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Review Article

EPR effect: Promising approach for tumor targeted drug delivery

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ABSTRACT

Targeted drug delivery to tumor sites is one of the ultimate goals in drug delivery. Recent advances in nanoparticles engineering has certainly improved drug targeting, however clinical effects are disappointing. Present review addresses challenges in cancer therapy and current status of tumor targeted drug delivery. Most of the efforts have been directed towards design and surface manipulation of nanoparticles with relative little attention to other aspects. EPR effect based drug delivery offers lower cost of therapy, greater therapeutic effects on more types of tumors and fewer adverse effects. Present review focuses on basic understanding of EPR effect, critical attributes of nanoparticles influencing success of EPR based drug delivery and factors involved in EPR effect. Tumor biology diversity is responsible for heterogeneity of EPR effect which reduces its universal validity. Augmentation of EPR effect overcomes heterogeneity, thus widening its application on more types of tumors. Clinical outlook of SMANCS, clinically successful macromolecular drug is presented to support the concept of EPR effect in tumor targeted drug delivery.

Keywords: EPR effect, tumor targeted drug delivery, nanoparticles, SMANCS

INTRODUCTION

Cancer remains one of the most urgent concerns in the world today. It is among the leading cause of deaths worldwide accounting for 8.2 million deaths in 2012. It is expected that annual cancer cases will rise from 14 million in 2012 to 22 within next two decades [1]. Cancer can be reduced and controlled by implementing evidence -based strategies for cancer prevention, early detection of cancer and management of patients with cancer. Many cancers have a high chance of cure if detected early and treated adequately. Research and development in the areas of nanoscience and nanotechnology promise to provide innovative, and more effective, approaches for targeted delivery to tumor. Hence there is need to focus on unsolved problems that impede progress. This review presents brief overview on cancer pathogenesis, treatment and challenges in cancer therapy. Review addresses multiple aspects of the enhanced permeability and retention (EPR) effect in cancer to explore full potential of EPR effect based cancer diagnosis, tumor targeted drug delivery or both.

Challenges in cancer therapy: With the increasing technical possibilities for evaluating diverse cancer tumors, accumulated evidence is suggesting that cancer tissue is heterogeneous at both the intratumoral and intertumoral level. Intratumoral heterogeneity is seen across many cell properties, including morphology or phenotypic expression, exhibition of primary or acquired drug resistance; and capacity for initiating new tumor growth. This heterogeneity can be attributed to random fluctuation of protein expression levels; however reasons for such extensive diversity are not fully revealed. These diverse conditions are further complexed by factors in the tumor microenvironment such as paracrine signalling from hypoxic environment or from associated stromal cells. Therefore, cancer is now not recognised as a single disease, but as many, each with varying causes, prognoses and appropriate treatments [2,3]. Also, the resistance that neoplastic cells manifest to cytotoxic drugs is a major challenge in the treatment of the disease. The resistance can be 'primary' (present when the drug is first given) or the 'acquired' type which may result from either adaptation of tumor cells or

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mutation with the emergence of the cells that are less susceptible or resistant to the drug. Because cancer cells can evolve in response to the selection of cancer therapy, the goal of eradicating the entire cell within a tumor by targeting a specific pathway unique to cancer cells becomes a major task. When this task is not completed, the cells that do not respond to the drugs may grow and reconstitute the tumor in the body [4]. Early detection of cancer is another important challenge. It is crucial process to detect the point at which the treatment can be successfully administered [5].

Current status of targeted drug delivery to tumors

Conventional approaches to treat cancer are chemotherapy, surgery or radiation. Surgical intervention is limited to removal of early stage tumors that are small and confined to a limited area, without metastasis. So the ultimate resort to control cancer is chemotherapy and to some extent radiotherapy. However; it is difficult to get tumor-selective toxicity because the biological events taking place in cancer cells are essentially as same as that of host cells. There is little difference in biochemical or molecular machinery between cancer & normal cells at cellular or molecular level. Also most conventional low molecular weight anticancer drugs have inherent character to transverse in and out of blood vessels freely. Consequently, their undesirable indiscriminatory distribution in normal tissues causes severe systemic side effects making therapy disastrous to patients [6].

Tumor targeted delivery has received great attention due to increased anticipation of achieving it using nanotechnology-based delivery systems and to overcome difficulties associated with conventional anticancer drugs including rapid clearance, insolubility under aqueous condition, lack of selectivity resulting in nonspecific toxicity towards normal cells and lowering the dose of drug to cancer cells. For simplicity, all nanostructures including drug-polymer conjugates, drug-protein conjugates, polymer micelles, liposomes, dendrimers, DNA polyplexes and drug nanocrystals will be referred to as nanoparticles. The following Table No. 1 classify much discussed targeting approaches in literature as active, passive, inverse and combined targeting based on drug targeting strategy used [7]. However, nanoparticles based targeted drug delivery has not fulfilled its expectations even after efforts of whole decade of 2000s. Recent article by Kinam Park et al explains the uncomfortable facts in targeted drug delivery [8]. According to authors, present targeting approaches does not result in targeted biodistribution of nanoparticles as they rely on blood circulation to reach the target as conventional drugs do. Ligand targeted drug delivery may increase the cellular uptake of drug if the cells in contact with nanoparticles happen to have overexpressed receptors. However, overexpression of molecular signatures on tumor cells is relative to normal cells which suggest that normal cells also express the receptors to some extent. This leads to capture of considerable amount of ligand bound drug by normal cells as total number of normal cells are much larger than number of cancer cells. Also great diversity exists with kinetics of drug uptake and receptor recycling which needs to be considered. Thus efficacy of such therapy is only 4-5% which is only useful as adjuvant/ supplementary. Thus low efficacy & high cost makes therapy unacceptable. The benefits for patients undergoing these treatments is a 1-2 month extension of the usual 3-10 years overall survivals. Furthermore, neither the intensity of adverse effects nor the frequencies of medical emergencies are reduced. Most cases demonstrated resistance after several months of its use [8]. Thus, rationale for drug-targeting method based on enhanced permeability & retention (EPR) effect is lower cost of therapy, with greater therapeutic effects on more types of tumors and fewer adverse effects.

UNIQUE FEATURES OF TUMOR VASCULATURE CONTRIBUTING TO EPR EFFECT

Rapid growth of tumor is due to the phenomenon called angiogenesis in which new tumor blood vessels are formed. These newly formed blood vessels have defective architecture and produce extensive amounts of various vascular permeability factors such as bradykinin, prostaglandins, vascular endothelial growth factor (VEGF) which causes enhanced vascular permeability; so that sufficient supply of nutrients & oxygen to tumor tissues for rapid growth is ensured [9,10]. Enhanced retention is attributed to the fact that tumor tissues exhibit poor lymphatic drainage. Small molecules do not show the EPR effect, because they can freely pass through the blood vessels into tumor as well as the normal tissue, & diffuse back into blood capillaries. In contrast, nanoparticles pass through the blood vessels around the tumor & do not diffuse back into blood capillaries or end up in lymphatic system. In addition, lymphatic metastasis is one of the most formidable consequence of cancer progression although the lymphatic system does not function properly in tumor tissues. Lymphotropic accumulation of nanoparticles thus can be utilized for effective diagnosis and treatment of lymphatic metastasis. These anatomical and architectural characteristics of tumor blood vessels constitute the foundation of the EPR effect [9-11]. Therefore nanoparticles exhibiting tumor targeting characteristics can be directed for targeted drug

delivery using principles and understanding of EPR effect.

CRITICAL ATTRIBUTES OF NANOPARTICLES INFLUENCING EFFICACY OF EPR EFFECT

Molecular size: Since molecular weight is an important determinant of EPR effect, particles or molecules larger than 40 kDa (threshold of renal clearance) show an active EPR effect with prolonged circulation time, increased plasma half life as a result of their very slow clearance from body [12]. Prolonged circulation time allows molecules to gradually permeate into tumors and remained accumulated in tumors for a relatively long time (e.g. several days) . As discussed earlier not only macromolecules but also drug-polymer conjugates, liposomes, micelles, nanoparticles, lipid particles and DNA polyplexes show EPR effect. For e.g. investigation of copolymer N-(2-hydroxypropyl) methylacrylamide HPMA with molecular size upto 778 kDa and protein α-macroglobulin (720 kDa) suggested that both macromolecules exhibits the EPR effect [13].

Biocompatibility: Not only molecular size but also biocompatibility of nanoparticles is critical to achieve long plasma half life and in turn, functional EPR effect. Biocompatibility issues are predominant in case of protein therapeutics. Most proteins, when they are denatured or highly chemically modified become less biocompatible and therefore cleared rapidly from the circulation via scavenger receptors or other mechanism [14]. Ligand targeted drug delivery system utilizes active targeting wherein nanomedicine is coupled with ligands which have specific affinity towards molecular signatures expressed on the surface of cells requiring clinical intervention.

Among the proteins and peptide class of ligands antibodies, antibody fragments and diabodies are widely investigated. Other protein based targeting moieties include carrier proteins such as transferring [15] & low density lipoprotein (LDL) [16], natural ligands of cell receptors such as hormones & lectins [17], and molecules derived from toxins and pathogens such as cell permeating peptides [7]. Other classes of ligands include small molecules such as folate [18] & vitamin B12, nucleic acids such as aptamers and certain sugars such as the monosaccharide mannose [7].

However only proteins and peptides, antibodies are widely investigated and used. These ligands are coupled with nanomedicines using various coupling strategies like co-synthesis, chemical conjugation [19] or bioconjugation [20]. These coupling strategies make conformational changes in native macromolecule or even in unmodified polymer leading to their identification by RES system and rapid clearance from plasma which is undesired for functional EPR effect. However, this challenge can be overcome by coupling with PEG [21,22]. Another newer approach is by coupling to peptides derived from protein CD47 which binds to SIRPα on leukocytes thereby preventing phagocytosis and prolonging plasma circulation time [23].

Ideally, the drug carrier should be eliminated after drug release. But, unless the nanocarriers are biodegradable, it will remain in the body and be dealt with as a foreign body. Foreign body causes activation of macrophages which in turn phagocytose & attempt to degrade the nanocarriers in its lysosomal compartment. If macrophages fail to do so, then foreign body giant cells will be formed by fusion of multiple macrophages or monocytes & ultimately forms lesions resembling granulomas [24]. Also, prolonged inflammation & frustrated phagocytosis can lead to induction of malignancy on chronic accumulation of non-biodegradable materials [25].

Surface charge: The presence of many sulphated & carboxylate sugar moieties on luminal surface of blood vessels gives them negative charged surface. This fact leads to rapid clearance of polymeric drugs with high positive charges as they will non-specifically bind to luminal surface of vascular walls leading to reduced plasma half life. Whereas particles with high negative charges not only get trapped in liver but also trigger coagulation cascade which ultimately leads to blood coagulation (clotting). In worst cases, clot can block brain capillaries, which may lead to stroke & finally patient may die [26]. Therefore, desired surface charge is weakly negative to near neutral. Figure 1 explains the effect of surface charge.

Release rate: The ultimate goal of targeted drug delivery is to deliver most of the administered drug to the target site at optimal release rate. Too slow release rate results in subtherapeutic drug level at target site whereas too fast release rate would lead to a high concentration of free drug in circulation but no drug accumulation at target site resulting in undesired systemic toxicity and lower therapeutic effect. Basically nanoparticles can be formed by either encapsulation (micelles, liposomes) or conjugation (drug-polymer conjugate) [27,28]. Encapsulation based micelles shows very rapid drug release (micellar burst) after injection e.g. nearly 50% release within about 30 minutes for several drugs whereas liposomes with adequate shelf life or stability in solution shows too slow

release rate as they possess cholesterol-enriched harder lamellar structures. Conjugate based nanoparticles are synthesized through chemical bonds between drug and polymer for e.g. amide, ester, urethane, azide, imide and hydrazone etc. Release of drug from its conjugate depends on temperature, pH or enzymatic cleavage. For e.g. ester linkages ensures a rapid release due to abundance of esterases in plasma whereas amide linkage will ensure slower release profile. Therefore, choice of linker in case of conjugates and selection of suitable encapsulation based nanoparticles is critical determinant of rate of drug release.

FACTORS INVOLVED IN EPR EFFECT

Most of these factors are common mediators in inflammation & cancer and are highly expressed (as shown in Figure 2). A major difference between the two pathological lesions is the clearance rate of extravasated macromolecules such as plasma proteins, nanoparticles which results in a prolonged retention time in tumor tissue compared with that in inflamed tissue [13, 14, 30] .Consideration and studies of these factors will help the development of new strategies to modulate the EPR effect, angiogenesis and thus tumor growth.

Bradykinin: Bradykinin is a major mediator of inflammation that induces extravasation & accumulation of body fluids in inflammatory tissues leading to edema & major cause of pain in inflammation. In addition to bradykinin, tumors have hydroxypropyl bradykinin, which is a derivative of kinin that has the third amino acid replaced by hydroxyproline. High levels of derivative were found in blood plasma & in peritoneal & pleural fluids in carcinomatosis in patients with advanced cancer. Recently it was found that bradykinin also activates endothelial cell-derived nitric oxide synthase; which ultimately leads to an increase in nitric oxide (NO), which is an important mediator of tumor vascular permeability [31].

Nitric oxide (NO) and its derivatives: NO has multiple direct & indirect roles as a signalling messenger & hence it is a vital molecule in living creatures. NO is produced from L-arginine by nitric oxide synthase (NOS) in presence of oxygen. NO is extensively produced using iNOS from a greatly increased number of infiltrated leukocytes. Oxidized products of NO including $ONOO^-$ & nitrogen dioxide potentiate the EPR effect [11,32]. ONOO- is a strong oxidizing and nitrating agent, which forms via the reaction of NO with superoxide anion (O^-_2).

Collagenase (Matrix Metalloproteinase (MMP): ONOO- activates matrix metalloproteinase. MMP's are zinc-dependent neutral endopeptidases which are overexpressed in tumor cells. Activated MMP's cause disintegration & remodelling of extracellular matrix as a result of collagenolytic action and thus facilitates vascular permeability. Further, activated MMP's cause activation of plasminogen & in turn activated prekallikrein, thus activates bradykinin cascade. In addition to activation of MMP's, $ONOO^-$ has other roles such as decomposition of $ONOO^-$ to generate NO and nitration of amino acids in protein (e.g. nitrotyrosine) & nucleic acids (e.g. nitroguanosine) as a result of its high reactivity. These nitro compounds release nitrite (NO^-2) which may serve as source of NO [11,33].

Prostaglandins (PGs): PGs are enzymatically derived form arachidonic acid by the action of cyclooxygenases (COXs) & are lipidic in nature. PGs are important mediators in inflammation & are upregulated by inflammatory cytokines & bradykinin. Among the various PGs, prostaglandin E & prostaglandin I2 exhibit effects similar to those of NO [11,33].

Angiotensin-converting enzyme (ACE) inhibitors: ACE inhibitors are important class of antihypertensive drug which acts by preventing conversion of angiotensin I (AT-I) to angiotensin II (AT-II). Since the amino acid sequence of angiotensin I is similar to that of bradykinin at the C-terminal end, ACE inhibitors blocks degradation of bradykinin as well. Therefore, ACE inhibitors potentiate the pharmacological actions of kinin & acts as vascular permeability factor [32].

Vascular endothelial growth factor (VEGF): Solid tumors are characterized by the angiogenesis which is important for rapid growth of tumor. VEGF plays important role in any angiogenesis, enhancement of vascular permeability; both facilitate & sustain rapid growth of tumor. VEGF is highly upregulated in most tumors [33]. Recently, inhibitors VEGF (angiogenesis inhibitors) are approved to treat cancer for e.g. bevacizumab (Avastin) [34], ranibizumab (Lucentis)[35].

HETEROGENEITY OF THE EPR EFFECT

The EPR effect is not perfect or effective for all solid tumors, because tumors of different patients vary greatly in actual clinical setting. Therefore, one can say that heterogeneity of the EPR effect reduces its universal validity. The heterogeneity is due to the tumor biology diversity.

Tumor doubling time (TDT): While designing EPR based anticancer nanoparticles, TDT is crucial

factor to consider as it is highly heterogeneous. It exhibits heterogeneity at both intratumor and intertumor level with respect to different tumor types, tissue origins, stages and grades. Therefore, while designing EPR based anticancer nanoparticles one should consider doubling time variation when planning the release mechanism of active chemotherapeutic agents from its nanocarriers, as well as internalization rate of macromolecular complexes into tumor cells [36] for e.g. polymer-drug conjugates with slow releasing amide bond, or slowly internalized liposome, could be good choice for tumors with a slow TDT. In contrast, fast releasing micelle or an ester bond linkage can be a better fit for rapidly dividing tumors as described in above section.

Variations in tumor vascular density: Vascular density is largely dependent on the type of cancer & varies largely within each tumor type. Also, metastatic tumors tend to posses higher vascular density compared to non-metastatic tumors. Also; it has seen found that large variation in expression of vascular permeability factor like VEGF exists. Thus, while designing the nanoparticles, the properties of the targeted tumor tissue such as cancer type, the microvascular density & the secretion of tumor vascular permeability factors such as VEGF should be considered to take full advantage of EPR effect. Tumor diameters can be less than 1 cm to larger than 10 cm; & tumors can be highly hypoxic to normoxic, can have different pathological classes, are genetically diverse, can have partial or extensive necrosis, can have occluded or compressed vascular systems with or without blood coagulation in or around the tumor mass [13,36]. This heterogeneity can be overcome in a number of ways as discussed in following section.

APPROACHES TO POTENTIATE THE EPR EFFECT

As we have identified many permeability factors such as NO, bradykinin, & prostaglandins, that facilitates extravasation or the EPR effect in cancer tissues. These factors are not generated in normal benign tumors and therefore these systems are not activated under normal circumstances. Therefore, augmentation of these factors will affect only tumor or influenced tissues, with the results being tumor- selective enhanced vascular permeability & improved delivery of drugs to tumors. In this way, heterogeneity of EPR effect can be overcome. Following section describes two approaches by which EPR effect can be potentiated namely by targeting tumor vasculature or stroma and reducing tumor cells barrier to drug delivery by killing them.

Targetting Tumor Vasculature or Stroma

Increased delivery of nanoparticles to tumors under Angiotensin-II induced hypertension

Tumor blood vessels lack a smooth layer or pericytes needed for vasoconstriction, so tumor blood vessels on infusion of AT-II show very little vasoconstriction, whereas blood vessels of normal tissues show constriction. Therefore; by inducing hypertensive state by using AT-II, normal blood vessels would constrict while tumor blood vessels will be open, thus facilitating vascular leakage. The final result would be increased blood flow volume in tumor tissues & hence increased drug delivery [37].

Use of nitroglycerin for enhanced delivery to tumors

As described in factors involved in EPR effect, NO is major facilitator of EPR effect. Hence, tumor targeted delivery of macromolecules can be enhanced by use of NO or NO-releasing compounds such as nitroglycerin. These NO releasing agents generate NO from NO2 selectively in hypoxic tumor tissue compared with normoxic tissues. Thus, such nitro agents facilitate the EPR effect via local NO generation in tumors, with enhanced drug delivery upto 2-to 3-fold & improved therapeutic effect [38]. Clinically used agents which are investigated include nitroglycerin and isosorbide dinitrate.

Using ACE inhibitors

As described in the above section, solid tumors generate bradykinin which would aid the EPR effect. ACE inhibitors inhibit degradation of bradykinin, thus raising the local bradykinin concentration in tumor tissues in the body [39].

Generating carbon monoxide (CO)

Enzyme heme oxygenase-1 (HO-1) is upregulated in most solid tumors & serves as important role by producing CO which has physiological function similar to the vasodilator function of NO. Therefore CO also has a key function in EPR effect. Therefore, use of OH-1 inducers like PEGylated hemin or similar agents or CO-releasing agents (e.g. Carbon monoxide-releasing molecule, CORM2), can facilitate the EPR effect [40]. Other possible options include use of prostaglandin (PG) I2 analogue such as beraprost sodium or use of TGF-B inhibitor (TGF-B is tumor growth & differentiation factor. It facilitates production of extracellular matrix.)

Reducing Tumor Cells Barrier to Drug Delivery by Killing Them

Tumor cells themselves acts as a barrier to deeper penetration of nanoparticles. Short term application of radiation, photo-dynamic therapy or chemotherapeutic agents to kill cancer cells can

negatively impact nanoparticles delivery to tumor by vascular shut down. Vascular shut down is result of killing normal cells along with cancer cells leading to damage to blood vessels. These methods can also damage tumor vasculature resulting in thrombotic occlusion from the bystander effect [41].

Recently, more selective method to kill tumor cells has been described by et al as photo-immunotherapy (PIT) [42]. PIT can specifically kill cancer cells exposed to near infra red by inducing immediate necrosis without damaging normal cells. 24-fold tumor targeted delivery of nanoparticles was observed when compared to untreated control tumor as most of the cells in the perivascular tumor stealths are killed. Increase in vascular permeability was observed as soon as near infra red light is exposed. Results of the study were supported by histology studies and dynamic fluorescence imaging. Thus, PIT has potential to increase drug delivery to tumor dramatically without causing thrombotic occlusion [43].

EPR EFFECT AND THERANOSTICS

Theranostics is concept of combining a drug with a diagnostic and is also known as personalized medicine, integrated medicine, Dx/Rx partnering, predictive medicines and pharmacodiagnostics. It utilizes molecular diagnostic tests which are used to determine targeted therapy for particular patient. Molecular diagnosis by imaging used to guide targeted therapy as well as to examine patient's response to treatment. Thus, it can be used to treat patients with right therapy, right dose of drug at right time [44]. Nanoparticles based platforms with large surface to volume ratio can be utilized to loaded with both imaging and therapeutic agents. However, some nanoparticles based platforms possess added advantage and behave as imaging agents, increasingly employed for imaging applications. By harnessing the well-developed surface chemistry one can load therapeutic moieties onto them and systems can be thus promoted as theranostic nanosystems. Quantum dots, carbon nanotubes and nanoparticles of iron oxide, gold, silica have been well investigated for imaging studies and are capable nanoplatforms for building up nanoparticles based therapeutics. Therefore, by utilizing concept of active targeting and EPR effect, it is possible to exploit full potential of theranostics in cancer. However, challenges involved such as toxicity, economic implications, environmental issues and issues with healthcare resources needs to be addressed for further advancements [43, 44]. Successful development of theranostics nanomedicine requires significant advances in imaging and nanomaterials, and considerable research with respect to their safety.

CLINICAL OVERVIEW OF MACROMOLECULAR DRUG: SMANCS

Antitumor proteins such as neocarzinostatin (NCS) are highly potent antitumor agents which has surpassed widely used conventional antitumor agents such as 5-fluorouracil, adriamycin & cis-platinum. NCS inhibit tumor cell growth at the nanomolar range, whereas many of the low molecular weight compounds do so at micromolar/millimolar range. Major limitation of NCS for wide clinical use is its severe toxicity (bone marrow suppression) & their very short half life. NCS was differentiated from conventional low molecular weight drugs as they predominantly accumulated in the regional lymph nodes when administered subcutaneously. This lymphotropic behaviour exhibited by NCS is important since lymphatic system is route by which tumor cells frequently metastasise & most therapeutic failures in cancer treatment occur because of lymphatic metastasis. Therefore, NCS was used to target lymphatics [45]. Maeda, later in 1979 conjugated poly(styrene-co-maleic acid-half-butylate) copolymer (SMA) with neocarzinostatin. It was approved in 1994 in Japan for use in treatment of hepatoma. SMA contains alternating linkages of styrene & maleic acid with about 30-50% of maleic acid in reactive anhydride form & half of the free carboxyl groups are butylated. This design of SMA was selected on the basis of optimum hydrophobicity, binding affinity of SMANCS to albumin and biological function of the covalently attached drug [46].

SMANCS exhibited unique properties compared with the parental NCS. These properties include prologation of plasma t ½ (by 20 fold); improved tumor-targeting capacity because of EPR effect, i.e. a markedly higher (10-to 20-fold) intratumor concentration compared with concentration in plasma, no immunogenicity; high lipophillicity. High lipophilicity enabled its solubilisation and formulation with a lipid contrast agent Lipiodol as a carrier [47]. Lipiodol is the ethyl ester of iodinated poppy seed oil which contains about 37% w/w iodine. It is used for lymphography due to its lymphotropic nature and its detection by X-rays. SMANCS/Lipiodol usually administered via tumor feeding artery for e.g. hepatic artery for hepatoma, renal artery for renal cancer. Visibility of Lipiodol under X-ray allowed accurate quantification and optimization of dose and tumor image by X-ray computed tomography. This lipid formulation allows truly selective tumor targeting such that drug concentration in tumor as much as 2000 times the concentration in blood (2000: 1) can be achieved. Retention of this macromolecular drug

for very long periods, several weeks or months gave sustained drug delivery with marked therapeutic effect [48].

Extended applications of SMANCS/Lipiodol therapy

With SMANCS/Lipiodol therapy it is now possible to treat advanced, difficult to treat solid tumors. This includes massive and multiple metastatic liver cancers, pancreatic cancers and their metastatic nodules in the liver, or bile duct carcinomas and cholangiocarcinomas. Advancements in tumor targeting was achieved by infusing SMANCS/lipiodol intra arterially under conditions of AT-II induced high blood pressure (e.g. from 100 Hg mm to 150 Hg mm). The blood pressure of 150-160 Hg mm was achieved via slow i.v. infusion of 0.5 µg/ml AT-II, which is set in a 20 ml infusion syringe-pump. Benefits of this method are improved therapeutic effect & diagnostic value. The improved diagnostic value is due to highly sensitive detection, by means of computed tomography (CT) of the tumor selective uptake of lipiodol; even in small tumor nodules with diameters of 3-5 mm. Improved therapeutic effect can be obtained on more types of tumors. Poor drug delivery to tumors due to heterogeneity of EPR effect can be overcome. Another benefit of this method is the reduced time required for tumor regression & less frequent drug administration needed [48,49].

PATENT LITERATURE ON EPR EFFECT BASED DELIVERY OF DRUGS OR IMAGING AGENTS

Li SD *et al* [50] has described the polymer conjugates comprising acetylated carboxymethyl cellulose (cmc-Ac) covalently linked via ester bond to polyethylene glycol and hydrophobic moiety. Hydrophobic moiety can be either drug or imaging agent. This polymer conjugate was encapsulated in a self-assembling nanoparticles composition having critical micelle concentration of 0.1 mg/ml. Thus formed nanoparticles possess average diameter of about 49-278 nm. Such innovative design not only provides tumor targeted delivery but also overcomes RES clearance of nanoparticles, improved pharmacokinetics and pharmacodynamic profile, improved stability of polymer conjugates and increased aqueous solubility of most of hydrophobic moieties. David A [51] has provided innovative targeting strategy for the selective delivery of diagnostic agents into solid tumors. He describes the use of polymer-NIR fluorochrome conjugates modified with targeting ligands for active targeting via receptors targeted delivery to tumor. Present invention fulfills need for effective cancer diagnosis as conventional, low molecular weight imaging probes exhibits limited tumor: background ratio. Doris E *et al* [52] describe use of polymeric micelles of size less than 100 nm for cancer diagnosis. Polymeric micelles comprises of fluorescent diagnostic agent and an amphiphilic polymer. Amphiphilic polymer can be obtained by the polymerization of an amphiphilic monomer consisting of lipophilic polymerizable vinylic or di-acetylenic group and hydrophilic polyoxyethylene or polyoxypropylene chain. These polymeric micelles possess satisfactory blood residence time, tumor uptake, imaging contrast and reproducible synthesis. Chung JH *et al* [53] describe that water soluble nanoparticles shows anticancer effect via EPR effect. These unique nanoparticles comprises of a multidentate metal chelating organic polymer and metal moiety between hydrodynamic size of 2 and 500 nm.

CONCLUSION

In the current arena of anticancer drug development, the need for a wide range of knowledge about cancer genomic & tumor biology is not fully explored. Drug development based on EPR effect is certainly an important first step, but some problems still remain. Even after a drug is delivered to cancer tissues, it must be taken up by tumor cells, & free active drug must then be released & interact with target molecules to achieve full potential of EPR effect based targeted drug delivery.

TABLE NO. 1: DRUG TARGETING STRATEGIES [8]

PASSIVE	Relies on physiological body features e.g.: -reticulo-endothelial system(RES) -monocyte-macrophage system -enhanced permeability and retention (EPR) effect
INVERSE	by blockade/saturation of passive targets -RES blockage by sugar polymers/ lipid microemulsions
ACTIVE	Based on imposing targeting properties to drug: -intrinsic: drug designed to target a specific molecule -extrinsic :drug coupled to targeting features *physical targeting :programmed release (pH ,temperature etc) *ligand based :coupling to affinity moieties: as conjugates or through carriers
COMBINED	By combining any of the above strategies

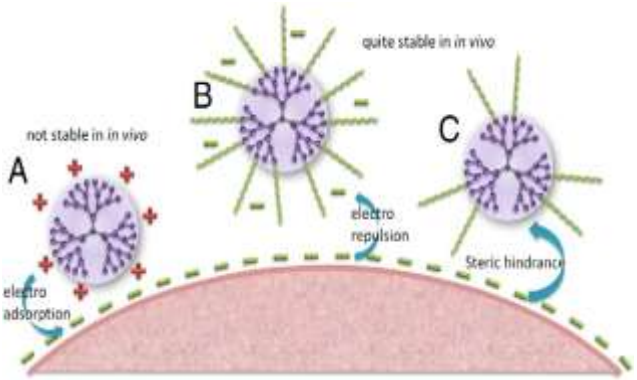

Figure 1: (A) Quick internalization due to electrostatic adsorption, but are not suitable in vivo; (B) negatively charged carriers are quite stable in vivo, but cause biocompatibility problems; (C) PEGylated nanoparticles are internalized slowly because of steric hindrance [29]. (Reprinted with permission from author)

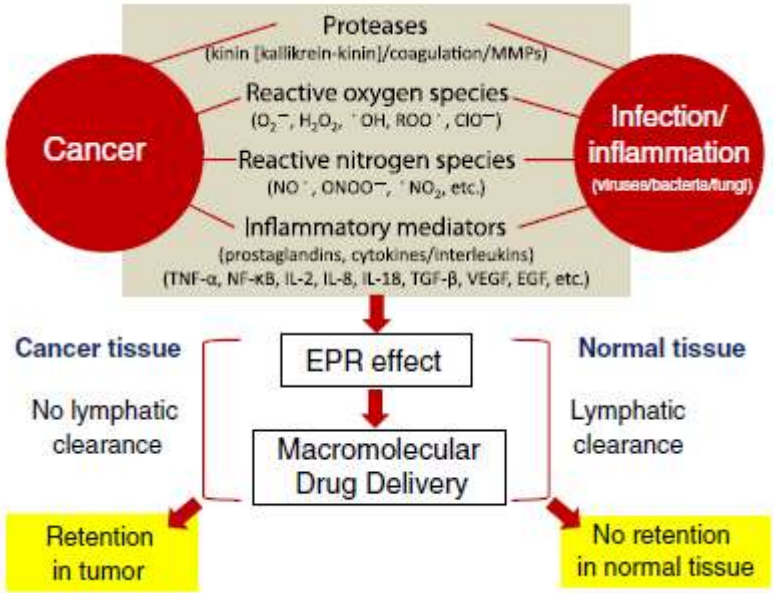

Figure 2: Various vascular mediators commonly found in inflammation and cancer that contribute to the EPR effect [30]. (Reprinted with permission from author)

REFERENCES

1. World Health Organization. http://www.who.int/mediacentre/factsheets/fs297/en/(2014) (Accessed on June 22, 2014).
2. Denison TA, Bae YH. Tumor heterogeneity and its implications for drug delivery. J Controlled Release 2012; 164:187-91.
3. Marusyk A, Polyak K. Tumor heterogeneity: causes and consequences. Biochim Biophys Acta 2010; 1805: 105-17.
4. Zahreddine H, Borden KLB. Mechanism and insights into drug resistance in cancer. Front Pharmacol 2013; 4:1-8.
5. American Cancer Society. http://www.cancer.org/research/cancerfactsfigures/cancerpreventionearlydetectionfactsfigures/cancer-prevention-early-detection-facts-figures (2013) (Accessed on June 24, 2014).
6. Florence AT. Pharmaceutical nanotechnology: more than size. Ten topics for research. Int J Pharm 2007; 339: 1-2.
7. Muro S. Challenges in design and characterization of ligand-targeted drug delivery systems. J Control Release 2012; 164:125-37.
8. Park K et al. Analysis on the current status of targeted drug delivery to tumors. J Control Release 2012; 164: 108-14.
9. Narang AS, Varia S. Role of tumor vasculature in drug delivery. Adv Drug Deliv Rev 2011; 63: 640-58.
10. Acharya S, Sahoo SK. PLGA nanoparticles containing various anticancer agents and tumor delivery by EPR effect. Adv Drug Deliv Rev 2011; 63:170-83.
11. Maeda H et al. The EPR effect: Unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. Adv Drug Deliv Rev 2011; 63:136-51.
12. Fang J et al. Factors and mechanism of "EPR" effects and enhanced antitumor effects of macromolecular drugs including SMANCS. Adv Exp Med Biol 2003; 519:29-49.

13. Greish K et al. Anticancer nanomedicine and tumor vascular permeability; Where is the missing link? J Control Release 2012; 164:265–75.
14. Maeda H et al. The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. Adv Drug Deliv Rev 2013; 65:71–79.
15. Pardridge WM. Drug targeting to brain. Pharm. Res 2007; 24(9): 1733-44.
16. Zheng G et al. Rerouting lipoprotein nanoparticles to selected alternate receptors for the targeted delivery of cancer diagnostic and therapeutic agents. Proc Acad Sci U.S.A. 2005; 102(49): 17757-72.
17. Bies C et al. Lectin-mediated drug targeting: history and applications. Adv Drug Deliv Rev 2004; 56(4): 425-35.
18. Xia W, Low PS. Folate-targeted therapies for cancer. J Med Chem 2010; 53(19): 6811-24.
19. Carter PJ. Introduction to current and future protein therapeutics: a protein engeneering perspective. Exp Cell Res 2011; 317(9): 1261-69.
20. Vaidya A et al. Bioconjugation of polymers: a novel platform for targeted drug delivery. Curr Pharm Res 2011; 17 (11):1108-25.
21. Immordino ML et al. Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential. Int J Pharm 2006; 1: 297-315.
22. Owens DE, Peppas NA. Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. Int J Pharm 2006; 307: 93-102.
23. Willingham SB et al. The CD47- Signal regulatory protein alpha (SIRPa) interaction is a therapeutic target for human solid tumors. PNAS Early Edition. 1-6.
24. Anderson JM et al. Foreign body reaction to biomaterials. Semin Immunol 2008; 20:6–100.
25. O'Neill LAJ. How frustration leads to inflammation. Science 2008; 320:619–20.
26. He C et al. Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles. Biomaterials 2010; 31: 3657-66.
27. Peer D et al. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol 2007; 2(12): 751-60.
28. Davis ME. Nanoparticle therapeutics: an emerging treatment for cancer therapy. Nat Rev Drug Discov 2008, 7(9), 771-82.
29. Shen Y et al. Challenges in design of translational nanocarriers. J Control Release 2012; 164:156-69.
30. Maeda H et al. Polymeric drugs for efficient tumor-targeted drug delivery based on EPR-effect. Eur J Pharm Biopharm 2009; 71:409–19.
31. Matsumara Y et al. Involvement of the kinin-generating cascade in enhanced vascular permeability in tumor tissues. Jpn J Cancer Res 1988; 79:1327-34.
32. Hori K et al. Tumor-selective blood flow decrease induced by an angiotensin converting enzyme inhibitor, temocapril hydrochloride. Jpn J Cancer Res 2000; 91:261-69.
33. Maeda H et al. Vascular permeability enhancement in solid tumor; Various factors, mechanism involved and its implications. Int Immunopharmacol 2003; 3:319-28.
34. www.avastin.com (Accessed on June 25, 2014).
35. www.lucentis.com (Accessed on June 26, 2014).
36. Yusuda K et al. Tumor doubling time and prognostic assessment of patients with primary lung cancer. Cancer 1994; 74:2239-44.
37. Nagamitsu A et al. Elevating blood pressure as a strategy to increase tumor targeted delivery of macromolecular drug SMANCS: cases of advanced solid tumors. Jpn J Clin Oncol 2009; 39:756-66.
38. Seki T et al. Enhanced delivery of macromolecular antitumor drugs to tumors by nitroglycerin application. Cancer Sci 2009; 100:2426-30.
39. Fang J et al. Carbon monoxide, generated by heme oxygenase-1, mediates the enhanced permeability and retention (EPR) effect of solid tumor. Cancer Sci 2012; 102:535-41.
40. Xie J et al. Nanoparticles-based theranostics agent. Adv Drug Deliv Rev 2010; 62:1064-79.
41. Kobayashi H et al. Improving Conventional Enhanced Permeability and Retention (EPR) Effects; What Is the Appropriate Target? Theranostics 2014; 4(1): 81-89.
42. Mitsunaga M et al. Cancer cell-selective in vivo near infrared photoimmunotherapy targeting specific membrane molecules. Nat Med 2011; 17: 1685-91.
43. Sano K et al. Markedly enhanced permeability and retention effects induced by photo-immunotherapy of tumors. ACS Nano 2013; 7: 717-24.
44. Lammers T et al. Theranostic nanomedicine. Acc Chem Res 2011; 44:1029-38.
45. Maeda H et al. Lymphotropic accumulation of an antitumor antibiotic protein, neocarzinostatin. Eur J Cancer 1980; 16:723–31.
46. Maeda H et al. A lipophillic derivative of neocarzinostatin. A polymer conjugation of antitumor protein antibiotic. Int J Pept Protein Res 1979; 14: 81-87.
47. Maeda H. SMANCS and polymer-conjugated macromolecular drugs: advantages in cancer chemotherapy. Adv Drug Deliv Rev 2001; 46: 169-85.
48. Maeda H. Macromolecular therapeutics in cancer treatment: The EPR effect and beyond. J Control Release 2012; 164:138–144.
49. Nagamitsu A et al. Elevating blood pressure as a strategy to increase tumor targeted delivery of macromolecular drug SMANCS: cases of advanced solid tumors. Jpn J Clin Oncol 2009; 39:756-66.
50. Li SD, Ernsting MJ, Tang WL. Cellulose based nanoparticles for drug delivery. U.S. patent 20140079644, March 20, 2014.
51. David A. Targeted delivery systems for diagnostic applications. U.S. patent 20130142734 A1, June 6, 2013.
52. Doris E, Duconge F, Gravel E, Mackiewicz N, Tavitian B. Polymerized micelles for diagnosis. U.S. patent 20130230467 A1, September 5, 2013.
53. Chung JH, Hong SH. Water-soluble organometallic nanoparticles and method for preparing the same. WO2007108618A1, September 28, 2007.