

BRAIN TUMOR AND PASSIVE TARGETING***Ojaswi Ghadge¹, Anubha Khale², Ajit Wankhede³**¹Lecturer, H. K.College of Pharmacy, Oshiwara, Jogeshwari (W), Mumbai- 400102²Principal, H. K.College of Pharmacy, Oshiwara, Jogeshwari (W), Mumbai- 400102³Asst. Professor, SandipFoundation 'sSandip Institute of Pharmaceutical Sciences,
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ABSTRACT

Insufficient drug delivery across the blood–brain barrier (BBB) has been a major challenge in the chemotherapy of brain pathologies. Various novel approaches have been developed for the accurate detection of early-stage of cancer like brain tumor and for its targeted therapies. Newly developed targeted therapies limits the unwanted toxicity to healthy cells. Various strategies of drug targeting like passive targeting are in limelight today. Main aim of this article is to review pathology of brain in presence of tumor, how the passive targeting has improved the survival rates in patients with brain tumors, understanding the phenomenon of active and passive targeting and to

explore different parameters of drug candidate to be modified for its best use in passive targeting through nanoformulation.

KEYWORDS: Brain tumor, passive targeting, blood brain barrier, targeted drug delivery, nanoparticles.

INTRODUCTION

One of the most popular chronic and fatal diseases in the world is named as Cancer. The number of new cases increases each year. Overall survival rate from cancer has not improved substantially over the past few decades [1, 2]. Therefore, it is needed to develop novel approaches for the accurate detection of early-stage of cancer and for targeted therapies. For enhancing intratumoral drug concentrations while limiting the unwanted toxicity to healthy tissue, recent advances in nanomaterials have explored passive and active targeting strategies

[3, 4]. The targeted delivery by nanoparticles overcomes difficulties associated with conventional free anticancer drugs, like solubility problems in aqueous medium, fast clearance, lack of selectivity, which results in nonspecific toxicity toward normal cells and lower the dose of drugs delivered to the cancer cells [5]. Nanoparticles simply get accumulated and entrapped in tumors and thus targeting cancer, it is termed as passive targeting. The phenomenon is called the enhanced permeation and retention effect, caused by leaky angiogenetic vessels and poor lymphatic drainage and has been used to explain why macromolecules and nanoparticles are found at higher ratios in tumors compared to normal tissues[6,7].

Developing a drug to treat a disease or to formulate the same is not a big deal in pharma world but the real challenge lies in distributing the drug to Central Nervous System (CNS) across Blood Brain Barrier (BBB). Insufficient drug delivery across the blood–brain barrier (BBB) is the major challenge in the chemotherapy of brain pathologies. Researchers have been trying various formulation techniques for anticancer drugs to cross blood brain barrier. Scientific community worldwide has been working toward discovering “nanoscale” solutions to treat chronic diseases like brain cancer by using nanoparticle-based drug delivery systems.

Tumor Grades and Types

In human body formation of new cells take place when normal cells grow old/ get damaged/ they die. When this process goes wrong, or when this cycle is abrupted, sometimes, new cells generate when the body actually doesn't need them, and old or damaged cells don't die/ degenerate as they should. This accumulation of extra cells frequently forms a mass of tissue called a tumor.

Brain tumors can be benign or malignant: Benign ones do not contain cancer cells, so referred as noncancerous. Usually, they can be removed but they can still grow back. Cells from benign tumors hardly rooted in tissues around them, therefore easy to remove by surgery. However, benign tumors can press on sensitive areas of the brain and cause serious health problems. Benign tumors occurring in most other parts of the body are less life threatening or do not cause major health problem as compared to benign brain tumors. Benign brain tumors may become malignant. Malignant brain tumors (also called brain cancer) contain cancer cells. They spread faster than benign ones. These are generally more serious and often is life threatening. They grow rapidly and aggressively invade the nearby healthy brain tissue. These cancer cells possibly will break away from tumour and spread to other parts of the Central

Nervous System (CNS) but rarely to other parts of body. Secondary brain tumours are malignant (majority). Secondary Brain Tumours can be at-Lung, Breast, GI etc.[8,9, 29]

Types of Brain Tumours include- Primary: benign or malignant (rare) and Treatment for Cancer related Oedema, Pain and Nausea includes- steroids, analgesia, antiemetics [8,9].

Tumor Grade

Brain tumors are graded as follows depending on the way cells look under a microscope:

Grade I

1. Presence of benign tissue.
2. Cells look nearly like normal brain cells,
3. Slow growth.

Grade II

1. Presence of malignant tissue.
2. Cells look less like normal cells as compared to Grade I tumor.

Grade III

1. Very different look of malignant tissue from normal cells.
2. Actively growing abnormal cells (anaplastic).

Grade IV

1. Most abnormal look of malignant cells
2. Faster growth.

Cells from low-grade tumors (grades I and II) look more normal and generally grow more slowly than cells from high-grade tumors (grades III and IV). As time passes a low-grade tumor may develop into a high-grade tumor. However, adults are more susceptible to this change than children.

Surgery, radiation therapy, and chemotherapy are the treatment options and treatment happens in the same sequence. As mentioned earlier for a benign brain tumor, surgery can be the only treatment needed. Chemotherapy and radiation therapy can be given at the same time in many situations. Sometimes, chemotherapy may be given before the radiation therapy. The biggest challenge in front of chemotherapy is delivery impediments related to the blood-brain barrier (BBB). Glioma, the most common and aggressive primary brain tumor, requires administration of anticancer drugs orally or intravenously but delivery of effective concentrations is not possible with these routes. In addition chemotherapy is usually a long

treatment. Therefore, transient disruption of the BBB is likely insufficient to deliver effective intratumoral concentrations of anticancer drugs.[8, 28, 30]

BBB (Blood Brain Barrier)

Blood Brain Barrier is a naturally occurring protective mechanism and is selective and semipermeable. It protects the brain and spinal cord from harmful chemicals entering those structures through the bloodstream. It is formed by the brain capillaries where endothelial cells have tight intercellular junctions (Zonulaeoccludens). There is selective passage of drugs from the bloodstream to the brain so that drug molecules are unable to penetrate through cell membranes. Therefore BBB is considered as one of the main factors affecting success of chemotherapy in brain tumour. The BBB allows passive diffusion of small lipid-soluble molecules, whereas hydrophilic substances or molecules with high molecular weight have minimal passive permeation. To improve CNS penetration of drugs, variety of techniques like chemotherapy (high-dose), intrathecal, intraarterial injections, induction of hyperosmolarity have been used where researchers found delivery of targeted nanoscaled formulations will be more suitable [9, 31, 33, 37].

However, research in the past two decades of. Better understanding of response of tumors to chemotherapy, and advances in targeted delivery has significantly improved the survival rates in patients with brain tumors. It has increased the life span and quality of life for many.

Drug-delivery systems

An ideal drug-delivery system should have the ability to target and to control the drug release. Targeting ensures high efficiency of the drug and reduces side effects, particularly when dealing with anticancer drugs which can also kill healthy cells when delivered to them. Controlled release reduces or prevents side effects. Nano Particulate Drug Delivery System (NPDDS) provides a better penetration of the particles inside the body as their size allows delivery via intravenous injection or other routes. The irritant reactions at the site of injection can also be minimized by Nano scale size of these particulate systems [10, 38].

Targeted drug delivery

The development of targeted drug delivery accelerated dramatically when the first biopharmaceuticals products were launched in the 1980's. These protein-based drugs could not be delivered by the oral route because the physiological conditions in the gut would cause

their destruction. Many academic groups worked to develop innovative ways of delivering these new medicines and these innovations have been widely adopted.

Drug delivery is the science of optimizing the administration a pharmaceutical or biopharmaceutical product. The aim is to maximize the therapeutic effect in the patient whilst minimizing the potential side effects of the intervention and increase patient compliance and satisfaction with the therapy. Historically therapies were administered via an oral route (tablets, pills and oral liquids) or via injections. These traditional drug delivery systems have certain disadvantages[10].

- a. The drugs are delivered to the entire body via the blood circulation and only a small proportion of the total dose reaches the site where it is required
- b. High doses required to treat the patient.

Targeted drug delivery can overcome these shortcomings by delivering the drugs right where it is needed, with minimal side effects. It allows far lower dose of drug to be used. It has the potential to reduce the side effects associated with conventional chemotherapy. It significantly enhances the penetration of the drugs into the tumors. This allows far lower doses to be used in order to achieve the same therapeutic effect.

Table 1: Active and Passive targeting

Active targeting (Grafting biorecognition molecules (ligands) onto the nanoparticles refers to active targeting and aims to increase specific cell uptake.)	Passive targeting (Nanoparticles that exhibit localization to specific organs or to sites of disease via biological mechanisms, such as the RES or the EPR effect, are known as 'passive targeting agents'.)
<ul style="list-style-type: none"> • These drugs are designed to target and interact with specific biological sites e.g. cancer specific antigens. • The drugs are designed to select and interact only with the intended target, minimizing side effects. • eg. Monoclonal antibodies and RNA interference therapies are examples of this 	<ul style="list-style-type: none"> • These are drugs that have been designed to be delivered to a specific site of action generally using innovative drug delivery devices. • In order to be effective the drugs may have to be specially formulated for sustained delivery of the drug or to enhance their permeability.

<p>category. [7,8]</p> <ul style="list-style-type: none"> • Monoclonal antibody therapy is used to treat a wide range of diseases such as cancer, multiple sclerosis, cardiovascular disease, inflammatory disease and transplant rejection. The antibodies bind only to the specific cells they were designed to target and they induce an immunological response against these target cells. Some monoclonal antibodies have been modified to deliver toxins, radioactive isotopes and other biologically active substances to the site of cancer cells in order to enhance the killing effects. RNA Interference (RNAi) therapies are still in development but hold great promise for the treatment of viral infections, cancer and macular degeneration[7-10]. • These therapies work by blocking gene transcription by targeting messenger RNA and blocking the synthesis of specific proteins. • Local delivery also makes it less likely that the drugs will have unanticipated, harmful effects elsewhere in the body. • This was therefore a case of an active targeted therapy delivered by a passive targeting. [7-12] 	<ul style="list-style-type: none"> • Nanomedical technologies are seeing increased use the development of these systems as nanoparticle engineering can help to maximize drug bioavailability at the sites of action. The main advantage of passive technologies compared to the active ones is to require a smaller dose of drug to be delivered directly to the site where it is needed. [7-9] • It involves preparing the delivery system of a definite molecular weight(>30Kda), molecular size(100-200nm), having hydrophilicity and neutral charge. • The physiological conditions in the tumor are considered. The physical properties of the target are studied. • Targeting by adjusting the delivery system sensitive to pH or temperature or charge or an enzyme. • The triggering mechanism may be supplied from outside the body also. • Eg. Targeting to the reticuloendothelial system or the circulatory system. • Methods of delivery include: nasal sprays, inhalation systems such as nebulizers, dry powder inhalers and metered dose inhalers, transdermal patches and creams, ophthalmic creams and suspensions and inter-uterine devices. [9-12]
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In passive targeting or physical targeting such a drug carrier complex is prepared so that it avoids the removal because of body defense mechanisms (metabolism, excretion and opsonisation, phagocytosis etc). This complex circulates in the blood stream and reaches to the target receptor taking help of body properties like pH, temperature, molecular size, shape.

Conventional surface nonmodified nanoparticles are usually trapped in the circulation by the RES (Reticulo Endothelial System), such as liver and spleen, depending on their size and surface characteristics. This can be avoided or controlled by adjusting size and surface characteristics of circulating nanoparticles. [13, 34]

To fulfill this requirement many of the drug properties are critical. Whether the device can destabilize the membranes of the body and whether it is sensitive to triggering signals like pH and temperature is also important.[13, 27] So the properties of the drug molecule and the properties of the drug, carrier complex are both important. Such properties are enlisted below and these parameters need to modify and design accordingly.

1. Molecular Weight
2. Molecular Size
3. Nature of surface
4. Surface Charge

1. Molecular Weight

- a. Total molecular weight of more than 30 Kda (drug+carrier)- can escape quick renal clearance and keep circulating in blood.
- b. Molecules which are less than 30 Kda and which are hydrophilic- microtubular cells of the kidney quickly filter and eliminate [27, 32]

2. Molecular Size

- a. Size of nanoparticles should be large enough to avoid rapid leakage into blood capillaries but small enough to escape capture by fixed macrophages that are lodged in the RES.
- b. Since, delivery system is expected to be able to penetrate through the openings or fenestrae of the endothelial cells of the capillaries, the size of the complex should not be more than 200 nm.
- c. For most normal capillaries the endothelial cells are attached to one another tightly (no chance for molecules to filter out or penetrate), on a continuous subendothelial membrane. Only small systems of sizes less than 10 nm can filter out or penetrate out of these junctions into extravascular tissues.
- d. Tumour tissues have blood capillaries with less tight junctions and are with bigger fenestrae, they are filters with big holes and they allow systems upto 200 nm. But only systems upto 200 nm can penetrate; bigger than that size cannot penetrate. Besides it is

also difficult to give IV injections of sizes more than 200 nm as they can cause blocking of blood capillaries.

- e. Under normal circumstances the liver, the spleen and the bone marrow have capillaries with large pores or openings. Solid tumors are also like this.

3. Nature of surface

- a. Surface of the nanoparticles should be ideally hydrophilic to avoid removal by the Monophasic Phagocytic System (MPS). If a hydrophobic body enters blood circulation, the MPS interprets it as a XENOBIOTIC and tries to attach to it. Opsonins (Body proteins) attach to it and they are like markers. Once they are marked by the opsonins, the macrophages in the blood imbibe them and remove them into the RES.
- b. There are two ways to escape this opsonisation and phagocytosis system (i) coating the surface of nanoparticles with a hydrophilic polymer (PEG) which protects them from opsonization by repelling plasma proteins (ii) nanoparticles can be formed from block copolymers with hydrophilic and hydrophobic domains. [27,35,36]

4. Surface Charge

- a. Surface charge of the delivery system (positive or negative or neutral) indicates the length of circulation time.
- b. Negative charge systems are removed from the circulation quickly by the Kupffer cells of liver. Whereas positive charges are recognized as foreign bodies by the Opsonins and tend to get removed from circulation.
- c. It is only the neutral system which has longer circulation time in the blood.

In addition we can attach a protein that can destabilize the natural biomembranes and penetrate them. Also a delivery system can be designed in such a way that drug release is triggered in response to physical properties like pH or temperature or charge in the body in the target area and drug release occurs only in that area and nowhere else. Such triggering mechanism can be supplied from outside the body. Eg. **Thermosensitive liposomes**: These are designed in such a way that its coat is stable during circulation in blood but when it goes into the capillaries in the tumor where the temperature is a little bit higher the coat must melt and release the drug which must accumulate in the tumor and cause its action. Or the drug can be attached to the carrier by means of a molecule called linker which is labile to a particular enzyme which is found only in the target tumor. [27, 32, 35, 36]

Limitations of passive targeting: Nanoparticles which are passively targeted release their therapeutic payload into the tumor environment rather than within cancer cells. (“PEG dilemma”) [11]. Also, for macromolecular drugs or drugs that are not readily retained in tumors or are not readily taken up by cancer cells, this extracellular release of drug may be less effective. It finds difficulty in at maintaining a differentially high tumor drug concentration over a prolonged period of time.

Nanoparticulate delivery system

NPDDSs are being discovered for the purpose of solving the challenges of drug delivery as most carriers are less than 100 nm in diameter and exist in different sizes and shapes. NPDDSs provide methods for targeting and releasing therapeutic compounds in much defined regions.

Definition: It is the drug delivery system where nanotechnology is used to deliver the drug at nanoscale.

Materials exhibit different, more desirable physical, chemical, and biological properties below 100 nm. Given the enormity and immediacy of the unmet needs of therapeutic areas such as CNS disorders, this can lead to drugs that can extend life and save untimely deaths (38, 39).

General properties of nanoscaled particles

- Diameter = 1 to 100 nanometers
- Surface area to volume ratio- High
- Interaction with biomolecules on the cell surface
- Well Absorption of drugs
- Ability to diffuse readily
- Drug release at an experimentally predetermined rate over a prolonged period of time
- Drugs release preferentially at target sites with the possibility of controlled release rates
- Maintenance of drug concentrations within therapeutically appropriate ranges in circulation and within tissues
- Protection of drug from hepatic inactivation, enzymatic degradation and rapid clearance *in vivo*.

Advantages of nanoparticles: Research on cancer Nano therapeutics has started to solve several limitations of conventional drug delivery systems which were nonspecific biodistribution and targeting, lack of water solubility, poor oral bioavailability, and low

therapeutic indices; thus reducing the achievable dose within the tumor and also resulting in suboptimal treatment due to excessive toxicities.[15, 18] Some other advantages are:

- Protection of drugs against degradation
- Targeting the drugs to specific sites of action, organ or tissues
- Delivery of biological molecules such as proteins, peptides, and oligonucleotides.
- Anticancer nanoparticles are being specially designed which are able to carry their loaded active drugs to cancer cells by selectively using the unique pathophysiology of tumors, such as their enhanced permeability and retention effect and the tumor microenvironment.
- Reduction in drug resistance.
- Increased intracellular concentration of drugs because of their ability to accumulate in cells without being recognized by P-glycoprotein, which is one of the main mechanisms of drug resistance. This is possible because NPs are enveloped by endosomes via receptor-mediated endocytosis[18].
- NPs use both passive and active targeting strategies and thus can enhance the intracellular concentration of drugs in cancer cells while avoiding toxicity in normal cells [3, 17].

Next generation of nanoparticles, which can also be called as multifunctional and multiplex nanoparticles, are getting actively investigated. Soon it will be available for patients facilitating personalized and tailored cancer treatment [14].

There are still many limitations to be solved such as poor oral bioavailability, instability in circulation, inadequate tissue distribution, and toxicity. Due to the pathophysiology of tumor blood vessels, most nanoparticles are expected to get accumulated in tumors. Delivery of nutrients to this actively growing tumor with a volume greater than 2 mm³ becomes diffusion-limited. Therefore, formation of new blood vessel is required to supply nutrients and oxygen [19]. The incomplete tumor vasculature causes leaky vessels with enlarged gap junctions of 100 nm to 2 μm, depending on the tumor type, and macromolecules easily access the tumor interstitium [20, 21]. Since tumors lack a well-defined lymphatic system, they have a compound retention time higher than normal tissues [22, 23]. These features provide an enhanced permeability and retention (EPR) effect, which constitutes an important mechanism for the passive targeting and selective accumulation of nanoparticles in the tumor interstitium. However, the development of resistance in cancer cells can evade the cytotoxicity not only of conventional chemotherapeutics but also of this newer molecularly targeted therapeutics [14, 15, 16].

Polymer-Based NPDDS

Some polymeric materials used for this purpose are gelatin, chitosan (CS), sodium alginate, poly(alkyl) cyanoacrylates, poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol-co-(lactic-glycolic acid)), poly(caprolactone), and polymethyl methacrylate [40-43]. Another approach to modify the biological response is based on the incorporation of suitable adjuvants in the NPs, like proteins such as albumin, invasins, and lectins, and polymers such as poloxamers and poloxamines[43]. Different manufacturing methods can also enable modifications of the physicochemical characteristics of NPs such as size, shape, structure, morphology, texture, and composition (40).

1. Hydrogel-Based Nanoparticulate Drug-Delivery Systems
2. Dendrimer-Based Drug-Delivery Systems
3. Calcium Carbonate Nanoparticles
4. Proticles: Protamine-Based Nanoparticulate Drug Carriers
5. Chitosan-Based Nanoparticulate Drug-Delivery System
6. Silicone Nanopore-Membrane-Based Drug-Delivery System
7. Polyester Polysaccharide Nanoparticles
8. Albumin and Gelatin Nanospheres
9. Polymeric Nanocapsules as Drug Carriers
10. Polystyrene Nanospheres

Types of Nanoparticles Used as Drug Delivery Systems

- Liposomes
- Polymeric Nanoparticles
- Dendrimers
- Fullerenes
- Quantum dots
- Magnetic nanoparticles

CONCLUSION

Pharmaceutical and Oncological research is a rapidly growing area. Especially, in the area of Brain tumour, BBB remains to be a big challenge in achieving efficacy of pharmaceuticals. Today technology has already developed safe brain-targeted long-circulating nano-DDSs. The practical use of simpler polymeric or lipid nanoparticles also resulted in favorable results. The mechanisms underlying their still hypothetical translocation through the BBB need

further investigations for validation. So, real achievements in nano-DDS brain delivery, but the way is still long from bench results to clinical applications. Current concerns over the safety of nanoparticles have led to the development of many new dimensions in research. There is still scope in expansion of knowledge about nanoparticle interactions within cells. In the years ahead, discovery of new nanoparticles will continue and new applications to Nano medicines will be found. There are quite a few examples of successful cancer diagnostic and therapeutic nanoparticles. Many of them have moved to clinical trials. However there is still a possibility for optimization in the area of the nanoparticle kinetics like improving their plasma circulation, bioavailability of tumour and understanding the effect of targeting ligands on their efficiency to treat cancer. The necessity for the development of novel and efficient ligands is always growing so, the use of proper conjugation chemistry is mandatory.

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