Review Article

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Drug targeting strategies for liver cancer and other liver diseases

Abstract

Treatment of liver cancer and other diseases are a challenging task for the current researchers in the pharmaceutical field. There are some physiological barriers like RES uptake, opsonization and first–pass metabolism of therapeutics present that make drug therapy more complex. Generally, conventional cancer therapy approaches give low response rate or remain un–success due to multi drug resistance (MDR), high clearance rate, severe adverse effect due to unwanted drug distribution and in–adequate concentration reached in cancer cells. Therefore it is a need to develop novel strategies which will target drug molecule specific to affected liver cells. In the current era of research and development, various approaches have been utilized to improve the drug delivery and drug targeting. The new targeting approaches are based on the use of targeting ligands which can conjugate with nanocarrier or with drug molecules. These conjugates systems are accumulate passively or actively. This review focus on some liver cancer cells specific targeting ligands such as manos6 phosphate, asialoglycoprotein, galactoside, lactobionic acid, PDGF, antibodies, aptamers, avimers which have been utilized to target cancer cell after conjugation with the therapeutic system. This review also describes the novel targeting approaches including latest targeting molecules and targeted drug delivery system used for the treatment of liver cancer.

Keywords: reticuloendothelial organ, hepatocellular, kupffer cells, hepatocytes, liposomes

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Introduction

Liver diseases, particularly hepatitis–B (viral HBV infections), liver fibrosis and hepatocellular carcinoma (HCC) are the major causes of disability and mortality worldwide.[1] These require long–term drug therapy. Liver–specific drug delivery is helpful in decreasing side effects by reducing drug distribution in non–target organ and improves the therapeutic efficacy by concomitantly increasing the drug concentration in target cells.[2] As the liver is the largest reticuloendothelial organ in the body, macrophages in the liver (i.e. kupffer cells) are attractive candidates to serve as the effector cells for therapy of hepatic diseases.[3,4] The body distribution and opsonisation of colloidal drug carrier systems by macrophages seem to be influenced by their particle sizes and surface characteristics.[5] The feasibility of particles ranged in 50–200nm to arrive at fenestration in the hepatic sinusoidal endothelium might lead to hepatic accumulation after IV injection. Although the carrier systems like nano particles or liposomes could not directly reach the hepatocytes, the uptake of IV injected particulate drug carriers by macrophages cells might as well be the main limiting factor in the efficient targeting of a drug to the kupffer cells in the liver.[6]

Basic epidemiology of liver diseases

Hepatitis–B

Hepatitis–B is caused by a DNA virus; it is transmitted through parenteral or mucosal exposure to infected blood, serous fluids and other body fluids such as seminal and vaginal fluids. Perinatal transmission (from an infected mother to infant during birth), unsafe needle sharing, blood transfusion practices and sexual contact are common routes of infection. Hepatitis–B is of two types acute and chronic. Chronic HBV infection can be divided in to three major phases based on virus host interactions, immune tolerant, immune clearance and inactive carrier phase.[7] Characteristic symptoms of immune tolerant phase is seropositivity of hepatitis–B antigen (HBe Ag), active viral replication (elevated HBV DNA blood levels) with little or no evidence of liver inflammation [i.e. Normal serum alanine aminotransferase (ALT) levels. In the immune clearance phase, liver inflammation occurs (elevated serum ALT levels). In this phase, anti–viral drugs are used to reduce the risk of developing liver cirrhosis and HCC. Clearance of HBeAg and the development of anti–HBeAg antibodies (HBeAg seroconversion), occurs in inactive carrier phase which is accompanied by a concomitant reduction in HBV DNA levels and normalization of serum ALT levels with improvement in liver fibrosis and inflammation over time.[8] Chronic hepatitis B also can lead to a type of liver cancer known as hepatocellular carcinoma.

Liver fibrosis

Chronic liver injury and persistent wound healing occur in liver fibrosis that could lead to cirrhosis i.e fibrosis is a wound healing process of liver in the body in which extracellular matrix (ECM) proteins and collagen fiber are formed that deposits and retain in liver tissue and responsible for causing tissue scar. Some other complications are also encountered during tissue injury such as loss of hepatic functions, ascites, portal hypertension, increased risk for esophageal varices and HCC. HCC is a most serious complication that often fatal. Drug or toxin–induced injury, chronic alcoholism, viral hepatitis, prolonged biliary obstruction and inherited metabolic disorders such as Willson's disease and hematochromatosis are main causes of liver fibrosis.[8] Cellular and molecular mechanisms of liver fibrosis has greatly advanced. Activated hepatic stellate cells (HSCs),

portal fibroblasts, and myofibroblasts of bone marrow origin have been identified as major collagen–producing cells in the injured liver. These cells are activated by fibrogenic cytokines such as TGF–β1, angiotensin II, and leptin. Cytokines such as transforming growth factor–β (TGF–β) and platelet–derived growth factor (PDGF) contribute to HSC activation and proliferation responsible for activation of myofibroblasts. Some other cytokines, transcription factors and intracellular signaling are also involve in this process. Liver fibrosis can be controlled by inhibiting the activation of HSCs.

Hepatocellular carcinoma

Liver diseases such as chronic hepatitis–B or C virus infections, alcoholic cirrhosis and non–alcoholic steatohepatitis, if occurs for a long time, results in HCC.[9] The development of HCC from each of the chronic liver diseases is a complex and multistep process with an accumulation of genetic and epigenetic alterations, which culminates in the aberrant activation of molecular signalling pathway connected to cellular proliferation and survival, as the central themes.[10] Hepatitis–C is a single–stranded RNA virus, having high genetic variability. There are six different genotypes of HCV isolated i.e genotype–I, II, III, IV, V and VI. Simultaneously infection of Hepatitis–B and Hepatitis–C virus in a patient liver who already suffered from cirrhotic has increases the chances of HCC.[11–18]

Treatments of various liver diseases

Hepatitis–B therapy

HBeAg seroconversion and by reduction of the circulating HBV DNA levels results in retardation of active HBV replication, which is a helpful tool to treat liver injuries and liver disease progression. The seven U.S. Food and Drug Administration (FDA) approved anti–HBV drugs can be broadly categorized as interferons (IFN) (IFN–α2b and pegylated IFN–α2a), nucleoside (lamivudine, entecavir and telbivudine) and nucleotide (adefovir and tenofovir) analogs. Proposed the use of monostearin containing solid lipid nanoparticles (SLN) as an effective drug delivery system for Adefovir and Dipivoxil in HepG2.2.15 cells, leading to significant down regulation of DNA levels in HBsAg, HBeAg and HBV in comparison to the free drug which leads to an enhanced reduction in the viral loads in the liver. Direct drug delivery to liver shown less systemic side effects. Reported that incorporation of a derivative of the nucleoside analogue iododeoxy–uridine into recombinant chylomicrons leads to selective targeting to liver parenchymal cells that was achieved effective and higher intracellular drug concentrations in liver. These drug carrier complexes represent a conceptual advance in the development of an effective and safe therapy for hepatitis–B. demonstrated a hepatocyte targeted system i.e. N–acetylgalactosamine–conjugated melittin–like peptide (NAG–MLP) with potent cholesterol–conjugated siRNA (a targeting coagulation factor VII) that are capable to target conserved HBV sequences resulted in multilog repression of viral RNA, proteins, and viral DNA. These conjugates holds great promise as a new therapeutic for patients chronically infected with HBV.

Liver fibrosis therapy

No standard treatment is available for liver fibrosis due to the complex and varied etiologies of liver fibrosis. Anti–fibrotic therapy differs depending on the type of liver disease. Combination of pegylated IFN and ribavirin used in the treatment of liver fibrosis.[11] Alcohol asceticism is an efficient way to recover alcohol–

induced liver fibrosis.[12] For treatment of autoimmune hepatitis and acute alcoholic hepatitis, corticosteroids are used because of inflammatory nature of the disease.[13] Rennin–angiotensin system inhibitors, IFN–γ, peroxisomal proliferator–activated receptor (PPAR)–γ ligands, pirfenidone, colchicines and other herbal medicines are also used for the treatment of liver fibrosis.[13,14] Reported that combined delivery of sorafenib and a MEK inhibitor via CXCR4–targeted nano particle to prevent activation of ERK in activated hepatic satellite cells (HSCs). It was shown anti–fibrotic effects in the CCl4–induced murine model. Targetable HSCs represents a promising strategy to prevent the development and progression of fibrosis–associated HCC. Demonstrated that silibinin and siCol1α1 loaded Vitamin A–decorated biocompatible micelles which was safe and efficient HSCs–targetable chemogene–delivery system for inhibiting fibrous collagen I and may serve as a novel and effective clinical option for treatment of liver fibrosis. Prepared polypeptide pPB–modified stable nucleic acid lipid nanoparticles (pPB–SNALPs) to selectively deliver siRNAs against heat shock protein 47 to the liver for hepatic fibrosis therapy. The study was shown increased uptake of prepared system by LX–2 cells and primary hepatic stellate cells (HSCs) of mice. It was shown increased liver distribution and HSC uptake in vivo. In addition, pPB–SNALP also exhibited an enhanced inhibitory effect on TAA–induced hepatic fibrosis in mice model with high gp46 mRNA expression.

Anti–HCC therapy

Surgical resection and liver transplantation is only treatment of HCC in the early stage of the disease. Nonsurgical local ablation techniques such as percutaneous ethanol injection and radiofrequency ablation are also used in HCC treatment.[15] The single liver tumor can be removed by surgical resection but an inadequate supply of donor organ is a limiting factor for it. In the case where surgical resection or transplantation is not suitable, nonsurgical local ablation treatments are very useful. Percutaneous ethanol injection is an intra–tumoral injection of absolute ethanol is given with use of ultrasound. It causes tumor mass dehydration and necrosis. RFA is the percutaneous delivery of radiofrequency energy via single or multiple electrodes which results in thermal necrosis of the cancerous tumor.[16] Some drugs also used in the treatment of HCC like doxorubicin, cisplatin and 5–fluorouracil, used alone or in combination. Grafted Xyloglucan with the doxorubicin (DOX) and galactosamine to target liver hepatocytes[1]. In vitro cytotoxicity experiment revealed that similar cytotoxicity as free DOX against HepG2 cells and when it incubated with HeLa cells there was no significant cytotoxicity change while in case of a human tumor xenograft nude mouse model, the prepared conjugates shown higher therapeutic effect than without conjugated and plain doxorubicin. They revealed that the prepared conjugates was improved the transfection efficiency and hepatocyte specificity that can be for tumor therapy. Demonstrated mechanism of GG–8–6 (1) induced apoptosis, G2/M arrest of, and the activation of caspase pathways in HCC cells. *In vivo* anti–tumor experiments showed that GG–8–6 (1) could significantly inhibit the growth of tumor in the mouse xenograft tumor model. At the dose of 40mg/kg of GG–8–6 (1), the inhibition ratio was 67.9% without weight loss. These finding suggested that GG–8–6 (1), a new cyclic peptide, might be a potential candidate for developing new anti–HCC drug in the coming future. Prepared antibody conjugated nano particle containing Sorafenib which was shows higher cellular uptake by HepG2 cells than without antibody–conjugated nanoparticles. The prepared system was down regulated the expression of anti–apoptosis molecule MCL–1, which resultant in polymerization of Bax. It was promoted the mitochondrial release of cytochrome C, resulting in cellular apoptosis. Moreover,

the antibodies conjugated nanoparticles significantly inhibited the growth of HepG2 xenograft tumors in nude mice without producing evident side effects. Developed calcium phosphate nanoparticles were loaded with FTY720 and siRNA. These NPs were stable in systemic circulation and easily up taken by affected cells due to their nano metric size. Furthermore, co–delivery of FTY720 and Beclin 1 siRNA significantly increased cytotoxicity *in vitro* and *in vivo* compared with that caused by treatment with the free drug alone.

Liver–specific targeting

Different carrier systems like liposomes, nano particles when entering into the bloodstream, nonspecific interaction occur with serum proteins and surface deposition of antibodies/complement proteins, this process called opsonization. This interaction results reduction of overall dose and reduction of circulation time of carriers via mechanical entrapment of aggregates in the alveoli and clearance by reticuloendothelial system in liver, spleen and bone marrow especially if the size is greater than 200nm and a large surface negative charge is present. The endothelial cells lining the liver sinusoids are another component of the RES h scavenger receptors that can internalize particles up to 0.23μm *in vivo*. Steric stabilization and shielding of carriers by incorporating PEG moieties minimizes protein binding and reduce non–specific scavenging of carriers by RES.[17] Generally targeting achieved by passive and active targeting. Passive targeting is obtained through desirable size and surface modified nanocarriers. Site–specific delivery of therapeutics can be obtained by passive targeting which increases the local concentration of the drug and reduces undesirable side effects. Active targeting is obtained by surface modification of nano particles with specific ligands such as carbohydrates, peptides, proteins and antibodies, which has the benefit of facilitating uptake in a liver cell type–specific manner, hence minimizing alterations to the physiological functions of other liver cell types.

Passive targeting

Nano particle therapeutics accumulates at specific body site due to certain anatomic or patho physiological features, this type of targeting known as passive targeting.[18] Liver sinusoids capillaries have two special characteristics first 100–200nm fenestrations present along the endothelial wall and second is the absence of basal lamina. These characteristics facilitate the passive accumulation of nano particle therapeutics. Nano particle therapeutics having a diameter less than 200nm facilitates passive liver targeting because these particles can extravasate through the slightly larger sinusoidal fenestrations, due to this process nano particle therapeutics obtained high local concentration at space of disuse, where distribution to the various liver cell types can occur.[19] EPR effect that was first described by Matsumura and Maeda in 1986, also helps in passive accumulation of nano particle therapeutics in the liver. Tumour has some distinctive features which increase EPR effect; these are leaky tumour vasculature leading to rapid and incomplete tumour angiogenesis to meet the elevated demands for oxygen and nutrients that results in enhanced permeability and extravasation of macromolecules, and impaired lymphatic drainage, which favors the retention of nano particle therapeutics in the tumour tissues.[20] Endothelial cells have gap junction size of between 400 and 600nm, therefore nano particle therapeutics expected to be extremely efficient at extravagating from the tumour microvasculature to result in a high local tumour interstitial concentration.[21,22]

Active targeting

Site–specific delivery of therapeutic system increases the local concentration of the drug, due to which therapeutic efficiency of drug increases and unwanted side effects decreases. Non–parenchymal sinusoidal endothelial cells (SECs), kupffer cells (KCs), hepatic stellate cells (HSCs) and the predominant parenchymal hepatocytes are responsible for diverse physiological functions of the human liver. HSCs are the main target in liver fibrosis because they are responsible for 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 are the main target for HBV infections and HCC.[23] Targeted drug delivery systems is very useful treatment of many liver diseases such as chronic viral hepatitis, liver fibrosis and hepatocellular carcinoma and it also beneficial to reduce side effects by reducing drug distribution in non–target cells and improve the therapeutic efficacy by concomitantly increasing the drug concentration in target cells. Sugar moieties are commonly used to target asialoglycoprotien receptors in hepatocytes mannose receptors in kupffer and liver endothelial cells. Some ligand–mediated approaches for liver cell targeting

1. Mannose 6–phosphate receptors present on hepatic stellate cells, by using mannose 6–phosphate as ligand we can target the liver, this strategy used in the treatment of liver fibrosis.[24–25].

2. Retinol binding protein receptors present in hepatic stellate cells, vitamin–A used as a ligand to treat liver fibrosis.[26]

3. Type VI collagen receptor and PDGF receptor present on hepatic satellite cells are highly expressed in liver fibrosis, Cyclic RGD and PDGF respectively used as a ligand in the treatment of liver fibrosis.[27]

4. Scavenger receptor class A is targeted by human serum albumin present on hepatic stellate cells also useful in liver fibrosis treatment.

5. Asialoglycoprotien receptors present on hepatocytes. Asialoorosomucoid, Galactoside,[28,29] Galactosamine.[30] Asialofetuin,[31] Sterylglucoside,[32] Lactose/lactobionic acid,[33,34] PVLA.[35] used as a ligand to target these receptors, this strategy used in the treatment of HCC.[36]

6. Scavenger receptor class B type I receptors present on hepatocytes, which are highly expressed in hepatoma cells and tumors, Apolipoprotein A– I used as a ligand to target these receptors.[37]

7. Plasma membrane fatty acid binding protein (Putative) are targeted by Linoleic acid, these receptors also present on hepatocytes and used in the treatment of HC.[38]

8. Glycyrrhizin receptors are targeted by Glycyrrhizin and Heparan sulphate by Acetyl–Cknekknkiernnklkqpp–amide ligand; both receptors present on hepatocytes and used to treat HCC.[39]
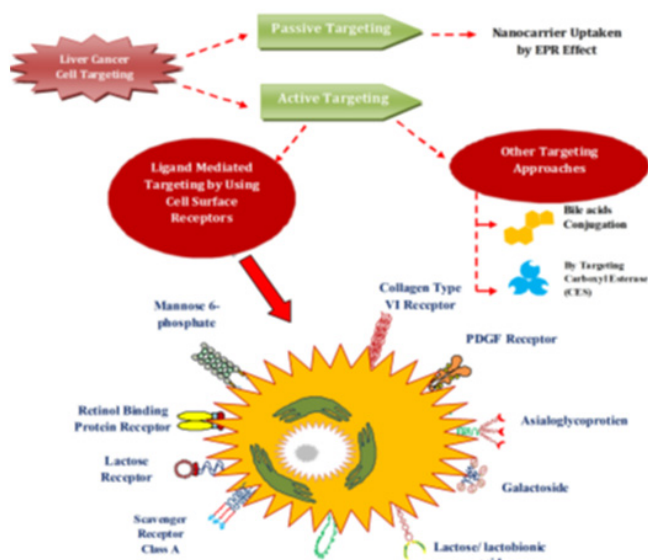
Other approaches for targeting liver cancer

Targeting carboxyl esterases (CES) over expressed by cancerous liver cells

Since in liver cancer cells carboxyl esterase–1 (CES–1) & carboxyl esterase–2 (CES–2) are over expressed, the derivative of the drugs converted to active form by CES–1 or CES–2 in the liver, thus

an active form of the drug is available to target cancer cells of the liver. Carboxyl esterases are serine esterases that cause hydrolysis of various esters and carbamates. Therefore carbamate prodrugs of some anticancer drugs are used to target liver cancer.[40] Some examples of prodrugs are Carbamate prodrugs of doxazolidine, 5–FU, and camptothecin Figure 1.

Figure 1 Targeting approaches for liver cancer cell.

Tethering bile acids

Another approach for targeting liver cancer is the use of bile acids, which are efficiently taken up by hepatoma cells via sodium–independent transport carriers. A series of bile acid–platinum conjugates have been synthesized and their cytotoxicity investigated both *in vivo* and *in vitro*. Conjugate Bamet–UD2 exhibited enhanced uptake in hepatocytes and has got ability similar to that of cisplatin to inhibit tumor growth and tendency to prolong survival time.[41]

Treating liver cancer with glass beads

Treating liver cancer with tiny radioactive glass beads is a new method which has been recently introduced. In this treatment, radioactive glass beads injected into the main artery which supplying blood to the liver. The blood carries the beads into the liver, where they deliver localized radiation to malignant cells in liver tumors.[42]

High density lipoprotein particles based targeting

Use of targeting moiety increases the therapeutic effect of the drug and decreases side effects of drug and decreases side effects of the drug.[43–45] Various targeting carriers are used in targeting to organs in which lipoproteins are potential drug carrier. Lipoproteins are spherical particles which are consisted of a polar lipid core surrounding phospholipid monolayer, cholesterol and apoproteins are embedded in it. Because core consists of a polar lipid highly hydrophobic drugs can be easily incorporated in to it.[46] Lipoprotiens are endogeneous in origin so these particles are completely biodegradable and non–immunogenic and also RES system is unable to recognize them. HDL is an important class of lipoproteins which is transport cholesterol from various tissues to the liver. There is specific recognition receptor SR–BI of apolipoprotien A–I (apo A–I) on hepatocyte surface. HDL takes up cholesterol from peripheral tissues and delivers it back to the liver via apoA–I, which binds to its receptor on the liver cell membrane,[47] due to this reason, HDL–drug complex is a very useful carrier for delivering antitumor drugs to hepatocyte cells.

Liver targeting micelles

Polymeric micelles have some remarkable advantages, such as small size and narrow size distribution,[48] long term survival in blood circulation and sulubilization of hydrophobic drugs.[49,50] Due to these advantages polymeric micelles are very useful to deliver the anticancer drug. These particles have the nano metric range and formed from self–assembled amphiphilic block co–polymers having core–cell architecture in aqueous solution. Therapeutic agents remain in this hydrophobic core and avoid possible degradation during *in vivo* transportation and hydrophilic shell of micelle maintain a hydration barrier that can effectively stabilize the drug–loaded micelles in the blood circulation.[51,52] Silibinin and siCol1α1 loaded Vitamin A–decorated biocompatible micelles were developed to deliver therapeutic molecules safely and efficiently to HSCs to inhibiting fibrous collagen–I for treatment of liver fibrosis.

Surface modified liposome

A liposome is a novel drug delivery system consisting of lipid layer containing water phase. Both hydrophilic and lipophilic drugs can encapsulate in to it. By modification of the surface of the liposome, drugs can be targeted to desired site or organ in the body.[53,54] When liposomes administered intravenously, it is cleared by RES.[55] Kupffer cells in the liver are part of RES, that's why liposome is mostly accumulated in the liver specially in non–parenchyma cells.[56] ASGP–receptor present on hepatocytes, these receptors recognised by galactose moiety attached as a ligand on the surface of liposome and they incorporated in to cells by endocytosis. Galactose terminated compounds such as lactosylceramide,[57] asialofetuin, or synthetic glycolipids, are used to modify liposomes. For example sterylglucoside containing liposome accumulated in the liver, especially in hepatocytes.[58,59]

Chylomicron emulsion

Natural spherical macromolecular emulsion particles called lipoproteins, these lipoproteins are involved in intercellular lipid and cholesterol transport in the circulation.[60] Chylomicrons are triglyceride–rich lipoprotein emulsions, dietary lipids absorbed through the intestine membrane in to blood circulation are packed into chylomicrons.[61,62] Lipase is a lipoprotein enzyme which can hydrolyze the core triglycerides of the chylomicron in blood circulation. On the surface of the chylomicron, many different apolipoprotiens are anchored by their receptors receptors these modified chylomicrons are taken up by liver parenchymal cells. When chylomicrons are reconstituted by natural lipids (100nm) was preferentially taken up by liver hepatocytes.[63] When a drug is incorporated in to chylomicron emulsion it can be easily targeted to the liver. Drug inside the emulsion is chemically more stable than a free drug because it is protected from enzymatic degradation. Also, the drug shows significant release when it is inside chylomicron emulsion.[64] Nucleoside analogue iododeoxy–uridine was successfully delivered by recombinant chylomicrons to target liver parenchymal cells for hepatitis B treatment. It was delivered encapsulated therapeutics in higher concentrations in liver.

Magnetoliposomes

It is a novel drug delivery carrier system.[65,66] Generally used anticancer drugs exhibit severe side effects that limit their effective therapeutic use.[67] Magnetoliposome is an approach to increase the beneficial/adverse effect ratio of such drugs, which is used to target diseased organs or tissues by using a magnetic field.

Conclusion

There are many limitations are present in conventional approaches for the treatment of liver cancer. In this conditions, required some new strategy to treat or manage advanced liver cancer. This review concluded that there are some new strategies available such as ligand mediated active targeting, nano carrier based RES uptake and some molecular conjugates of targeting ligands and active drugs that applicable to target effectively and killed the actively proliferating cancer cells,[68,69] and the available targeting ligands such as asialoglycoprotein, lactose and mannose, liver cancer cell specific antibodies, aptamers, avimers, bile acid conjugation, Collagen Type VI Receptor, Collagen Type VI Receptor and others ligands were successfully used for targeting liver cancer and further can be used for targeting and deliver anticancer drug or drug which are used to treatment of liver diseases.[70]

Conflict of interests

Author declares that there is no conflict of interest.

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