

USE OF LIPOSOMES FOR EXPERIMENTAL CHRONIC
LIVER INSUFFICIENCY THERAPY

Rinat A. Mukhamadiyarov, Nelly L. Vorontsova

Experimental and Clinical Cardiology Department, Research Institute for Complex Issues of
Cardiovascular Diseases SB RAMS, 6 Sosnovy bulvar, Kemerovo 650002 Russia

Abstract

The aim of the study was to evaluate the use of lecithin/cholesterol liposomes in experimental chronic liver insufficiency (CLI) therapy. The effects of the empty liposomes, ATP and phosphocreatine-containing liposomes and α -tocopherol and superoxide dismutase-containing liposomes were studied. It was found that all the liposomes decrease serum concentrations of liver damage enzyme markers and reduce oxidative stress.

Key words: *liposomes, liver insufficiency, lipid peroxidation.*

1. INTRODUCTION

Liposomes are lipid membrane-bound vesicles (Barsukov, 1998). Liposomes, being a convenient model of elementary biological membrane, are widely used in fundamental research and are known for their potential for practical application in pharmacology, medicine, cosmetology and food industry.

A delivery of a variety of molecular complexes to a diseased organ or tissue (Barsukov, 1998; Seifulla, 2010; Allen, 2007; Torchilin V.P., 2005, 2010) is quite a promising medical application. Chronic liver insufficiency is a very common condition, which develops if a patient has some chronic liver diseases. One of the reasons for CLI development is the venous congestion caused by circulatory impairment due to heart failure or Chiari-Budd syndrome (Boyarinov, 1999; Abraldes, 2006). Hypoxia and the derangements of inflow and outflow metabolite transport induce lipid peroxidation processes, which constitute an important stage in CLI pathogenesis. (Boyarinov, 1999; Sergeeva, 2006). Lipid peroxidation is associated with the destruction of essential phospholipids and lecithin as the most important one (Bilenko, 1989).

A characteristic feature of liposomes is that they can be rapidly - within 1 or 2 hours - absorbed by *reticuloendothelial* system, particularly by the liver (Barsukov, 1998) and the intravenous administration of liposomes can be regarded, accordingly, as a method of target drug delivery. Thus, one of the possible ways to treat liver disorders is to use liposomes to deliver drugs to diseased liver cell membranes; moreover, lecithin, which occupies the outermost *part* of the *liposome*, *can act as a substitute for the molecules damaged during lipid peroxidation.*

The aim of the study was to evaluate the efficacy of *liposomal drug use in experimental chronic liver insufficiency therapy.*

2. MATERIAL AND METHODS

2.1. Liposomal drugs

The study used 3 types of liposomes and the contents of lipid bilayers and liposomal inner compartments are shown in Table 1. Liposomes were prepared by forcing suspensions of lipid through

polycarbonate filters with the LIPEX extruder (Lipex Biomembranes Inc., Canada). Before the extrusion Heidolph rotary evaporator (Germany) was used in order to obtain a film of lipids consisting of egg lecithin (Lipoid, Germany) and cholesterol (Sigma-Aldrich) in a 7 to 5 ratio on the wall of an one-liter flask. Alpha-tocopherol was added to the film of lipids for antioxidant-containing liposomes.

Table 1.

Liposomal contents in the given experiment

Type of liposomes	Lipid bilayer. Molar ratio of the contents Lecithin:Cholesterol:Alpha-tocopherole	Liposomal inner compartment
Empty liposomes (EL)	7:5:0	0.9% NaCl
Liposomal macroergic (MPL) phosphates	7:5:0	0.9% NaCl, 2% ATP, 2% PCr
Liposomal antioxidants (AOL)	7:5:0.5	0.9% NaCl, 2 mg/ml SOD, 2 mmole GSH

ATP - adenosine triphosphate, PCr – phosphocreatine, SOD - superoxide dismutase, GSH – reduced glutathione

The film of lipids was then hydrated with an aqueous vehicle and shaken to obtain multilamellar vesicles. The aqueous vehicle for empty liposomes (EL) consisted of 0.9% NaCl; 2% ATP (Sigma-Aldrich) and 2% PCr (Sigma-Aldrich) were used to obtain liposomal macroergic phosphates (MPL) and 2 mg/ml SOD and 2 mmole GSH (Sigma), based on 0.9% NaCl, for liposomal antioxidants. The vesicles were then subjected to a freeze-thaw procedure repeated 10 times to improve hydration and hydrophilic drugs encapsulation.

The liposome suspension was extruded 10 times through Costar polycarbonate filters of 100 nm pore size. The purification of liposomes was done by gel-filtration chromatography using a sterile column filled with Sepharosa 2B (Sigma-Aldrich). Liposome-containing fraction was diluted in 0.9% NaCl until the lipid concentration reached 25 mg/ml.

2.2. Chronic liver insufficiency simulation by partial hepatic vein ligation

The experiments were conducted on **mature wistar male rats weighing 250-300g in accordance with the principles of the European Convention (Strasbourg, 1986) and the WHO Helsinki Declaration (1996).**

The rat abdominal cavity was opened under ether anaesthesia in aseptic conditions. CLI was simulated by the ligation of right and central hepatic veins distal to their entrance into the inferior vena cava. The ligation was done step by step during 2-3 min. CLI developed due to venous congestion within 8 weeks.

The experimental rats were administered with liposomal drugs, such as EL, liposomal antioxidants and liposomal macroergic phosphates (the contents are shown in Table 1), to treat the condition. 25 mg of lipids/kg were injected 15 times into the tail vein at 48h intervals.

In total, 4 groups of rats took part in the experiment: control rats (intact, injected with 0.9% saline) and rats with simulated CLI receiving either saline (CLI+saline), EL (CLI+EL), liposomal macroergic phosphates (CLI+MPL) or liposomal antioxidants (CLI +AOL). Each group included 10 to 12 rats.

2.3. Biochemical studies

The blood and the liver tissue were used for the study. The liver tissue was washed and frozen by placing it into the liquid nitrogen. The activity of organospecific enzymes in the blood *serum* and liver *homogenates was a criterion for liver damage. The liver tissue was cooled to 4 °C* to produce homogenates and centrifuged at 3000 rpm for 10 min. The activity of gamma-glutamyltransferase (GGT), sorbitol dehydrogenase (SORD) and arginase was measured by [spectrofluorimetric](#) methods using KONE DIAGNOSICS kits (Finland). Pseudochoolinesterase activity was assessed by its reaction with dithiobisnitrobenzoic acid.

The concentrations of [malondialdehyde](#) (MDA) –the main peroxidation product – were measured using a color reaction with [thiobarbituric acid](#) (Stalnaya, 1977) to evaluate the lipid peroxidation processes. Total antioxidant activity in the liver tissue was measured by a method based on the lower MDA production in arachidonic acid solution (Promyslov, 1990); besides, the catalase activity was measured (Aebi, 1984). All the [spectrofluorimetric](#) studies were carried out with Ultrospec plus [spectrophotometer](#) (Pharmacia, Sweden).

SPSS Statistics 6.0 software was used for statistical processing of the data. Data are presented as means± SD.

3. RESULTS

CLI+saline group showed a significant increase in cytolysis enzyme marker activity in the blood serum compared with the control group following partial ligation of hepatic veins (Table 2). [SORD](#), arginase and GGT activity increased by 87.2%, 21% and 61.7%, respectively. At the same time pseudochoolinesterase activity was found to decrease (Table 2). Serum MDA concentrations and antioxidant activity in this group did not differ from the controls. [SORD](#) activity in liver homogenates was found to decrease by 21%, the increase in MDA concentrations made up 83% and catalase and antioxidant activity decreased by 14% and 28%, respectively (Table 3).

The administration of all the liposomal drugs resulted in the decrease of serum liver enzyme activity compared with CLI+saline group and the effect depended on the drug type (Table 2). The maximum decrease in all the cytolysis markers under study was observed in CLI+AOL group; however, the activity of the given enzymes was still higher than in the control group. These changes in the enzyme activity can be regarded as an indicator of reparative processes in the liver. The administration of liposomal drugs allows better hepatocyte preservation and restores the structural integrity of a cell membrane.

Pseudochoolinesterase activity increased in CLI+MPL and CLI+AOL groups by 33% and 18%, respectively, compared with CLI+saline group.

Serum antioxidant activity decreased in CLI+EL group and increased in CLI+MPL and CLI+AOL groups following liposome administration (Table 2).

All the groups demonstrated a significant increase in serum MDA concentrations following liposomal drug administration compared with the controls and CLI+saline group. CLI+EL, CLI+MPL and CLI+AOL groups showed an MDA increase of 162%, 148% and 128%, respectively, compared with CLI+saline group, and this difference was statistically significant (Table 2).

Table 2.

Enzyme activity, MDA concentrations and antioxidant activity in the rat blood serum after simulating CLI and the administration of liposomes (15 injections at 48h intervals)

	SORD , mmole/ml·min	Arginase, mkmole /ml·h	GGT, U/l	Pseudocho- linesterase , U/l	Antioxidant activity , %	MDA, mkmole/l
Controls	19.23±0.95	2.59±0.11	28.86±1.17	427.8±12.56	3 [#] 3.28±1.32	6.54±0.35
CLI+saline	36.0±0.95*	6.14±0.09*	46.66±1.15*	241.8±5.24*	34.76±1.31	6.56±0.28
CLI+EL	32.34±0.99* [#]	4.78±0.19 [#]	41.10±1.59 [#]	227.8±8.9* [#]	23.33±1.08* [#]	17.21±0.68* [#]
CLI+MPL	33.19±1.23* [#]	3.36±0.14* [#]	36.71±1.67* [#]	321.3±9.06* [#]	37.53±1.46 [#]	16.3±0.70* [#]
CLI+AOL	28.38±0.98* [#]	2.86±0.10 [#]	34.77±0.96* [#]	284.3±9.52* [#]	31.11±0.86	14.9±0.24* [#]

* - statistical significance of the differences from CLI+saline group when P>0.05, [#] - statistical significance of the differences from CLI+saline group when P>0.05. [SORD](#) – sorbitol dehydrogenase, GGT - gamma-glutamyltransferase, MDA - [malondialdehyde](#). Groups: control animals (intact, injected with 0.9% saline) and animals with simulated CLI receiving either saline (CLI+saline), EL (CLI+EL), liposomal macroergic phosphates (CLI+MPL) or liposomal antioxidants (CLI+AOL). 25mg/kg of liposomal drugs were injected 15 times at 48h intervals.

Table 3.

[SORD](#) and MDA concentrations, catalase and antioxidant activity in the rat liver homogenates after liposomal drug administration

	SORD , Mole/g/h	MDA, mkmole/g	Catalase, mkmole/g min	Antioxidant activity, %
Controls	221.0±9.2	5.02±0.11	44.02±1.05	36.5±1.29
CLI+saline	175.6±6.6*	9.22±0.17*	37.62±0.56*	25.83±0.87*
CLI+EL	193.1±10.1 [#]	5.34±0.27 [#]	37.45±1.27*	27.84±0.75* [#]
CLI+MPL	217.7±8.2 [#]	8.26±0.32 [#]	35.70±1.10*	20.38±1.02* [#]
CLI+AOL	218.3±7.8 [#]	4.6±0.16 [#]	37.37±0.94*	35.15±1.10 [#]

The * and [#] signs stand for the same values as in Table 2.

Liver homogenates (Table 3) showed an increase in [SORD](#) activity by 10% and 24% in CLI+EL, CLI+MPL and CLI+AOL groups, respectively, compared with CLI+saline group. MDA concentrations in liver homogenates decreased by 42%, 10% and 50% in CLI+EL, CLI+MPL and CLI+AOL groups, respectively, after the liposomal drug administration. Liver antioxidant activity in CLI+EL and CLI+AOL groups increased by 8% and 36%, respectively, and decreased in CLI+MPL group by 20% compared with CLI+saline group. Catalase activity in all the treatment groups did not differ significantly from CLI+saline group.

4. DISCUSSION

The obtained data show that 8 weeks after partial ligation of hepatic veins the rats in CLI+saline group demonstrate a significant increase in the activity of serum enzyme markers of hepatocyte cytolysis, e.g. [SORD](#), arginase, GGT, compared with the control group. The activity of different enzymes showed a 1.5-2.5-fold increase which evidences the selective permeability defect of the plasma membranes of liver parenchymal cells and cell necrosis. At the same time this group was found to experience a decrease in pseudocholinesterase activity which can be regarded as a sign of *hepatocyte protein* synthesis inhibition. This is also proven by the decrease in [SORD](#) activity in liver homogenates. In general, all these results show that partial hepatic vein ligation leads to a deterioration in liver function and the impairment of hepatocyte structure and function. These changes can be regarded as signs of CLI caused by circulation impairment.

An increase in MDA, being an informative marker of peroxidation activity, and a simultaneous decrease in general antioxidant activity and catalase activity were registered in CLI+saline group compared with the control group. These data suggest the induction of lipid peroxidation in the liver cells and a concurrent antioxidant system depletion. The reason for lipid peroxidation induction in the given experiment can be energy deficiency and hypoxia caused by venous congestion.

The found changes in enzyme activity are similar to those in patients with mitral valve disease (Boyarinov, 1999; Sergeeva, 2006); therefore, our model of venous congestion in laboratory rats can be selected for liver dysfunction evaluation in the treatment of mitral valve patients.

Three types of liposomal drugs were used to treat CLI caused by venous congestion. EL are the liposomes containing lecithin - the most important essential phospholipid. Lecithin makes up to 50% of all the liver phospholipids and is one of the substrates for lipid peroxidation; therefore, EL can be regarded as a lecithin carrier to hepatocyte membranes or even a membrane "patch" for damaged cell membranes (Ipatova, 2005). Liposomal [macroergic phosphates](#) have ATP and PCr as parts of the inner compartment. These liposomes can be used to deal with energy deficiency due to hypoxia (Korb, 2008). Liposomal antioxidants contain alpha-tocopherol, SOD and GSH which present in different chains of antioxidant cell protection system (Bilenko, 1989). Liposomes are regarded as antioxidant carriers to the damaged cells in order to enhance antioxidant protection.

EL administration led to a decrease in the serum activity of the enzymes under study compared with CLI+saline group which tells about the hepatocyte cytolysis reduction; the increase in pseudocholinesterase activity demonstrates the enhancement of a liver synthetic ability which is further proven by the increase in [SORD](#) activity. The administration of empty liposomes decreased the intensity of lipid peroxidation and the enhancement of antioxidant system activity shows the decrease of oxidative stress due to circulation impairment. Thus, the administration of empty liposomes had a marked therapeutic effect.

Likewise, the administration of liposomal [macroergic phosphates](#) decreased the activity of the serum liver enzymes in CLI rats compared with CLI+saline group, but the efficacy was higher than after EL administration. A significant increase in serum pseudocholinesterase activity indicates the higher rates of its synthesis and, consequently, the improvement in liver function. [SORD](#) activity in homogenates also increases. These data show that the administration of liposomal [macroergic phosphates](#) in CLI rats induce hepatocyte biosynthesis and in this has the best effect among the studied liposomal drugs. However, the oxidative status improvement was the lowest among the other liposomal drugs under study. Thus, liposomal [macroergic phosphates](#) protect hepatocytes from lysis and induce cell biosynthesis but have an insignificant effect on oxidative stress.

Augmentation of intracellular *antioxidant* enzymes via *liposomal* delivery decreased the serum levels of enzyme cytolysis markers at most, led to an enhancement of pseudocholinesterase and [SORD](#)

synthesis, decelerated the synthesis of peroxidation products and enhanced antioxidant activity (Table 2 and 3). Thus, liposomal antioxidants had the highest therapeutic effect among the drugs under study.

A significant increase in serum MDA following the administration of any of the liposomal drugs under study requires a special attention. However, CLI+saline group did not demonstrate the increase in serum MDA concentrations. It suggests that the liposomal lipids probably act as additional substrates for lipid peroxidation and put blood antioxidant system under additional load. Even when the rats were administered with liposomal antioxidants and serum antioxidant activity increased significantly, there was no evident decrease in MDA concentrations.

Thus, the conducted studies showed fairly high efficacy of liposomal drug delivery in the treatment of liver dysfunction caused by venous congestion. The use of experimental CLI let study not only serum metabolic derangements but also liver tissue impairments and evaluate the efficacy of some liposomal drugs. The administration of liposomal drugs, containing lecithin and cholesterol of 100 nm, can restore damaged cell membranes. There is no doubt that the further study of liposomal effects is necessary, but at this point it is already possible to declare that the use of liposomal drug delivery to target diseased liver cells is highly promising.

5. CONCLUSIONS

1. The use of liposomal drugs demonstrates marked hepatoprotective activity in experimental liver insufficiency due to venous congestion.
2. Empty liposomes are found to restore the structural integrity of a cell membrane and can be regarded as a lecithin carrier.
3. Liposomal antioxidants delivery not only allows the treatment of liver dysfunction but also reduces oxidative stress which is a critical phenomenon in chronic liver insufficiency.

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