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Abstract

Introduction.

Glioblastoma (GBM) is the most common and aggressive primary malignant brain tumors in adults. The blood brain barrier (BBB) is a major limitation reducing efficacy of anti-cancer drugs in the treatment of GBM patients.

Areas covered.

Virtually all GBM recur after the first-line treatment, at least partly, due to invasive tumor cells protected, from chemotherapeutic agents, by the intact BBB in the brain adjacent to tumor. The passage, through the BBB, by antitumor drugs is poorly and heterogeneously documented in the literature. In this review, we have focused our attention on: (i) the BBB, (ii) the passage of chemotherapeutic agents across the BBB and (iii) the strategies investigated to overcome this barrier.

Expert commentary.

A better preclinical knowledge of the crossing of the BBB by antitumor drugs will allow optimizing their clinical development, alone or combined with BBB bypassing strategies, towards an increased success rate of clinical trials.

Keywords: glioblastoma, blood-brain barrier, cytotoxic chemotherapy, pharmacokinetics, delivery.

1. Introduction

Glioblastoma (GBM) is the most frequent primary brain cancer in adults. Indeed, GBM has an annual incidence from 0.6 to 3.7/100,000 individuals, with the highest incidences in European countries, United States, and Australia [1]. The median overall survival of newly diagnosed GBM patients is 12 to 18 months despite very intensive therapeutic regimens. The standard of care in newly diagnosed GBM patients, under 70 years old and in good clinical conditions, is maximal safe resection surgery followed by concurrent radiochemotherapy and adjuvant treatment with temozolomide (TMZ), an alkylating agent [2].

Virtually all GBM patients experience tumor recurrence. Several issues are known to limit the immediate and long-term efficacies of anti-cancer drugs in GBM: (i) the blood-brain barrier -BBB- limiting penetration of drugs within the tumor and the brain adjacent to tumor -BAT-, (ii) primary or intrinsic molecular resistance, and (iii) secondary or acquired resistance after drug exposure.

In this review, we will focus on the BBB in the setting of primary brain cancers. Indeed, the BBB is a physical and biological barrier limiting drug penetration within the brain, and therefore within GBM cells. Although the BBB is disrupted in the tumor core, allowing a partial penetration of anti-tumor drugs, the BBB is widely intact around the BAT where invasive/escaping GBM cells can be found [3]. Reaching efficiently and safely these invasive/escaping GBM cells is one of the main challenges in GBM treatment, and developing strategies to overcome this limit will undoubtedly open new therapeutic perspectives using well-known cytotoxic drugs or innovative drugs.

In this review, we have focused our attention on: (i) the BBB, (ii) our knowledge of the passage of chemotherapeutic agents across the BBB and (iii) the strategies investigated to overcome this physico-biochemical barrier.

2. Methods

Our review of public data was performed using: (i) Pubmed (http://www.ncbi.nlm.nih.gov/pubmed), (ii) Google (https://www.google.fr/), (iii) Google Scholar (https://scholar.google.fr/) and, (iv) University library.

Data related to the ability of drugs to cross the BBB were searched using the following formula (e.g. for CCNU): (CCNU OR belustine OR lomustine) AND ("brain/blood" OR "brain/plasma" OR "CSF/blood" OR "CSF/plasma" OR "brain:blood" OR "brain:plasma" OR "CSF:blood" OR "CSF:plasma") ratio.

Data related to the physicochemical characteristics of drugs were collected using public databases chEMBL (https://www.ebi.ac.uk/chembl/) and drugbank (http://www.drugbank.ca/)

I*n silico* data prediction was performed using http://www.cbligand.org/BBB/index.php.

Data related to cytotoxicity of anti-cancer drugs were collected from chEMBL database (https://www.ebi.ac.uk/chembl/).

The figures were made using the Servier Medical Art (http://www.servier.fr/smart/banque-dimages-powerpoint.

3. Brain barriers

3.1. The normal BBB

The BBB is a physical and biological barrier: (i) protecting the brain from pathogens and toxic molecules circulating in the blood flow and, (ii) regulating hydrometabolic exchanges between the brain and blood to maintain brain homeostasis.

The BBB includes several cellular and molecular actors: (i) endothelial cells, (ii) pericytes, (iii) astrocytes, and (iv) extracellular matrix (Figure 1B). The barrier function of the BBB is mainly endorsed by the endothelial cells of blood vessels. The BBB functioning is also influenced by neurons, oligodendrocytes and microglial cells that belong to the neurovascular unit [4].

There are five main mechanisms or pathways driving molecular penetration through the BBB: (i) passive paracellular pathway, (ii) transcellular lipophilic pathway, (iii) transcytosis pathway, (iv) transport protein pathway, and (v) efflux pumps pathway (Figure 2).

Passive paracellular diffusion of molecules between endothelial cells is hampered by the tight junctions (TJ) and adherens junctions (AJ). Only few small highly liposoluble molecules can cross the BBB by passive paracellular diffusion [4].

Some small gaseous or lipophilic molecules are also able to cross the BBB by passive transcellular diffusion across endothelial cells themselves [4].

The transcytosis pathway refers to successive endocytosis from one side and exocytosis from the other side of endothelial cells. The three main transcytosis types are: (i) constitutive and non-specific -*i.e.* fluid-phase endocytosis: micropinocytosis, macropinocytosis-, (ii) ligand's charges mediated and non-specific -*i.e.* adsorptive endocytosis- and (iii) specific receptor-mediated. The non-specific transcytosis mechanisms are less represented in the BBB than in peripheral blood vessels [4,5].

The transport protein pathway is an active and specific transport mechanism of molecules across the BBB. This transport pathway is predominant in the BBB. A large variety of transporters are expressed by endothelial cells including transporters from the solute carrier family (SLC). SLC2A1 (GLUT-1), involved in the crossing of glucose, is one of the most abundant transport of the SLC family [4,6,7].

The last mechanism is the efflux pumps pathway, a crucial mechanism for detoxification. Mainly ABCB1 (P-gp), ABCG2 (BCRP) and MRP 1 to 5 reject potential harmful xenobiotics from the endothelial cells to the blood (Figure 2) [4,8].

The BBB is disrupted in restricted zones of the brain close to the 3rd and the 4th ventricles: the circumventricular organs. These organs are isolated from CSF by tightly attached ependymal cells (tanycytes) and from the brain by a dense layer of astrocytes, tanycytes and extracellular matrix [9–11] (figure 1E).

3.2. The blood-tumor barrier

In GBM, the tumor bulk is schematically organized in three major parts: (i) the necrotic central area, (ii) the proliferative/angiogenic forehead, and (iii) the BAT including invasive/escaping tumor cells (Figure 3).

The blood tumor barrier (BTB) refers to a histologically and/or biologically altered BBB with increased permeability. In the BTB, the blood vessels are anarchic, disorganized, sinuous, irregularly shaped, large and leaky, mainly due to an imperfect angiogenesis and inflammation [12,13].

These modifications are due to both: (i) pro-angiogenic and immune-modulating factors secreted by GBM cells, and (ii) tumor-induced micro-environment changes [14–16].

3.3. CSF-related barriers

As discussed by Saunders *et al.*, CSF is both isolated from the blood and the brain. In the ventricular system, CSF is isolated from the blood in the choroid plexus by epithelial cells that play a barrier role similar to the endothelial cells in the BBB (figure 1C). Even if the mechanisms are similar, specific transporters and efflux pumps are different from the ones expressed in the BBB [17,18]. Ependymal cells lining the ventricle are not tightly attached in adults. The ependyma is therefore not thought to hamper the diffusion from the CSF to the brain (figure 1C). However, transport systems and CSF flow limit diffusion to 1-2 mm [17,19]. CSF is also isolated from brain and blood by the arachnoid and pia matters that both present tightly packet cell layers that prevent diffusion from the blood to CSF and from CSF to the brain [20] (figure 1D).

4. The BBB limits drug penetration to both normal brain and tumors

Currently, the most frequently used chemotherapy agent in GBM is temozolomide (TMZ), a drug that is able to cross the BBB [2]. Table 1 indicates several drugs according to their clinical use and their relevance in treatment of central nervous system (CNS) tumors.

Table 2 shows experimental brain and CSF penetration data for several drugs. Recently, Jacus *et al.* reviewed the pharmacokinetic properties of several anticancer agents, and assessed their penetration in CSF and/or in brain tissue of patients with CNS tumors [21].

Several physicochemical parameters are involved in the ability of drugs to cross the normal BBB: (i) size, (ii) liposolubility, (iii) charge, (iv) interactions with plasma proteins, and (v) interactions with efflux pumps and transporters. According to these parameters, several groups have suggested a way to predict *in silico* their ability to cross the BBB. The rule of 5 developed by Lipinski is the theoretical basis of these predictions [22]. According to this rule, "poor absorption or permeation is more likely when: (i) > 5 hydrogen bond donors, (ii) MWt > 500, (iii) logP > 5, (iv) > 10 hydrogen bond acceptors, and (v) substrates for biological transporters are exceptions to this rule". Although this modeling has been significantly improved over time, predictions are not always consistent with the experimental data [23]. Table 3 shows several parameters used for prediction of BBB crossing by drugs. As an example of the limits of predictive models, irinotecan is predicted not to cross the BBB and cisplatin is predicted to cross the BBB (Table 3), while the *in vivo* data reported in Table 2 shows that irinotecan is more likely to cross the BBB than cisplatin.

Predicting accurately the ability of anti-cancer drugs to cross the BBB and to penetrate in brain patients, based on our currents preclinical models, remains challenging. Combining in silico, in vitro and in vivo predicting approaches may help for better prediction.

5. Overcoming the BBB for better drug delivery within the tumor core and the BAT

Table 4 indicates *in vitro* efficacy of chemotherapeutic cytotoxic agents investigated against GBM cells. Significant inter-laboratories variability is observed (*e.g.* the IC50 for paclitaxel on U87 cells ranges from 80 to 90000 nM). TMZ, the most commonly used chemotherapy agent in GBM, is inconsistently cytotoxic on GBM cell lines, while vincristine, vinblastine, paclitaxel and doxorubicin are up to 10,000 – 100,000 times more cytotoxic than TMZ. Integrated therapeutic strategies including improved brain delivery of the most efficient drugs and molecular biomarkers of response to these drugs (*e.g.* MGMT for TMZ) will significantly improve the outcome for GBM patients [24]. Several approaches have been developed or are still under development.

5.1. Intra-tumor injection

Direct delivery of chemotherapy within the tumor and the BAT require insertion of a catheter within the tumor site. Imaging prior to drug administration is thus necessary to locate specifically the target site. Any molecule, regardless of its physicochemical characteristics, is deliverable using this method. The main limitations are local injuries: (i) infection, (ii) inflammatory reaction, and (iii) direct neurotoxicity (Table 1) [25,26]. Indeed, neurotoxicity of chemotherapeutic agents is a major issue when increasing local delivery (*e.g.* vincaalcaloids may induce seizure, encephalopathy, ataxia, and/or movement disorders; taxanes may induce seizure; and platinum derivatives may induce seizure, encephalopathy, stroke, ataxia and/or myelopathy) [27,28]. The main advantage of catheter-based drug delivery is an increase of local drug concentration without increasing systemic concentration and drug toxicity. The use of this method for nitrosoureas (*i.e.* BCNU and CCNU) has shown efficacy and few side effects in mice and patients [29,30]. A stabilization of the tumor was observed for 72% of patients treated by DTI-015 (BCNU in 100% ethanol) (NCT00038441) [29]. However, the invasiveness and the direct exposure of the brain to the drug toxicity limit the use of this method.

5.2. Convection enhanced delivery (CED)

Convection Enhanced Delivery (CED) is based on a catheter inserted, during a neurosurgical procedure, within the tumor or the BAT. The catheter is linked to an Ommaya/Rickham reservoir or to an external pump maintaining a positive pressure and flow. It allows a slow drug delivery over a longer period of time to reduce the potential acute neurotoxicity of chemotherapy [31]. CED has been tested for cisplatin, methotrexate, paclitaxel, nimustine, topotecan and carboplatin [32]. The capacity of a drug to diffuse within the brain parenchyma is heterogeneous and depends on : (i) the drug, (ii) the tumor site, and (iii) the administration parameters [32–35]. Cisplatin was reported to diffuse 1 cm around the needle tip in 1982 [36]. A more recent study indicated the mean volume of distribution is between 12.8 to 22.9 cm^3 for paclitaxel [35]. Neurotoxicity was reported in some trials (*e.g.* paclitaxel). Despite the limitations of this invasive procedure, clinical benefits were observed brain tumor patients in some trials (*e.g.* nimustine, topotecan, carboplatin) [32]. Recently, an implantable catheter system was recently developed and tested with carboplatin in a recurrent GBM patient, and induced a 58% tumor shrinkage and a stabilization of the patient’s clinical condition (NCT01317212) [37].

5.3. CSF delivery

Drugs can be directly injected within the CSF. This method is mainly used to treat spinal cord tumors, leptomeningeal tumors, and tumor meningitis. As the CSF volume is lower than the blood volume, the intrathecal injection of chemotherapy leads to a higher concentration of chemotherapy, with a minimum risk of systemic toxicity [38]. However, the limited diffusion from CSF to the CNS parenchyma reduces the impact of this strategy in the treatment of intraparenchymal GBM patients [38]. Although some drugs are commonly used through the CSF route (e.g. methotrexate, cytarabine) with acceptable side effects, other drugs (e.g. vincristine) are contra-indicated for direct CSF delivery due to the high risk a severe neurotoxicity or death. [39,40].

5.4. In situ biodegradable polymer, gels, microships or microcarrier

After surgical resection of the brain tumor, a cytotoxic agent-impregnated biodegradable polymer can be deposited in the tumor resection cavity. Carmustine impregnated wafers (Gliadel®) continuously deliver the drug directly in the brain parenchyma over 3 weeks [41]. Although initial results were promising, more recent data suggest a limited survival benefit in GBM patients (NCT00003876) [42–44]. Increasing drug concentration within the wafers might increase efficacy with acceptable toxicity as shown in a phase I clinical trial [45]. Adverse effects such as seizures, convulsions, confusion, brain edema, infection, hemiparesis, aphasia, and visual field defects were reported with this treatment [41–44,46,47]. High dose BCNU was detected 5 to 6.1 mm around the wafer on day 1, and between 1.1 to 3.6 mm from days 3 to 30. Several drugs were used within this delivery system. They were detected at low concentrations up to 5 cm around the wafer, but their concentration dropped below the LC90 (lethal concentration) within 1 cm around the wafer [46,48,49]. However, tumor recurrence was reported to occur mainly in the 2 cm around the BAT. The use of gels to fill the postsurgical cavity, micro-chips and micro-carriers instead of wafers has also been evaluated with quite similar efficacy and limitations mainly in murine models [50–53]. Gels have been investigated also in cancer patients (NCT00479765) [51].

5.5. Transnasal epithelium drug delivery

A drug can also cross the nasal epithelium at least in some regions of the brain [54], and reach CSF and brain. Transnasal drug delivery has been tested in animals for various treatments including methotrexate or 5-FU [54–56]. However, it has not been used in human to treat brain tumors so far.

5.6. High-dose and dose-dense chemotherapy delivered using intravenous (i.v.) peripheral route

As mentioned above, some drugs are very efficient against GBM cells *in vitro*, but exhibit limited effects *in vivo* due to their low ability to cross the BBB (*e.g.* doxorubicin, vincristine, vinblastine, paclitaxel). High-dose and dose-dense chemotherapy regimens aim, with or without bone marrow transplant, increasing drug concentrations within the brain tumor using higher dose of chemotherapy delivered in a peripheral vein. The benefits of these procedures, associated with significant toxicity, is debated and heterogeneous across patients (e.g. NCT00304031 and NCT01364064) [57–62].

5.7. Intra-arterial (i.a.) drug delivery

The *i.a.* delivery of drugs via the carotid artery has been shown to improve drug delivery within CNS. *I.a.* injection of cisplatin and etoposide led to a 2- and 4- fold increase of drug delivery to the brain compared to *i.v.* injection, respectively [63,64]. Penetration of methotrexate, aminoisobutyric acid and dextran 70 within the brain was 2 to 2.5 higher with *i.a.* delivery compared to i.v. delivery to tumor-bearing rats [65]. BCNU *i.a.* delivery achieved an 50-fold improvement of delivery in glioma patients [66]. ACNU *i.a.* delivery was not associated with an improvement of survival in glioma patients but showed a lower chemotherapy-related toxicity compared to HeCNU delivered by an *i.a.* injection. Overall, this route of injection is associated with a high risk of neurological, ophtalmological, and vascular toxicities limiting its use [67–73].

5.8. Efflux pumps inhibition

P-gp and BCRP can be inhibited by various drugs (*e.g.* cyclosporin A, elacridar, valspodar, tariquidar, or zosuquidar trihydrochloride) [74–76]. The association of paclitaxel and valspodar reduced the tumor volume up to 90%, while paclitaxel alone had no effect. A prolonged 1.7 fold increase of brain concentration of paclitaxel was observed when combined with several of these inhibitors [77–79]. Colchicine and vinblastine uptake was enhanced 8.42- and 9.08-fold, respectively, when they were co-injected with valspodar in rats [80]. Cyclosporin A treatment also increases brain delivery of doxorubicin in rats [81]. However, cyclosporin A injection in non-human primates did not appear to improve the CSF delivery of doxorubicin [82]. Docetaxel brain concentration was also increased by elacridar, valspodar and cyclosporine A in mice [83]. Such inhibitors showed no or poor effect on different non-CNS tumors expressing P-gp in clinical trials enrolling patients (NCT00069160) [75]. However, due to the BBB and to high expression of efflux pumps in brain normal cells, this approach might be interesting. For example, combination of verapamil to an antiepileptic treatment in a patients with pharmacoresistant seizures doubled the time interval between hospitalizations, improved the overall control of seizures and the quality of life of patients [84]. An improved brain/plasma ratio was also obtained for loperamide when associated with tariquidar and elacridar [85]. Moreover, even if a method allows a molecule to cross the BBB, the therapeutic impact would be decreased by the efflux of the drug to the blood if it is substrate of efflux pumps [86]. Any method developed to delivery drugs to the brain could benefit the addition of an adjuvant efflux pumps inhibitors.

5.9. BBB opening

Interestingly, beside their direct antitumor effect, some anti-tumor therapeutic strategies already used in clinics are able to open the BBB (*e.g.* etoposide, morphine and radiotherapy) [2,87–89]. The opening of the BBB can also be obtained by *i.a.* injection of hypotonic solutions or hyperosmotic solutions (*i.e.* mannitol). These two methods induce a water flow from the endothelial cells to the blood, leading to shrinkage and subsequent opening of TJs [90–92]. Interestingly, complete tumor response was reported for patients receiving carboplatin and etoposide after *i.a.* administration of mannitol [93]. Methotrexate, aminoisobutyric acid and dextran 70 delivery to the brain was improved by 2.5 to 7.6 fold by mannitol-induced BBB disruption [65]. In the same line, intra-carotid hyperosmolar perfusion in rats allowed 240-500% increase for antibodies [94]. Bradykinin or its agonist (*i.e.* RMP-7) or histamine also opens the BBB [95–97]. Intra-carotid infusion of RMP-7 improved the delivery of carboplatin by 2.7 fold in rats [98]. For methotrexate, aminoisobutyric acid and dextran 70, Neuwelt *et al.* reported an increase of drug delivery to the tumor and the BAT in rats by: (i) 2.2 to 2.5-fold after *i.a.* injection compared to *i.v.* injection, (ii) 2.5 to 7.6-fold after mannitol-induced BBB disruption compared to saline injection and, (iii) 6.3 to 16.7-fold combining both methods (*i.a.* + mannitol *vs* *i.v.* + saline) [65].

Ultrasounds can also be used to open the BBB [92,99]. Indeed, association of low frequency ultrasounds with microbubble contrast agents was shown to open the BBB, a technics that was described to be minimally/non-invasive and safe [100–102]. The ultrasound-induced opening of the BBB was reported to improve the TMZ CSF/plasma ratio from 22.7% to 38.6% in tumor bearing rats [103]. Irinotecan delivery was increased by 206% to 331% in healthy rabbits [104]. In a primate model, the mean platinum brain distribution was 5.2-fold higher in the US field (0-5mm section) than in the contralateral hemisphere [105]. A phase I clinical trial (NCT02253212), testing non-focused ultrasounds plus carboplatin, is currently enrolling recurrent GBM patients. Thermal ablation of GBM on patients has also been performed with transcranial high-frequency focused ultrasound [106]. The use of the same device with different ultrasound parameters could therefore lead to a BBB opening [107].

5.10. Drug design, modification and encapsulation

Biochemical modifications (*e.g.* addition of ligand to receptor mediated transcytosis, lipophilic molecules, nanovectors and/or positively charged molecules) of existing drugs are explored to improve their capacity to cross the BBB and their anti-tumor efficacy.

Doxil®/Caelix® consists of doxorubicin encapsulated in a PEGylated liposome. A stabilization in malignant gliomas patients was obtained with Doxil® [108]. The modification of Doxil® with glutathione groups led to a 4.8-fold increase of the brain-to-blood ratio compared to Doxil®/Caelix® (~0.08% *vs.* ~0.02% respectively) in preclinical models [109,110]. Various other methods have been tested, such as the modification of drugs with fatty acids to increase their liposolubility and to improve their diffusion through the BBB [111]. The modification of a drug with a molecule that is recognized by specific receptors/proteins on endothelial cells can also promote its passage through receptor mediated transcytosis (*e.g.* complexation of drugs with transferrin) [5,112,113].

Interestingly, drug modification can be combined with other strategies to increase drug delivery within the brain, improve its stability, or reduce its elimination [114].

5.11. Magnetic delivery

Magnetic nanoparticles can be included in liposomes, forming thus magnetoliposomes. These magnetoliposomes can be modified with molecules such as transferrin to promote their interaction with the brain endothelium. Drugs can be loaded in these magnetoliposomes. Therefore, the application of a magnetic field around the brain can attract these particles out of the blood vessels, through the BBB, and deliver the drug in the brain parenchyma [115]. The use of paclitaxel-loaded anti-GPNMB antibodies-decorated magnetoliposomes improved the brain delivery of paclitaxel by 4 fold in rats. Paclitaxel concentration was still high 48h after treatment for the liposomal form, while it was not detected after 6h in animals treated with unmodified paclitaxel [116].

5.12. Electric fields and Electromagnetic fields

In 1977, application of low intensity electric fields to the brain was shown to induce BBB opening and to improve passage of dyes and drugs from the blood to the brain. This passage of drugs can also implicate multiple putative mechanisms : (i) iontophoresis (charge-mediated displacement of charged molecules), (ii) electro-osmotic, (iii) convection flows, and/or (iv) electroporation [117]. Recently, the use of intracranial irreversible electroporation in rats' brain was shown to induce a BBB opening [118].

The Novocure device delivers "tumor-treating fields" (TTF) to the brain, and was shown to induce tumor cells death in preclinics and to increase survival of newly diagnosed GBM patients (NCT00916409) [120]. The mechanism of tumor cells death remains unclear, but it is at least partly due to interaction of TTF with the cytoskeleton. Indeed, TTF impede polymerization and functions of tubulin resulting in abnormal mitoses and cytokinesis. Finally, the electroporation of tumor cells was also observed. Electroporation was proposed to explain the synergic effect of TTF and chemotherapy observed in some patients [120]. Moreover, a BBB opening was observed after non-thermal irreversible electroporation as a tumor ablation method [92].

Interactions between electromagnetic fields and BBB are still unclear and under investigations. [121].

6. Discussion

GBM is a lethal disease and more efficient therapeutic strategies are urgently needed. Despite the fact that multiple efficient cytotoxic chemotherapeutic agents are available, as demonstrated in *in vitro* preclinical models (*i.e.* GBM cell lines) and *in vivo* preclinical models without BBB (*i.e.* GBM subcutaneous xenografts), their efficacy is dramatically reduced in GBM orthotopic xenografts and in GBM patients [122]. Several reasons might explain this reduced efficacy in patients, one of them being the limited penetration of drugs within the tumor and the BAT.

The passage of cytotoxic drugs across the BBB is commonly admitted to be limited in patients and to be well-documented in the literature. However, data are scarce and heterogeneous in the literature, limiting comparisons across studies. Our review of the literature reports, in Table 2, the ability of anti-tumor drugs to cross the brain barriers (*i.e.* brain/plasma and CSF/plasma ratio). However, preclinical and clinical studies are heterogeneous in terms of material and methods: (i) heterogeneity of models and patients -tumor or not, tumor type, CNS involvement or not- and, (ii) heterogeneity of methods - *i.e.* route of administration of the drug, total dose, time between treatments, biological samples management, assays, cell lines used-. These heterogeneities, also raised by Jacus *et al.*, highlight the difficulties to compare and to interpret studies in robust manner [21]. Therefore, major efforts need to be conducted by the community to standardize preclinical evaluation of drug efficacy and preclinical evaluation of drug penetration within the tumor, the BAT and the CSF.

Indeed, these data are critical to optimize drug delivery of current cytotoxic agents and to take advantage of efficient drugs that would be otherwise disregarded due to their incapacity to reach GBM cells. Indeed, one of the major therapeutic advances that have been accomplished over the last years in neuro-oncology came from very old cytotoxic drugs [123,124]. Therefore, "old drugs" might have unexpected efficacy if used in the right indication, in the right therapeutic regimen, at the right moment and in the right patients.

Assessment of brain distribution is challenging in practice in preclinical and clinical settings. *In silico* prediction is the most efficient approach to perform a high throughput analysis. However, the limited accuracy of this method hampers the benefits of such evaluations. On a smaller scale, a limited number of molecules can also be analyzed using *in vitro* models of BBB. Various models exist, and they all rely on the transwell system. Endothelial cells are cultured on a porous membrane delimiting two compartments, representing the blood and the brain [4,125]. The drug can then be deposited in one compartment, and measured in the two compartments to quantify the passage of the drug. The most commonly used human endothelial cells are the immortalized human brain endothelial cells hCMEC/D3 cells [126]. These cells were shown to retain the normal gene expression pattern of endothelial cells of the BBB, making them one of the easiest to use, the most reproducible and the most reliable models. More accurate models are available but cannot be used in medium to large-scale studies [127]. The optimal animal model remains the non-human primate (*e.g.* Rhesus monkey). The BBB of rodents (mice, rats) is different from the human BBB. More specifically, the expression of efflux pumps such as ABCB1 (P-gp) and ABCG2 (BCRP) is qualitatively similar, but quantitatively different [128]. The pathway of *in silico*, *preclinical in vitro*, *preclinical in vivo* analysis allows preselection of the best candidate drugs at each step, and reduces the cost of drugs screening [129].

In clinical trials, most often, the passage of drugs is measured in the CSF. However, the drug concentration is not always well-correlated between the CSF and the brain. The most robust data are obtained with biopsies of the brain after chemotherapy administration. However, biopsies are mainly used for diagnosis rather than for drug dosage, and are thus performed prior to initiation of treatment. Phase 0 clinical trials may help to better understand CNS pharmacokinetics and pharmacodynamics of anti-cancer drugs [130].

7. Expert commentary

Treating brain diseases including glioblastoma is challenging due, at least partly, to the BBB. It will be virtually impossible to test, in human patients, for each anti-GBM candidate drug and for each drugs combination, their ability to cross the BBB, their therapeutic efficacy and their toxicity. Therefore, robust, reproducible and consensual models to assess the ability of anti-GBM candidate drugs to cross the BBB in preclinical settings (i.e. in vitro and in vivo) needs to be optimized and validated-admitted across the research teams involved in the field. These models, including the complexity of the human BBB, will undoubtedly better rationalize our selection of anti-GBM candidate drugs to be tested, alone or combined with a BBB-opening procedure, in clinical trials enrolling GBM patients. This strategy will improve our success rate in clinical trials dedicated to GBM patients and will benefit to GBM patients.

8. Five-years view

Currently, two major axis of therapeutic research are converging to increase efficacy of anti-cancer treatments in the field of primary malignant brain tumors. Innovative smart anti-tumor drugs are developed and some of them have demonstrated dramatic efficacy in systemic cancers raising hope in the treatment of GBM patients. Some of these promising drugs are large molecules (*e.g.* monoclonal antibodies) or highly hydrophilic. Obviously, although these anti-cancer drugs are efficient, they will not be able to reach GBM cells located in the BAT. Significant efforts are ongoing to increase, using chemical or physical approaches, bioavailability of these drugs with the GBM bulk and the BAT. Merging these two axis of therapeutic research will undoubtedly improve treatments of GBM patients.

9. Conclusions

The blood brain/tumor barrier is a major obstacle limiting efficacy of anti-cancer agents in GBM. Multiple classic cytotoxic agents and innovative drugs showed promising therapeutic activity in GBM cells in the absence of the BBB or BTB (i.e. *in vitro* experiments or subcutaneous xenografts). Their ability to cross the BBB and the BTB has been poorly or heterogeneously documented in the literature. A better comprehensive and standardize evaluation of the ability of drugs to cross the BBB and their anti-tumor efficacy is needed. In parallel, multiples innovative physical and chemical strategies are under development to bypass the BBB and the BTB particularly in the BAT. A better knowledge of the ability of drugs to cross the BBB and a better ability to open the BBB will undoubtedly improve treatments of GBM patients.

10. Key issues

- Escaping/invasive GBM cells, located in the BAT which is protected by intact BBB, are often the source of GBM recurrence
- The BBB remains a major obstacle to obtain therapeutic drug bio-availability within the GBM bulk and the BAT
- Systematic, comparable and comprehensive pharmacokinetics and pharmacodynamics data for anti-cancer drugs are lacking.
- Physical strategies to open the BBB in a reproducible, large, transient and safe are under investigations.
- Chemical strategies to increase drug penetration through the BBB are under investigations

11. References

Papers of special note have been highlighted as:

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Legends to figures

Figure 1: Structure of the brain barriers. Overview in Panel A. Adapted from Saunders et al. 2013 [18] and Langlet et al. 2014 [131].

Panel B: The actors of the Blood Brain Barrier. Endothelial cells are the main actors of the BBB. Pericytes are closely attached to the endothelial cells via gap and adherens junctions. Astrocyte end-feet strongly surround the basal matrix.

Panel C. The choroid plexus and ependyma. The choroid plexus is at the interface between blood and CSF in the 3rd and 4th ventricles. The fenestrated endothelium is covered by tightly attached Choroid plexus epithelial cells. The ependyma surrounding the 3rd and 4th ventricle is constituted of a loosely attached ependymocytes contacted by astrocytes.

Panel D. The meningeal barriers. CSF in the subarachnoid space is protected by the arachnoid barrier cell layer and is isolated from brain by the pia matter. Pia matter and the arachnoid barrier also covers blood vessels.

Panel E. The circumventricular organs. Due to their particular role, circumventricular organs require a direct contact to the blood. Therefore, the BBB does not exist or is altered in these organs. However, they are isolated from the brain by a dense matrix and astrocytes/tanycytes layer. These organs are close to the 3rd and 4th ventricle, but a particular ependyma in these organs with tightly attached tanycytes prevent diffusion to the CSF. Tanycytes also contact the blood vessels in and out of these organs, and replace astrocytes endfeet.

Figure 2: Transport mechanisms through the blood brain barrier

Five pathways are distinguished: (i) paracellular pathway, (ii) transcellular pathway, (iii) transcytosis pathway, (iv) transport protein pathway and (v) efflux pumps pathway

Figure 3: Left temporal glioblastoma

Panel A1, MRI T1SE weighted images; Panel A2, same panel as A1 with central necrotic area indicated in yellow; Panel B1, MRI T1SE weighted images with gadolinium infusion showing contrast enhancement; Panel B2, same panel as B1 with the tumor proliferative forehead indicated in red; Panel C1, MRI T2 FLAIR weighted images; Panel C2, same panel as C1 with the brain adjacent the tumor indicated in green.

Table 1. Cytotoxic chemotherapeutic agents used in neuro-oncology

Drug	Tested as monotherapy in clinical trial dedicated to GBM patients	CNS toxicity at high dose or in direct exposure to the CNS	Currently used in neuro-oncology	References
Busulfan	No	Medium	++	[28]
Thiotepa	No	Low-medium	++	[28]
CCNU (lomustine)	Yes	Low	+++	[28,132,133]
BCNU (carmustine)	No	Low-medium	+++	[28,42,132]
ACNU (nimustine)	Yes	Medium	++	[28,69,70]
Temozolomide	Yes	Low	+++	[28,134–136]
Methotrexate	Yes	Medium	+++	[28,132,137]
Topotecan	Yes	Low	-	[28,32,138,139]
Cisplatin	No	High	++*	[28,132]
Etoposide	Yes	Low-medium	++	[28,140]
Irinotecan / SN-38	Yes	Low-medium	+	[28,141–143]
Carboplatin	Yes	Medium	++	[28,132,144–146]
Doxorubicin	No (Yes for liposomal form)	Medium-high	-	[28,108,132]
Vinblastine	No	Medium	+	[28,132]
Vincristine	No	Medium-high	++	[26,28,132]
Procarbazine	Yes	Medium-high	++	[28,132,147]
Paclitaxel	Yes	Medium-high	-	[32,132,148–150]
Fotemustine	Yes	Low-medium	+	[151–154]
Ifosfamide / 4-Hydroxyifosfamide / aldoifosfamide	Yes	High	-	[28,132,136]
Bevacizumab**	Yes	No	++	[133,155–158]
5-FluoroUracil	No	Medium-high	-	[28,132]
Bleomycin	Yes	Low	-	[28,159]
Hydroxyurea	Yes	Low	-	[28,132,160]
Docetaxel	Yes	Medium-high	-	[28,161–163]
Cytarabine (cytosine arabinoside, ara-C) / ara-U	No	Low	+	[28]

Legend: -: not commonly used in neuro-oncology; +: poorly used or used in limited or specific situations; ++: commonly used in neuro-oncology; +++: highly used in neuro-oncology; *, mainly in pediatric neuro-oncology; **, not a cytotoxic agent but a monoclonal antibody targeting VEGF-A used in neuro-oncology.

Table 2. Blood brain barrier crossing: experimental data for chemotherapeutic agents used in neuro-oncology

Drug	Species	Normal Brain/ Plasma ratio	CSF/ Plasma ratio	References
Busulfan	H	NA	95-99%	[164–167]
	P	NA	NA	
	R	74-77%	NA	
Thiotepa	H	NA	101%/95% **	[168,169]
	P	NA	93% (ventricular) ; 113% (lumbar)	
	R	NA	NA	
CCNU (lomustine)	H/P	NA	NA	[170,171]
	R	High ; 20% ****	NA	
BCNU (carmustine)	H/P	NA	NA	[171,172]
	R	High; 30%	NA	
ACNU (nimustine)	H/P/R	NA	NA	
Temozolomide	H	18%*; ***	20-40% **	[103,173–180]
	P	NA	33%	
	R	22-41% (include ****)	20-23% **	
Methotrexate	H	NA	0.5-2.5%	[181–188]
	P	NA	1.5%	
	R	3%* - 21%	0.5%	
Topotecan	H	NA	18-42% **	[189–194]
	P	NA	19-24%	
	R	5.5% ****	NA	
Cisplatin	H	NA	3% **	[195–198]
	P	1-3% *	3-5% (include **)	
	R	10% (include ****)	NA	
Etoposide	H	NA	0.5-5% ; 9%***	[171,199–203]
	P	NA	NA	
	R	Very low; 3-8%; 36%	NA	
Irinotecan/SN38	H	NA	NA	[173,204]
	P	NA	13%/ND	
	R	9-13%/1-6%	NA	
Carboplatin	H/R	NA	NA	[105,196,197]
	P	2/3-4%*	1-5%	
Doxorubicin	H/P	NA	NA	[171,205]
	R	Very low; 0-0.5%	NA	
Vinblastine	H/P	NA	NA	[127,206]
	R	ND**-10%	NA	
Vincristine	H	NA	ND	[152,193, 227–229]
	P	NA	NA	
	R	Very low****-18%	NA	
Procarbazine	H/P	NA	NA	[171]
	R	medium	NA	
Paclitaxel	H	ND****	ND	[209–211]

	P	NA	NA	
	R	ND – ≈19%	NA	
Fotemustine	H	NA	17-30%	[152]
	P/R	NA	NA	
Ifosfamide / 4-Hydroxyifosfamide / aldoifosfamide	H	NA	23- 53% / NA / NA	[212–214]
	P	NA	NA / 13% / NA	
	R	NA	NA	
Bevacizumab	H	0.2% (general for IgG)	NA	[215,216]
	P	NA	NA	
	R	ND	NA	
5-FluoroUracil	H	NA	ND-low**	[171,217–219]
	P	NA	48% (bolus); 11-20% (infusion, depending upon infusion rate)	
	R	low - 18*	NA	
Bleomycin	H/P	NA	NA	[171]
	R	Very low	NA	
Hydroxyurea	H	NA	33% (HIV patient)	[220–222]
	P	NA	NA	
	R	9-25%	6%	
Docetaxel	H	NA	0-9%**	[83,211,223,224]
	P	NA	NA	
	R	4.4- ≈8% ; 29-35%	NA	
Cytarabine (cytosine arabinoside, ara-C) / ara-U	H	NA	3-15% / 15-25% (include **)	[225–228]
	P/R	NA	NA	

Legend: H, human; P, primate; R, rodent –mice or rat-; *, extracellular fluid by microanalysis; **, CNS involvement; ***, Brain adjacent to tumor; ****, Normal brain of animals/patients with brain tumors. ND, not detected. Data above 100% indicate accumulation of the drug, or a faster clearance in the plasma than in the brain or CSF. Many drugs also showed a similar effect with large scales –*e.g.* methotrexate, paclitaxel, docetaxel-. We tried to show penetration with minimum impact of accumulation or differential clearance.

Table 3. Parameters used to predict crossing of the blood brain barrier by chemotherapeutic agents

Drug	M. weight	Ex-LogP	Polar SA	P. charges	Rot. bound	HB acceptor	HB donor	Protein binding	P-gp	Rule of 5	*In silico prediction**
Busulfan	246	-0.5	87-104	0	7	4	0	32-79%	No	Yes	Yes
Thiotepa/tepa	189/173	0.5/NA	9-51/36	0/0	3/3	3-1/1	0/0	NA/NA	No/NA	Yes/Yes	Yes
CCNU (lomustine)	234	2.8	62	0	4	2	1	50%	No	Yes	Yes
BCNU carmustine)	214	1.5	62	0	5	2	1	80%	No	Yes	Yes
ACNU (nimustine)	273	NA	114	0	5	NA	NA	NA	NA	Yes	Yes
Temozolomide	194	-2.8	106	0	1	5	1	15%	No	Yes	Yes
Methotrexate	454	-1.9	211	-2	9	12	5	50%	Yes	No	No
Topotecan	421	0.8	103	0	3	6	2	35%	Yes	Yes	No
Cisplatin	298	-2.2	NA	0	1	5	1	90% (Free P)	No	Yes	Yes
Etoposide	589	0.6	161	0	5	12	3	97%	Yes	No	No
Irinotecan / SN-38	587/392	3.2/NA	113/100	1/0	5/2	6/5	1/2	30-68%/NA	Yes/Yes	No/Yes	No/No
Carboplatin	371	NA	NA	0	0	2	0	90% (Free P)	No	Yes	Yes
Doxorubicin	544	1.3	206	1	5	12	6	74-76%	Yes	No	No
Vinblastine	811	3.7	154	2	10	9	3	98-99%	Yes	No	No
Vincristine	825	2.8	171	2	10	9	3	~ 75%	Yes	No	No
Procarbazine	221	0.1	53	0	5	3	3	NA	No	Yes	Yes
Paclitaxel	854	3.0	221	0	14	10	4	89-98%	Yes	No	No
Fotemustine	316	NA	107	0	9	3	1	NA	No	Yes	Yes
Ifosfamide / 4-Hydroxyifosfamide / aldoifosfamide	261 / 277 / 277	0.9 / NA / NA	51 / 71 / 77	0 / 0 / 0	5 / 5 / 10	2 / 3 / 3	1 / 2 / 2	Low / NA / NA	No / NA / NA	Yes / Yes / Yes	Yes / Yes / Yes OR No***
Bevacizumab	149,000	NA	NA	NA	NA	NA	NA	NA	NA	No	NA
5-FU**	130	-0.89	58.2	0	0	2	2	8-12%	No	Yes	Yes
Bleomycin	1415	NA	627	2	36	28	20	1%	Yes	Yes****	No
Hydroxyurea	76	-1.8	75	0	0	2	3	NA	No	Yes	Yes
Docetaxel	808	2.4	224	0	13	10	5	97%	Yes	No	No

Cytarabine (cytosine arabinoside, ara-C) / ara-U	243 / 244	-2.8 / NA	129 / 119	0 / 0	2 / 2	7 / 6	4 / 4	13% / NA	No / NA	Yes / Yes	Yes OR No / Yes OR No

Legend: M weight, Molecular weight (g/mol); Ex-LogP, experimental LogP; Polar SA, polar surface area (Å²); P charges, physiological charges; Rot. Bound, Rotatable bound count; HB acceptor, hydrogen bond acceptor count; HB donor, hydrogen bond donor count; Prot. binding, Protein binding; P-gp, P-glycoprotein substrat; *, http://www.cbligand.org/BBB/index.php; NA, not available on the used databases; Free P, free platinum; **, no information for active forms after hepatic metabolism; ***, depending on the algorithm; **** found in database, but inconsistent with the size of the molecule. Data obtained from chEMBL (https://www.ebi.ac.uk/chembl/) and drugbank (http://www.drugbank.ca/).

Table 4. In vitro efficacy of chemotherapeutic cytotoxic agent used in neuro-oncology

Drug	GI50	IC50	LC50*
Busulfan	234000 nM[U251]	NA	NA
Thiotepa / Tepa	58749 nM[U251] / NA	NA / NA	NA / NA
CCNU (lomustine)	31550 nM[U251]	NA	328000 nM
BCNU (carmustine)	52119 nM[U251]	15000 nM[U251]	173000 nM
ACNU (nimustine)	NA	NA	179000 nM
Temozolomide	100000 nM[U251]	250000 nM[U251]; 49000 nM[U87]	NA
Methotrexate	92 nM[U251]	NA	2400 nM
Topotecan	18 nM[U251]	60 nM - 3000[U251]; 160 nM[U87]	NA
Cisplatin	1918.67 ; 100000 nM[U251]	490 - 16193.6 nM[U87]; 11580.08 nM[U251]	3670 nM
Etoposide	NA	500-25000 nM[U251/U373]; 145 - 12400 nM[U87]; 14880 nM (8,76 µg/mL)	NA
Irinotecan / SN-38	3741 nM[U251] / 1 nM[U251]	NA / NA	NA / NA
Carboplatin	68076.94 nM[U251]	NA	80000 nM
Doxorubicin	40 - 125 nM[U251]	18 - 790 nM[U87]; 10 - 600 nM[U251/U373]	500 nM
Vinblastine	0.7 nM[U251]	5,02 - 8.1 nM[U251]; 1 - 29.98 nM[U87]	240 nM
Vincristine	132 nM[U251]	NA	80 nM
Procarbazine	336512 nM[U251]	NA	NA
Paclitaxel	20 nM[U87]; 3 - 3.98 nM[U251]	80 - 90000 nM[U87]; 30 - 128 nM[U251]	LC90 7.2 nM
Fotemustine	NA	NA	NA

Ifosfamide / 4-Hydroxyifosfamide / aldoifosfamide	310456 nM^{U251} / NA / NA	NA / NA / NA	NA / NA / NA
Avastin	NA	NA	NA
5-FU	912.01 nM^{U251}	NA	NA
Bleomycin	NA	4363.14 nM^{U251} ; 11057.16 nM^{U87}	NA
Hydroxyurea	580764.42 nM^{U251}	NA	NA
Docetaxel	10 nM^{U251}	2.5-25647 nM^{U251} ; 3.55 nM^{U87}	NA
Cytarabine (cytosine arabinoside, ara-C) / ara-U	NA / NA	1129.99 nM^{U251} ; 1742.57 nM^{U87} / NA	NA / NA

Legend : GI50, drug concentration inducing 50% of growth inhibition; IC50, drug concentration inducing 50% of inhibition; LC50, drug concentration inducing 50% of cell death ; *, median LC50 obtained from multiple glioma cell lines [229]. Data obtained from chEMBL (https://www.ebi.ac.uk/chembl/)

Figure 1

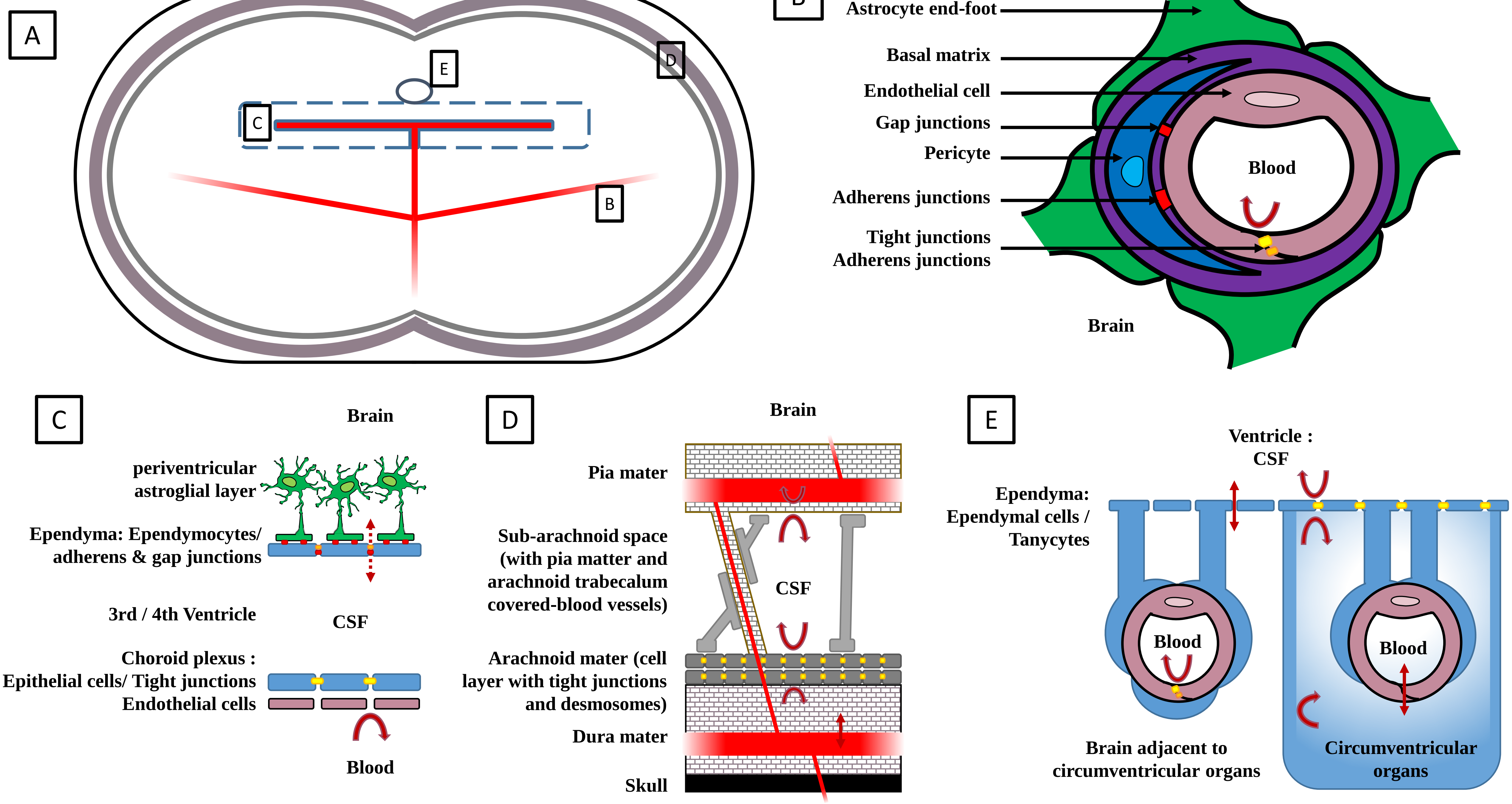
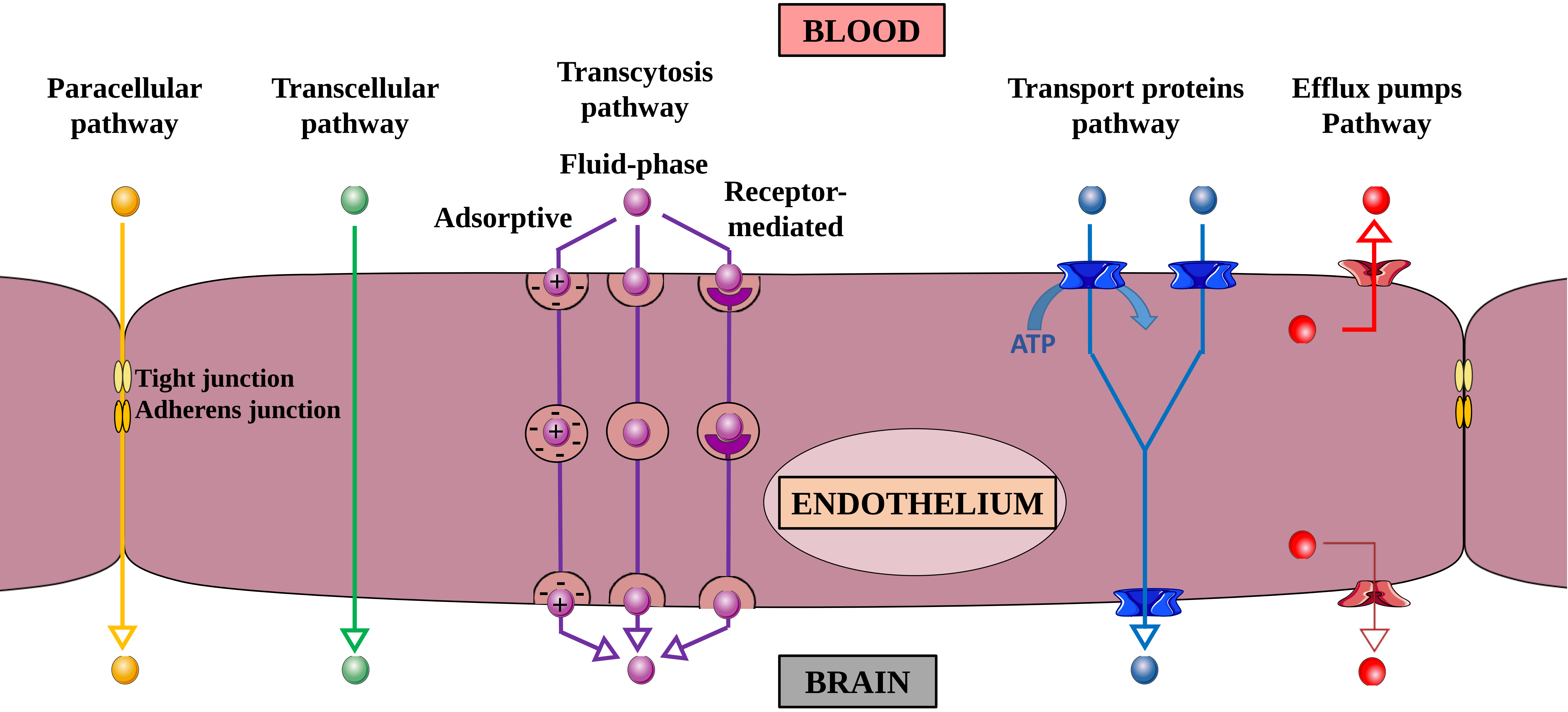

A
B
C
D
E
Astrocyte end-foot
Basal matrix
Endothelial cell
Gap junctions
Pericyte
Adherens junctions
Tight junctions
Adherens junctions
Blood
Brain
Brain
periventricular
astroglial layer
Ependyma: Ependymocytes/
adherens & gap junctions
3rd / 4th Ventricle
CSF
Choroid plexus :
Epithelial cells/ Tight junctions
Endothelial cells
Blood
Brain
Pia mater
Sub-arachnoid space
(with pia matter and
arachnoid trabecalum
covered-blood vessels)
CSF
Arachnoid mater (cell
layer with tight junctions
and desmosomes)
Dura mater
Skull
Ventricle :
CSF
Ependyma:
Ependymal cells /
Tanycytes
Blood
Blood
Brain adjacent to
circumventricular organs
Circumventricular
organs

Figure 2



BLOOD
Paracellular pathway
Transcellular pathway
Transcytosis pathway
Fluid-phase
Adsorptive
Receptor-mediated
Transport proteins pathway
Efflux pumps Pathway
Tight junction
Adherens junction
ATP
ENDOTHELIUM
BRAIN

Figure 3