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HEPATIC CELL TARGETED DRUG DELIVERY SYSTEM: A REVIEW

Karunakar Prasad Dwivedi*, Amresh Gupta, Arpita Singh and Swarnima Pandey

Goel Institute of Pharmacy and Sciences, Lucknow Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow, UP, India.

***Corresponding Author: Karunakar Prasad Dwivedi**
Goel Institute of Pharmacy and Sciences, Lucknow Affiliated to Dr. APJ Abdul Kalam Technical University, Lucknow, UP, India.
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ABSTRACT
Few of the hepatic diseases that cannot be effectively cured by some reasons because there are various receptors existed in the liver. In this time, several passive targeted drug delivery systems have been used in the drug/gene delivery in the treatment of hepatic diseases. More importantly, different measures would be taken in accord to the specified cell that was lesioned or dysfunctioned via interaction between homing ligands and target receptors so as to improve accumulation of drugs in the target cell and to reduce nonspecific toxicity towards other cells or organs. Many serious liver diseases affecting millions of people world-wide cannot be treated despite many efforts which warrant a search for new therapeutic strategies. Potent drugs may not be effective enough in-vivo or exhibit adverse effects and enhanced delivery into the target cells may improve this significantly. We aim to summarize the available options for drug delivery to the different intrahepatic cell-types.

KEYWORDS: Liver targeted drug delivery, Hepatic disease, Nonspecific toxicity.

INTRODUCTION
In last 10 years, significant improvement have been made in the development of herbal hepatoprotective drugs mostly because the lower toxicity and a multi-factorial approach in improving health, finding equilibrium in mind, body & environment and placing a greater emphasis on the multidirectional elements of health than on pathology alone. Along with regular medications, phytomedications have mostly prescribed in the treatment of of many hepatic diseases. However, phytotherapeutics goes under a scientific approach to deliver the components in a regulated manner to improve patient compliance and avoid re-administration. It can be found by designing novel drug delivery systems (NDDS) for herbal components, in addition to the drugs already found in the market. Novel drug delivery systems not only overcome the re-administration (due to its sustained-release properties) and noncompliance but also help to improve the therapeutic value by reducing toxicity, increasing the bioavailability, stability, and targetability to a targeted cell or organ. In a long period of time, herbal medicaments were not considered for development as novel formulations due to lack of scientific justification and processing difficulties, such as pharmacodyanamik and pharmacokinetic parameters of individual drug components in complex polyherbal systems. However, modern phytopharmaceutical remove the hurdels of the scientific needs in modern medicine(herbal), which gives solutions about developing of novel formulations such as nanoparticles, microemulsions, matrix systems, solid dispersions, liposomes, and solid lipid nanoparticles. However, for targeted delivery to specificed cell of liver, novel drugs delivery system in herbal drugs yet needs modification like attaching of ligand or targeting moiety that can recognize and interact with targeted cell of liver. In this review, we enumerate all the techniques for attaching targeting moiety to delivery system and different factors which could follow in account while designing NDDS for hepatic cell which will be of valuable in future. This review will be helpful to explain the importance of both the drugs and herbal medications to the liver so as to ensure successful treatment outcomes.

Morphological Study of Liver
While discussing the different methods of targeting, it is necessary to knowledge about the morphology of liver (especially vascular supply) with the molecular scale of targeted tissue in order to design of novel drug delivery system. Numerous metabolic, immunological, and endocrine functions engagedby the liver. Hepatic system receives blood (oxygenated and deoxygenated) from the gut and heart via the portal vein and hepatic artery. Blood circulates through a permeable discontinuous capillary network term as the sinusoids to reach the central and hepatic veins. The sinusoids are small blood vessels (5 to 10 μm wide) between the radiating rows of hepatocytes having fenestrations of size 100–150 nm (depending on the type of animal species). They allow almost unrestricted passage of plasma components to the

perisinusoidal space, where the cords of parenchymal cells called as hepatocytes are situated. Inside the sinusoid capillaries, the Kupffer cells are responsible for phagocytic activity of the liver.[1,2]

LIVER

The liver, an organ only found in vertebrates, detoxifies various metabolites, synthesizes proteins, and produces biochemicals necessary for digestion.[3] In humans, it is located in the right upper quadrant of the abdomen, below the diaphragm. Its other roles in metabolism include the regulation of glycogen storage, decomposition of red blood cells and the production of hormones.[4] The liver is an accessory digestive gland that produces bile, an alkaline compound which helps the breakdown of fat. Bile acids in digestion via the emulsification of lipids. The gallbladder, a small pouch that sits just under the liver, stores bile produced by the liver.[5] The liver's highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions.[6] Estimates regarding the organ's total number of functions vary, but textbooks generally cite it being around 500.[7] Terminology related to the liver often starts in hepat- from ἡπατο-, the Greek word for liver. There is currently no way to compensate for the absence of liver function in the long term, although liver dialysis techniques can be used in the short term. Artificial livers are yet to be developed to promote long-term replacement in the absence of the liver. As of 2017, liver transplantation is the only option for complete liver failure.

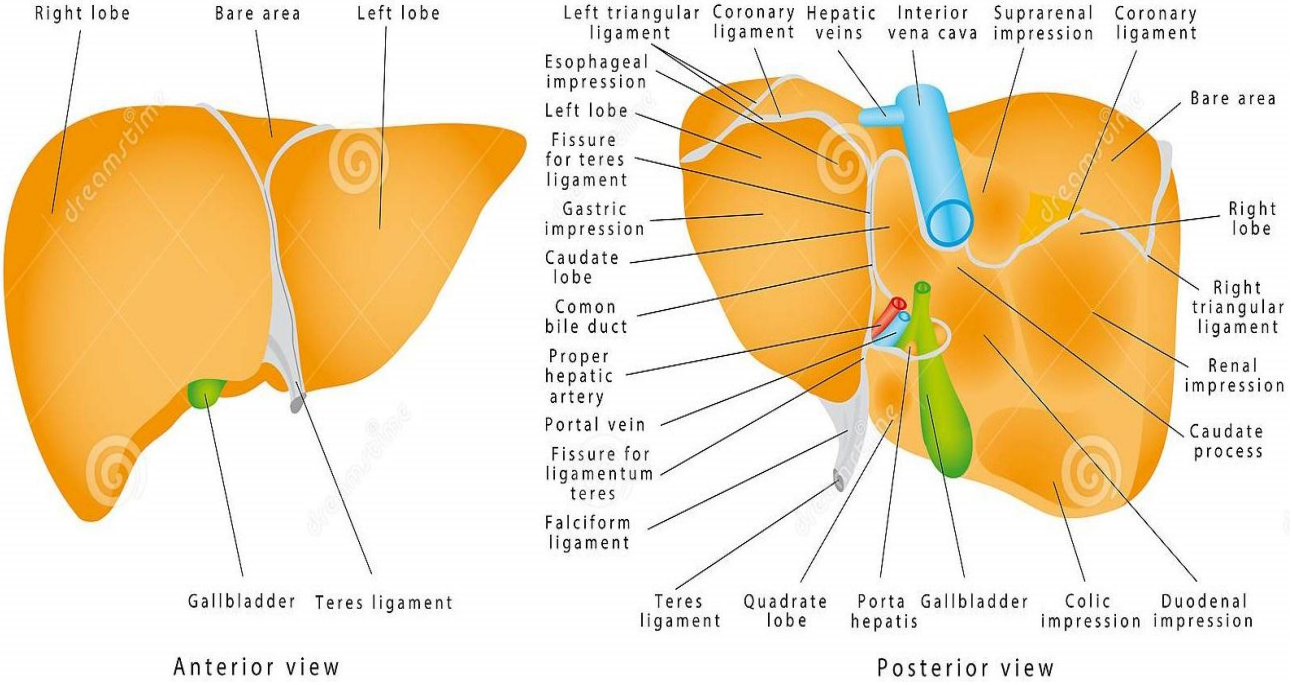

Fig 1: Liver Anatomy and Physiology.

Function of liver

The various functions of the liver are carried out by the liver cells or hepatocytes. The liver is thought to be responsible for up to 500 separate functions, usually in combination with other systems and organs. Currently, there is no artificial organ or device capable of reproducing all the functions of the liver. Some functions can be carried out by liver dialysis, an experimental treatment for liver failure.

Blood supply

The liver receives a dual blood supply from the hepatic portal vein and hepatic arteries. The hepatic portal vein delivers approximately 75% of the liver's blood supply, and carries venous blood drained from the spleen, gastrointestinal tract, and its associated organs. The hepatic arteries supply arterial blood to the liver, accounting for the remaining quarter of its blood flow. Oxygen is provided from both sources; approximately half of the liver's oxygen demand is met by the hepatic portal vein, and half is met by the hepatic arteries.[8]

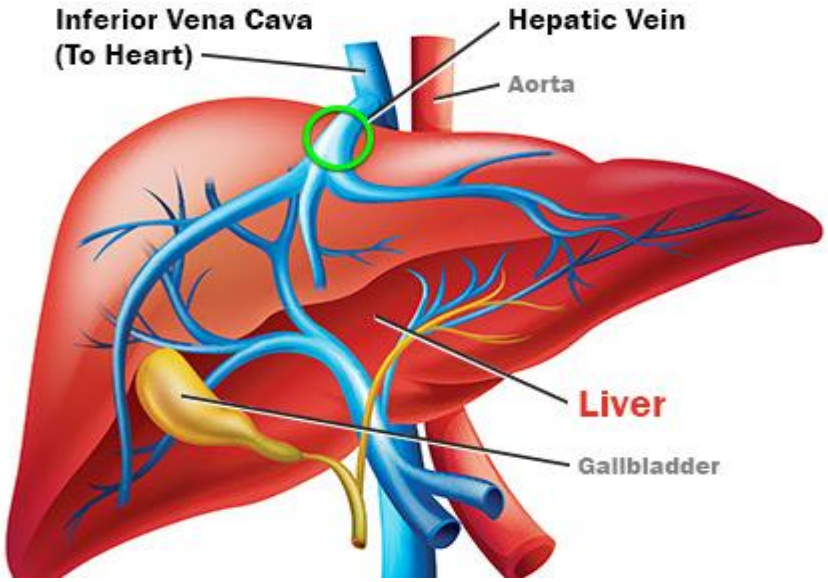

Fig 2: Liver Veins.

Bilary flow

The biliary tract is derived from the branches of the bile ducts. The biliary tract, also known as the biliary tree, is the path by which bile is secreted by the liver then transported to the first part of the small intestine, the duodenum. The bile produced in the liver is collected in bile canaliculi, small grooves between the faces of adjacent hepatocytes. The canaliculi radiate to the edge of the liver lobule, where they merge to form bile ducts. Within the liver, these ducts are termed intrahepatic bile ducts, and once they exit the liver they are considered extrahepatic. The intrahepatic ducts eventually drain into the right and left hepatic ducts, which exit the liver at the transverse fissure, and merge to form the common hepatic duct. The cystic duct from the gallbladder joins with the common hepatic duct to form the common bile duct.[9]

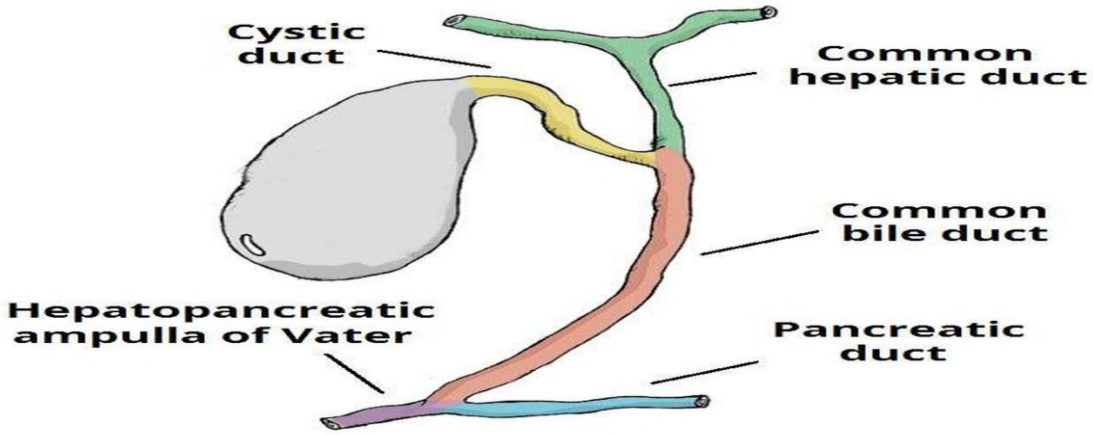

Fig 3: Bilary Tract.

Synthesis

The liver plays a major role in carbohydrate, protein, amino acid, and lipid metabolism. The liver performs several roles in carbohydrate metabolism: The liver synthesizes and stores approximately 100g of glycogen via glycogenesis, the formation of glycogen from glucose. When needed, the liver releases glucose into the blood by performing glycogenolysis, the breakdown of glycogen into glucose. The liver is also responsible for gluconeogenesis, which is the synthesis of glucose from certain amino acids, lactate or glycerol. Adipose and liver cells produce glycerol by breakdown of fat, which the liver uses for gluconeogenesis.[10]

The liver is responsible for the mainstay of protein metabolism, synthesis as well as degradation. It is also responsible for a large part of amino acid synthesis. The liver plays a role in the production of clotting factors as well as red blood cell production. Some of the proteins synthesized by the liver include coagulation factorsI (fibrinogen), II (prothrombin), V, VII, VIII, IX, X, XI, XIII, as well as protein C, protein S and antithrombin. In

the first trimester fetus, the liver is the main site of red blood cell production. By the 32nd week of gestation, the bone marrow has almost completely taken over that task. The liver is a major site of production for thrombopoietin, a glycoprotein hormone that regulates the production of platelets by the bone marrow.

Breakdown

The liver is responsible for the breakdown of insulin and other hormones. The liver breaks down bilirubin via glucuronidation, facilitating its excretion into bile. The liver is responsible for the breakdown and excretion of many waste products. It plays a key role in breaking down or modifying toxic substances (e.g., methylation) and most medicinal products in a process called drug metabolism. This sometimes results in toxication, when the metabolite is more toxic than its precursor. Preferably, the toxins are conjugated to avail excretion in bile or urine. The liver breaks down ammonia into urea as part of the urea cycle, and the urea is excreted in the urine.[11]

Liver diseases and its treatments: Different types of liver diseases are hepatitis B virus (HBV) infections, liver fibrosis, hepatocellular carcinoma, liver cirrhosis, choleostasis, acute liver failure, nonalcoholic fatty liver disease (NAFLD) and alcoholic liver disease. Hepatitis B Virus (HBV) Infection: Hepatitis B virus infection is a major global public health problem. HBV infection accounts for 500 000 to 1.2 million deaths each year and is the 10th leading cause of death worldwide. Approximately 2 billion people who have been infected worldwide, more than 350 million are chronic carriers of HBV. Approximately 15-40% of infected patients will develop cirrhosis, liver failure, or hepatocellular carcinoma (HCC). HBV is a highly contagious DNA virus that is transmitted through parenteral or mucosal exposure to infected blood, serous fluids and other body fluids such as seminal and vaginal fluids. Common routes of infection include perinatal transmission (from an infected mother to infant during birth), unsafe needle sharing, blood transfusion practices and sexual contact.[12,13] Chronic HBV infection can be divided into three major phases based on virus-host interactions: immune tolerant, immune clearance and inactive carrier phases.[14] The U.S. Food and Drug Administration (FDA) approved anti-HBV drugs can be broadly categorized as interferons (IFN-α2b and pegylated IFN-α2a), nucleoside (lamivudine, entecavir and telbivudine) and nucleotide (adefovir and tenofovir) analogs.[15]

Liver fibrosis: Liver fibrosis is defined as the building up of excessive amount of extracellular matrix, also known as scar tissue, in the liver parenchyma.[16] Liver fibrosis is the final pathway for most chronic liver disease and is the main reason for increased mortality in affected patients. The extent of liver fibrosis displays great individual variation, even after controlling for age (at infection), gender & exogenous factors. Thus, host genetic factors are likely to play an important role in the process of liver scarring.[17] Loss of hepatic functions, ascites, portal hypertension with an increased risk for esophageal varices and HCC are among the most serious complications that are often fatal. As activation of the hepatic stellate cells (HSCs) is the central event in fibrogenesis, various candidate drugs including rennin-angiotensin system inhibitors, IFN-γ, peroxisomal proliferator activated receptor (PPAR)-γ ligands, pirfenidone, colchicine and herbal medicines that have demonstrated potential in inhibiting HSC activation, proliferation and collagen synthesis have been proposed for the treatment of liver fibrosis. In addition, antioxidants such as vitamin E, silymarin, phosphatidylcholine and S-adenosyl-L-methionine have also been investigated for protection against oxidative stress that may induce hepatic injury and fibrogenesis.[18,19]

Hepatocellular carcinoma (HCC): Hepato-cellular carcinoma (HCC) is the most frequent primary malignancy of the liver and ac- counts for as many as 1 million deaths annually worldwide. In some parts of the world it is the most common form of internal malignancy and the most common cause of death from cancer. El-Serag and Mason I have described an increase of about 80% in the incidence of HCC in the United States over the past 20-30 years and it is estimated that approximately 15,000 new cases occur each year.[20] HCC typically occurs in the milieu of long standing liver diseases such as chronic hepatitis B or C virus infections, alcoholic cirrhosis and non-alcoholic steatohepatitis, the nature of which follows a distinct geographical distribution.[21, 22] In the early stages of HCC, the disease is potentially curable by surgical resection, liver transplantation and nonsurgical local ablation techniques such as percutaneous ethanol injection and radiofrequency ablation (RFA). Patients with advanced HCC can be treated by conventional systemic chemotherapeutic agents such as doxorubicin, cisplatin and 5-fluorouracil, sorafenib used alone or in combination.[23-25]

Cholestatic Liver Diseases: Cholestasis (reduced bile duct excretion) is another well-known cause of liver fibrosis. Cholestasis triggers the proliferation of the cholangiocyte lining of the intrahepatic and extrahepatic bile duct systems through a complex regulatory milieu that involves both autocrine and paracrine factors.[26] Cholestasis i.e., blockage of bile flow, is due to either intrahepatic disorders such as cystic fibrosis, granulomatosis or drug side effects. In Cholestasis, the bile canaliculi are enlarged, the fluidity of the canalicular cell membrane is decreased (cholesterol embedding, bile salt effect), their brush border is deformed (or totally absent) and the function of the cytoskeleton, including canalicular motility, is disrupted.[27] The dihydroxy bile acid, ursodeoxycholic acid (UDCA), is increasingly used for the treatment of chronic cholestatic liver diseases.

Liver cirrhosis: Cirrhosis of the liver refers to scarring of the liver which results in abnormal liver function as a consequence of chronic liver injury. Cirrhosis is a leading cause of illness and death in the United States. The most common causes of cirrhosis are excess alcohol use, chronic infection with hepatitis viruses (such as hepatitis B and hepatitis C), cirrhosis can be caused by other conditions including fatty liver disease, inherited disorders, drug-induced injury, bile duct disorders and autoimmune diseases. A large portion of patients (up to 20%) do not have an identifiable cause for cirrhosis this is known as cryptogenic cirrhosis.[28] Two goals in the management of compensated cirrhosis are.

1. Treatment of the underlying liver disease (e.g., hepatitis C or B, alcohol, non-alcoholic steatohepatitis), and;
2. Prevention or early diagnosis of the complications of cirrhosis.

Acute Liver Failure: Acute liver failure (ALF) is a rare condition in which rapid deterioration of liver function results in altered mentation and coagulopathy in previously normal individuals. U.S. estimates are placed at approximately 2,000 cases per year.[29] The most prominent causes include drug induced liver injury, viral hepatitis, autoimmune liver disease and shock or hypoperfusion; many cases (~20%) have no discernible cause.[30] Acute liver failure often affects young persons and carries a high morbidity and mortality. The causes of chronic liver failure that is accompanied by fibrosis (cirrhosis) of the liver are; inflammation, chronic persistent viral hepatitis; alcohol abuse, the most common cause in susceptible patients, side effects of drugs such as, folic acid antagonists and phenylbutazone. Liver transplant is the best way to manage the liver failure.[31]

Nonalcoholic Fatty Liver Disease (NAFLD): NAFLD and its subtype, Non-Alcoholic Steatohepatitis, or NASH, are usually seen in individuals with metabolic syndrome (MS) or its components such as obesity, type-2 diabetes (DM), dyslipidemia, and insulin resistance. NASH rarely manifests as inflammation and/or apoptosis/ necrosis only, more often than not it is also accompanied by liver fibrosis.[32] It refers to the accumulation of fat, mainly triglycerides, in hepatocytes so that it exceeds 5% of the liver weight. Treatment strategies for NAFLD have revolved around.

1. Identification and treatment of associated metabolic conditions such as diabetes and hyperlipidaemia;
2. Improving insulin resistance by weight loss, exercise, or pharmacotherapy;
3. Using hepatoprotective agents such as antioxidants to protect the liver from secondary insults.

Alcoholic Liver Disease: Excessive and chronic alcohol consumption is an important causal factor of liver fibrosis and cirrhosis. The process of the breakdown of ethanol produces two profibrotic agents, acetaldehyde and reactive oxygen species (ROS).[33] Alcoholic liver diseases are often grouped into three histological stages of ALD: fatty liver or simple steatosis, alcoholic hepatitis, and chronic hepatitis with hepatic fibrosis or cirrhosis. These latter stages may also be associated with a number of histological changes including the presence of Mallory's hyaline, mega mitochondria, or perivenular and perisinusoidal fibrosis. Fatty liver develops in about 90% of individuals who drink more than 60 g/day of alcohol, but may also occur in individuals who drink less.[34] Treatment approaches includes inhibition of tumor necrosis factor, antioxidant therapy, stimulation of liver regeneration, and stimulation of collagen degradation.

Drug targeting: Drug targeting is the ability of the drug to accumulate in the target organ or tissue selectively and quantitatively, independent of the site and methods of its administration. Ideally, under such conditions, the local concentration of the drug at the disease site(s) should be high, while its concentration in other non-target organs and tissues should be below minimal level to prevent any negative side-reactions.[35]

The following advantages of drug targeting are.

1. Drug administration protocols may be simplified;
2. Drug quantity required to achieve a therapeutic effect may be greatly reduced;
3. The cost of therapy reduced;
4. Drug concentration in the required sites can be sharply increased without negative effects on non-target compartments. The same is, for the great extent, true for the use of many diagnostic agents.

Currently, the concept of magic bullet includes a coordinated behavior of three components.

i) Drug;

ii) Targeting moiety and;

iii) Pharmaceutical carrier used to multiply the number of drug molecules per single targeting moiety.

Pharmaceutical carriers include soluble polymers, microcapsules, microparticles, cells, cell ghosts, lipoproteins, liposomes, and micelles. All of them can be made targeted in one way or another.

The recognition of the target can occur on the level of a whole organ, on the level of certain cells specific for a given organ, or even on the level of individual components characteristic of these cells, such as cell surface antigens. The most universal form of target recognition is the recognition on the molecular level, based on the fact that every organ or tissue certain compounds (antigens) can be found that are specific only for the organ of interest. For successful targeting, another compound can be used as a transporting unit, which is capable of the specific interaction with the specific target component (for example, a monoclonal antibody against the target antigen). Basing on this principle, numerous systems for drug targeting have been constructed capable of the delivery of pharmaceuticals to the variety of

tissues and organs.Currently, the whole set of targeting protocols is under development that includes many different approaches to targeted drug delivery. Not necessarily these approaches involve the use of specific targeting moieties. In certain cases various physical principles and/or some physiological features of the target area may be utilized for a successful targeting of pharmaceuticals and pharmaceutical carriers.

Principal schemes of drug targeting currently investigated in various experimental and clinical settings include

1. Direct application of the drug into the affected zone (organ, tissue);
2. Passive accumulation of the drug through leaky vasculature (tumors, infarcts, inflammation);
3. Physical targeting based on abnormal pH and/or temperature in the target zone, such as tumor or inflammation (pH and temperature-sensitive drug carriers);
4. Magnetic targeting of drugs attached to paramagnetic carriers under the action of external magnetic field;
5. Use of vector molecules possessing high specific affinity toward the affected zone.

Liver targeting: The liver is a critical target tissue for drug delivery because many fatal conditions including chronic hepatitis, enzyme deficiency, and hepatoma occur in hepatocytes. In general, liver targeting systems employ passive trapping of microparticles by reticuloendothelium or active targeting based on recognition between hepatic receptor and ligand-bearing particulates.[36]

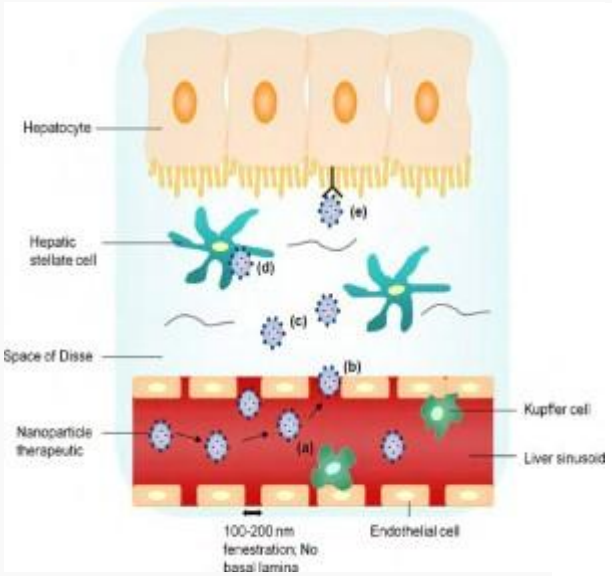

FIG 4: Passive and Active Liver Targeting Strategies of Nanoparticle Therapeutics.[37]

Passive targeting

Passive targeting refers to NP transport through leaky tumor capillary fenestrations into the tumor interstitium and cells by passive diffusion or convection or also refers to the accumulation of nanoparticle therapeutics at a specific body site due to certain anatomic or pathophysiological features.[38] The liver sinusoids are highly specialized capillaries characterized by.

1. the presence of 100-200 nm fenestrations along the endothelial wall and;
2. Absence of basal lamina. As a result of these characteristics, rapid and passive liver accumulations are frequently observed with nanoparticle therapeutics following intravenous (i.v) administration.

Following systemic administration, the defining size properties (typically < 200nm in diameter) of nanoparticle therapeutics greatly facilitates passive liver targeting in the absence of significant self-aggregation or aggregation with serum proteins as it allows for their extravasation through the slightly larger sinusoidal fenestrations. This effectively builds up a high local concentration of nanoparticle therapeutics in the space of Disse, where diffusion to the various liver cell types can occur.

Interestingly, evidence has also suggested an opportunity for deformable nanocarriers of up to 400 nm to extravasate through the sinusoid endothelial fenestrations via a mechanism of forced extrusion, possibly aided by transient interactions with the sinusoidal endothelial cells.[39] In HCC, passive accumulation of nanoparticle therapeutics in the liver can also be achieved by EPR effect that was first described by Matsumura and Maeda in 1986.[40]

The EPR effect can be observed in almost all human cancers with the exception of hypovascular tumors like prostate cancer or pancreatic cancer. For such a passive targeting mechanism to work, the size of the nanoparticles must be controlled to avoid uptake by the reticuloendothelial system (RES). The EPR effect stems from distinctive features of the tumor microenvironment including.

1. Leaky tumor vasculature brought about as a consequence of the rapid and incomplete tumor angiogenesis to meet the elevated demands for oxygen and nutrients, leading to enhanced permeability and extravasation of macromolecules, and;
2. Impaired lymphatic drainage, which favors the retention of nanoparticle therapeutics in the tumor tissues.[41]

As the size of the gap junction between endothelial cells is reported to vary between 400 and 600 nm, nanoparticle therapeutics are therefore expected to be extremely efficient at extravasating from the tumor microvasculature to result in a high local tumor interstitial concentration. Indeed, the EPR effect has been credited with the selective deposition and targeting of zein nanoparticle (ZP) encapsulated 5-fluorouracil in HCCs following intravenous injection. In these studies, the drug loaded ZPs could be efficiently targeted at the

liver by intravenous delivery observed in patients with liver cancer.[42]

The method and site of administration of nanoparticle therapeutics are also known to influence distribution patterns within the liver. In the area of gene delivery, for instance, hydrodynamic injections of naked DNA led to increased accumulations of DNA in the livers of rodents as the increased intrahepatic pressure results in a transient increase in the diameter of the sinusoidal fenestrate to cause a leakage of DNA-containing solutions from hepatic sinusoids into the space of Disse.[43]

Active targeting

The specific delivery of the therapeutic system to the diseased cell type allows for the capitalization of the therapeutic effects of the cargo and also minimizes unwanted side effects on normal liver cells resulting from non-specific cellular uptake. The diverse physiological functions of the human liver are achieved through the specific activities of various cell types, including the non-parenchymal sinusoidal endothelial cells (SECs), Kupffer cells (KCs), hepatic stellate cells (HSCs) and the predominant parenchymal hepatocytes. In liver fibrosis, HSCs are considered to be the main target for therapeutic interventions due to their major roles in the secretion and maintenance of copious amounts of extracellular matrix (ECM) in response to various biochemical stimuli produced by the injured hepatocytes, SECs and KCs.

Hepatocytes, on the other hand, are implicated in the development of HBV infections and HCC and therefore, are being targeted for the treatment of these diseases. As each of the two liver cell types has distinct morphologies, physiological activities and pathoanatomical characteristics that are reasonably established, unique targeting opportunities of therapeutics by ligand-mediated approaches to the HSCs and hepatocytes are abound.

Table 1: Ligand Mediated Approaches for Liver Targeting.

Liver cell type	Cellular target	Targeting ligand	References
Hepatic stellate cells	Mannose – 6 –phosphate receptor	Mannose-6-phosphate	44
	Type VI collagen receptor	Cyclic RGD	45, 46, 47
	PDGF receptor	PDGF	48
	Scavenger receptor class A	Human serum albumin	49, 50
Hepatocytes	Asialoglycoprotein receptor	Galactoside	51, 63, 67
		Galactosamine	68
	Plasma membrane fatty acid binding protein (Putative)	Linoleic acid	54
	Scavenger receptor class B type I	Apolipoprotein A-I	55
	Heparan sulfate	Acetyl CKNEKKNKIERNNKLKQPP-amide	56
	IL-6-receptor and/or immunoglobulin A binding protein (Putative)	Pre-S1	57
	Glycyrrhizin receptors	Glycyrrhizin	58, 62

RGD: Arg-Gly-Asp; PDGF: platelet-derived growth factor.

Drug targeting to Hepatic stellate cells (HSCs)

The five main strategies make the use of features of the pathological development of liver fibrosis that is initiated by the activation, proliferation and the subsequent transformation of HSCs into myofibroblasts. Activated HSCs are known to have upregulated expression of mannose-6-phosphate/ insulin-like growth factor II (M6P) receptors to facilitate the activation of the cytokine, transforming growth factor β (TGF-β), which stimulates collagen production by HSCs.[44] Capitalizing on this phenomenon, the direct conjugation of M6P via a short peptide linker to a N-(2- hydroxypropyl) methacrylamide (HPMA) copolymer showed a majority uptake (~80%) by the HSCs in dimethylnitrosamine (DMN)-induced liver fibrotic rats.[45] To exploit native interaction between collagen type VI receptors and its ligand, researchers have covalently attached a cyclic octapeptide C*GRGDSPC* (C* denotes the cyclizing cysteine residues) to the lysine groups of human serum albumin (HSA) and observed selective internalization by activated rat HSCs.[46] A further modification was made to the peptide by substituting cysteine with lysine (C*GRGDSPK*) in order to replace the less stable cyclizing disulfide (—S—) bond with a more stable peptide bond (—NH—CO—) in the latter, without adversely influencing targeting efficacy.[47,48] Receptors for platelet-derived growth factors (PDGFs), which mediate many of the HSC responses to cytokines, are generally upregulated during liver injury. Expression of the PDGF receptor type, in particular, is acquired at high levels during the myofibroblastic transformation of HSC.[49] The scavenger receptors (ScRs) present on HSCs act as an alternative endocytotic uptake route for nanoparticle therapeutics, particularly for the HSA-based therapeutic systems due to their polyanionic nature.[50, 51]

Drug targeting to Hepatocytes

Targeting to the asialoglycoprotein receptor (ASGP-R) is the most universally employed method to enhance clathrin mediated endocytotic uptake of nanoparticle therapeutics by hepatocytes. This approach takes advantage of the innate binding affinity of the ASGP-R to a broad range of molecules exposing galactose and N-acetyl-galactosamine residues, such as asialoorosomucoid, asialofetuin (AF), sterylglucoside, lactose and poly-(N-ρ-vinylbenzyl-O-β-Dgalactopyranosyl-[1- 4]-D-glucosamine (PVLA) for target in to hepatocytes.In polymeric systems, the most commonly seen approach is through coupling of lactobionic acid or lactose to the nanocarrier through carbodiimide chemistry, with the final product retaining functional galactose moieties.L. Li and his group has recently synthesized a series of amphiphilic polycarbonate-based copolymers bearing carbohydrate pendant chains as targeted drug carriers and found significantly higher uptake of doxorubicin (DOX)-loaded galactose-containing micelles by the ASGP-R positive HCC cell line HepG2 compared to the ASGP-R negative HEK293 cell line.[52] The specificity of galactose-mediated uptake of the DOX-loaded nanoparticles by HepG2 was evidenced by the inhibition by AF in a dose-dependent manner. Interestingly, although the conjugation of most galactose-bearing moieties to the polymer backbone occur at the 1-position of the pyranose ring, Li and his co-workers results demonstrated that the ASGP-R can recognize galactopyranosides appended at the 6-position. Simultaneous expression of ASGP-Rs in normal hepatocytes and HCC cells, however, could restrict the clinical applicability of this class of receptors for targeting purposes.In fact, studies have discovered a decrease in ASGP-R expression in HCC, particularly in the poorly differentiated state.[52,53] suggesting that the normal hepatocytes may internalize the nanoparticle therapeutics to a greater extent compared to their diseased counterparts. The tumor levels of the galactosylated poly (HPMA)-DOX is nevertheless substantially higher than the background levels, implying that the galactose moiety does provide some form of targeting, albeit with lower specificity, to the tumors. The fatty acid metabolism and cholesterol storage function of the liver is another avenue that has been explored to enhance hepatocyte uptake of nanoparticle therapeutics. For example, linoleic acid, an essential polyunsaturated fatty acid that is taken up by hepatocytes via its putative plasma membrane transporter[53] has been used to drive the uptake of self-assembled superparamagnetic iron oxide nanoparticles-loaded chitosan-linoleic acid/DNA complexes by hepatocytes for imaging and gene delivery purposes.[54] Additionally, various liposomes containing apolipoprotein A-I (apo A-I), the major protein of the high-density lipoprotein (HDL), have exploited the natural mechanism of uptake of HDL cholesteryl ester via the class B type I scavenger receptor, CLA-1 (human) or SR-BI (rat) to enhance internalization in the hepatocytes.[55] Besides the frequently over expressed cell surface receptors such as transferrin, folate and epidermal growth factor receptors in solid tumors, other ligand mediated targeting strategies have also exploited natural hepatic invasion mechanisms by protozoa through the use of acetyl-CKNEKKNKIERNNKLKQPP-amide to bind to heparin sulfate proteoglycans on the hepatocyte surface[56] and the use of pre-S1, a hepatitis B viral envelope protein sequence known to mediate virus entry into hepatocytes.[57] In addition, the hepatic glycyrrhizin (GL) receptors have also been targeted through GL surface modifications.[58]

Liver targeting drug carriers

Table 2: Liver Targeting Drug Carriers.

Carriers	Model drug	Polymers/ lipids	Method
Liposome	30-stearyl glycyrrhizin	HEPC,CH	Ether Injection
	Probucol	DSPC, CH	Ether Injection
Nanoparticles	Oridonin	BSA	Desolvation
	Adriamycine	Chitosan	Ionic gelation
	Antifibrotic drug	HAS	Desolvation
	Paclitaxel	γ-PGA-PLA	Emulsion/solvent evaporation
	Norcantharidin 5-fluorouracil	Chitosan Zein	Ionic gelation Phase separation
Polymeric micelles	Diammoniumglycyrrhizinate	Chitosan	-
Phytosome	Silymarin	Phospholipids	Solvent evaporation

HEPC: hydrogenated egg phosphatidylcholine; CH: cholesterol.
DSPC: Distearoylphosphatidylcholine; γ-PGA-PLA: poly (γ-glutamic acid)-poly (lactide).
BSA: Bovine serum albumin; HSA: Human serum albumin.

Liposomes

Liposomes are small vesicles composed of unilamellar or multilamellar phospholipid bilayers enclosing an aqueous space. Soluble drugs can readily be incorporated into this aqueous space and lipophilic drugs can be incorporated into the lipid bilayers. Elimination from the circulation is dependent on the lipid composition, charge, and size of the liposomes. Common liposomes such as

neutral and negatively-charged liposomes, are however, primarily cleared by the phagocytotic processes of the cells of the reticuloendothelial system (RES), the KCs having the greatest responsibility for this process.It has been shown for instance that the targeting of cytostatic agents such as adriamycine to tumours is associated with loss of KC function, thereby contributing to the immunosuppressed status of patients. The high KC uptake has been surprisingly under-exploited in drug targeting approaches to treat liver diseases.[59] Liposomes have been used for the targeting of anti-Leishmania drugs[60] andimmunomodulators[61] and have greatly increased the efficacy of these drugs in Leishmania infections and metastatic tumor growth, respectively. Hepatocytes selective targeting of liposome can be achieved through introduction of cells recognizing ligands on the liposomal surface. There is galactose receptor on the surface of hepatocytes which recognizes the galactosyl residues of desilated serum glycoproteins. So, galactose-terminated compound such as asialofetuinlactosylceramide have been used as the ligand on liposomes for targeting to hepatocytes.[62] M. Hashida and his co-workers synthesized the galactosylated liposomes for hepatocyte targeting and elucidate the relationship between the movements of galactosylated liposomes.[63] The glycyrrhizin derivative is also used as the ligand on liposome for targeting to hepatocytes.H. Kiwada and his co-workers developed the glycyrrhizin modified liposome for hepatocyte targeting.[62] PEG liposomes, also called stealth liposomes because when modifying the SUV liposome membrane by adding polyethylene glycol can markedly reduce the interaction of the vesicles with the stationary macrophages in the liver and spleen after i.v. application and this increases the circulation half-time. Pohlenet al prepared the 5-flurouracil enclosed in Stealth Liposome for the treatment of Liver Metastases.[64]

Nanoparticles (NPs): Biodegradable nanoparticles (NPs) are effective drug delivery devices. Various polymers have been used in drug delivery research as they can effectively deliver the drug to a target site and thus increase the therapeutic benefit, while minimizing side effects.[65] The controlled release (CR) of pharmacologically active agents to the specific site of action at the therapeutically optimal rate and dose regimen has been a major goal in designing such devices. Liposomes have been used as potential drug carriers instead of conventional dosage forms because of their unique advantages which include ability to protect drugs from degradation, target the drug to the site of action and reduce the toxicity or side effects.[66] However, developmental work on liposomes has been limited due to inherent problems such as low encapsulation efficiency, rapid leakage of water-soluble drug in the presence of blood components and poor storage stability. On the other hand, polymeric NPs offer some specific advantages over liposomes. For instance, NPs help to increase the stability of drugs/proteins and possess useful CR properties. Nanoparticles generally vary in size from 10 to 1000 nm. In the NPs drug is dissolved, entrapped, encapsulated or attached to a NPs matrix and depending upon the method of preparation, nanoparticles, nanospheres or nanocapsules can be obtained. For targeting of polymeric nanpoarticle to liver various ligands such as folic acid and asialoglycoproteins, galactosyl residues, glycyrrhizin derivative, have been introduced into drug carriers. C. Li et al designed albumin nanoparticles with surface modification by galactose residues to achieve the effectively targeting delivery of Oridonin into liver cancer cells.[67] Ping et al conjugated glycyrrhizin (GL) to the surface of chitosan nanoparticles (CS-NPs), prepared by an ionic gelation process.[58] These nanoparticles were developed for a drug delivery system targeting the liver through a specific interaction between GL and hepatocytes. The cellular uptake of GL-CS-NPs was dependent on incubation time and dose of nanoparticles, suggesting that internalization of these nanoparticles into hepatocytes was mostly mediated by a ligand receptor interaction. Liang et al prepared Paclitaxel-loaded poly (γ-glutamic acid)-poly (lactide) nanoparticles as a targeted drug delivery system for the treatment of liver cancer and they studied, the distribution of the particle size, the zeta potential, the drug loading content and the drug loading efficiency of the prepared nanoparticles, and their release profile and cytotoxicity on HepG2 cells (a liver cancer cell line) were investigated in vitro.[68] Additionally, biodistributions of the prepared nanoparticles were studied in vivo in normal mice and hepatoma-tumor-bearing nude mice. Q. Wang et al developed Norcantharidin-associated galacto-sylated chitosan nanoparticles for hepatocyte-targeted delivery and confirm its targeting characteristics.[69]

Polymeric micelles

Polymeric micelles have recently emerged as a novel promising colloidal carrier for the targeting of poorly water soluble and amphiphilic drugs. Polymeric micelles are considerably more stable than surfactant micelles and can solubilize substantial amounts of hydrophobic compounds in their inner core. Due to their hydrophilic shell and small size they sometimes exhibit prolonged circulation times in vivo and can accumulate in tumoral tissues. Polymeric micelles also used in liver targeting, Yang KW and his co-worker designed Diammoniumglycyrrhizinate (DG)-loaded conventional PIC micelles (mPIC micelles) and lactose-modified PIC micelles (Lac-PIC micelles) and they found that Lac-PIC micelles could deliver more DG to liver than mPIC micelles.[70]

Phytosomes

The term "Phyto" means plant while "some" means celllike. The phytosome structures contain the active ingredients of the standardized plant extract or its constituents bound to phospholipids, mainly phosphatidylcholine producing a lipid compatible molecular complex. Phytosomes have improved pharmacokinetic and pharmacological parameter which

in result can advantageously be used in the treatment of the acute and chronic liver disease of toxic metabolic or infective origin or of degenerative nature. It can also be used in anti-inflammatory activity as well as in pharmaceutical and cosmetic compositions.[71] Phytosomes are prepared by reacting the herbal extract in an aprotic solvent such as methylene chloride, dioxane and ethyl acetate with the phospholipid such as phosphatidylcholine, phosphatidylethanolamine or phosphatidyiserine dissolved in the same solvent. After solubilization has been completed, the complex compounds are isolated by removing the solvent under vacuum, by freeze drying or by precipitation with non-solvents such as n-hexane. Thus, the obtained complexes are lipophilic in character and soluble in a polar and aprotic solvent, in which the individual components of the complex are normally insoluble.[72] The phytosome process has also been applied to many popular herbal extracts including Ginkgo biloba, grape seed, hawthorn, milk thistle, green tea, and ginseng. The flavonoid and terpenoid components of these herbal extracts lend themselves quite well for the direct binding to phosphatidylcholine.[73] Ravarottoet al reported silymarinphytosome show better antihepatotoxic activity than silymarin alone and can provide protection against the toxic effects of aflatoxin B1 on performance of broiler chicks.[74]

Medications

There are so many drugs and chemicals that are used to cure the liver diseases and in treatment of injury to the liver. Probably the best-known medication that can damage the liver is acetaminophen, also known as Tylenol®. However, medications used to treat insomnia, nail fungus, high cholesterol, hypertension, cancer, seizures, pain, infections and many other conditions put an excessive strain on the live.[75]

Table 3. Allopathic medicine can also induce hepatotoxicity.[76]

Chemical	Consequence
Acetaminophen	Cytochrome P-450-2E1 generates a toxic metabolite NAPQI and this produces hepatic necrosis.
Amoxicillin	Moderate rise in SGOT and SGPT level, hepatic dysfunction including jaundice, hepatic cholestasis and acute cytolytic hepatitis.
Chlorpromazine	Infectious hepatitis with laboratory features of obstructive jaundice.
Ciprofloxacin	Cholestatic jaundice elevated SGPT, SGOT and alkaline phosphatase level.
Diclofenac	Elevation of ALT and AST level, liver necrosis, jaundice and fulminant hepatitis.
Erythromycin	Increased level of SGPT, SGOT, hepatocellular and/or cholestatic hepatitis with or without jaundice.
Fluconazole	Elevated transaminase level, hepatitis, cholestasis and fulminant hepatic failure
Isoniazid	Elevation of serum transaminase level, severe and fatal hepatitis
Oral contraceptives	Intrahepatic cholestasis with pruritus, jaundice, benign neoplasm, rarely neoplasm of the liver and hepatic vein occlusion.
Rifampin	Hepatitis, hyperbilirubinemia and cholestasis.

CONCLUSION

Targeted drug delivery can be a highly desirable strategy to improve the therapeutic outcome, with significantly decreased toxic side-effects compared to traditional chemotherapy. Previously most studies were based on conjugating carriers or drugs with targeting ligands, such as antibodies and sugars. The recent strategies use site specific drug carriers such as antibodies, peptides, natural and modified or synthetic polymers. Beside these, prodrugs are also investigated that are designed to cleave in a site-specific manner. Some prodrugs gain cell specificity whereas others gain specificity by using cell-specific surface receptors (e.g., bile acid transporter) that facilitate prodrug transport into liver cells. Till today very few delivery systems are marketed as liver targeted drug delivery system.

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