1.1 Introduction

Cancer is a disease in which group of genetically altered cells display uncontrolled growth, invasion and sometimes metastasis. This can develop in almost all tissues. One of the most critical and challenging tumours is that of the malignant brain cancer which arises in the tissues of the brain and spinal cord and associated with very poor prognosis (Bondy et. al., 2008). Malignant tumour can be both primary and secondary (metastatic). Secondary (metastasis) tumours are more common than primary and are named by the location of origin (“General Information about Adult Brain Tumours”, National cancer Institute, 2014).

According to the 2014 fact sheet of the Central brain tumour registry of the United States (CBTRUS, http://www.cbtrus.org) overall 68,470 new cases of primary malignant and non-malignant brain and CNS tumours are expected to be diagnosed in 2015. The rate is higher in males (5.44 per 100,000) than females (5.40 per 100,000). Adolescent & Young Adult (AYA) incidence (Ages 15-39 Years) rate is 10.08 cases per 100,000 for a total count of 51,118 incident tumours (Liao et al., 2014). The average annual mortality rate in the US between 2007 and 2011 was 4.26 per 100,000 (CDC Wonder Online Database 2014). In 2015 it is estimated to cause death of 13,770 people in USA. The risk of developing CNS tumour for males and female in US is 0.69 and 0.55%, and the risk of dying is 0.51 and 0.41% respectively (SEER Stat Database).

1.2 Classification of Brain Tumour

Brain tumour has been classified from grade-I to IV based on histology and the rate at which it is likely to grow and spread (Armstrong and Gilbert, 1996; Louis et al 2007). Depending on the type of tissue involve they have been classified as Meningioma’s, astrocytoma’s, oligodendroglia’s and ependymoma’s (Janny et al., 1994, Piepmeier et al., 1996). Glioblastoma multiform is the deadliest and most common form of glioma (i.e. grade IV) and median survival is only 12–17 months from the time of diagnosis (Lefranc et al., 2006). Metastatic or secondary tumours are more common than primary ones. These cancerous neoplasms circulate through the bloodstream, and are deposited in the brain. Among adults, the most common primary sites of brain metastases are lung cancer, breast cancer, colon cancer, kidney cancer and melanoma. Metastatic tumours are as malignant as the cancerous primary tumour from which it originate and grow rapidly. Metastases are common among middle-aged and elderly men and women and median survival ranges from 2 to 16 months (Norden et al 2005, Eichler and Loeffler 2007).
1.3 Approaches for Brain Tumour Treatment

Treatment of brain tumour is dependent on age and general health of the patient. Type, location and size of the tumour are important factors, which are also considered prior to selection of therapies. The standard treatment for brain tumours consists of surgery, radiotherapy, chemotherapy and currently developed innovative targeted therapies.

1.3.1 Surgery

Surgery is the treatment of choice for brain tumours, if the location of a brain tumour is accessible for surgery. Surgery can help to refine the diagnosis, remove as much of the tumour as possible, and release pressure within the skull. Several techniques are increasingly used including image-guided surgery, intraoperative MRI/CT and functional brain mapping to precisely locate the tumour and improve the advantage/risk ratio of surgery (De Witt Hamer et al., 2013; Gil-Robles et al., 2010; Sanai et al., 2010). The risks of surgery might be too high for certain tumours when they are located near critical areas in the brain and some tumours that tend to spread diffusely are also not cured by surgery. Radiation or chemotherapy may be used on the remaining tumour cells (Nunez et al., 2009; Chamberlain et al., 1998).

1.3.2 Radiation

Radiation therapy uses controlled high-energy rays to treat brain tumours. It works by damaging the DNA of tumour cells and is more beneficial in the treatment to control malignant cells (Nunez et al., 2009; Chamberlain et al., 1998). Although radiation is applied to the majority of gliomas, relapse at the primary site and surrounding brain is a limitation of this approach. In spite of the advances in radiotherapy, recurrences occur frequently. The etiology of failure is multifactorial including relative radio resistance of tumour cells, failure to administer a cytotoxic dose to the entire tissue and limitations in the ability to increase dose owing to potential injury to surrounding structures. However combination of chemotherapy delays the recurrence and prolongs survival of patient (Sminia et al., 2012).

1.3.3 Chemotherapy

Chemotherapy is the use of drugs which interfere with the quickly dividing tumour cells to prevent growth of the tumour. Due to malignant transformation, recurrence and toxicity associated with radiotherapy, chemotherapy has gained an intensive concern for treatment of progressive low grade glioma (LGG), especially in young children and most often for higher-grade glioma (HGG) (Scheinemann et al., 2011; Ater et al., 2012). Usually chemotherapy for brain tumours is administered following surgery or radiation therapy and is
not considered efficient for treating brain tumours. This is mostly because of the difficulty of crossing BBB and the brain efflux system that limit the access of chemotherapeutics at tumour site in brain.

Some chemotherapeutic including temozolomide permeate partially across BBB. However, temozolomide should be administered in high systemic doses to attain therapeutic brain levels due to its short half-life of about 1.8 h in plasma (Baker et al., 1995) Many other antineoplastic agents including anthracyclines, platinum (II) complexes, paclitaxel, etoposide, irinotecan, topotecan and methotrexate have some success against brain tumour (DeAngelis et al., 2005). However, most of these chemotherapeutic agents do not penetrate into brain in appropriate amounts and high doses of drugs are required systemically for obtaining effective brain tumour concentrations. At higher dose most of these drugs suffer from toxicity that limits their efficacy.

1.4 Barriers against Chemotherapy

1.4.1 Blood Brain Barrier

BBB is situated at the interface of the blood and the brain to maintain the brain homeostasis. It is the most important influx barrier that regulates the transport of exogenous and endogenous substrates into the brain (Begley et al., 2004a). It is formed by capillary endothelial cells of the brain with continuous tight junctions and exhibit very low pinocytic action (Wolburg et al., 2002). Other component of BBB includes the pericytes, basal membrane; neuronal cells and astrocyte end-feet which enclose the endothelial cell surface (Fig- 1) and control its permeability (Abbott et al., 2006). The possible transport mechanisms at BBB include transcellular passive diffusion and active endogenous transport systems. Passive diffusion is the process in which transport of the substrates from blood to the brain parenchyma and is restricted to small (<400-500 Da) lipid soluble molecules (Pardridge W.M., 2003). However many drug molecules fail to permeate as predicted by their lipid solubility due to lack specific physicochemical features (Golden and Pollack., 2003).
1.4.2 Metabolic barrier

BBB expresses a variety of enzymes such as monoamine oxidases (MAO), catechol O-methyl transferase (COMT), γ-glutamyltransferase, cholinesterases, GABA transaminase, aminopeptidases, endopeptidases, Alkaline phosphatase, adenosine deaminase and purin nucleotidase which have higher activities at the BBB compare to the brain tissue (Johnson and Anderson, 1996; Agundez et al., 2014). These enzymes evidently remove the molecule from the blood brain interface when drugs pass through cellular route of BBB (Ghersi-Egea et al., 1995). The drug-inactivating enzymes at BBB prevent access of drugs to brain (Pereira et al., 2014). In addition to phase I and Phase II enzymes expressed at BBB, ATP-dependent active efflux transporter can also be consider as phase III enzyme that prevent the drug from attaining a therapeutic concentration in brain.

1.4.3. High drug Efflux from brain

Earlier, it was presumed that uptake of nutrients are mediated via specific transporters existing at brain capillary endothelial cells, whereas lipophilic drugs cross the barrier by cellular passive diffusion. Accordingly, hydrophilic drugs should scarcely enter brain (Levin...
However, low permeation of lipid soluble and large molecules, suggested the presence of active efflux transporter P-gp. The most considerable evidence of efflux mediated by P-gp was demonstrated in mice that lack P-gp encoded by the MDR1a gene which significantly increased brain distribution of various anticancer drugs, immunosuppressant, and antiviral drugs (Schinkel et al., 1994; Schinkel, 1999). The active efflux transport system at the BBB is P-glycoprotein (P-gp) which belongs to ABCB1 gene family. Other members of the ABC family are multidrug resistance protein (MRPs; ABCC) and breast cancer resistance protein (BCRP; ABCG2) which exist at the BBB and may participate in active efflux transport (AET) (Pardridge, 2005; Anderson, 2009). The efflux of drug from brain to blood is mediated by transporters including ATP-binding cassette (ABC) gene family expressed at luminal membrane. Solute carrier (SLC) gene family and organic anion transporter (OAT) are example of the energy independent exchanger expressed at abluminal membrane of the brain capillary endothelial cell (Pardridge 2007a).

1.5. Approaches to overcome the BBB and drug delivery

1.5.1 Disruption of the BBB

BBB Disruption (BBBD) is an advanced method associated with modulation of the tight junction to open the paracellular route of the brain for drug delivery. It could be considered as a therapeutic strategy for systemic administration of chemotherapeutic drug for brain tumour treatment. An ideal technique should be temporary and reversible so that protective function of BBB does not loose permanently for the brain. The first attempts were done by infusion of dimethyl sulfoxide or ethanol and metals such as aluminium X-irradiation. Furthermore, inductions of pathological conditions such as hypertension, hypoxia or ischemia also cause BBBD (Misra et al., 2003). However, all these techniques are unacceptably toxic and clinically not useful.

For the treatment of high grade glioma osmotic opening of BBB was developed. In the 1970s Rapport et al., demonstrated the osmotic disruption of the BBB in which an inert hypertonic solution of mannitol or arabinose was injected via the carotid artery. Mannitol initiates shrinkage of endothelial cells by drawing fluid out of them, and opens the tight junctions for few hours to allow therapeutic drugs entering to brain tumour (Neuwelt et al., 1989). This method was found to be successful in patients who failed systemic chemotherapy (Rautio and Chikhale 2004). Furthermore, allyl glyceroles, particularly monoacetyl and diacetyl glycerols, were observed to modulate the permeability (Erdlenbruch et al. 2003a; Lee et al., 2002). Using this approach, methotrexate was delivered to the rat brain (Erdlenbruch et
al. 2003b). However, the opening of the BBB is nonselective and a large area of non-neoplastic brain is exposed to chemotherapeutic agents, which at times lead to unreasonable toxicity. So toxic vs. therapeutic ratio is a major concern in adopting BBBD.

The opening of the BBB using biochemical techniques is potentially safer and more reliable alternative to hypertonic solutions. This includes selective opening of brain tumour capillary with an intracarotid infusion of leukotriene C4 which did not affect the adjacent normal brain capillaries (Chio et al., 1992). This was attributed to abundant presence of γ-glutamyltranspeptidase (γ-GTP) in normal brain capillaries unlike in brain tumour endothelial cells, which inactivates leukotriene C4 (Black et al., 1994). The synthetic bradykinin analog RMP-7 infusion has been shown to selectively open BBB in animal model (Cloughesy and Black 1995; Begley 2004a). Promising results has been reported for enhanced tumour drug delivery in preclinical models with carboplatin and RMP-7 (Emerich et al., 1999). Nevertheless, this opening is relatively unselective and result in unwanted toxic side effects.

1.5.2 Direct intracerebral delivery

The most obvious way of increasing drug concentrations inside the brain is the direct injection of the drug into the brain or in case of a solid tumour into the brain tumour parenchyma. Several methods including infusion of active agents or implantation of drug loaded polymer wafers are available. The advantages of these techniques are the circumvention of the BBB and the low systemic toxicity. Several infusion systems have been demonstrated to deliver chemotherapeutics in brain. Ommaya reservoir (Ratcheson and Ommaya 1968) system used an inert plastic reservoir implanted subcutaneously in the scalp for delivery of drug. Some other pumps that were also used include Infusaid pump (Chandler et al., 1988) and solenoid pump (Lord et al., 1988). Although these infusion systems successfully delivered chemotherapeutics including doxorubicin and cisplatin (Walter et al., 1995) Infection and damage of brain parenchyma along the catheter, enhanced neurotoxicity and high discomfort for the patient limit the applicability of infusion techniques.

1.5.3. Brain drug uptake using the olfactory pathway

An alternative and interesting approach to deliver drugs to the brain is the use of the olfactory pathway (Illum 2002). The intranasal route is based on the fact that olfactory neurons that penetrate the cribriform plate are surrounded by a part of the arachnoid membrane. A fraction of the CSF inside the arachnoid membrane flows into the local lymphatic system, but another part of the CSF seems to be recirculated back into the subarachnoid CSF. Compounds that are attached to the olfactory mucosa may be transported
via this CSF fraction into the subarachnoid space (Begley and Brightman 2003; Begley 2004b). Besides this extraneuronal route, an intraneuronal pathway is postulated by Illum (2003). According to this, the olfactory nerves themselves may carry drugs by the retrograde axonal cytoplasmic flow into the brain. The intranasal approach is a non-invasive and very fast transport way to the brain, as compounds administered to the nose are detected in the brain within a few minutes (Sakane et al., 1995). Various drugs have been transported to the brain using the olfactory pathway including sulfonamides (Sakane et al., 1991) and polypeptides such as insulin and hyaluronidase (Fehm et al., 2000). Lipophilicity of a compound increases the intranasal transport indicating the participation of a transmembrane movement in the drug transport process (Eskandari et al. 2011). Further investigations are required to elucidate the exact delivery process. However, it is questionable, if the achieved brain concentrations are sufficient to achieve therapeutic effects.

1.5.4. Colloidal drug carriers

Colloidal drug carriers include micelles, liposomes and nanoparticles. They have particle size of between 1 and 1,000 nm in diameter (Kreuter J., 2001). Many of these drug carrier nanoparticles can be effectively transported across the BBB by endocytosis or transcytosis, and have shown early preclinical success for the management of brain tumours (Wong et al., 2012). Currently, NPs are gaining extensive interest as a carrier for the CNS delivery because NPs offer more stability to the encapsulated drug in biological fluids and against enzymatic metabolism as compared with other colloidal systems such as liposomes or micelles (Yoo et al., 2005). Nanoparticles of loperamide, tubocurarine, and doxorubicin have been successfully reported in brain delivery. NP encapsulated drug have been shown to have CNS entry of drug by reducing efflux process (Tosi et al. 2007). However, NPs can permeate the BBB, their transport efficiency is not high enough to result in therapeutic effects in the CNS. Therefore, functionalizing the NPs by surface modification and conjugation with transporters of the BBB has been proposed to enhance their transport efficiency into the brain (Bhaskar et al., 2010; Kanwar et al., 2012; Kulkarni et al., 2011).

1.5.5. Disabling efflux transporters

Active efflux of chemotherapeutics contributes to brain tumour chemo-resistance (Demeule et al., 2002a; Ito et al., 2005; Schaich et al., 2009). Efflux pumps such as multidrug resistance associated protein (MRPs) and P-glycoprotein (P-gp) are mainly responsible to restrict drug permeation into the brain (Kruh and Belinsky 2003). The resistance of tumour to anticancer drug is due the expression of efflux transporter in both low and high grade gliomas
(Demeule et al 2001). It has been evidenced that active efflux at the BBB is hurdle for drugs that are substrate for MRPs and Pgp. This mechanism limits the concentration of the drug in tumours and basis for the clinical failure of various molecular targeted therapies (Agarwal et al., 2011 and 2012). To circumvent this hurdle, one strategy is to co-administration of chemotherapy with inhibitors of the active efflux transport.

Verapamil, quinidine, cyclosporine A and nifedipine are the first generation P-gp blockers. Clinical studies have been performed to determine the potential of P-gp inhibitors to improve CNS permeability of the drug. However, these compounds lack specificity and are required in high concentration for inhibitory activity which causes in-vivo toxicity (Sikic et al., 1997; Shukla et al., 2011). Second generation inhibitors, such as, valsapodar has relatively higher inhibitory potency. These inhibitors suffer from side effects. Additionally, these inhibitors are substrate of cytochrome P450 and partly block the metabolism of co-administered drugs, resulting increased side effects (Xia et al., 2012). Currently the third generation inhibitors include elacridar, tariquidar, zosuquidar and laniquidar. Preclinical studies indicate that these compounds can potentiate the cytotoxicity of anticancer drugs (Breedveld et al., 2006).

1.5.6. Cell penetrating peptides

A relatively new research field regarding drug delivery to the brain is the development of cell penetrating peptides. The mechanism of this cell penetration is for the most part unknown. Several hypotheses are described in the literature. According to Torchilin et al., (2001), the peptides initiate the formation of reverse micelles in an energy independent process. In contrast, Richard et al., (2003) suggested, that endocytic events are induced at the plasma membrane similar to the interactions observed at adsorption mediated transport (AMT). The improvement in chemotherapeutic concentration by conjugation to peptides has been demonstrated for doxorubicin by linking it to penetrating in a rat in situ brain perfusion model (Rousselle et al., 2000).

1.5.7. Receptor mediated transport

Receptor-mediated endocytosis promotes the selective uptake of large molecules into the brain. In general, this process is initiated through endocytosis of substrate to its respective receptor expressed on the luminal side, followed by forming vesicles and movement towards abluminal side of endothelial cells and exocytosis of substrate into brain. With respect to BBB, various kinds of receptors are expressed on brain microvascular endothelial cells
(BMEC) membrane. Receptor for CNS drug delivery includes transferring receptor (TfR), insulin receptor and the low density lipoprotein (LDLR) receptor (Pardridge, 2002).

1.5.7.1 Transferrin receptor

Transferrin receptors are mainly expressed on choroid plexus epithelial cells and neuron in the brain (Moos and Morgan, 2000) and endothelial cells of the BBB (Ponka and Lok, 1999). Visser et al., (2005) has shown in vitro drug delivery to BBB endothelial cells by liposomes tagged with transferrin. Furthermore, Zhang et al., (2012) have demonstrated brain-targeting efficiencies of transferrin-modified paclitaxel-loaded micelles (TPM). However in vivo application of transferring is limited because transferrin receptors are more often saturated with endogenous transferrin (Qian et al., 2002).

1.5.7.2. Insulin Receptor

Insulin receptor is also expressed on brain microvascular endothelial cells (BMEC) membrane and characterized as receptor mediated transcytosis system for targeted drug delivery to the brain (Duffy and Pardridge; 1987). When insulin binds to the receptor, it undergoes for conformational changes to form tunnel to allow the entry of molecules in the cells, such as glucose. Insulin receptor is a tyrosine kinase linked receptor and undergoes phosphorylation which is necessary to promote complex cellular response and internalize into endosomes (Bottaro et al., 1989). As intended for transferrin, in vivo application of insulin as carrier protein is also limited because high concentration of insulin required which resulting in lethal overdosing (Bickel et al., 2001).

1.5.7.3. Lipoprotein receptor family

The low density lipid receptor (LDLR) and its related proteins (LRP1 and 2) have been also exploited as a target for drug delivery to the brain. LRP1 is expressed on brain, at high levels in both glial and neuronal cells suggested that it may be good target for drug delivery to primary and secondary brain tumour. LRP1 act a transporter of ligands such as lactoferrin and melenotransferrin across the BMEC (Demeule et al., 2008; Fillebeen et al., 2008). Malanoferrin is an endogenous iron-binding protein, which crosses BBB faster than transferring and lactoferrin (Demeule et al., 2002b). Malanotransferrin has been covalently conjugated to doxorubicin for treating brain tumour in mice and it was found equally effective against tumour compared with free doxorubicin (Karkan et al. 2008). Angiopeps a designed library of peptides comprised of aprotinin (known human protein substrate for LRP1) with KPI domain (Kunitz protease- inhibitor) constructed to transport drug into brain by targeting LRP receptor (Kounas et al., 1995). Paclitaxel-angiopep-2 conjugate was found to enhance
drug concentration in brain and is under clinical trial in patients with primary or secondary tumour (Regina et al., 2008).

1.5.8 Optimization of the physicochemical properties

Drugs generally cross the BBB if molecular mass is between 150 and 500 Da and partition coefficient log Po/w between 0.5 and 6.0 (Bodor and Buchwald 2003). Furthermore, the drug has to be partially ionized at physiological pH 7.4. Molecular features like polar surface area in excess of 80Å, high Lewis bond strength and high potential for hydrogen bond formation reduce molecule penetration through the BBB (Doan et al., 2002). Small chemical modifications to a drug molecule may improve the characteristics for penetrating the BBB, such as increase in log Po/w values, circulatory half-life, AUC in plasma, resistance to enzymatic hydrolysis in circulation and make available the drug in higher concentrations for brain uptake (Begley 1996). The correlation between BBB penetration and drug partition into lipid of the cell membrane, suggested a clear relationship between lipophilicity and CNS penetration (Levin 1980). Thus, chemical modification to convert water soluble drugs that do not cross BBB into lipid soluble drug to enable BBB permeability has been extensively studied (Bodor et al. 1992). However, lipidization is difficult to execute in actual practice, additionally it increases the uptake in periphery organ of the body, resulting in decrease in plasma AUC (Grieg et al 1990). Additionally, lipidization increase penetration but decrease in solubility and bioavailability by increase in protein binding.

1.5.9 Carrier mediated transport

Many endogenous transports systems have been recognized that play an important role to maintain the BBB integrity and homeostasis in brain and also persuade drug transport to the brain (Pardridge W.M., 1999; 2003). Transport system at BBB includes carrier mediated transport system (CMT) that assists solute transfer by specific substrate-transporter interaction. Small molecules transported in this manner including sugars, amino acids, oligopeptides, organic anions and organic cations. This plays important role in various cellular physiological processes, such as importing or exporting nutrients, neurotransmitters and metabolites (Hediger et al., 2004; Huang and Sadee, 2006). They also permit the drive of hydrophilic and large compound resembling structural features of their substrates across the BBB (Tsuji and Tamai, 1999; Pardridge, 2007b). Some transporters are unidirectional and some are bidirectional, transport of solutes can be enabled in either direction into or out of the
CNS subjected to the concentration gradient across the BBB (Meier et al., 2002; Begley, 2004b; Tsuji, 2005; Pardridge, 2001).