Chapter 6
Hepatic Targeting: Physiological Basis and Design Strategy

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6.1 Introduction

The mammalian liver plays a stalwart role in the metabolism of carbohydrates, fats, and proteins and detoxification of organic by-products, cellular debris, drugs, pesticides, xenobiotics, foreign particles, etc. from the systemic circulation. It is also involved in anabolism of cholesterol, steroid hormones, biochemicals, and proteins. Being the largest and strategically located internal organ with a plethora of functions, it is prone to many contaminants, injuries, and disorders. Diseases afflicting the liver continue to be the fifth most common cause of death and are ever-increasing [1]. Grave hepatic disorders range from liver fibrosis or cirrhosis, fulminant hepatitis or viral hepatitis (A, B, C, D, E, G), primary liver cancer, hepatic cholangiocarcinoma, severe congenital liver failures, metabolic genetic disorders, and hepatocellular carcinoma (HCC). HCC has one of the lowest (1-year) survival rates among all cancers [2] with about 5,00,000 new cases diagnosed every year, especially in developed nations [3]. While surgical interventions are resorted to in benign cancers, chemotherapy is the preferable treatment in cancers [4].

Most drugs achieve high hepatic concentration after administration. However, drugs for treating liver disorders have often experienced circumscribed success with a high relapse rate, due to limited efficacy and poor sensitivity at conventional doses. Dose escalation is often hindered by patient tolerability, hepatic and off-target safety concerns, and high resistance due to efflux pumps (P-glycoprotein) which limit their efficacy [5]. Moreover, different hepatic conditions obligate high degree of specificity and accumulation within the proper intrahepatic cells for addressing optimal therapeutic potential. At the receptor level, the treatment varies with varying

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harboring sites of disorders. This has fueled an exigent need for effective, safe, yet affordable liver targeted drug delivery systems.

The past two decades have seen an increase in nanotechnology based liver targeted drug delivery, which relies on altered biodistribution for enhanced therapeutic efficacy. The unprecedented development relies on the unique size and surface characteristics, overcoming anatomical and physiological barriers coupled with enhanced penetrability. Enabling early diagnosis is yet another feature [6]. Nanoparticulates in the size range of 50–250 nm are easily accumulated in the liver [7]. Besides, engineering of these nanoparticulates for site specific hepatic delivery is amenable.

The present chapter comprehensively reviews the liver architecture, various cell types, and approaches for targeted drug delivery to liver. Active and passive targeting strategies with a focus on the hepatic receptors are detailed.

6.2 Liver Architecture: Normal and Pathological State

The liver is a histologically complex organ with four types of substantial target cells—hepatocytes, Kupffer cells, sinusoidal endothelial cells, and hepatic stellate cells. The histological unit of the liver is the lobule. Primarily, about 80 % of the liver comprises parenchymal cells (PC) or hepatocytes (Fig. 6.1) [8]. The hepatocytes and discontinuous hepatic sinusoidal endothelial cells (SEC) are physically demarcated by the space of Disse also known as peri-sinusoidal space containing dispersed fat containing lipocyte, or hepatic stellate cells (HSC). Plasma is preferentially filtered through the sinusoids into the space of Disse, and provides body lymph.

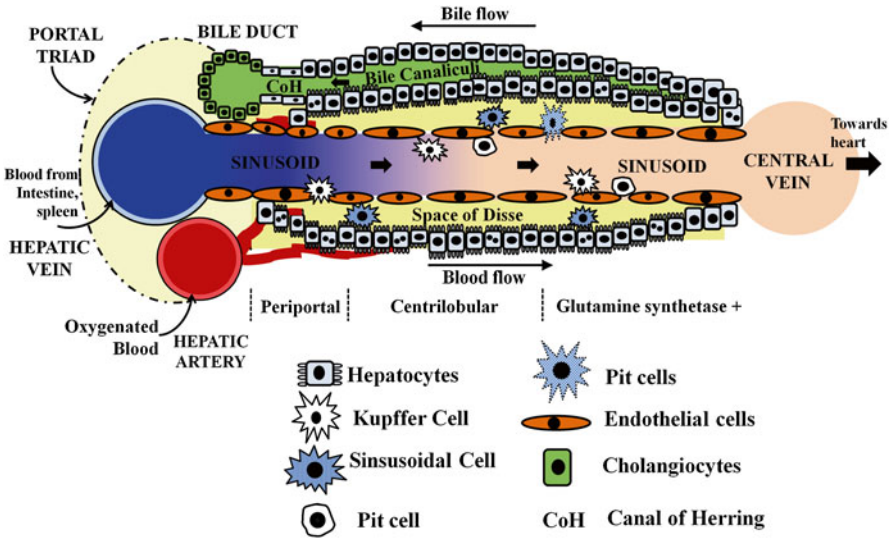

Fig. 6.1 Pictorial representation of a liver lobule

Hence, exchange of nutrients, proteins, and wastes between hepatocytes and blood occurs in this microenvironment of space of Disse. In obliteration of this space as in alcoholic liver diseases, uptake by hepatocytes is hindered. The widened space of Disse increases resistance to sinusoidal blood flow thereby raising portal pressure [9]. The Kupffer cells (KC) or the resident non-parenchymal liver macrophages (~18 %) are located along the luminal side of the EC in the sinusoidal area with no specialized contacts [10]. Also located on the endothelial lining are the pit cells that correspond to large granular lymphocytes with natural killer activity. The RES (reticuloendothelial system) of liver consists of SEC and KC [11, 12]. The liver receives oxygen rich blood through the hepatic artery and hepatic portal vein (shunted capillaries from spleen and intestine). The space connecting the biliary ductules and hepatocytes is the Canal of Hering [13]. Canal of Hering plays an important role in carcinogenesis [14].

6.2.1 Reticuloendothelial System (RES) Cells of the Liver

Reticuloendothelial system (RES) is a part of human body defense, derived from bone marrow contributing to both nonspecific and specific immunity. Till recently the RES was considered synonymous with the mononuclear phagocytic system (MPS). However, it has now been established that RES constitute both wandering and sessile phagocytic cells, e.g., monocytes, SEC, KC, polymorphonuclear leucocytes, dendritic cells, histiocytes. whereas the MPS is restricted only to macrophages like KC. The role of the liver RES can be summarized as follows:

- Engulfment and ingestion (phagocytosis) of abnormal cells, pathogens and foreign substances.
- Presentation of antigens or foreign invaders to lymphocytes which secrete antibodies.

6.2.1.1 Kupffer Cells (KC)

KC form only 6.5 % of liver volumes, but contribute to 80–90 % of tissue macrophages present in the human body [15]. KC are found in high number in rats over ~20 months old [16] following partial hepatectomy, or a single intravenous injection of zymosan [17] and in alcohol related hepatitis and liver diseases [18]. Depletion of KC is seen on administration of gadolinium chloride [19], clodronate liposomes [20], and in HCC [21].

Phagocytosis of IgG-coated erythrocytes also decreases the complement receptor of KC [22]. KC exhibit abundant lysosomes and pronounced phagocytosis as they are specialized macrophages of the reticuloendothelial system (RES). The typical macrophage activity of KC plays a crucial role in innate immune defense, ischemia, resection, acute and chronic responses to toxic compounds and removal of particulate,

damaged debris, bacterial and viral infections, endotoxins, etc. [23]. Activation of KC produces pro-inflammatory mediators, e.g., nitric oxide, prostanoides, signaling molecules (cytokines, TNF-alpha), macrophage colony-stimulating factor, reactive oxygen species, other growth factors (innate immune defense) and prevents liver inflammation [15]. Balance of these secretions is necessary to maintain a harmonious environment for the hepatic cells and the extracellular matrix. Exposure to lipopolysaccharide endotoxins leads to damage of hepatocytes and liver injury [24]. KC is responsible for pathogenesis of non-alcoholic steatohepatitis, viral hepatitis, fibrosis, intrahepatic cholestasis, alcoholic liver disease, rejection of liver during liver transplantation, etc. [18]. Besides macrophagic activity, KC plays a key role in arresting circulating tumor cells and controlling metastasis [25] as well as in the clearance of erythrocytes by scavenger receptors [26].

6.2.1.2 Sinusoidal Endothelial cells (SEC)

The roles of KC and SEC exhibit some overlap and are at times controversial [11, 12]. SEC are considered dormant of phagocytosis; however, on impairment of KC, SEC acquire phagocytotic competence. SEC constitutes about 40 % of hepatic cells, and represents a barrier between blood and hepatocytes. SEC form small fenestrations (50–200 nm)and are grouped together to form sieve plates permitting filtration, thereby allowing diffusion of many substances but not of chylomicron size (80–500 nm) [27]. Compared to KC, SEC uptake colloids of size <0.23 μm or soluble materials, while KC can take up larger particles up to 15 μm [28]. However, impairment of KC facilitates uptake of large particles by SEC [11, 12]. Contrary to the above, colloidal carbon is reported to preferentially accumulate in KC upon intravenous injection. Smaller colloid particles fail to reach SEC due to aggregation in plasma or adherence to platelets resulting in their phagocytosis by KC [29]. In non-alcoholic fatty liver disease, simple infiltration of fat and chylomicrons is enhanced and accumulated in liver. Liver SEC exhibit huge receptor endocytotic capacity for extracellular matrix components, e.g., hyaluronic acid, collagen (especially in SEC not expressing Endo180) and play a major role in metabolism of the extracellular matrix [30, 31]. Damage to SEC is associated with graft versus host disease, veno-occlusive disease and sepsis [32]. Deposition of extracellular matrix leads to thickening of SEC causing defenestration of SEC followed by fibrosis. Overall, SEC plays an important role in regulation of hemostasis, inflammatory reactions, microcirculation, and immunity [33, 294].

6.2.1.3 Hepatic Stellate Cells (HSC) (Ito Cells or Lipocytes)

HSC house 80 % of retinoid found in the entire body. Cellular retinol-binding protein, type 1 (CRBP) binds to retinol and undergoes receptor mediated endocytosis of the complex complex containing retinol and Retinol Binging Protein (RBP), to maintain plasma retinol [34]. Besides these, platelet-derived growth factors, epidermal and fibroblast activation protein, adhesion molecules, cytokines, vascular cell integrins, etc. activate HSC [35].

Under normal physiological conditions, HSC are in the quiescent state [36]. In the activated state they act as antigen presenting cell and stimulate proliferation of natural killer T cells [37]. HSC secrete fibronectin and vascular endothelial growth factor stimulating production of nitric oxide. As a result retinol is lost from the cell and HSC undergo morphological change. This leads to increased proliferation and trans-differentiation to fibrogenic myofibroblast-like cells [38] which secrete collagen scar tissue and fibrogenic and inflammatory cytokines (extracellular matrix). Fibrosis and cirrhosis therefore result [39].

6.2.1.4 Pit Cells/Large Granulated Lymphocytes

Pit cells, large granulated lymphocytes are present in lower numbers, approximately 10 % of KC. They function as natural killer cells. These cells are 0.2–0.5 μm in diameter and majorly contain acid phosphatase. Pit cells possess much higher cytotoxic activity and higher grade of activation with diverse immune phenotypic features. Situated in the sinusoidal lumen, their cytoplasmic processes adhere to KC and with microvilli of hepatocytes through the endothelial sieve. When triggered by biological response modifiers, proliferation of pit cells occurs with migration towards the Space of Disse to exhibit viricidal activity. Interleukin-2 released during viral infections and neoplasms is also known to trigger such transit [40]. Pit cells exhibit spontaneous antitumor activity by adhering to tumor cells [41] and also kill hepatitis virus-infected cells [42].

6.2.2 Non-reticuloendothelial System Cells (RES) of Liver

6.2.2.1 Hepatocytes

Hepatocytes are principally involved in the metabolism of carbohydrates, fat, and proteins as well as in secretion of bile, clotting factors, and cholesterol and protein transporters. They comprise ~80 % of liver volume with distinct nucleoli, both rough and smooth endoplasmic reticulum, mitochondria, and Golgi apparatus [43]. These highly metabolic active cells break down toxic chemicals, drugs and hormones which are easily eliminated from circulation. This is also known as “first pass effect.” Hepatocytes lining the bile canaliculi possess numerous Golgi vesicles. Hepatocytes are critical in synthesis of molecules supporting homeostasis of glucose and cholesterol and maintaining energy levels. They are storage sites for glucose, vitamins (A, D, E, K, folate, B12), and minerals (Cu, Fe). Metabolic activities in the liver lobule although compartmentalized are highly integrated. The periportal hepatocytes involve themselves in gluconeogenesis and glycogenolysis, while the centrolobular hepatocytes are responsible for glycogen synthesis and glycolysis. The glutamine synthetase positive centrolobular hepatocytes are involved in metabolism of ammonia and the periportal hepatocytes are responsible for removal of ammonia. The microenvironmental signal for the differential positions is the differences in

oxygen gradient [44]. Hepatocytes are well differentiated with high and unlimited capacity of replication and longevity. The rapid growth of liver (60–70 %) after resection is mainly dependent on hepatocyte proliferation and hyperplasia [45]. Extensive proliferation of hepatocytes and cellular damage is observed in liver injury, hepatocellular carcinoma, chronic hepatitis, and exposure to certain chemicals [46] and continues till cirrhosis. Decreased hepatocyte number is seen in chronic consumption of ethanol [47], decreased hepatocyte growth factor activity and impaired liver regeneration, ischemia–reperfusion, etc. [48].

6.2.2.2 Biliary Cells: Cholangiocytes

The biliary cells are a part of hepatic cell lineage developed during embryogenesis along with hepatoblasts and form 1 % of the liver [49]. They exhibit heterogeneity in both morphological functions, extending from the liver hilum to the bile duct. The function and phenotype properties vary with hepatocytes though derived from the same lineage. The biliary epithelial cells maintain contact with the hepatoblasts and express markers for hepatocytes (albumin and alpha-fetoprotein) and bile duct epithelium (cytokeratins 7 and 19, carcinoembryonic antigen, carboanhydrase, glutamyl transpeptidase) [14, 40]. They regulate bile formation, liver inflammatory process, fibrogenesis, and angiogenesis. Bile duct cells are affected in bile duct cancer (cholangiocarcinoma) predominantly observed in women [50].

6.2.2.3 Stem Cells

Recently, an unsettled discussion has been the detection of progenitor cells/ hepatic stems. The origin of these cells at the junction of the hepatic cords (Canal of Hering) and bile ducts has been debated as either migration from bone marrow to liver or being the real hepatic resident cells [51]. Stem cells are non-specialized cells with the abilities of self-renewable, limitless proliferation and resistance to chemotherapy. These stem cells generate oval cells on exposure to carcinogens with dual characteristic of hepatocyte and biliary cells, bipotential progenitor cells, which can generate hepatocytes and bile duct cells when the hepatocytes and cholangiocytes fail to regenerate [14]. Mutations in the stem cells are suggested to be responsible for growth and maintenance of cancer [52–54]. Research in hepatic stem cells is in its infancy with size and morphology yet not clear.

6.3 Hepatic Targeting

Targeting to the liver can be achieved through direct intraportal, intra-tumoral, intra-arterial route injection. Direct administration to the site prevents unnecessary exposure to other non-target organs. Retrograde intrabiliary infusion for genetic

delivery of nanoparticulates or complexes has been one way wherein the hepatocytes could be specifically targeted easily via the biliary system [55–57]. The entire process necessitates the need for cannulation [58]. Targeting to the liver could be achieved by generalized organ based targeting or could be directed to one or more cell types detailed above. Approaches to target to the liver would essentially be dictated by the cells being targeted and could be achieved using two strategies, active or passive targeting. The practical approaches for passive and active targeting to the liver are detailed below.

6.3.1 Passive Targeting

Passive targeting relies on the basic defense mechanism of the RES to target foreign invaders like bacteria, viruses, etc., and this strategy can be widely explored for conditions wherein the RES is the target site of action [59, 60]. Understanding the conditions that trigger such targeting provides useful information to design passive targeting strategies outside the RES. The processes responsible for RES uptake are opsonization and phagocytosis.

6.3.1.1 Opsonization and Phagocytosis

Opsonization is fouling of invading particulates by deposition of plasma proteins mainly fibrinogens, fibronectin, lipoproteins, etc. [61]. Once opsonized, the foreign object or nanoparticulates are activated, recognized, and engulfed by macrophages via phagocytosis [61]. Opsonization of particulates by complements (C3, C4, and C5) and immunoglobulins makes the particulates more recognizable by the KC. Phagocytosis is initiated by attachment of the foreign body with the KC, followed by invagination and spreading of cell membrane covering the particle to form a vacuole called phagosome. Phagosomes coalesce with intracellular organelles to mature into phagolysosomes. Phagolysosomes have an acidic environment with many digestive proteins which finally degrades the internalized material. Phagocytosized material is eliminated by exocytosis. In case the particulate cannot be digested, it remains sequestered in residual bodies within the cell. The process of phagocytosis can be explained as given in Fig. 6.2.

Phagocytosis is a nonspecific uptake mechanism influenced by many factors such as shape, size, charge, rigidity, etc. [62].

1. Particle size
   Size and radius of particles affect the biodistribution profile and internalization by KC. Optimal phagocytosis occurs with particles of 1 and 3 μm. Smaller particles (<35 nm diameter) escape the interaction contacts with KC but are easily removed by the kidney and provide more easy access to hepatocytes [63]. Nanocarriers with a particle size limit of 80 nm have physical access to hepatocytes [64]. Targeting to hepatocytes necessitates design particles of less than 100 nm

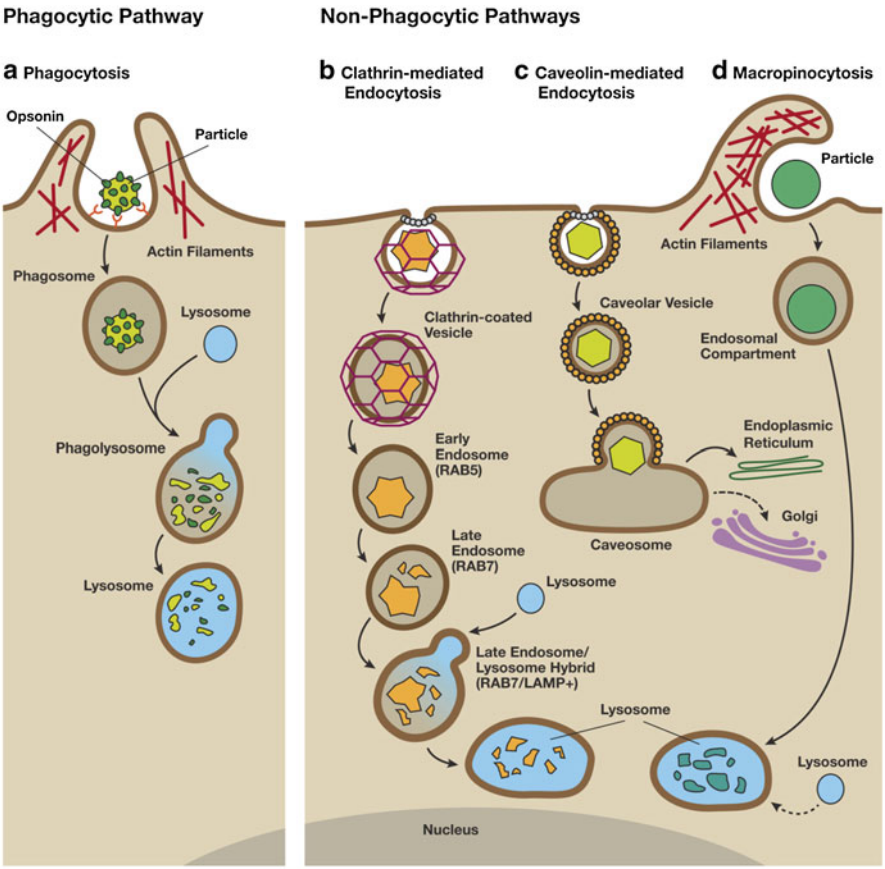

Fig. 6.2 Different uptake mechanisms for particles (reproduced from [279])

to diminish KC uptake. Large sized rigid particulates up to a size of 20 μm or those with three times the volume of macrophages are removed by the RES system, typically liver and spleen macrophages [65, 66]. Excessively larger particles cannot be internalized easily as it requires strong and extensive cytoskeleton remodeling [67]. The upper size limit for phagocytosis has been determined around 20 μm in vitro or whenever the size exceeds more than three times than that of KC [68]. Liu et al. [69] investigated the biodistribution of different sized (30–400 nm) liposomes. Particles greater than 250 nm in size irrespective of PEGylation are rapidly removed from the RES [70]. Excessive and maximum stretching of KC membrane causes frustrated phagocytosis wherein the system is not fully engulfed [71].

2. Surface charge
   Cationic nanoparticulates with a zeta potential >25 mV amplify complement activation and deposition of opsonins than those below 15 mV [72, 73].

Self-aggregation and opsonization of nanoparticulates with anionic serum protein causes passive accumulation in the RES cells. Neutral charged nanocarriers decrease the KC uptake [74]. Positively charged nanoparticles therefore exhibit a higher cell uptake than hydrophilic neutral or negatively charged particles [75]. Long half-life of anionic carriers could be due to less opsonin adsorption [76]. Intravenous administration of extracellular superoxide dismutase plasmid as polycationic liposome resulted in reduced peroxidation of lipids and enhanced levels of hepatic glutathione and serum superoxide dismutase [77]. Coatings of hyaluronic acid prolonged circulation times [78]. Nucleic acids have been successfully delivered to hepatocytes through cationic and PEGylated liposomes (80–100 nm) with higher suppression of HBV, attributed to longer half-life of nucleic acids [79].

3. Particle shape
   The effect of shape on phagocytosis has been recognized and particle shape has been recently reported as an influencing factor for MPS uptake [80, 81]. The ability of irregular shaped polymer–lipid hybrid nanoparticles (LIPOMER) to bypass the KC and accumulate in the spleen has been demonstrated (~400 nm) [82]. Non-spherical shaped particles bypassed phagocytosis due to incomplete actin structure formation. Uptake of rod shaped particles is unachievable if they macrophages attack them on their major axis [81]. Likewise oblate (disk-like) particles effectively adhered to cell surfaces compared to spherical particles of comparable volume to bypass phagocytosis [83]. High aspect ratios (i.e., ratio of larger surface dimension over smaller surface dimension) hinder actin membrane spreading and hence internalization [67]. Spherical nanoparticulates of sub 100 nm displayed higher uptake than rod shaped particles [84].
4. Flexibility and deformability
   The effect of flexibility and deformability on uptake by macrophages is also cited [85]. Stiffness of the particles influences the shape of phagocytic cup formed after activation of actin recruitment [67]. Particles should be either small or deformable to be able to penetrate through sinusoidal fenestrations for hepatocyte targeting. Reports of deformable nanoparticulates sized 400 nm being extravasated via forced extrusion mechanism bypassing KC and RES cells enabled localization in the hepatic parenchyma [86]. Fc-receptor mediated phagocytosis internalizes large rigid opsonized particles preferentially over softer particles. It influences the activation of actin recruitment to shape the phagocytic cup [87].
5. Hydrophilicity
   Particles with a hydrophobic surface are rapidly removed from circulation. PEGylation masks the particle appearing more like water body and prevent RES uptake [74]. Surface modification with hydrophilic coatings enables particles to masquerade as water bodies and by pass the RES. The hepatic B virus is considered to be a best example of stealth, as it escapes the RES [88]. PEGylated tamoxifen nanoparticles bypassed the liver compared to non-PEGylated nanoparticles [89]. Hydrophilic coating recommended to enable decreased KC uptake with higher parenchymal uptake include dextran, phosphatidylinositol,

monosialoganglioside, pullulan, poloxamers, polyvinylpyrrolidone, and cellulose derivatives [90–92]. This is the popularly known "Stealth" technology. Stealth technology can be exploited for effective delivery of drugs to the liver in infections and inflammations. Under such stress, increase in vascular permeability or increase in dimension of fenestrations enable leukocyte extravasation and accumulation at the inflamed site. Thus, particulates of lower dimensions can easily pass through these pores which are generally inaccessible.

6.3.2 Active Targeting

In active targeting, therapeutics are transported selectively and specifically to relevant cells with the help of ligands through receptor mediated endocytosis [93] or through stimuli responsive nanocarriers, e.g., temperature, ultrasound, magnetic field [94]. Ligands such as carrier proteins, metabolites, saccharides, peptides, vitamins, lectins, hormones, antibodies, aptamers, neurotransmitters, etc. are grafted on nanoparticulates and thus selectively target specific receptors. Addressing drug delivery systems can prevent non-desired accumulation in the body and exert precise effects especially on cells with low expression [95]. Designing strategies to target receptors thus holds intriguing promise in therapeutic interventions, bypassing multidrug resistance [96].

Delivery of charged molecules and genetic materials intracellularly is better facilitated by nanoparticulates attached with fusogenic agents or ligands for active targeting [97]. Besides, the higher the valency of binding, the higher the binding potential [98]. Readers are requested to make a note that the receptors dealt below are mostly transmembrane in nature rather than intracellular receptors as they would play an important role in transportation of carriers to intracellular environment of cell.

6.3.3 Receptor Mediated Active Targeting

Different receptors are present on cell membranes responsible for specific interaction with neighboring cells. These receptors also facilitate specific interaction with carrier system. Receptor mediated endocytosis follows adsorptive pinocytosis. Mechanisms of binding and internalization vary from clathrin-mediated endocytosis, caveolae-mediated endocytosis and clathrin- and caveolae-independent endocytosis depending on the size of the endocytic vesicle, the nature of the cargo and the mechanisms of vesicle formation [99]. For details on these uptake mechanisms readers are directed to the following references [91, 100, 101]. Macropinocytosis is a transient process while micropinocytosis (clathrin-dependent, caveolae mediated, and clathrin- and caveolae-independent endocytosis) is a constitutive pathway. Clathrin-coated vesicles and macropinosomes fuse with endolysosomes whereas caveolae-coated vesicles can escape endolysosomes and lead to direct exocytosis [102].

Several endocytotic mechanisms often take place simultaneously [91]. Clathrin-mediated endocytosis is one of the best characterized and widely studied endocytosis pathways. The best known receptors adopting this mechanism are ASGP-R, low density lipoprotein receptor, epidermal growth factor receptor (tyrosine kinase receptor), β2-adrenergic receptors, etc.

Clathrin-mediated endocytosis a common pathway for the internalization of a variety of ligand–receptor complexes. These processes are relatively slower than that of phagocytosis. As for phagocytosis, binding of ligand to the receptor is also dependent on size, geometry of ligand, charge, density of ligands, etc. [103]. Various receptors found on different types of liver cells are summarized in Table 6.1.

Table 6.1 Receptors on hepatic cells and their ligands

Receptor	Ligand	References
Kupffer cells		
Mannose/*N*-acetylglucosamine receptors	Mannose and *N*-acetylglucosamine	[280]
Fucose recognition receptors	Fucose	[105, 149, 281]
Fc receptors (FcγII-B2)	Antibodies/IgG	[282, 283]
Scavenger receptors (SR-BI, MARCO, dSR-C1, CD36, 95 kDa receptor Macrosialin)	Modified or acetylated LDL, polyanionic, lipopolysaccharides	[113, 284, 285]
Cannabinoid receptors (CB2)	Endocannabinoid	[195]
LDL receptors	LDL	[179, 286]
Fibronectin receptors		[287]
Sinusoidal Endothelial cells		
Scavenger receptors (SR-AI, AII, B, H)	Oxidized LDL, polyinosinic and polyguanosinic acid, polyanionic ligands	[288, 289]
Mannose receptors/*N*-acetyl glucosamine receptors	Mannose, lysosomal enzymes, tissue plasminogen activator, immune complex	[11, 12, 31]
Fc receptor	Antibodies/IgG	[119, 290, 291]
Stabilin receptor	Hyaluronic acid, chondroitin sulfate	[289, 292]
Collagen receptor	Denatured collagens	[40]
Laminin receptor	Laminin/nidogen	[40]
Hepatic stellate cells		
Retinol-binding protein receptor	Retinol	[187]
Cytokine receptors	–	[35, 293]
Transferrin receptor	Transferring	[294]
Growth factors—platelet-derived, cell–matrix interactions, epidermal and fibroblast	C*SRNLIDC* peptide, Arg-Gly-Asp (RGD) peptide	[299]
Tyrosine kinase receptors	–	[295, 296]
Uroplasminogen receptors	–	[297, 298]
Vasopressin receptors		[294, 299]
Integrin/complement receptors (CR1, CR3, CR4 C3b and C1q)	Opsonized components	[22, 300, 301]

(continued)

Table 6.1 (continued)

Receptor	Ligand	References
Hepatocytes		
Asialoglycoprotein receptors	Galactose terminated glycoproteins, arabinogalactan, pullulan, sitoG	[146, 302]
Glycyrrhizin(GL)/glycyrrhetinic acid (GA) receptors	Glycyrrhizin, glycyrrhetinic acid	[303]
HDL receptors	High density lipoprotein	[304]
LDL receptors	Low density lipoprotein	[305–307]
Scavenger receptors (SR-BI, CD36)	Native and modified lipoproteins, anionic phospholipids, apoptotic cells	[308, 309]
Transferrin receptors	Transferrin and its derivatives	[310–312]
Insulin receptors	Insulin analogues	[313, 314]
Ionotrophic purinergic receptors (P2X)	–	[315]
Glucagon-like peptide-1 receptor (GLP-1)	Exendin-4	[202]
Cannabinoid receptors (CB1)	Endocannabinoids	[195, 316]

6.3.3.1 Kupffer Cells

(a) Mannose receptors or *N*-acetyl glucosamine receptor/GlucNAc R
Mannose/*N*-acetylglucosamine receptor recognizes and clears off glycoproteins with mannose, glucose, and N-acetylglucosamine residues in exposed positions regardless of SEC and Kupffer cell. Mannose receptors are major receptor responsible for removal of denatured collagen from blood [31]. Mannosylated – human serum albumin selectively targets the KC and the EC [104–106]. Anti-inflammatory actives such as dexamethasone, immunosuppressive, enzymes like superoxide dismutase in chronic or acute hepatic inflammatory disorders, alcohol-induced hepatitis have been actively targeted to KC through mannose receptors [106, 107]. A drawback of targeting mannose receptors is activation of signaling processes sensitizing the immune system [108, 109]. Genetic delivery through mannose receptors have also been reported [93]. Though mannose receptor possesses eight carbohydrate recognition domains, only one is actively involved in binding. Mannose receptors differ from ASGP-R receptors in terms of binding. High mannose glycans are poor ligands for the mannose receptor. Liposomes possessing mannosylated ligands have exhibited enhanced targeting to macrophages, both in vitro and in vivo, than the non-ligand ones [110]. Lei Dong mentions the presence of one more macrophage lectin β-glucan receptor which binds glucose or glucan polymers. Hence, chitosan, a glucosamine polymer has considerable affinity for macrophages [111].

(b) Fucose receptors
Fucose receptors are responsible for clearance of glycoproteins bearing terminal fucose sugar. In vitro studies revealed that fucose and mannose receptor both

regulate uptake of fucosylated BSA. Nevertheless, fucosylated BSA is more Kupffer cell-selective because it exhibited a lower sinusoidal endothelial cell uptake than mannosylated BSA [105]. Fucosylation is more commonly explored in diagnosis than in therapeutics [112].

(c) Scavenger receptors
Scavenger receptors on KC constitute the scavenger receptors class SR-A (type I, II, and MARCO) and SR-B (type I, CD36, and CD68/macrosialin) [113]. It is known to be downregulated in animal models of Nonalcoholic steatohepatitis [114]. The CD68 is partially expressed in endolysosomal compartments and also on also on the transmembrane of macrophages [115]. Plasma proteins are removed from circulation by inducing a negative charge on its surface by succinylation to the lysine groups [26, 116]. The coated pits create a cationic surface charge permitting endocytosis of highly negatively charged molecules [117]. Particles up to 0.23 μm can be easily internalized [28]. Among the various receptors, scavenger receptors class A binds to varied polyanionic ligands but with varied affinity [113]. Expression of SR-A varies with the presence of ligands; lipopolysaccharides decrease the expression while oxidized LDL increases the expression [118].

Ligands such as fucoidan, polyinosinic acid, phosphatidylserine, oxidized low-density lipoprotein have a high affinity for scavenger receptors. Scavenger receptors play a major role in discrimination between foreign and self [118]. Weak negatively charged compounds show only a small degree of hepatic uptake whereas strongly anionized ones, e.g., phosphatidylserine-containing liposomes, PLGA have been considered to be taken up by liver non-parenchymal cells, via scavenger receptor mediated endocytosis of KC and SEC due to the direct recognition of their negative charge.

(d) Fc receptor
Fc receptors eliminate the soluble circulating immunoglobulin G immune complexes by receptor mediated endocytosis. Fc receptors exhibit delayed degradation of ligands than that internalized by scavenger expressed on SEC [119]. Fc receptors are unaltered till the necrotic foci are infiltrated, excessive injury with D-galactosamine in chronic inflammation. In such conditions, Fc receptors are minimized [120].

6.3.3.2 Hepatic Endothelial Cells

HSC express similar receptors as those found on KC, e.g., mannose receptors [11, 12], scavenger receptors internalizing advanced glycation end-products-Alb, maleylated bovine albumin, and fucoidan [121] and Fc receptors internalizing immune complexes of IgG and IgA. In addition, to these receptors, SEC possesses stabilin receptors and receptors for removal of extracellular matrix, e.g., laminin, hyaluronic receptor. Stabilin receptors are responsible for regulating the extracellular concentration of the matrices and their concentration in blood [122].

6.3.3.3 Hepatic Stellate Cells

HSC are involved in liver fibrosis or liver cirrhosis. Targeting HSC achieves decreased secretion of extracellular matrix [123].

(a) Phosphomannosyl receptor/Mannose 6 phosphate receptors
Phosphomannosyl receptor receptors are intracellularly located in the membranes of the endoplasmic reticulum, Golgi apparatus, and the lysosomes; only 10 % of the receptors are identified on the plasma membrane [124]. Delivery of newly synthesized lysosomal enzymes from the Golgi apparatus to lysosome in HSC requires the recognition of mannose 6-phosphate on these enzymes by a specific receptor—phosphomannosyl receptor/Mannose 6 phosphate receptors [125]. Targeting mannose 6 phosphate stimulates cytokines, converting growth factor β (TGF-β) stimulating production of collagen. Direct conjugation of mannose 6-phosphate with HPMA showed maximum uptake in diethyl nitrosamine induced liver fibrosis [54]. Albumin modified with mannose 6-phosphate selectively binds to hepatic stellate cells in fibrosis and accumulates up to 58 % of the injected dose after intravenous injection by endocytosis [126]. Inactivated hemagglutinating virus of Japan with a plasmid DNA tagged with luciferase has been targeted using liposomes decorated with albumin modified mannose-6-phosphate [127].

(b) Miscellaneous receptors
Retinol binding protein receptors (RBP) are also found unaltered in liver disorders and hence an important target in fibrosis [128]. A pro-drug Bexarotene (Targretin®) targets retinoic acid receptors in cancer. Cell surface integrins integrate with the matrix collagen type VI protein through Arg-Gly-Asp (RGD) dependent interactions via αvβ3 receptor. Intravenous injection of liposomes encapsulating siRNA responsible and conjugated with vitamin A suppressed collagen secretion as well as reduced fibrosis [129]. Covalent interaction of a cyclic octapeptide bearing "RGD" peptide to HSA increased the selective uptake by HSC [130]. Similarly cyclic peptide C*SRNLIDC* is recognized by platelet derived growth factor receptor (PDGF). RGD labeled liposomes have efficiently delivered interferon alpha-1b in fibrosis [131]. Conjugation to Human serum albumin incorporating an apoptotic drug led to accumulation on HSC [1].

Hepatitis C virus induces liver fibrosis and cirrhosis. Wang and coauthors have recently reported the presence of hepatitis C virus co-receptors responsible for promotion of liver fibrogenesis and engulfment of hepatocytes apoptotic bodies. In addition, many cytokine receptors, growth factor and transcription receptors, etc. are present on HSC, nevertheless with rare applications to liver targeting [39].

6.3.3.4 Hepatocytes

Hepatocytes are active targets in hepatic cellular carcinoma, hepatitis, steatohepatitis, genetic disorders, and metabolic disorders [132]. Hepatocytes receive the systemic circulation born substances after diffusion across the SEC separating blood

and hepatocytes. The particulates have foremost to pass through fenestrations of the sinusoid intravascular space generally of 100–200 nm [28, 133]. For active targeting in hepatocytes, introduction of steric stabilization, charge shielding techniques such as PEG layer reduce the opsonization and degradation in the lysosomes of KC.

(a) Asialoglycoprotein receptors (ASGP-R) or *N*-acetyl galactosamine receptor/ GlcNAc–R
ASGP-R clears off serum desialylated glycoproteins from the systemic circulation having non-reducing galactose or acetylgalactosamine residues, exposed at the end of their oligosaccharides, and through receptor mediated endocytosis [134]. The desialylated glycoproteins are subsequently processed through the liver lysosomes. The binding affinity of N-acetyl galactosamine residues to ASGP-R is 10- to 50-fold higher than ligands with only terminal galactose residues [135, 136]. A higher expression and density of 500,000 ASGP-R per cell has been reported on the basolateral side of hepatocytes [137] and on the side facing the sinusoidal area [138]. The ASGP-R possesses three Ca^{+2} dependent carbohydrate recognition domains and hence exhibits strong interaction—"cluster effect" with multivalent ligands (tri- or tetra-antennary *N*-linked glycans) [139]. Consequently, this results in lesser possibility of ligand escape towards other receptors [140, 141]. However, at higher surface density of galactose residues complete shift in uptake from hepatocytes to KC is observed due to ready recognition by the galactose receptors on Kupffer [142, 143]. Expression of ASGP-R in conditions like hepatocellular carcinoma is still of debate with reports of overexpression [144] as well as decreased expression [145]. Ligands ranging from asialofetuin soybean derived sterylglucoside, sito-G, arabinogalactan, pullulan, lactobionic acid to synthetically synthesized galactosylated ligand have been widely studied as ligands for ASGP-R. D'Souza et al. [146] and coauthors performed an in silico screening of various ligands (arabinogalactan, pullulan, and kappa carrageenan) for targeting ASGP-R and observed good correlation with liver distribution in healthy rats on intravenous administration of nanocarriers anchored with the ligands.

KC also express galactose receptor distinct from ASGP-R [147]. Functionally, both the receptors have affinity for galactose residue of lactose. However, specificity depends upon the degree of lactosylation. High substitution of lactosylated lipoprotein delivery targets the Kupffer cell (>300 lactose/LDL) despite being in minority, while at lower substitution (60 lactose /LDL); hepatocytes are targeted [148]. Lactosylated high density lipoprotein with diameter of 10 nm showed hepatocyte-specific targeting [142]. Galactose particle receptor has a high affinity for galactose, exposing particles with ligand size between 15 and 20 nm [145, 149, 150]. Liposomes with a lower degree of tri-antennary galactoside modification (5 % tri-antennary galactosides) were taken up by the ASGP-R on hepatocytes while those containing 50 % tri-antennary galactosides were taken up by KC [64, 143, 151]. Hence, an optimum balance of galactose density is desirable to prevent a shift in uptake from hepatocytes to galactose particle receptor on KC [142, 143].

(b) Glycyrrhizin (GL) and glycyrrhetinic acid (GA) receptors
Negishi et al. [152] demonstrated the presence of Glycyrrhizin and glycyrrhetinic acid receptors on the cellular membrane of hepatocytes. The binding sites for GA receptors surpass that of glycyrrhizin receptors [153]. GA is a metabolite of glycyrrhizin obtained from liquorice [154]. In vitro studies revealed 3.3-fold and 4.9-fold higher uptake for chitosan nanoparticles and poly(ethylene glycol)-b-poly(γ-benzyl L-glutamate) micelles, respectively modified with GA compared to unmodified nanoparticulates [155, 162]). Chemical conjugation of GA to nanoparticles increased internalization by liver cancer cells [156–161]. Derivatives of glycyrrhizin—30-stearyl glycyrrhizin [162]—also increased hepatic uptake. GA conjugated to hyaluronic acid has been suggested as a double targeting strategy for liver cancer by [163]. Recently the presence of GA receptors on HSC and tumor cells have also been reported [164].

(c) Integrin receptor
Vectors in Viral mediated delivery, viz., adenoviruses, retrovirus, hemagglutinating virus, lentiviral vectors innately are transduced through the adenoviral and integrin receptors [165–169]. The vector binds to the coxsackievirus-Ad receptor and is subsequently internalized by integrins. However, for hepatocyte transduction, this type of interaction between vector and CAR is not mandated [170, 171]. Uptake in KC causes degradation of genetic material. Immunogenicity and off-target effects have been improved by designing vectors derived from human immunodeficiency virus and pseudotyped with Sendai virus fusion protein F [166]. Though beta 1-integrin collagen receptors are reported to be present in hepatocytes, αVβ5 integrin receptor in KC have also been studied [172]. Integrin receptors are also associated with tumor blood vessels and are widely used for delivery of thrombolytic agents and anticancer drugs [173–175]. Specific targeting to hepatocytes through integrin is rare. Disruption of extracellular matrix related integrin signaling leads to termination of liver regeneration [101].

(d) Low-density lipoprotein receptor (LDLR)
Low-density lipoprotein receptor is an endocytotic type I transmembrane cell surface receptor and contributes to uptake of circulating cholesterol-rich LDL particles [176]. Uptake occurs via the clathrin-mediated receptor endocytosis system and is triggered by binding to the signaling proteins [177]. It maintains lipidic homeostasis as well as regulates fibrogenesis. Overexpression of LDLR is observed in non-alcoholic fatty liver disease and increases with advancement in fibrosis [178]. LDL metabolism has been associated with both hepatocytes and KC and follows saturation kinetics. Degradation of LDL is 18-fold higher in KC than in hepatocytes [179]. However, as in case of integrin receptors, LDLR are also known to be overexpressed in several tumors and have been widely studied for targeted delivery to malignant cells [180]. Hepatocyte specific delivery of disease-related genes using siRNAs has been cited [181, 182].

(e) Miscellaneous receptors
Targeting of apolipoprotein E (high density lipoprotein) and apolipoprotein A-1for delivery of siRNA and miRNA has been demonstrated via the class B type I scavenger receptor (SR-B1) [183, 184]. CD36 receptors are found to

retain inflammatory cells [185]. Lipid nanoparticles of acyclovir palmitate-recombinant HDL complex (~33 nm) revealed fourfold enhanced hepatic accumulation on intravenous injection [186, 187]. Scavenger receptors also have their existence on KC associated [118]. Transferrin receptor increases in patients with alcoholic liver diseases with excessive iron accumulation [188, 189] and is expressed in all nucleated cells [190].

Qin He incorporated hepatocyte specific AFP (α-fetoprotein) promoter to recombinant plasmid encapsulated in PLGA nanoparticles and achieved targeted delivery to hepatoma cells. AFP promoter exhibited specific activity only in cells containing α-fetoprotein [191]. Other techniques for hepatocyte targeting have been by conjugating with bile acids which exhibit hepatotropism to specific transport systems on the sinusoidal plasma membrane of hepatocyte [192]. A ligand activated nuclear receptor, Farnesoid X receptor, known to regulate lipid and glucose metabolism exhibits affinity to bile acids [193]. Other hepatocyte-specific transgene expression promoters are albumin, alpha 1-anti trypsin, enhanced transthyretin, etc. [194]. Receptors like cannabinoid receptor-1 though expressed on hepatocytes are also expressed in myofibroblasts, and adipose tissue and intestine bearing extrahepatic CB1 receptors [195]. Conditioning of KC with acetylated LDL or HDL increases the number of HDL receptors [196]. Recently receptors for mosquito-borne dengue viruses consisting of three proteins: heparan sulfate, the 37/67 kDa high-affinity laminin receptor, and prion protein have been reported [197, 198]. An association between the laminin receptor, a part ofDENV (Dengue virus) receptor and prion proteins was observed in HepG2 cells. Readers are directed to the following references for details [199, 200]. Glucagon like peptide receptors and δ opioid receptor have also been exploited [201–205]. The disposition of nanoparticulates in the liver is schematically depicted in Fig. 6.3 following intravenous injection.

6.3.3.5 Stem Cells

Cancer stem cells are generally resistant to chemotherapeutic drugs due to the presence of active transmembrane adenosine triphosphate-binding cassette (ABC) transporter family [206]. However, Wnt receptor, transmembrane Frizzled (Fzd) receptors, etc. are known to be stimulated on cancer stem cells [207, 208], while Notch and hedgehog signaling pathway inhibitors etc. can also serve as molecular target for cancer prevention by increasing the cell sensitivity to drugs and inhibiting drug efflux in both tumor cells and stem cells. Targeting to cancer stem cells has been proposed to be more efficient in eradicating and providing cure mainly to HCC [209, 210].

6.3.4 Stimuli Responsive Active Targeting

Drug release at specific sites can be triggered by external and/or internal stimuli. While magnetic [211], photo-irradiation, ultrasound [212], electric field, etc. are external stimuli [213, 214], internal stimuli include changes in pH [215], and

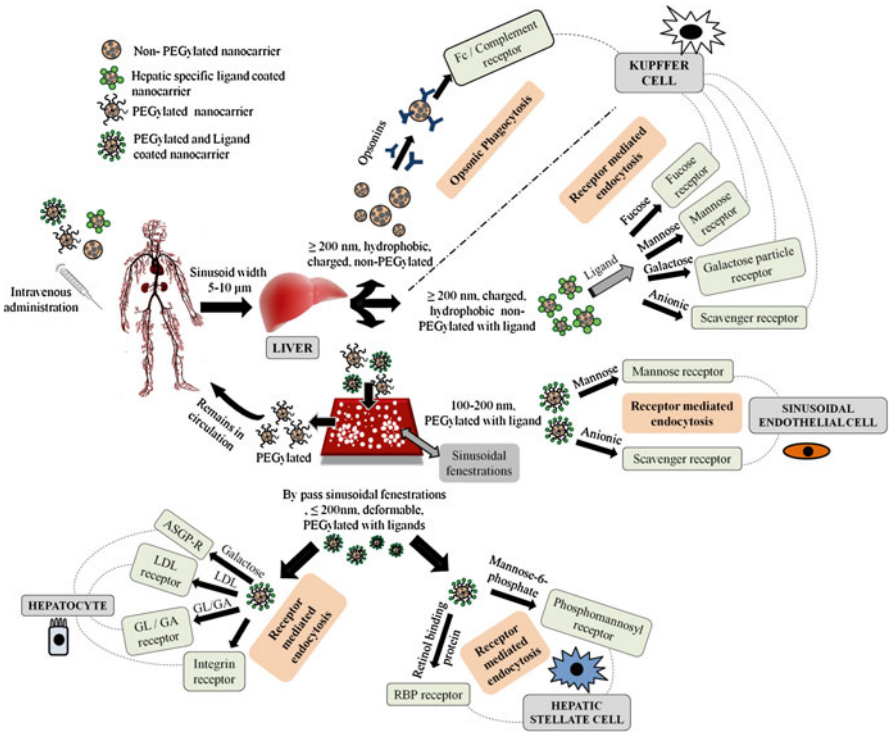

Fig. 6.3 Physiological fate of nanoparticles following intravenous administration (schematic)

temperature [216, 217] which can occur within organs during disorders or tumors. Dual stimuli—thermal and pH—responsive self-assembled structures of poly(*N-isopropylacrylamide*)-b-poly(L-histidine) were designed for controlled release of doxorubicin in liver carcinoma [218]. Thermodox®, doxorubicin containing PEGylated liposomes, is a temperature sensitive nanocarrier which releases drug only upon externally applied heat, i.e., radiofrequency ablation at the site of the tumor to raise the temperature above 39.5 C, or upon EPR-mediated passive tumor accumulation [219].

6.3.5 Antibody Mediated Active Targeting

Targeting with antibodies capable of recognizing and binding with affinity to antigens present on tumors as targeting strategy is widely explored [220, 221]. Immunoliposomes are widely exploited for delivery of anticancer agents [222]. ASGP-R single chain variable fragments on conjugation to immunotoxins exhibited an increased cytotoxicity in HepG2 and Huh7 cells compared to non-conjugated immunotoxin scFv fragments [223]. Interferon alpha genetically fused to a domain

antibody (dAb), an asialoglycoprotein receptor specific antibody, increased the in vivo targeting to liver [224]. Four Glypican-3 antibodies—GC33, YP7, HN3, MCX-1414—have been developed for cancer therapy and are under investigation [225]. Antibody mediated targeted delivery to CD133, an important surface marker of liver cancer stem cells, has shown good promise in cancer therapy [226]. Human recombinant single chain antibody (C1-3 scAb) to synaptophysin, a HSC selective expression, is being explored for targeting in anti-fibrotic therapy [227, 228].

6.4 Targeted Delivery Systems and Applications

Drugs administered orally or as injectables are efficiently removed from systemic circulation by KC due to first pass effect of drug causing metabolic transformation, detoxification, and excretion of drugs [229]. Nevertheless, KC largely contributes to uptake of particulate matter. Thus, drugs are precluded from reaching the desired cell type [230].

Covalent binding of therapeutically active drug to a liver targeting polymer improves liver targeting potential, circulation time and increases specificity [231]. Many polymers such as poly-lactic acid poly-glutamic acid (PLGA) have been galactosylated and have shown improved biodistribution over the conventional polymers [232]. Hydrolysis of drug from conjugates using enzymatic or environmental as triggers could also modify the drug kinetics. PEGylated conjugates are well reported for the success in treating various disorders including hepatitis C [233]. Protamine-asialofetuin lipoplexes contained asialofetuin as a natural targeting ligand to ASGP-R [234]. Oligonucleotide poly-L lysine polyplexes inhibited the expression of hepatitis B virus gene expression with increased hepatocyte uptake [235].

Nanotechnology has enabled systematic and site-specific delivery of drugs. Reviews on the same are abundant. Readers are directed to recent reviews [236]. Nanoparticles are high engineerable with integration of different physicochemical functionalities such as size, shape, hydrophobicity, etc. Further modification of surface properties such as charge, anchoring ligands, modulating ligand density for achieving selectivity with desired systemic effects has been explored. Exploitation of active and passive targeted strategy relies on the characteristics of nanocarriers. Nevertheless, administered drug achieve high and nonspecific accumulation in liver due to first pass effect for metabolism, while nanoparticles are efficiently removed from systemic circulation by the macrophages of RES especially liver (first-order targeting) [229] (Fig. 6.3). Though KC occupies only 40 % of liver cells, they are a major site of accumulation of nanoparticles (higher than parenchymal cells occupying 80 % and SEC occupying 6.3 %). Details of passive targeting to KC attributed to phagocytosis and factors contributing to phagocytic uptake are discussed earlier in Sect. 6.3.1.

Once passive targeting to KC occurs, disorders or conditions wherein accumulation of nanoparticles in hepatocytes or SEC is desirable are never achieved. Nevertheless, tailored nanoparticulates can be designed for efficient uptake by individual hepatic

cells. As mentioned in Sect. 3.2. Different strategies based on specificity of each receptor can be engineered on nanoparticles so as to achieve active targeting to different cells of liver. Another well-known technique is to administer a dose of blank particulates to saturate KC [67].

The advantages of each approach need to be weighed prior to designing nanoparticulates for active or passive targeting [237]. One such example in tumors and cancer is as follows: EPR permits inherent passive accumulation of nanoparticulates in tumor, thereby precluding the need for active targeting [238]. The nanoparticles evade the RES system either by manipulation of size, charge or by stealth coating and concurrently using an active targeting strategy enables specific cellular targeting. A combination of active targeting technique is also used to increase targeting efficiency. Hongfen Wei et al. prepared galactosylated docetaxel nanoparticles targeting hepatocytes in HCC combined with exposure to ultrasound to increase vascular permeability [239].

We present below the possible applications of targeted delivery to the liver for various liver afflictions in Table 6.2.

Nanoparticles targeting to liver using either passive or active targeting have been frequently used in the following disorders or conditions:

6.4.1 Hepatocellular Carcinoma or Hepatoma

Hepatocellular carcinoma (HCC) is a globally concerned disorder with a high mortality and accounts for 85 % of primary liver cancers [240, 241]. Physiology of the liver is altered and could be exploited for passive targeting [242]. As observed in other tumors, HCC also exhibit leaky vasculature with discontinuous endothelial cell lining with pores (600–800 nm and at times up to 2 μm) [243–245]. Nanoparticulates up to 400 nm can easily extravasate through the leaky vasculature and result in increased drug concentration in the tumor [245, 246]. Macromolecules larger than 40 kDa and smaller than the fenestrations can easily accumulate in tumor tissue [244]. This condition is also a boon for active targeting using pH dependent release. Tumor cells show an increased glycolysis causing an acidic tumor microenvironment [247]. The acidic microenvironment of tumors due to increased glycolysis also permits pH triggered drug release from liposomes. Active targeting of hepatocellular carcinoma has been achieved mainly by targeting the ASGP-R [248], retinoic acid receptor [249], glycyrrhetinic receptors [250], LDL receptors [251], etc. Most of the targeting strategies for treatment of HCC are based on binding to ASGP-Rs utilizing galactose as targeting agents [252]. But to date, very few nanocarriers have been developed [187].

SMANCS, a conjugate of Poly(styrene maleic acid)—SMA—and the protein antitumor agent neocarzinostatin—NCS—in Lipiodol—lipid contrast agent, an oily formulation has been selectively used in the treatment of HCC in Japan since 1993 [253, 254]. It is devoid of side effects caused by conventional chemotherapeutic agents and suitable for X-ray computed tomography [255].

Table 6.2 Receptors, ligands, and nanocarriers for liver targeted delivery

Diseases	Nanocarriers	Ligand	Receptor	Reference
Hepatocellular carcinoma	Albumin–chitosan nanoparticles	Retinoic acid	Retinol binding protein receptor on HSC	[249]
	DOX loaded in glycyrrhetinic acid tagged chitosan–PEG nanoparticles	Glycyrrhetinic acid	Glycyrrhetinic acid receptors on hepatocytes	[157, 160]
	IL-6-receptor and/or immunoglobulin A binding protein	Pre-S1	IL-6-receptor on hepatocytes	[317]
	Doxorubicin loaded in glycyrrhetinic acid tagged alginate nanoparticles	Glycyrrhetinic acid	Glycyrrhetinic acid receptors on hepatocytes	[250]
	Doxorubicin delivery by chitosan-galactosylated modified polymer microbubbles	Galactose	Asialoglycoprotein receptor	[248]
	G5.0 PPI dendrimers to construct LDL- and HDL-conjugated dendrimeric nanoconstructs	LDL- and HDL	LDLR and HDLR	[251]
	Sulfated chitosan	Glycyrrhetinic acid	Glycyrrhetinic acid on hepatocytes	[159]
	Polymer conjugates (Phase I clinical trial)	Galactoseamine	ASGP-R on hepatocytes	[145]
	Oridonin loaded galactosylated bovine serum albumin nanoparticle	Galactosylated bovine serum albumin	ASGP-r on hepatocytes	[318]
	Trans-resveratrol loaded chitosan nanoparticles	Biotin and avidin	Biotin and avidin receptors specifically on hepatic cancer cells	[319]
Hepatic fibrosis	DOX loaded Poly(benzyl malate) bearing biotin	Biotin	Biotin on hepatic cancer cells	
	Sodium ferulate loaded on Albumin nanoparticles		Mannose 6-phosphate in hepatic stellate cells	[164]
	Liposomes encapsulating interferon alpha-1b	Cyclic RGD	Type VI collagen receptor on HSC	[130, 131, 320, 321]
	15-deoxy-delta12,14-prostaglandin J2	PDGF	PDGF receptor on HSC	[322]
	Hemagglutinin virus of Japan in liposomes	Mannose 6-phosphate	Mannose 6-phosphate in hepatic stellate cells	[127]
	Liposome containing siRNA against gp46	Vitamin A	Retinol binding protein receptor	[129]
	Liposomes containing quercetin	Galactose	ASGP-R	[323]

(continued)

Table 6.2 (continued)

Diseases	Nanocarriers	Ligand	Receptor	Reference
Genetic diseases	*N*-acetylgalactosamine functionalized polyethylene glycol -b-Poly(episol-caprolactone) micelles	siRNA targeting apolipoprotein B	ASGP-R on Hepatocytes	[328]
	Nanoassociates of DNA and hyaluronic acid coupled to polymer	Gene delivery	hyaluronic receptor on HSC	[324, 325]
	PLGA nanoparticles modified with mannan PE for gene delivery	Mannan-based PE-grafted ligands	Mannan receptor	[93]
	Galactosylated Polyethylenimine-graft-poly(ethylene glycol) conjugated with gene	Galactose	ASGP-R	[326]
	Mannose Polyethylenimine DNA	Mannose	Mannose receptor	[327]
	Galactosylated micelles for ribavirin delivery	Galactose	ASGP-R	[268]
	Doxorubicin delivered through cross-linked micelles	Galactose	ASGP-R	[328, 329]
	Naked pCMV DNA entrapped in PLGA nanoparticles	Asialofetuin	ASGP-R	[330]
Infectious diseases	Lactose modified LDL, Tri-gal-chol-LDL Galactose coated dendrimer of primaquine phosphate Apolipoprotein E anchored chylomicron of primaquine phosphate Nanoemulsion of primaquine phosphate	Galactosylated/ lactosylated low density lipoprotein Galactose Apolipoprotein E Apolipoprotein E	LDL on KC ASGP-R Apolipoprotein E Receptor Apolipoprotein E Receptor	[176, 264, 331, 332]
	Ribavirin linked nanoparticles by self-assembly of amphiphilic copolymer	Lactose	ASGP-R on hepatocytes	[320, 321]

6.4.2 Infectious Diseases

The liver being home for many transport machineries and almost 80 % of the macrophage population, foreign bodies and large therapeutic molecules (Molecular weight ~50 kDa) achieve high hepatic concentration on administration [256]. Nano drug delivery systems also render high uptake of particulates by the macrophagic KC [246, 257]. This physiological phenomenon could be an advantage for treatment of macrophage related infections (Leishmaniasis, AIDS, Brucellosis, Listeriosis, Mycobacteria, and Salmonella infections).

In parasitic infections like malaria, the sporozoites of Plasmodium selectively infect erythrocytes and human hepatocytes. This erythrocytic stage of Plasmodium causes increased gametocyte production and subsequently sequestration in systemic circulation [258]. Though the size of sporozoites exceeds the size of fenestrations, the sporozoites from blood sequester hepatocytes using proteoglycans by squeezing through the endothelial fenestration [259]. Targeted delivery of Primaquine to the liver has been evaluated following intravenous administration of liposomes [260, 261] and gelatin and albumin nanoparticles [262]. Preferential delivery of Primaquine to the hepatocytes was achieved using an artificial chylomicron emulsion [263] and galactosylated liposomes [139]. Dendrimeric nanoparticles of PQ coated with galactose, a ligand for the ASGPR receptor on hepatocytes, also favored high accumulation of PQ in the hepatocytes [264].

6.4.3 Nucleic Acid Delivery

Chemotherapies at times cannot address issues which have caused specific mutations or alterations, and hence genetic delivery becomes mandatory. However, nucleic acids possesses large size, anionic charge repulsion, hydrophilic highly charged and possesses short half-life due to nucleases and metabolic nature of liver violating the Lipinski's rule of 5 [265]. Cellular targeting of genetic material is often construed a herculean task. The journey begins from protection of the genetic material in systemic circulation. Cationic liposomes and nanoparticles conceal the genetic material while facilitating cellular uptake [72]. Further, cationic polymers exhibit strong buffering capacity between pH 5–7 causing osmotic swelling and finally vacuole disruption releasing the genetic material into cytoplasm [266]. Genetic transfer specifically to hepatocytes by incorporation of hepatocyte-specific promoters (albumin, alpha 1-anti trypsin, or enhanced transthyretin, α-fetoprotein, etc.) in lentiviruses (retroviral mediated genetic delivery) is reported. This reduces the expression on non-parenchymal cells [194]. High efficient transfer of siRNA using polyconjugates [267], polymeric micelles [268], and self-assembled amphiphilic cationic copolymers [269] have been studied. Gene targeting of human Factor FVIII using gamma retroviral vectors to hepatocytes in hemophilia A has been prompted for Phase I clinical trials in patients. Synthesis of F factors occurs primarily in the liver and is supplied to the blood. Majority of the patients exhibited good tolerance to the

treatment. Targeting of these genes to antigen presenting cells increases the immunity and decreases long term expression. Hence, a hepatocyte specific promoter is mandated. Micro-RNAs especially for liver diseases, inflammation, and cirrhosis are under investigation [270].

The inability of sinusoids to form a barrier for proteins has been long known. Hence, viral vector mediated gene delivery successfully exhibit its expression in hepatocytes rather than non-parenchymal cells. Also, the genetic material tends to degrade in the lysosomes of KC. Exploitation of protein for hepatocyte targeting has thus been widely studied. Giri explored this strategy for delivery of interferon-α using cationic PLGA nanoparticles with hepatitis B surface antigen (HBsAg) adsorbed onto its surface. The author proposed the system as an artificial viral vector [271]. Similarly, PEGylated interferon (Pegasys, PEG-Intron) has been successfully targeted to hepatocytes in hepatitis by passive targeting [272]. The stealth property imparted by PEGylation increases the circulation time thereby favoring high uptake. Since the uptake is not attributed to specific receptors, uptake in non-desired sites has also been observed [273]. Jung and coauthors designed core shell nanoparticles with a hollow core and a shell made of HBV envelope (bio-nanocapsule) which had pre-S1 peptide as a ligand for hepatocytes. The bio-nanocapsule was conjugated to liposomes for peptide delivery [187, 274].

Efficiency of gene delivery can also be enhanced by temporarily depleting the KC. Depletion of KC can be achieved by administration of clodronate liposomes. The technique is however risky as the reappearance of KC would take up to 1 week [275]. Depressed blood flow, endotoxemia, and bacteremia are also associated with decreasing clearance activity in Kupffer cell.

6.5 Imaging and Diagnosis

Over the decades, considerable advancement has evolved in diagnostic detection of various liver disorders. Common techniques for detection of HCC are quantifying the serum αfetoprotein or magnetic resonance imaging. The most common being the later, except in tumors less than 2 cm [276]. Radiopharmaceuticals containing galactose, lactose, or *N*-acetyl galactosamine recognizing ASGP-R on hepatocytes are used as nuclear imaging radiopharmaceuticals targeting hepatocytes [277].

Table 6.3 summarizes some of the major approaches for diagnosis of liver conditions.

6.6 Future Directions

Nanocarriers for targeted delivery in liver afflictions are in clinical investigation for therapy and diagnosis (Table 6.4). Polyisohexylcyanoacrylate nanoparticles encapsulating doxorubicin was the first nanoparticulate to enter in clinical trials for HCC. However, associated pulmonary adverse effects resulted in suspension of the

Table 6.3 Diagnostic interventions for liver targeted delivery

Diagnosis	Ligand	Receptor	Reference
Bioimaging of quantum dots	Hyaluronic acid derivative	Hyaluronic acid receptors	[333]
	D-galactose	ASGP-R	[334]
Fluorescence imaging	Glypican-3	Antibody mediated targeting specific for HCC	[335]
Radioactivity	99mTc hydrazino nicotinamide-galactosylated chitosan	ASGP-R	[336]
	99mTc- galactosylated chitosan	ASGP-R	[326]
	99mTc-gold nanoparticles capped with HYNIC-peptide/mannose	Mannose receptor	[337]
MRI	poly(propylene imine) dendrimers composed of GdDTPA and cyclic NGR	Cyclic NGR (similar to RGD) binding to collagen type IV protein	[338]
	PLA-PEG/Gd-DTPA	Passive accumulation	[339, 347]
	PLA-PEG-NH2 immobilized with FITC through biotin–avidin system and anti-alpha-fetoprotein	Biotin avidin receptors on hepatocytes affected with cancer	[339]
	LDLR-targeted amphiphilic gadolinium (Gd)-diethylenetriaminepentaacetic acid chelates	LDLR	
	Galactosylated manganese ferrite nanoparticles	ASGP-R	[340]
	Mannan-coated superparamagnetic iron oxide nanoparticles	Mannose receptors	[341]
	Gadolinium labeled LDL nanoparticles	LDL receptor	[342]
	Gadolinium labeled cholesterol-HDL nanoparticles	HDL receptor	[180]

Table 6.4 Clinical trial and commercialization status of liver targeted delivery system

Clinical trials	Brand name	Phase in study	Reference
Doxorubicin loaded poly(alkyl cyanoacrylate) nanoparticles	Transdrug for HCC	Phase II and III	[343]
Hepatic arterial infusion of nanoparticle albumin-bound paclitaxel	–	Phase I	[344]
HPMA bearing doxorubicin with galactosamine (PK2)		Phase I	[145]
PEG–arginine deiminase (i.v.)	Hepacid by Phoenix for HCC	Phase I/II	[231]
Virosomal hepatitis vaccine (Liposomal IRIV)	Epaxal Berna Hepatitis A	Marketed by Berna Biotech (Bern, Switzerland)	[345]
PEG–alpha-interferon 2a	Pegasys for Hepatitis C	Nektar (San Carlos, CA, USA), Hoffmann-La Roche (Basel)	[345]
PEG–interferon 2b	PEG-Intron for Hepatitis C	Enzon, schering-plough	[345]
Iron nanoparticles for imaging liver tumors	Resovist	Schering (Berlin)	[345]
Iron nanoparticles for imaging liver tumors	Feridex/Endorem	Advanced magnetics (Cambridge, MA, USA), Guerbet (Roissy, France)	[345]

Phase II study. Other unexplored areas for targeting parasites invading the liver include amoebic liver abscesses, hydatid cyst of the liver, fluke diseases, hemophilia, type I tyrosinemia, Wilson disease, etc. A recent upcoming area is the pharmacological modulation of the phenomenon autophagy for therapy of liver disorders. Autophagy is a process of lysosomal degradation of bulk cytoplasm or damaged organelles [278]. Improved therapeutic and diagnostic efforts have changed the status of hepatic cancer from dreadful to at least a treatable disease.

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