Potential and Promises of Phospholipid Structured Novel Formulations for Hepatoprotection

Silki, Kapoor Deepak¹, Malviya Sarvesh¹, Talwar Vaibhav, Katare Om Prakash*
UGC Centre for Advanced Studies, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh – 160014
¹Oniosome Research Centre (ORC), Oniosome Healthcare Pvt. Ltd., F-352, Phase VIII-B, Industrial Area, Mohali – 160071, Punjab

Abstract
Liver is highly vulnerable to damage because of its critical role in the transformation and clearance of chemicals. Hepatotoxicity is the prime factor responsible for drug withdrawal from the market. There is no doubt that hepatotoxicity requires considerable attention and that every viable measure be taken towards an efficient mechanism for hepatoprotection. The primary areas of focus for hepatoprotective therapies encompass better targeting, stability and better bioavailability. Such focus areas can be effectively met with the aid of lipid based delivery systems which can prove to be a breakthrough in the field of hepatoprotection.

*Corresponding author, Mailing address:
Katare Om Prakash
E-mail: drkatare@yahoo.com

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Hepatotoxicity and Hepatic damage
Hepatotoxicity refers to drug, or chemically-driven damage to the liver. The liver is prone target of xenobiotics, oxidative stress and drug-induced toxicity as it plays a crucial role in the metabolism and clearance of these chemicals. Such chemicals, when taken in an overdose, can afflict the liver. Various physicochemical reactions result in formation of free radicals and liver is prone to be attacked by these radicals to produce cell necrosis[4]. Load of these metabolic reactions and exposure to dangerous chemicals make liver vulnerable to a variety of disorders, such as acute or chronic...
inflammation, toxin- drug-induced hepatitis, cirrhosis, and hepatitis due to viral infection[1]. Liver injuries may be viral or caused by drugs, chemical, and alcohol. Drugs are an important cause of liver injury. Approximately one-half of the cases of acute liver failure are accounted to drug induced hepatotoxicity which also mimics all forms of acute and chronic liver disease[2]. More than 900 drugs, toxins, and herbs have indicated liver injury. There are certain specific conditions that lie within the general category of hepatotoxicity. These conditions include Hepatitis (inflammation of the liver), Hepatic necrosis (death of hepatocytes) and Hepatic Steatosis (fatty liver). Hepatic damage is first indicated by some indistinct symptoms such as fatigue, anorexia, nausea, discomfort in the right upper quadrant, and dark urine.

Mechanism of Hepatotoxicity

Various patho-physiological mechanisms of drug induced hepatotoxicity are still being studied which include both hepatocellular as well as extracellular mechanisms. These include disruption of the hepatocytes, disruption of the transport proteins, cytolytic t-cell activation, apoptosis of hepatocytes, mitochondrial disruption, bile duct injury etc. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages. Apart from lipid peroxidation, varieties of other mechanisms responsible for hepatotoxicity are induced by electrophilic drug metabolites and free radicals that may cause depletion of glutathione as well as covalent binding to proteins, lipids or nucleic acids. Consequently, there are direct effects on cell organelles such as mitochondria, endoplasmic reticulum, cytoskeleton, microtubules, or the nucleus[3]. Drug induced hepatotoxicity can be either immunogenic or dose dependent. Drug-induced immune-mediated liver injury occurs by hapten-like reaction in which low molecular weight drugs or their metabolites may covalently bind to macromolecules such as liver proteins, leading to their alteration and become immunogenic[4]. Dose-dependent hepatotoxicity is due to prolonged administration or a single toxic dose. Acute liver injury involves the parenchyma cells of liver, secretory function of bile or both[5]. In the case of a cholestatic disorder, the endogenously engendered bile acids produce hepatocellular apoptosis by actuating fat translocation from the cytoplasm to the plasma membrane, wherein, self-accumulation takes place to trigger apoptosis. Hepatocyte stress and/or damage could result in the release of signals that stimulate activation of other cells, particularly those of the innate immune system, including Kupffer cells (KC), natural killer (NK) cells, and natural killer T (NKT) cells. These cells contribute to the progression of liver injury by producing pro-inflammatory mediators and secreting chemokines to further employ inflammatory cells to the liver[6, 7]. Activation of certain enzymes in the cytochrome P-450 system such as CYP2E1 also leads to oxidative stress[8].

Hepatoprotection

The use of natural remedies for the treatment of liver diseases has a long history and medicinal plants and their derivatives are still used all over the world in one form or another for this purpose. Liver protective plants contain a variety of chemical constituents like phenols, coumarins, monoterpenes, glycosides, alkaloids and xanthenes[9]. Despite advances in modern medicine, there is no effective product available that stimulates liver function, offers protection to the liver from damage or helps to regenerate hepatic cells. However, there are a number of plant extracts being evaluated in order to establish their hepatoprotective activity.

Role of Phospholipids and lipid based drug delivery in Hepatoprotection
Phospholipids are the structural and functional building blocks of cell membranes and hence are of utmost importance. Phospholipids are crucial to the protection, and regeneration of damaged cells. In addition to the enhancement of bioavailability of drugs with low aqueous solubility or low membrane penetration potential, phospholipids also function for the improvement or alteration of uptake and release of drugs, protection of sensitive active agents from degradation in the gastrointestinal tract, reduction of gastrointestinal side effects of non-steroidal anti-inflammatory drugs and even masking of bitter taste of orally administered drugs. Phospholipids are amphiphilic molecules, which have a tendency to organize themselves in various structures where, the drugs could be entrapped. Phospholipids in their bilayer circular structured form (Liposomes) can serve the purpose of improving the efficacy of therapies by improving the solubility of hydrophilic as well as lipophilic drugs. The cell-liposomal vesicle interaction plays an important role in hepatoprotection, as in lipid based formulations the entrapped drugs have been easily transported across the bio-membranes (Fig. 1. Shows the interaction of lipid based vesicles and cell membranes during drug transportation). Phospholipids are functional carriers, which also exhibit hepatoprotective activity in case of fatty liver. Phospholipids are a source of phosphatidylcholine and choline, both of which tend to dissolve the fat deposited inside the liver in case of hepatic steatosis or fatty liver and exhibit other additional hepatoprotective effects. Various lipid based novel drug delivery systems which can be employed for delivery of herbal drugs are shown in fig. 2 and are discussed herein.

a) Phospholipid-Drug Complex

A phospholipid-drug complex is formed by interaction of the phospholipid with a functional group of the drug. Phospholipid complex formulations of several natural drugs, such as silymarin and dolichol, have been found to show improved bioavailability. Singh et al., have prepared quercetin-Phospholipid complex which has been proved to improve the water solubility of quercetin by 12 folds without any effect on its bioactivity. Priscilla D'Mello et al., evaluated the hepatoprotective activity of ethanolic extract of P. guajava and the phospholipid complex of the extract with phosphatidylcholine against paracetamol induced hepatic damage in albino rats. It was found that the Phospholipid complex of the extract showed better activity than the plain extract.

b) Liposomes in hepatoprotection

Liposomes have been used as drug delivery vehicles for several decades now and rightly so, purely because selective targeting and release rate control can be performed with the aid of appropriate modifications to the carrier itself and that too without altering the original structure of the drugs. Furthermore, the mainstay of liposomes rests on some vital factors such as their low toxicity and their relatively easier methods of preparation. Their properties can be altered to accommodate various effective molecules both lipophilic as well as hydrophilic, ranging from DNA to superoxide dismutase to alpha tocopherol. Although, the...
high affinity of liposomes to the liver has been the primary weakness in liposomal therapy, it would prove to be an asset in hepatoprotection. For instance, several formulations showed greater than 80% accumulation in the liver in less than 15 minutes after being intravenously injected\textsuperscript{[23]}. Furthermore, it has been demonstrated that changes in the concentration of liposomes lead to variation in the kinetics of liver uptake\textsuperscript{[24]}. The natural affinity for the liver shown by liposomes ensures easy target ability. Liposomes are able to enhance the performance of the products by improving ingredient solubility, bioavailability, intracellular uptake, altered pharmacokinetics as well as bio-distribution and \textit{in vitro} and \textit{in vivo} stability. Liposomes can easily incorporate fluorescent dyes and/or can easily be radio-labeled, which in turn can provide valuable information as to the location and distribution of the injected agent\textsuperscript{[25]}. Sonia Abrol \textit{et al.}\textsuperscript{[26]}, prepared lipid based formulations of silymarin using soyabean oil as the lipid carrier. It was proved that carriers like lipid microspheres can help to navigate and negotiate the silymarin molecules \textit{in vivo} in an effective way for hepatoprotection. Remarkably, oral administration of silymarin loaded lipid microspheres showed performance at par to that of \textit{i.v.} administration.

\textbf{Fig. 2:} Various Lipid based novel drug delivery systems

c) \textbf{Lipid Nanosystems in Hepatoprotection}

Nanosystems encompass various nanosized delivery systems like nanoparticles, nanospheres, nanocapsules, solid lipid nanoparticles (SLN), self-emulsifying drug delivery systems (SEDDS) and submicron/nanoemulsions\textsuperscript{[27, 28]}. Nanoparticle drug delivery systems offer many advantages such as, enhancement of solubility, stability as well as bioavailability, reduced toxicity, enrichment of pharmacological activities, improvement of tissue macrophage distribution, sustained delivery and protection from physical as well as chemical degradation\textsuperscript{[29, 30]}. Solid lipid nanoparticles (SLN), remain solid at room temperature and are advantageous in controlling drug release, targeting with reduced toxicity, increasing drug stability and high drug payload\textsuperscript{[30]}. Nanosuspensions have been used to increase the solubility, dispersity and homogenization, intravenous injectability, simple production process, universal adaptivity of poorly water soluble drugs\textsuperscript{[31]}. Emulsions and microemulsions have been used as templates to form nanosuspensions\textsuperscript{[32]} and SLNs\textsuperscript{[34]}. Nanocapsules have also been designed, to improve stability, absorption, quantitative tissue transfer and pharmacodynamic activity. Self nanoemulsifying drug delivery systems (SNEDDS) were reported to be a thermodynamically and physically stable formulation with high solubility, improved dissolution rate and extent of absorption, thus, resulting in more reproducible blood-time profiles\textsuperscript{[35]}. Meiwan Chen \textit{et al.}\textsuperscript{[36]}, have prepared various above mentioned nano-sized drug delivery systems for oleanolic acid, a triterpenoid with hepatoprotective and other medicinal properties. Oleanolic acid formulated in nanosystems was proved to be much better absorbed and bioavailable. Yen F\textit{L}et \textit{et al.}\textsuperscript{[37]} have prepared nanoparticulate formulation of \textit{Cuscuta chinensis} which provide hepatoprotective effect in acetaminophen-induced hepatotoxicity in rats. It was found that with the use of nanotechnology, dose of \textit{Cuscuta chinensis} could be reduced upto 5 times as compared to that of ethanolic extract.
d) Micromulsions In Hepatoprotection

As a drug delivery system, emulsion distributes in vivo in the targeted manner due to its affinity to the lymph. In addition, the drug can be sustained release in a long time because the drug is packaged in the inner phase and kept off direct touch with the body and tissue fluid. After the oily drugs or lipophilic drugs being made into O/W or O/W/O emulsion, the oil droplets are phagocytosized by the macrophage and get a high concentration in the liver, spleen, and kidney in which the amount of the dissolved drug is very large. The size of the emulsion particle has an impact on its target distribution hence enabling microemulsions to distribute throughout the body. Song Y. M. et al. [38], have prepared intramuscular, hepatoprotective nanoemulsions of Silybin to produce a sustained effect.

e) Phytosomes® in Hepatoprotection

Phytosomes® or Herbosome is a technology, developed to incorporate standardized plant extracts or water soluble phytoconstituents into phospholipids to produce lipid compatible molecular complexes, known as Phytosomes®. Since, the term ‘Phytosomes® has been registered by Indena S.P.A, Italy; the technology is being referred as ‘Herbosome’ by the researchers. In liposomes no chemical bond is formed and the phosphatidylcholine molecules simply encapsulate the water soluble drug. In contrast, with the Phytosomes® process the phosphatidylcholine and the plant components actually form a 1:1 or a 2:1 molecular complex depending on the substance(s) complexed, involving chemical bonds[25]. Suresh RN et al., have prepared Gingkoselect Phytosomes® which prevent inhibits lipid peroxidation (LPO) and stabilize the Reactive oxygen Species (ROS). Yanyu X, et al.[39], prepared silybin Phytosomes® which has been proved to enhance absorption of Silybin upto seven times.

Challenges

Lipid based drug delivery systems have become one of the key research areas in drug delivery research and this interest could be accounted to the numerous benefits these delivery systems offer. Some of the advantages offered by lipid excipients like phospholipids and lipid based delivery systems have already been discussed above. Although, these delivery systems have established significance in novel delivery of drugs, they also pose challenges from their development, manufacturing, biopharmaceutical as well as from a regulatory perspective[40]. Some of the limitations include, i) difficulty in handling, storage, and administration because of susceptibility to aggregation; ii) unsuitability for high dose drugs; iii) tendency of small sized vesicles such as nanocarriers to gain access to unintended environments with harmful consequences, e.g. crossing the nuclear envelope of a cell and causing unintended genetic damage and mutations[41, 42]. Lipid based delivery systems are able to entrap the drug within the vesicles or lipid matrix. However, as with dietary lipids, the lipid excipients can also be digested and dispersed in the GI tract. Therefore, whether the drug remains in solubilised form in the presence of changing phases after administration remains one of the questions for a lipid-based oral formulation. Moreover, several lipid-excipients and surfactants present in pharmaceutical formulations have also been reported to inhibit the CYP 3A metabolism or P-gp transport, which may give rise to certain concerns about drug absorption and bioavailability. Thus a critical scrutiny is essentially required while selecting the lipid carriers. Unlike conventional oral dosage forms, lipid based delivery systems have a complex absorption mechanism inside the body, which poses questions about their in vitro drug release evaluation. Since, the bio-processing of lipid vehicle (dispersion and digestion) and drug dissolution/release are related, it would be inappropriate to use conventional dissolution testing apparatus for evaluating drug release from complex lipid based systems. Thus, there is a need to develop some in vitro dissolution
technique which could mimic the in vivo complexities, involved with lipid based delivery systems, in order to predict reasonable and genuine drug release data. The physical and chemical complexity of lipid based drug delivery products can present unique challenges to the sterilization process. Lipid based products being sensitive to changes in manufacturing parameters, require identification and evaluation of critical manufacturing parameters during product development.

Conclusion
Considering the challenges in the area of hepatology it is very much in demand to generate new therapeutic options. While, several new phytochemical entities are being investigated extensively, little care is being given to the opportunity available in the form of drug carrier systems. Thus, the background and the analysis of information from different sources points conclusively towards phospholipids as one of the most promising novel material, which can be exploited intelligently and effectively to target liver related disorders and diseases as well as its protection.

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