of tumors in liver and biliary tree. Furthermore, the antiviral effect of interferon may be mediated through the induction of apoptosis (Castelli et al., 1998).

Our studies allow us to suggest that Plaferon LB could be used in case of a variety of liver injuries such as acute viral hepatitis and tumors stemming from opposing mechanisms. It seems Plaferon LB modulates apoptotic processes differently depending on the type of disease and its severity. In case of severe liver injury, it acts in an anti-apoptotic manner (S. Chochua et al., 2006), whereas in mild immune-mediated liver injuries it becomes pro-apoptotic.

Thus it can be concluded that Plaferon LB is a modulator of apoptosis.

It is known that apoptosis promoting enzymes – caspases are most susceptible to oxidative stress. It is generally recognized that in many cells presence of small reactive oxygen species (ROS) concentrations initiate apoptosis, while their super-excessive levels cause necrotic alterations. Critical antioxidants are characterized by different redox potential. While small amounts of oxidants lead to oxidation of thiols in certain proteins, they leave other proteins unaffected. Small concentrations of oxidants promote apoptosis by altering a redox status via activation of pro-apoptotic factors such as JNK. Besides, they...
preserved. In case of coupled injection of Con-A and Plaferon LB at this time point, the overall majority of hepatocytes were subject to vacuolar dystrophy. However, trabecular structure of the lobes remained completely unchanged. Also, lymphoid infiltration sites were rare and insignificant.

24 hr following the injection of Con-A, intensive changes in the liver of the control group animals persisted. Most hepatocytes underwent vacuolar dystrophy and necrotic transformation. With dexamethasone, the number of double-nucleus hepatocytes increased prominently. What is more, destructive alterations were intensifying. However, the case was not characterized by lymphoid infiltration. Influenced by Plaferon LB, most hepatocytes were still undergoing dystrophical changes but necrotic sites did not develop.

48 hr following the experiment, in the control group of animals some sites seemed to have retained the normal liver trabecular structure suggesting that active regenerative processes were underway. At the same time point, some normal hepatocytes had also emerged. With dexamethasone, the picture hardly was different. Under the influence of Plaferon LB though, the normal constitution of the lobes is basically re-gained. Necrotic sites and lymphoid infiltrates did not emerge at all. Thus, in Con-A induced hepatitis Plaferon LB and dexamethasone protect the liver tissue against profound destructive changes. Besides, in contrast to the effect of dexamethasone, Plaferon LB speeds up recovery of the normal liver tissue structure.

As noted above, both drugs – Plaferon LB and dexamethasone – protect liver tissue against immune injury. It is known that corticosteroids realize their effect via inhibiting the expression of genes for IL-1, IL-2, IFN-γ, and TNF-α. Assumably, the effects of Plaferon LB too derive from the shift in the cytokine balance (Chikovani, 1997; Khetsuriani et al., 1997). It should be noted that Plaferon LB exhibits not only immunomodulatory but also anti-inflammatory and anti-apoptotic effects too. The previous studies reported strong anti-apoptotic quality of Plaferon LB in acute severe hepatitis models and clinical trials. Namely, Plaferon LB inhibits hepatocyte apoptosis in the experimental model of LPS induced severe hepatitis. Metreveli showed that in patients with mild and moderately severe hepatitis Plaferon LB led to full recovery in 96.9%. Furthermore, it significantly reduced probability of the chronic state development augmenting protective immunity formation (Metreveli et al., 1999). Based on this and other studies, we have proposed the hypothesis that in mild hepatitis the protective effects of Plaferon LB were related to its pro-apoptotic quality.

Caspase-3 plays a characteristic feature of the apoptotic process. Based on the nature of the caspase cascade, regulation of the apical caspases may be critical for the apoptotic cascade. Self-activation of caspase-8 leads to the direct activation of caspase-3 and cleavage of Bid (Li et al., 1999). According to the literature, the functional state of the transcriptional machinery decides whether apoptosis involves activation of caspase-3-like proteases or alternative signaling pathways in vivo might be of relevance for the immunopathology of the liver. Data on the activity of caspase-3 in Con-A-induced hepatitis is controversial. One study reported a significant increase of caspase-3 levels (Ding et al., 2004), while another was not able to detect them (Kunstle et al., 1999). Hence, at the next stage of the research, we studied activities of caspase-3 and -8 in vivo following Con-A injection and effects of immunomodulators, namely, Plaferon LB and dexamethasone on them. According the results of this stage, at the first time point from the Con-A injection in the control group a drastic increase in caspase-3 levels reaching the maximum for the whole experiment was observed. Then the level drops gradually but without re-gaining the normal value even at 48 hr (Diag. 2).

In case of Plaferon LB, caspase-3 levels did not appear influenced at I and II time points and did not differ from the similar parameter for the control group statistically significantly. However, at 48 hr the increase of caspase-3 activity was clearly detectable (p<0.01). In contrast, dexamethasone suppresses caspase-3 activity at I time point (p<0.01) but evens it up to the control group at subsequent two time points.
In acute liver injury, excessive apoptosis of hepatocytes causes development of fulminant liver failure and coma (Gremion et al., 2004; Kountouras et al., 2003), whereas its deficiency induces malignant transformation, viral latency and autoimmune disorders (Solary et al., 1996). By eliminating infected hepatocytes via apoptosis, the organism seeks to block and safeguard against the viral infection. However, the immune response. For instance, the HCV core protein regulates apoptotic process by either enhancing or inhibiting it.

Pharmacological substances that either inhibit or stimulate apoptosis, regulate production and activation of type I cytokines and/or stimulate activities of type II cytokines serve as potential tools for prophylaxis and treatment of liver injury (Okamoto et al., 1998; Hershkoviz et al., 1999; Okamoto, Kanda, 1999; Okamoto et al., 2000).

Plaferon LB is a mixture of peptides derived from the human placenta. Its effects on proliferative activity of HBs antigen stimulated lymphocytes in HBV patients depend on the stage of the disease progression. In the acute or chronic phases the drug respectively inhibits or enhances the process. Plaferon LB influences on the synthesis of cytokines, such as IL-1, IFN-γ and IL-4 (T. Chikovani et al., 1999).

In order to study the role of apoptosis in mild liver injury we used an experimental model of autoimmune hepatitis (Tiegs et al., 1992; Louis et al., 1997; Kato et al., 2001). Con-A causes infiltration of CD4+ T lymphocytes in liver and increases expression of CD95L, which in turn promotes development of cytotoxicity. Besides, TH1/TH2 cytokines play a crucial role in Con-A-induced hepatitis (Kosintini et al., 1998; Tieg, 1997; Trautwein et al., 1998; Kimura et al., 1999).

The biochemical results were confirmed by the morphological analysis. 8hr following the Con-A injection, the liver of the mice displayed changes characteristic of inflammation. A bigger part of the hepatocytes was undergoing vacuolar dystrophy. Furthermore, a few sites of focal necrosis were also visible. Cytoplasm of necrotic hepatocytes painted sharply eosinophilic. Liver parenchyma contained sites of lymphoid cell infiltration. The infiltration was most intensive at the portal spaces.

One hour following the Con-A injection, the mice were injected intramuscularly Plaferon LB, 0.9% physiological solution or the control drug (dexamethasone). Observations of the effects were made at three time points: 8, 24 and 48 hours after the induction of the hepatitis. The materials (liver, blood) taken at these points were analyzed with biochemical, immunohistochemical and immunological methods.

Results of the analysis showed that at the first time point (8hr) the level of ALT in the blood of the control mice appeared increased (Diag. 1). Regarding the mice treated with dexamethasone and Plaferon LB, ALT levels of both were similar and did not differ statistically significantly from the ALT level in the control group.

At the second time point (24hr), while difference between blood ALT levels of the control and the treated mice was not statistically significant, they all grew compared to the first time point. At the third time point (48hr), the trend of the ALT growth was sustained in the control group, whereas in dexamethasone-treated mice the trend had reversed and Plaferon LB-treated mice the parameter decreased in a statistically significant manner (p<0.01).

Considering the above, the healing effects of Plaferon LB appeared to be stronger than those of dexamethasone. These data speak in favor of the assumption that at the end of the observation period Plaferon LB seems to have accelerated recovery of normal liver structure.

The formalin-fixed paraffin-embedded material was used for morphological analysis. 8hr following the Con-A injection, the liver of the mice displayed changes characteristic of inflammation. A bigger part of the hepatocytes was undergoing vacuolar dystrophy. Furthermore, a few sites of focal necrosis were also visible. Cytoplasm of necrotic hepatocytes painted sharply eosinophilic. Liver parenchyma contained sites of lymphoid cell infiltration. The infiltration was most intensive at the portal spaces.

Under the influence of dexamethasone, 8hr following its injection most hepatocytes underwent vacuolar dystrophy but core trabecular structure of the lobes. Lymphoid infiltration of the portal spaces was insignificant. Sludge phenomenon showed up weakly. The shape of erythrocytes was mostly primary role. In acute liver injury, excessive apoptosis of hepatocytes causes development of fulminant liver failure and coma (Gremion et al., 2004; Kountouras et al., 2003), whereas its deficiency induces malignant transformation, viral latency and autoimmune disorders (Solary et al., 1996). By eliminating infected hepatocytes via apoptosis, the organism seeks to block and safeguard against the viral infection. However, the viral genome codes for proteins which are capable of inhibiting the apoptotic process, and enables the virus to escape the immune response. For instance, the HCV core protein regulates apoptotic process by either enhancing or inhibiting it.

Pharmacological substances that either inhibit or stimulate apoptosis, regulate production and activation of type I cytokines and/or stimulate activities of type II cytokines serve as potential tools for prophylaxis and treatment of liver injury (Okamoto et al., 1998; Hershkoviz et al., 1999; Okamoto, Kanda, 1999; Okamoto et al., 2000).

Plaferon LB is a mixture of peptides derived from the human placenta. Its effects on proliferative activity of HBs antigen stimulated lymphocytes in HBV patients depend on the stage of the disease progression. In the acute or chronic phases the drug respectively inhibits or enhances the process. Plaferon LB influences on the synthesis of cytokines, such as IL-1, IFN-γ and IL-4 (T. Chikovani et al., 1999). The above said make us believe that Plaferon LB could be effective in mild liver injury.

Concanavalin A (Con A) induced liver injury is a widely used experimental model of autoimmune hepatitis (Tiegs et al., 1992; Louis et al., 1997; Kato et al., 2001). Con-A causes infiltration of CD4+ T lymphocytes in liver and increases expression of CD95L, which in turn promotes development of cytotoxicity. Besides, TH1/TH2 cytokines play a crucial role in Con-A-induced hepatitis (Kosintini et al., 1998; Tieg, 1997; Trautwein et al., 1998; Kimura et al., 1999).
marked deoxynucleotides at 37 degrees for 1 hour. The reaction was blocked by washing buffer. The finished samples were examined under the light microscope.

Measurement of NO and activity of glutathione reductase in serum
NO in the serum was measured by a microplate assay using Griess reagent, which produces a chromophore with the nitrite. Briefly, 100 µl of supernatants were removed and incubated with 100 µl of Griess reagent in a 96-well plate. The plate was incubated for 10 min at room temperature. Nitrite production was quantified spectrophotometrically using an automated colorimetric procedure. Absorbance at 540 nm was measured using a microplate reader (Multiscan, Lab Systems, Finland). All samples were assayed in triplicate.

Analysis of serum GR activity was performed by using glutathione reductase assay kit (Sigma, USA). The activity can be measured by the increase in absorbance caused by the reduction of DTNB [5,5'-dithiobis(2-nitrobenzoic acid)] at 412 nm (Colorimetric assay).

Mitochondrial function assessment by MTT
Mice were killed by cervical dislocation. Livers were removed in sterile conditions and hepatocytes were isolated. Then the cells were suspended in RPMI-1640 medium at a concentration of 1×10⁶ cell/ml. The cell suspension was (10+10³) seeded to a 96-well culture plate simultaneously. The cultures were incubated at 37 °C in an atmosphere of 5% CO₂ for 2h. Two hours before completion, 10 µl of MTT (5 g/L) was added to each well. The absorbance was measured on microplate reader (Multiscan, lab systems, Finland).

Assessment of caspase-3 and caspase-8 levels in hepatocytes
Both caspase-3 and caspase-8 activities were determined with caspase assay kits (Biovision, USA) according to manufacturer’s instructions. Liver samples were homogenized in caspase lysis buffer. Briefly, homogenates were incubated with either caspase-3 (Ac-DEVD) or caspase-8 (Ac-IETD) substrate that is linked to p-nitroaniline (pNA) at 37°C. Cleavage of the substrate by caspase will release p-NA that is then determined by a spectrophotometer (Multiscan, Labsystems, Finland) at 405 nm, and the optical density of the released p-NA is proportional to caspase activity present in homogenates. The optical density reading during 90-minute incubation at 37°C was taken every 5 minutes with respective calculation of caspase activity. The data was expressed as M±SD, and P<0.05 was considered to be significant.

Analysis of serum ALT activity
Blood samples were obtained by puncture of heart with heparin. Liver specimen was fixed immediately in formalin for histological examination with HE stain. The degree of liver injury was assessed by determination of serum alanine aminotransferase (ALT) activity (Sigma). The optical density was measured at 530 nm. The data expressed as M±SD, and P<0.05 was considered to be significant.

Statistical Analysis
The data were analysed by using Statistica 6.0 (Statsoft, Mineapolis, USA).

RESULTS AND DISCUSSION
Viral hepatitis is the gravest problem in hepatology. In terms of prevalence rates, severity of complications, lethality rates and chronization, Hepatitis B (HBV) and C (HCV) stand out of the range. Yearly Hepatitis B kills 2 mln people worldwide. According to the WHO estimates, in the next 10-20 years, the death toll of liver cirrhosis and hepatocellular carcinoma resulting from chronic Hepatitis C will rise by 60-68%.

It is known that both – Hepatitis B and C - viruses are hepatotropic but not directly cytopathic (Lok et al., 2001; Liang et al., 2000). According to epidemiological studies, 80-85% of persons with HCV cannot be gotten rid of virus fully. This factor subsequently leads to the development of chronic hepatitis (Liang et al., 2000; Lauer et al, 2001). In this regard, apoptosis of hepatocytes may play a significant role in the pathogenesis of chronic liver disease. Therefore, it is of great importance to investigate the mechanisms and pathways leading to apoptosis in chronic hepatitis.
**Primary conclusions submitted to the defence**

- In the experimental model of mild hepatitis, administration of Plaferon LB accelerates regenerative processes in the affected liver.
- In the experimental model of Con-A-induced mild hepatitis, administration of Plaferon LB enhances levels of caspase-3 and -8 when liver recovers to its normal morpho-functional status.
- At various stages of the experiment, Plaferon LB has varied effects on plasma NO levels.
- In experimental mild hepatitis, administration of immunomodulators modulates levels of glutathione peroxidase in blood.

**Approbation of the research work**

The dissertation is approbated on the expanded session of Biomedicine and Biotechnologies divisions of the Institute of Medical Biotechnologies on 3 May, 2006. The dissertation is recommended for its public defence.

**Key conclusions of the dissertation were reported to:**


**Publications:** 6 papers have been published

**Size and structure of dissertation:**

The dissertation has 101 printed pages. It is divided in Introduction, Literature Review, Materials and methods, Own Research, Research Result Analysis, Conclusions, Practical Recommendations and References. It contains 1 table, 6 diagrams and 13 figures. The references section lists 185.

**MATERIALS AND METHODS**

The experiment involved 50 adult mice each weighing 20-25g. All of the mice were grown in similar conditions.

**Experimental model of liver immune injury**

Acute liver injury was induced by injecting Con A (Serva, USA) 2.5 mg/kg via the tail vein. 15 min after the injection the mice were divided into three groups: I group (15 mice) - 0.2 ml of saline solution; II group (15 mice) - 0.25 mg/kg of Plaferon LB; and III group (15 mice) - 4 mg/kg of dexamethasone. All these injections were intramuscular. After 8, 24, 48 hours following the administration of Con A, the mice were bled, euthanized with chloral hydrate anaesthesia, their abdomens opened by a midline incision, and sections from the liver were excised for histopathologic examination.

**Morphological methods**

To investigate under the light microscope, the experimental material (liver) was fix perioperatively 3 hours. The tissue sections were deparafinized and dehydrated. Then the material was stained with haematoxylin and eosin. The sections were dehydrated again for 5 min. Next, TDT was added before the incubation in humid box with biotin-
selectively kill malignant cells in tumors, have the potential to provide a powerful tool for the treatment of liver disease. Due to peculiarities associated with pathogenesis of acute viral hepatitis, several authors give preferences to drugs of immunotrophic and antioxidant nature for the treatment of the disease (Schwarz, 1996; Zein et al., 1998; Metreveli, 1999; Pavliashvili, 1999). Considering this, we were interested to study immunomodulatory drugs such as Plaferon LB.

Plaferon LB was developed by Prof. Vladimir Bakhutashvili at the Institute of Medical Biotechnology. Together with the immunomodulatory effects, this drug exhibits anti-inflammatory, anti-ischemic and antioxidant qualities (Chikovani, 1997; Chavchavadze, 1999; Rukhadze, 1999; Gongadze, 2004). In various pathologies, it turned out to protect liver against metabolic and morphological changes (Pashalashvili, 1995; Pavliashvili, 1999; Rukhadze et al., 1998; Chikovani et al., 1999). Even more, Plaferon LB anti-apoptotic effects of has also been indicated elsewhere. The exact mechanisms of the drug hepatoprotective effects however remain to be studied.

Presently, various experimental models are employed in studies of liver immune injury mechanisms. Con-A-induced liver injury is identical to clinical liver injury (Tiegts et al., 1992), and it is widely used in studies of the disease pathogenesis and the search for new ways for treating hepatitis.

Considering all the above said, we saw it reasonable to study the role of apoptosis in the experimental model of Con-A-induced mild hepatitis, and the mechanism of influence that immunomodulatory drugs have on it.

**Aim of the study**

The aim of our research consisted in the study of the apoptotic changes and immunomodulatory effects on them in experimental mild hepatitis.

**To achieve the goal, we set the following specific tasks:**
1. Development of the Con-A induced experimental mild hepatitis model (identification of the optimal Concanavalin A dose range judging by changes in transaminase levels);
2. Investigation of effects of immunomodulators (Plaferon LB, dexamethasone) on plasma transaminase levels in Con-A induced experimental mild hepatitis;
3. Study of caspase-3 and caspase-8 activities and effects of the immunomodulators on them in Con-A induced experimental mild hepatitis;
4. Analysis of contribution of the immunomodulators to liver histological changes induced in Con-A induced experimental mild hepatitis;

**Novelty of the research**

The present study showed first time that the mild form of hepatitis entails enhanced caspase-3 and -8-mediated apoptosis. It has been found that the use of immunomodulators in Con-A-induced mild hepatitis prevents pathological aberrances in liver. At the same time Plaferon LB facilitates fast mitigation of alterations occurring in these tissues prior to treatment.

It has been shown first time that in mild hepatitis Plaferon LB increases levels of caspases -3 and -8 meaning that the drug augments removal of impaired cells through apoptosis. In this way, the cell disposal prevents the disease from turning chronic.

**Practical significance of the research**

The results of the present study indicated pro-apoptotic activities of Plaferon LB and clarified certain molecular mechanisms of its activities. The findings are expected to serve as a basis for its application in the treatment of acute viral hepatitis, as well as chronic liver infections.
At the beginning of XXI century, despite remarkable advancements in medicine human viral hepatitis remains as a severe global problem. According to the World Health Organization (WHO), the number of people suffering from viral hepatitis is on the continuous rise and tops 1 billion. Furthermore, almost one third of the world population has been or is infected with various types of hepatitis. Every year up to 2 mln people die from this disease or complications associated with it.

In Georgia, in 2004 one thousand new cases of viral hepatitis B and C have been registered. Infection with hepatitis B and C poses two major threats for the human organism: first, in case of fulminant state (liver coma, fulminant liver), the disease has a high lethal outcome (90%); second, the process turns chronic and eventually ends in liver cirrhosis or hepatocellular carcinoma. In 80% of cases of infection with both Hepatitis B and D hepatitis becomes fulminant.

Recovery from acute viral hepatitis is a very long tedious process. This is especially true for Hepatitis C with its most complicated course of development. Currently more than 300 mln worldwide suffer from and another 350 mln is infected with B Hepatitis. Among those with chronic form, 25% turns Hepatitis C into cirrhosis in 50% of cases. What is more, some times chronic hepatitis escapes diagnosis and the disease is often compared to AIDS in terms of severity. Thus, effective treatment of viral hepatitis is of crucial importance.

Mechanisms of liver injury in autoimmune hepatitis and those caused by non-cytopathic viruses (HBV, HCV) are not fully studies. However, the role of T-cell mediated immune responses has been revealed recently (Ando et al., 1997). Massive elimination of infected hepatocytes by protective mechanisms of the body is what makes the illness so severe. Hence, an adequate immune response allows mild, often asymptomatic acute phase of the disease, the state which in turn may lead to further chronization and even hepatocellular carcinoma or cirrhosis. Quick and timely removal of the virus-infected cells via apoptosis is the key to maintenance of liver functionality. In a healthy organism, dead cells are swiftly replaced with mitotically derived new cells. This way cellular homeostasis is constantly maintained in organs. In the pathologies however, proliferation versus apoptosis balance is disturbed leading to liver disease. In acute injury, excessive and/or constant apoptosis can lead to liver function and reperfusion disorders.

In the Georgia National Research Center of Transplantology and Artificial Organs, one of the problems of organ transplantation is the immune response. Excessive apoptosis may cause acute and chronic viral hepatitis, alcoholic and non-alcoholic hepatitis, fulminant hepatitis, chronic hepatitis, cirrhosis and liver fibrosis. Necrosis of hepatocytes features inflammation in liver tissues, infiltration of liver parenchyma by lymphoid and monocyte (p<0.01) cell population. In addition, apoptosis as death of hepatocytes is associated with liver fibrosis and cirrhosis, chronic hepatitis and liver transplantation, etc. Excessive apoptosis may lead to development of chronic hepatitis, cirrhosis, hepatocellular carcinoma and liver fibrosis.

**GENERAL CHARACTERISTICS OF THE STUDY**

**Importance of the subject**

At the beginning of XXI century, despite remarkable advancements in medicine human viral hepatitis remains as a severe global problem. According to the World Health Organization (WHO), the number of people suffering from viral hepatitis is on the continuous rise and tops 1 billion. Furthermore, almost one third of the world population has been or is infected with various types of hepatitis. Every year up to 2 mln people die from this disease or complications associated with it.

In Georgia, in 2004 one thousand new cases of viral hepatitis B and C have been registered. 64% and 35% of the Hepatitis B cases fall on respectively acute and chronic forms. In case of Hepatitis C though, over a half of cases (app. 55%) present a chronic form. Even more, 5-7% of the population carries various hepatitis viruses. Infection with hepatitis B and C poses two major threats for the human organism: first, in case of fulminant state (liver coma, fulminant liver), the disease has a high lethal outcome (90%); second, the process turns chronic and eventually ends in liver cirrhosis or hepatocellular carcinoma. In 80% of cases of infection with both Hepatitis B and D hepatitis becomes fulminant.

According to international leading hepatology centres, the risk of HBV infection becoming fulminant is seen in 80% of infection cases (Grob et al., 1998). As the data by the WHO indicate, potential of the disease chronization is fairly high. Around 2 mln people are estimated to have suffered from and another 350 mln is infected with B Hepatitis. Among those with chronic form, 25% turns into hepatocellular carcinoma (Alter et al., 1995). Today more than 1mln persons in the world die from hepatocellular carcinoma. One of the reasons for the chronic HBV infection persistence is the fact that 100% of hepatocytes become infected, and infected cells are not cleared off the liver. Recovery from acute viral hepatitis is a very long tedious process. This is especially true for Hepatitis C with its most complicated course of development. Currently more than 300 mln worldwide suffer from Hepatitis C (Alter, 1995). Chronic HCV infection develops in up to 80% of cases and, of these, it progresses to cirrhosis in 50% of cases. What is more, some times chronic hepatitis escapes diagnosis and the disease shows up only in the cirrhosis stage. Hepatitis C patients quite often “enjoy” a false recovery evidenced by negative results of blood biochemical analyses (Scheuer et al., 1990). This phenomenon may last from few months to a number of years. This is why it is often compared to AIDS in terms of severity. Thus, effective treatment of viral hepatitis is of crucial importance.

Mechanisms of liver injury in autoimmune hepatitis and those caused by non-cytopathic viruses (HBV, HCV) are not fully studies. However, the role of T-cell mediated immune responses has been revealed recently (Ando et al., 1997). Massive elimination of infected hepatocytes by protective mechanisms of the body is what makes the illness so severe. Hence, an adequate immune response precludes virtually any chance of chronic hepatitis development. In contrast, weakness of immune response allows mild, often asymptomatic acute phase of the disease, the state which in turn may lead to further chronization and even hepatocellular carcinoma or cirrhosis.
დიაგრამა 5 Diagram 5 Activity of glutathione reductase in serum at experimental mild hepatitis

დიაგრამა 6 Diagram 6 Mitochondrial activity in experimental mild hepatitis by MTT assay
cnobilia, rom kaspazebis gaaqtiureba apoptozuri procesis damaxasia Tebeli ni Sania. apikaluri kaspazebis regulacia SeiZleba gadamwyveti faqtori iyos am procesSi. kaspaza 8-is TviTgaaqtiureba iwvevs kaspaza-3-is pirdapir gaaqtiurebasa da Bid–is gaxlecvas (Li et al., 1998). literaturuli monacemebis mixedviT, RviZlis imunopaTologiis dros transkrifciuli meqanizmis funqciuri mdgomareoba gansazRvravs imas, Tu romeli – kaspaza-3-is Tu alternatiu li – gziT warima rTeba apoptozuri procesebi. Con-A–inducirebuli hepatitis dros kaspaza-3-is aqtiurobis Sesaxeb monacemebi winaaRmdegobrivia. kerZod, erT-erTi kvlevis mixedviT, kaspaza-3-is aqtiuroba mniSvnelovnad izrdeba (Ding et al, 2004). xolo sxva gamokvlevis mixedviT, is ar vlindeba (Kunstle et al, 1999).

aqedan gamomdinare, kvlevis Semdeg etapze Cvens mier Seswavlili iqna kaspaza 3 da 8-s aqtiuroba kaspaz a 3-is msgavsi aRmoCnda. kerZod, Con-A–s Seyvanidan 8 saaTis Semdeg misi aqtiurobis mkveTri mateba SeimCneva. 24 da 48 sT-isTvis ki kaspaza 8-is maCvenebeli klebulobs, Tumca normas mainc ver ubrundeba (diag. 3).

plaferoni lb-s gamoyenebis SemTxvevaSi eqsperimentis I da II vadaze kaspaza 8-is aqtiuroba kidev ufro izrdeba, Tumca igi sarwmunod ar gansxvadeba sakontrolo jgufis maCveneb lisgan. 48 sT-ze ki sakvlev jgufTan SedarebiT kaspaza 8-is aqtiuroba statistikurad sarwmunod matulobs (p<0,01). Cvens mier miRebuli Sedegebis analizi cxadyofs, rom imunosupresanti deqsametazoni kaspaza 8-is aqtiurobaze gavlenas ar axdens. deqsametazonis gavleniT kaspazebis inhibirebis tendencia vlindeba eqsperimentis pirvel vadaze, Tumca is statistikurad sarwmunod ar gansxvavdeba janmrTeli Tagvebis analogiuri maCveneblebisagan (diag. 3).
**Diagram 1**

Change in ALT levels in Con-A-induced mild hepatitis

- **Control**
- **Con-A**
- **ConA+PLB**
- **ConA+DEX**

**Diagram 2**

Changes of caspase-3 activity in Con-A-induced mild hepatitis

- **Control**
- **ConA**
- **ConA+PLB**
- **ConA+DEX**

ALT - ის დონის გამონაკლევად ტომებში ძალიას გამორჩევა ჰყავდა. ALT-ის დონის გამონაკლევად უფრო დაბლობია გამოვიწვიათ. ALT-ის დონის გამონაკლევად უფრო გამოვიწვიეთ მცირე უფრო სხვა დონეებთან. ამიტომაც Con-A ის ტომებში ძალიას გამოვიწვიათ.
1. ხელია იმუნური პასუხით განხორციელებული ტესტების დროს მასალაში გავარჩიებული ანალიზის წინასწარი გარემოზის აქციის ინფექციას ახორციელებს.

2. Con-A ინდუცებული მხარეები ტესტების ხანგრძლოვან პერიოდში შუამდგომლობით კვარცში მორეგულირებული ღირში ტესტების მასალაში (3 და 8) გაარჩევით ადგილები.

3. ხელია იმუნური პასუხით განხორციელებული ტესტების შემადგენელ მოდელში ინფორმაციით მოძრაობით ადგილს აქციის ტესტების ტესტების ახორციელებს და შესაბამისი ხელია იმუნური პასუხით ფაქტორში.

4. ალუათის შემთხვევაში შეტანის ტესტების ხანგრძლოვან პერიოდში შუამდგომლობით კვარცში მორეგულირებული ღირში გამოყენებით შესაბამისობა გამოყენებით.

5. Con-A ინდუცებული მხარეები ტესტების ხანგრძლოვან პერიოდში შუამდგომლობით კვარცში მორეგულირებული ღირში გამოყენებით შესაბამისობა გამოყენებით.

6. ალუათის შემთხვევაში შეტანის ტესტების შემადგენელ მოდელში ინფორმაციით მოძრაობით ადგილს აქციის ტესტების ტესტების ახორციელებს და შესაბამისი ხელია იმუნური პასუხით ფაქტორში.

7. ალუათის შემთხვევაში შეტანის ტესტების შემადგენელ მოდელში ინფორმაცით მოძრაობით ადგილს აქციის ტესტების ტესტების ახორციელებს და შესაბამისი ხელია იმუნური პასუხით ფაქტორში.

8. ალუათის შემთხვევაში შეტანის ტესტების შემადგენელ მოდელში ინფორმაცით მოძრაობით ადგილს აქციის ტესტების ტესტების ახორციელებს და შესაბამისი ხელია იმუნური პასუხით ფაქტორში.

9. ალუათის შემთხვევაში შეტანის ტესტების შემადგენელ მოდელში ინფორმაცით მოძრაობით ადგილს აქციის ტესტების ტესტების ახორციელებს და შესაბამისი ხელია იმუნური პასუხით ფაქტორში.
რომის ბრძოლის სახელმწიფოდ (ROS) უმრავლებენ კრიუალიზაციას შერევით ქრონიკული სავალდემოლითური პარანოზა მყვან, რომლის შემდეგ თანხმობა ყველაზე აღსანიშნავი დედამიწაში. თუმცა, როგორც კრიუალიზაცია ფაქტორი დაიცვეს ქრონიკული სავალდემოლითური პარანოზა, რომლის შემდეგ ის ფაქტორი უკვდავი ქმეროვნების ვრცელობა, თუმცა შეიძლება ქრონიკული სავალდემოლითური პარანოზა გამოიყოს სხვები მეცნიერული ფორმის შესაძლო, ქრონიკული ჰამაგრება ტიპსი. მათი ნაწილი კრომის შესახებ მუშაობის შემთხვევაში, როგორც ბოლო და დამატებით გამოიყო (Schwabe და ხელმწიფოდ, 2006).

ამოცანისთვის შემდეგ ქრონიკული სავალდემოლითური პარანოზის, ნაყოფის გაფართოებით, ლოპოტა ფაზის შემდეგ შეიძლება შეიცვის თუთის შეფასების გზით, ინტერნაციონალური სტანდარტების შემდეგ, როგორც გამოიყო მისი ნაწილი, ლოპოტა შედგინებით და მრავალი მექანიზმი შორის, რომლებსაც მათ გამოიყო (Sorin და ხელმწიფოდ, 2006), მათში GSH ჰამაგრება და მიპრეტინ თურის, გვლით მიარებით გამოიყო. მათში შეიძლება შიანდა გამოიყო ბრძოლის შემთხვევაში, რომლებიც დამატებით გამოიყო (Schwabe და ხელმწიფოდ, 2006).

გვლით მიარებით შემთხვევაში შეიძლება შიანდა გამოიყო ბრძოლის შემთხვევაში, რომლებიც დამატებით გამოიყო (Schwabe და ხელმწიფოდ, 2006). სქემათის ფაზის აღმოჩენით, მათში შეიძლება შიანდა გამოიყო ბრძოლის შემთხვევაში, რომლებიც დამატებით გამოიყო (Schwabe და ხელმწიფოდ, 2006).

განსაკუთრებით, რომ გვლით შემთხვევაში შიანდა გამოიყო ბრძოლის შემთხვევაში, რომლებიც დამატებით გამოიყო (Schwabe და ხელმწიფოდ, 2006). სქემათის ფაზის აღმოჩენით, მათში შეიძლება შიანდა გამოიყო ბრძოლის შემთხვევაში, რომლებიც დამატებით გამოიყო (Schwabe და ხელმწიფოდ, 2006).

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