Journal of Innovative Optical Health Sciences
Vol. 10, No. 5 (2017) 1730011 (12 pages)

DOI: 10.1142/S1793545817300117

Blood–brain barrier and laser technology for drug brain delivery

Oxana V. Semyachkina-Glushkovskaya*, Arkady S. Abdurashitov
and Elena I. Saranceva
Saratov State University 83 Astrakhanskaya Str.
Saratov 410012, Russia
**glushkovskaya@mail.ru*

Eketerina G. Borisova
Institute of Electronics
Bulgarian Academy of Sciences
TsarigradskoChaussee 72, Sofia 1784, Bulgaria
ekaterina.borisova@gmail.com

Alexander A. Shirokov
Institute of Biochemistry and Physiology of Plants and Microorganisms
Russian Academy of Sciences
13, Prospekt Entuziastov, Saratov 410049, Russia
shirokov_a@ibppm.ru

Nikita V. Navolokin
Saratov State Medical University
Saratov 410010, Russia
nik-navolokin@yandex.ru

Received 29 June 2017
Accepted 4 September 2017
Published 27 September 2017

Here, we discuss an important problem in medicine as development of effective strategies for brain drug delivery. This problem is related to the blood–brain barrier (BBB), which is a "customs" controlling the entrance of different molecules from blood into the brain protecting the normal function of central nervous system (CNS). We show three interfaces of anatomical side of BBB and two functional types of BBB — physical and transporter barriers. Although this protective mechanism is essential for health of CNS, it also creates a hindrance to the entry of drugs into the brain. The BBB was discovered over 100 years ago but till now, there is no effective methods for brain drug delivery. There are more than 70 approaches for overcoming BBB

*Corresponding author.

including physical, chemical and biological techniques but all of these tools have limitation to be widely used in clinical practice due to invasiveness, challenge in performing, very costly or limitation of drug concentration.

Photodynamic therapy (PDT) is usual clinical method of surgical navigation for the resection of brain tumor and anti-cancer therapy. Nowadays, the application of PDT is considered as a potential promising tool for brain drug delivery via opening of BBB. Here, we show the first successful experimental results in this field discussing the adventures and disadvantages of PDT-related BBB disruption as well as alternatives to overcome these limitations and possible mechanisms with new pathways for brain clearance via glymphatic and lymphatic systems.

Keywords: Blood–brain barrier; laser technologies; photodynamic therapy; brain drug delivery.

1. The First 100 Years of BBB Research and Nowadays

The history of blood–brain barrier (BBB) begins from the end of 19th century. In 1885, Paul Ehrlich studied anti-bacterial effects of different acidic vital dyes and observed that if dyes were injected intravenously, they would stain peripheral organs except brain and spinal code (the choroid plexus was stained, but cerebrospinal fluid (CSF) was not).[1] In 1909 and 1913, student of Ehrlich Edwin Godlman repeated this experiment but additionally made the injection of trypan blue into the CSF in rabbits and showed that the brain and spinal code were stained by dye but not by peripheral organs.[2,3] Despite the fact that Enrlich did not understand the paradoxical futures of BBB and Gordman made historical error owing to belief that anatomical side of the BBB is of choroid plexus (not parenchymal microvessels), the concept of BBB is attributed to these two names.[4] Currently, using of intravenous injection of Evans Blue is a classical test for the study of BBB permeability (Fig. 1).

In early 1920s, Lina Stern and Gautier proposed the term "BBB", which was widely accepted. She performed detailed studies of the permeability of wide range of different substances from blood into CSF (1921), into subarachnoid and ventricular CSF (1923) and into brain (1922).[6–8] This term shows both anatomical position and function of BBB: the BBB locates between blood and the brain on complex of endothelial cells of cerebral microvessels (the arteriole-capillary-venule level) and his main function is controlling the passage of blood-borne agents into the central nervous system (CNS) protecting the brain against pathogens.

In 1928, Stern and Rapoport clearly showed existing two routes of entry from blood into brain: one directly across the microvessels — the endothelial BBB and the other via the choroid plexus — the epithelial blood–CSF barrier.[9,10] The arachnoid barrier is also mentioned as special type of barrier. It is avascular epithelial barrier, which separates the sagittal sinus from the dura projecting arachnoid villi into this main cerebral vein, thereby allowing the CSF movement out of the brain to blood.

However, until the late 1950s, many researchers had doubts that the physical barriers exist and the progress in the study of BBB actively started after the 1960s due to progress in optical visualization of brain tissues and vessels.[11]

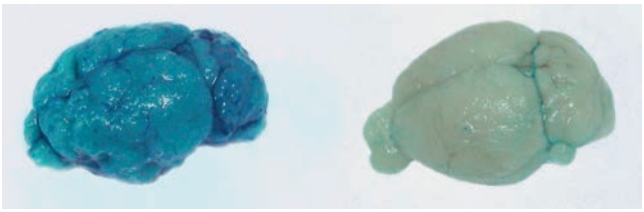

Fig. 1. The example of BBB disruption in adult rats after severe stroke (left) and the intact brain (right). The blue color of brain tissues suggest the high BBB permeability to *Evans blue* injected intravenously. Figure was taken from Ref. 5.

2. The Structure and Functions of Barriers Between Blood and Brain

Nowadays, it is widely well accepted that there are three principle interfaces of barrier between blood and brain: the BBB, the blood–CSF barrier and arachnoid barrier[12] (Fig. 2). All organisms with a well-developed CNS have the BBB. Mammalians such as rat, mouse, rabbit, guinea-pig, dog, sheep and human have similar mechanisms of BBB

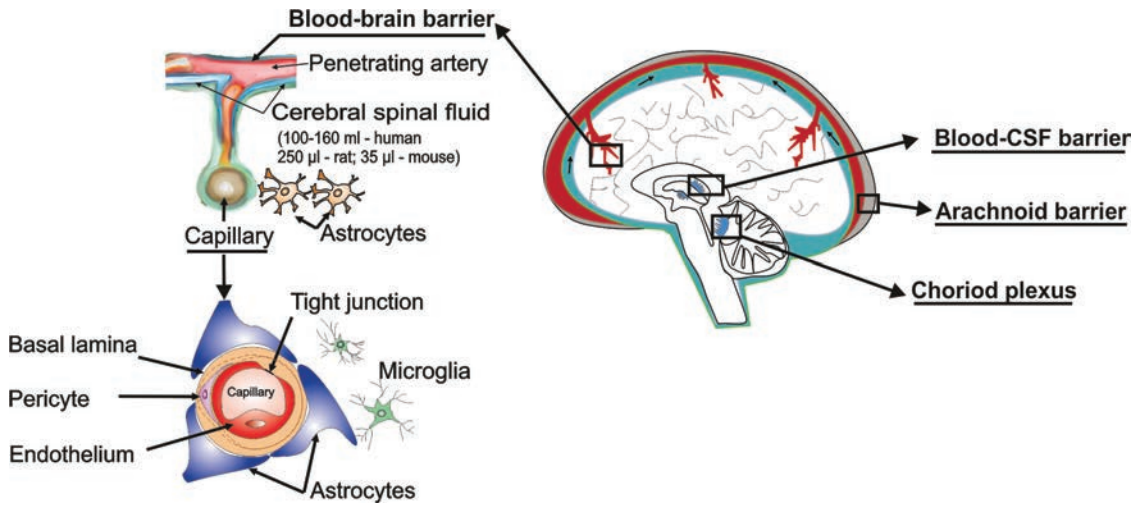

Fig. 2. Three interfaces of barriers: (1) the BBB, which locates between blood and the brain on complex of endothelial cells of cerebral microvessels (the arteriole-capillary-venule level); (2) the epithelial blood–CSF barrier lies at the choroid plexus of ventricular system of the brain; (3) the avascular arachnoid barrier separates the sagittal sinus from the dura projecting arachnoid villi into this main cerebral vein allowing the CSF movement out of the brain to blood.

function.[13] Therefore, researchers are free to choose a subject for the study of BBB.

The physical BBB form three elements: endothelial cells, embedded with them pericytes and covering them astrocyte end-feet (Fig. 2). The diffusion BBB presented by complex of intra- and transmembrane proteins[14,15] (Fig. 3). Tight junctions are located on the apical membrane of cerebral endothelium and contain the family of claudins, occludin, junctional adhesion molecules as well as in cytoplasm of endothelial cells including zonula occludens proteins (ZO-1, ZO-2, ZO-3 and cingulin) bound to actin cytoskeleton.[16–18] Adhesion junctions are located at the part of basement membrane of the paracellular space and are composed of cadherin, integrin and their associated proteins.[19]

Claudins as major component of tight junctions were identified by Furuse in 1998.[20] There are at least 24 members of claudin family in mouse and human.[21,22] Occludin was identified by Furuse in 1993

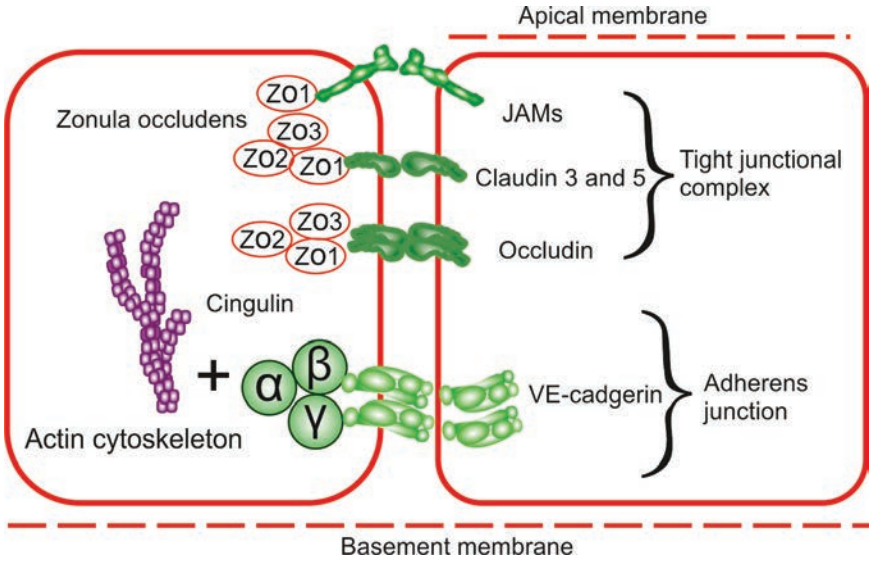

Fig. 3. The schematic illustration of tight junctions in cerebral endothelial cells. Tight junctions contain transmembrane proteins such as the family of claudins, occludin, junctional adhesion molecules as well as proteins in cytoplasm of endothelial cells including zonula occludens proteins (ZO-1, ZO-2, ZO-3 and cingulin) bound to actin cytoskeleton. Adhesion junctions are composed of cadherin, integrin and their associated proteins.

in chicken[23] and then by others in mammalians.[24–26] Occluding and claudin assemble are composed of intramembranous strains and together form the main component of the BBB. Both these proteins bind to complex of cytoplasmic scaffolding proteins (ZO-1, ZO-2, ZO-3) controlling the actin/myosin cytoskeleton system and diameter of paracellular space.[12,15,27]

The third type of tight junction protein — junctional adhesion molecules, which belong to immunoglobulin family, was identified by Martin-Padura in 1998.[28] These proteins are involved in cell-to-cell adhesion and monocyte transmigration through BBB.[29,30]

Cytoplasmic proteins (ZO-1, ZO-2, ZO-3, cingulin and others) known as membrane-associated guanylate kinase-like proteins and they play a role in organizing proteins at the cellular plasma. COOH-terminal of ZO-1 and ZO-2 bind actin, which is primary cytoskeleton protein. Thus, these complex cross-links transmembrane proteins provide supporting to architecture of endothelial cells.[31]

Adherens junctions assemble include cytoplasmic cadherin, which forms adhesive contacts between endothelial cells due to its connection with the actin cytoskeleton via intermediary submembranal proteins — catenins. Cadherin bind to β and γ-of catenin, which are linked to the actin via α-subunits of catenin.[17] Adherens junctions hold the endothelial cell together giving the BBB structure support.

Tight junctions are presented in cerebral blood vessels in the early embryonic development restricting the passage of low-molecular-weight molecules into the brain.[32] For instance, tight junctions appear on eight weeks of gestation in human, at 13 days in mice when the first vessels appear in the cortex.[33] But, gene expression of tight junctions undergoes changes from postnatal period to adulthood. However, the age-differences in the BBB remains poorly studied.

3. Transport Systems Across BBB

Transport barrier is a complex of proteins of endothelial cells, which are involved in mechanisms to enter different molecules from blood into brain. Figure 4 shows different routes of transport across the BBB. Passive diffusion into brain is generally correlated with lipid solubility. For instance, the diffusion of dissolved blood oxygen and carbon dioxide into brain is passive via their concentration gradient.

Molecules such as glucose, amino acids, nucleosides, small peptides, which are necessary for the brain metabolism, across the BBB via express specific solute transporters located both at luminal and apical membranes.[12,34,35] The orientation of transporters result the preferential direction of transport

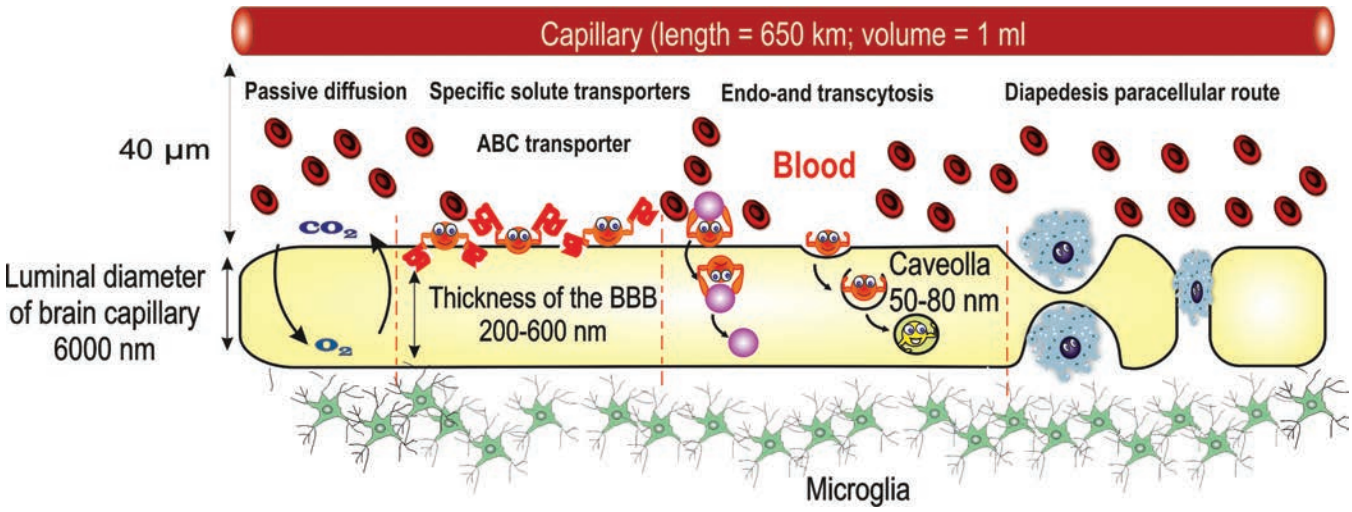

Fig. 4. The schematic illustration of transport across the BBB: (1) passive diffusion into brain for lipid solubility substances such as dissolved in blood oxygen and carbon dioxide; (2) specific solute transporters for metabolic molecules as glucose, amino acids, nucleosides, small peptides; (3) ABC transporter using ATP — binding cassette for many drugs via complex of proteins — P-glycoprotein, Multidrug Resistance Protein, the Multidrug Resistance-associated proteins; breast Cancer Resistance Protein; (4) endo- and transcytotic mechanisms for transport through the BBB of macromolecules such as proteins and peptides; (5) diapedesis and paracellular route for migration of immune cells from blood into brain.

into or across of endothelial cells and direction from blood into brain and from brain into blood.

The major transporter for many drugs and cholesterol is ABC transporter (ATP — binding cassette) that is family proteins containing 48 members grouped into seven sub-families.[36] Among proteins of ABP transport several sub-groups play greatest role: (1) P-glycoprotein (plays greatest significance); (2) Multidrug Resistance Protein; (3) the Multidrug Resistance-associated proteins: (4) breast Cancer Resistance Protein[37–39] Macromolecules (proteins, peptides, for instance, insulin, immunoglobulin G, transferrin, etc.) across the BBB via endo- and transcytotic mechanisms including two processes.[40–42] First, receptor-medicated mechanism, where the binding of macromolecular ligands and receptors on the surface of endothelial cell triggers an formation of endocytotic vesicles. Second, adsorptive-mediated transcytosis is related with excess positive charge on molecule, which renders cationic inducing endocytosis.

Over 100 years, it was believed that only in pathological state, the mononuclear leukocytes, monocytes and macrophages are not able to penetrate the BBB.[43–45] Recently, it was discovered that immune cells can across the BBB.[11,12,46] There is an evidence that in normal BBB, immune cells are able to enter into the brain via diapedesis but not through paracellular route without opening tight-junctions complex, which is more obvious during inflammation.[47]

4. Photodynamic Therapy as a Potential Tool for Brain Drug Delivery

These protective mechanisms, including physical and transporter barriers, strongly restrict the entrance of many drugs into the CNS. The last decade has been characterized by progress in different classes of medications, among which only two are used for the therapy of CNS diseases.[48] There are 7000 drugs, which are registered in the Comprehensive Medicinal Chemistry database, only 5% can treat the neurological diseases due to reason that antibodies, recombinant proteins, gene therapeutics and most small molecules do not cross BBB.[48–50] This is the reason why the approaches for overcoming of BBB have received significant attention from researches around the world in the last four decades. The search in PubMed for last five years using words "brain drug delivery" show more than 10.370 citation. If the first 100 years of history of the BBB (1880–1980) was focused on the study of anatomical structure and physiology of the BBB, nowadays, it is focused on the period for the development of technologies for the brain drug delivery. There are more than 70 different physical, chemical and biological methods for overcoming of the BBB.[51–54] However, no approaches for the opening of BBB, which are widely used in daily clinical practice due to their invasiveness and challenge in performing such as intra-arterial injection of mannitol, limitation of drug concentration using intranasal drug delivery or area of treatment (1–3 mm) using focused ultrasound.[55–57]

Here, we discuss technology, which is used in clinical practice but with completely new view on this method as novel approach for the brain drug delivery. Photodynamic therapy (PDT) with using 5-aminolevulinic acid (5-ALA)-PDT is a valuable method for surgical navigation of glioblastoma.[58–61] PDT is a form of therapy that combines a light source and nontoxic photosensitizing agents (photodynamic dyes). The systematically or topically administered photosensitizer is specifically accumulated in diseased tissue. When concentration of photosensitizer is sufficient, it is activated by exposure to light appropriated for its excitation. The excited photosensitizer interacts with molecular triplet oxygen ($^3O_2$) and produces $^1O_2$ resulting the tissue oxidation. The mechanisms of $^1O_2$ generation is the light-induced transition of one of electrons in a higher-energy orbital from the ground state (triplet oxygen) in a short-lived ($\tau_{fl} = 10^{-6}$–10^{-9} s) electronically excited state (singlet oxygen).

5-ALA is most preferable photosensitizer in medicine because it can be orally administered and is relatively safe.[58–61] 5-ALA itself does not produce fluorescence, after administration into blood, 5-ALA is metabolized in the cells to protoporphyrin IX that accumulates in tumor tissues giving photodynamic effect via light activation of the target tissue and allowing the neurosurgeon easily to detect and accurately resect tumor (Fig. 5).[58]

5-ALA naturally accumulates in the vascular endothelial cells including cerebral vasculature allowing "vascular targeted" PDT.[62,63] Compared to normal tissues, most types of cancers are especially active in both the uptake and accumulation of photosensitizers agents, which makes cancers especially vulnerable to PDT. Currently, in

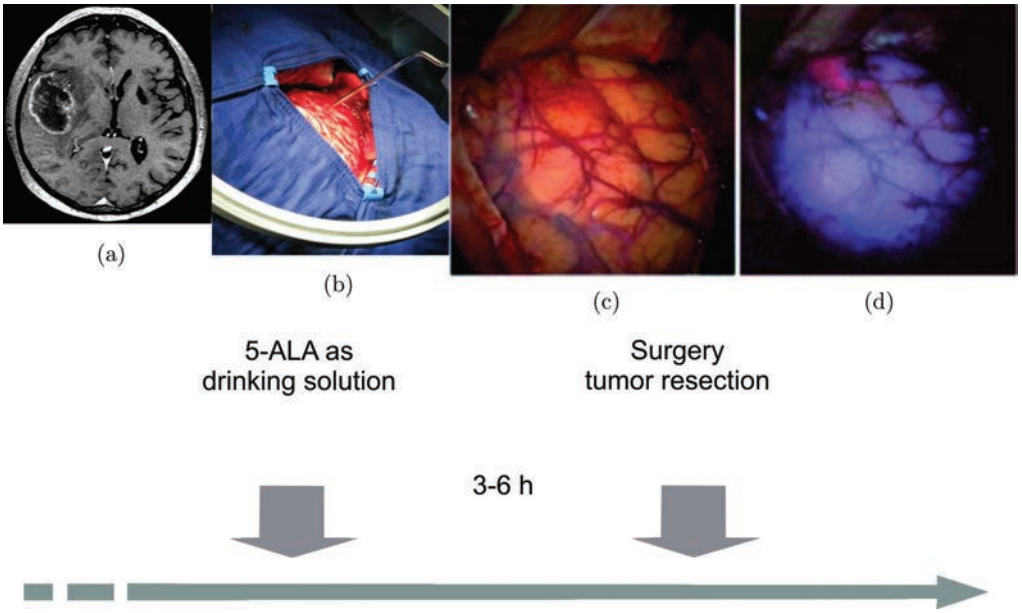

Fig. 5. The fluorescence guided resection of glioblastoma in patient: (a) — magnetic resonance imaging of glioblastoma (arrowed); (b) — preparation of surgical area; (c) — the surface of brain after craniectomy; (d) — specific fluorescence of 5-ALA in ultraviolet 3–6 h after 5-ALA administration per os. Figure presented with permission of Herbert Stepp, University Hospital of Munich, Germany.

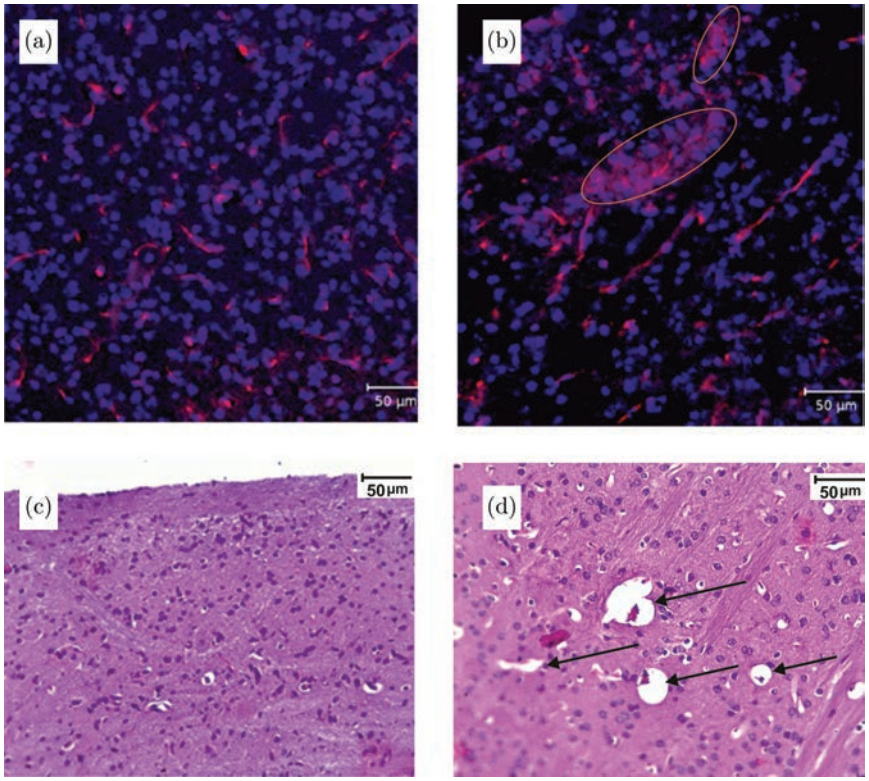

Fig. 6. The PDT-induced opening of the BBB using 5-ALA (laser: 635 nm, 20 J/cm^2, 60 mV, 333 s; 5-ALA — 20 mg/kg, i.v.). In the left — control (a and c), no laser effects; in the right — 90 min after PDT. (b) — the extravasation of dextran (circled) from cerebral vessels into the brain parenchyma. (d) — The perivascular edema (arrowed) in the area of laser application. Figure was taken from Semyachkina-Glushkovskaya Ref. 84.

PDT of brain tumors is used with various photosensitizers.[64]

However, PDT has serious consequence such as vasogenic edema, which appears after PDT performing.[59] The one reason of PDT-induced accumulation of water in perivascular space is the PDT-related opening of BBB.

So, the traditional explanation of anti-cancer PDT effect is $^1O_2$-induced damage of the endothelial cells resulting tumor microvasculature collapse.[65,66]

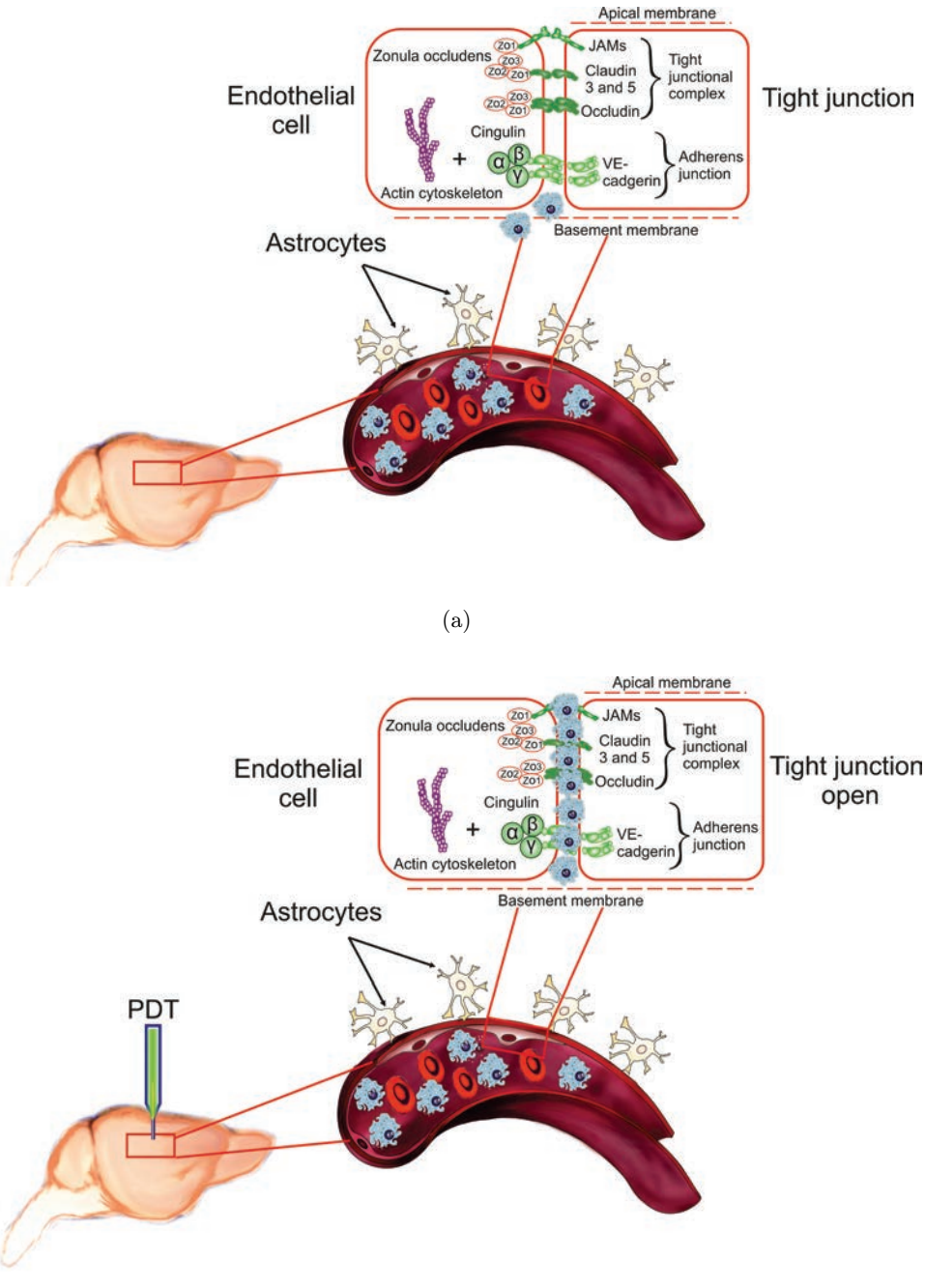

Fig. 7. The schematic illustration of PDT-mediated BBB disruption and macrophages migration from blood into brain. The photosensitizers binds in the cerebral endothelial cells, under light exposure photosensitizers stimulates $^1O_2$ generation, which causes the enlargement of endothelial gaps due to changes of the cytoskeleton, cell rounding and constriction via microtubule depolarization. (a) — the normal state (intact BBB), (b) — the PDT-induced opening of BBB for macrophages.

However, several studies show that PDF increases the BBB permeability temporally.[67–70]

Hirschberg *et al.* using 400 μm bare flat-end quartz fiber (635 nm) and stereotaxic procedure clearly show that the ALA-PDT causes the opening of BBB. At the low fluence levels of 9L, the ALA-PDT opens the BBB rapidly without any damages of brain tissues, the disrupted BBB is observed during 2 h following PDT and restored during the next 72 h.[67] The disruption of BBB is greater using the higher fluence level of 26 J but the damages of surrounding tissues (necrosis, edema, hemorrhage) are observed for 17 days.

We confirmed these results in our work. Using laser 635 nm (10–40 J/cm^2, 40–100 mV, 250–400 s) and 5-ALA (NIOPIK, Russia, 20 mg/kg, i.v.) we showed maximal increase in the BBB permeability in middle dose of laser influences on the brain tissues (15–20 J/cm^2, 40–60 mV, 375–333 s) while higher dose was not accompanied by more pronounced changes in the BBB disruption (Fig. 6).

The mechanisms responsible for PDT-mediated increase in BBB disruption remain poorly understood. Some authors believe that PDT has direct effect on the capillary endothelial cells[71] inducing the increasing of endothelial gaps[72] due to changes of the cytoskeleton, cell rounding and constriction via microtubule depolarization.[73,74]

Several authors show the efficiency of PDT-related BBB disruption for targeted macrophages migration from blood into brain with the aim to improve PDT of glioma using macrophages as transport system through the BBB for gold nanoparticles (Fig. 7).[68–70] The nanoparticles show great promise for PDT.[75] Madsen *et al.* in experiment *in vivo* showed that PDT (the photosensitizer —

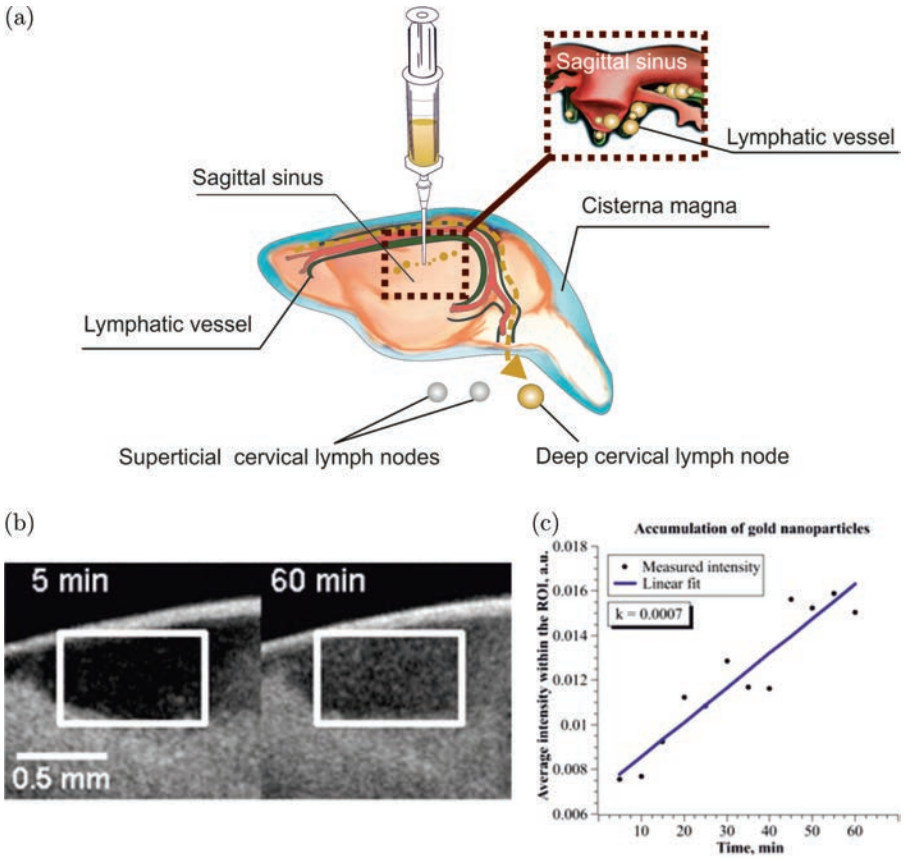

Fig. 8. The imaging of glymphatic pathway of brain clearance from GNRs. (a) — illustration of experiment: GNRs are injected into the brain parenchyma; GNRs drain from the brain via the meningeal lymphatic vessels; in 20 min after injection, GNRs accumulate in the deep cervical lymph node with maximal effect 1 h later; (b) — the OCT image of the deep cervical lymph node before injection of GNRs (left); the OCT images illustrating accumulation of GNRs in the deep cervical lymph node 60 min after injection of GNRs (right); (c) — average intensity of OCT signal within the ROI showing linear accumulation of nanoparticles in the deep cervical node with the slope $k = 0.0007$. Detailed information about k value interpretation is given in the text. Figure in modification was taken from Ref. 85.

aluminum phthalocyanine disulfonate ($AlPcS_{2a}$), $\lambda = 670$ nm; light dose = 2.5 J) is effective for the opening of BBB and the migration of systemically administered exogenous macrophages loaded with iron oxide nanoparticles (120–180 nm) in brain of rats.[68] Trinidad *et al.* in experiments *in vitro* using macrophages loaded gold nanoparticles (120 nm) and human FaDu cancer cell line (head and neck squamous cell carcinoma) showed reducing two-fold the cell viability after PDT ($AlPcS_{2a}$-mediated $\lambda = 670$ nm, fluence level 0.25 J/cm^2) combined with photothermal therapy ($\lambda = 810$ nm, at 14 W/cm^2 irradiance).[76]

Despite the promising effect of PDT on the transportation of macrophages through the BBB, there is an important question: "How the brain cleans from gold nanoparticles and is it possible to use this procedure for human?".

The latest discoveries of glymphatic and lymphatic systems, playing the role of clearance of the brain, allow to answer this question.[77–79] In our experiments, gold nanorods (GNRs) are injected directly into the brain parenchyma, which show the accumulation of GNRs in the deep cervical lymph node in 20 min after injection suggesting activation of clearance systems of the brain immediately after treatment (Fig. 8).

5. Conclusion

PDT-related reversable opening of BBB might be new promising step in the progress of treatment of CNS diseases. For example, the targeted effect of PDT on the BBB can be useful in anti-cancer therapy, when it is necessary to have focusing treatment. While PDT-non specific opening BBB is can be suitable for neurotropic drugs delivery and treatment CSN pathology such as Alzheimer's or Parkinson's diseases, depression etc. Although the PDT-related opening of BBB is attractive, it needs further studies for optimization of PDT condition and tissue oxygenation status, photosensitizer concentration and pharmacokinetics/distribution, scheme of laser illumination and personalized dosimetry (i.e., light, photosensitizer, $^1O_2$) to avoid vessel damages such as edema, constriction, thrombosis, hemorrhages.[80–82] To assess efficacy of PDT with curative intent, high quality comparative, randomized studies are needed. Palliative treatment with PDT seems to increase the quality of life in otherwise untreatable patients. Note, intrigue "gold key" has been found to overcome the limitations of PDT, such as low light penetration and high toxicity of photosensitizers, by direct generation of $^1O_2$ using quantum-dot laser diodes emitting in the near infrared spectral range (1067 nm, 1268 nm).[83]

Acknowledgments

The experimental results presented in this review was supported by Grant of Russian Science Foundation No. 17-15-01263.

References

1. P. Ehrlich, *Das Sauerstoffbedürfnis des Organismus, Eine Farbenanalytische Studie*, Hirschwald, Berlin (1885).
2. E. E. Goldmann, *Vitalfarbung am Zentral-nervensystem, Abh Preuss Akad Wissensch, Physkol Mathem Klasse*, Vol. 1, pp. 1–60, Konigl. Akademie der Wissenschaften (1909).
3. E. E. Goldmann, "The process of digestion: Illustrated by the action ofstains on the living tissues," *The Lancet* **185**, 69–71 (1913).
4. N. R. Saunders, J.-J. Dreifuss, K. M. Dziegielewska, P. A. Johansson, M. D. Habgood, K. Møllgård, and H.-C. Bauer. "The rights and wrongs of blood-brain barrier permeability studies: A walk through 100 years of history," *Front Neurosci.* **8**, 404 (2014).
5. O. V. Semyachkina-Glushkovskaya, "Blood-brain barrier and cerebral blood flow: Age differences in hemorrhagic stroke," *J. Innov. Opt. Health Sci.* **9**, 1–10 (2015).
6. L. Stern, "Recherchessurle liquid céphalo-rachidien.1.Les rapportsentrele liquid céphalo-rachidienetlacirculationsanguine," *Arch. Int. Physiol.* **17**, 138–192 (1921).
7. L. Stern, "Recherches sur le liquid céphalo-rachidien. II. Les rapports entrele liquid céphalo-rachidien et les élémentsnerveux de l'axecérébro-spinal," *Arch. Int. Physiol.* **17**, 391–448 (1922).
8. L. Stern, "Recherches sur le liquid céphalo-rachidien. II. Rapports entrele liquid céphalo-rachidien et des espacesventriculaires et cellui des espaces sous-arachnodiens," *Arch. Int. Physiol.* **20**, 403–436 (1923).
9. L. Stern, "Les rapports entre l'augmentation de la perméabilité de labarrièrehémato-encéphalique et les alterations de son substratum morphologique," *C. R. Soc. Biol.* **98**, 1515–1517 (1928).
10. L. Stern, "Les échangesentrele liquid céphalorachidien et lesélémentsnerveuxcérébro-spinaux," *C. R. Soc. Biol.* **98**, 1518–1519 (1928).

11. W. A. Banks, "From blood-brain barrier to blood-brain interface: New opportunities for CNS drug delivery," *Nat. Rev. Drug Discov.* **5**(4), 275–292 (2016).
12. N. J. Abbott, "Structure and function of the blood-brain barrier," *Neurobiol. Dis.* **37**, 13–25 (2010).
13. N. J. Abbott, Comparative physiology of the blood–brain barrier, *Physiology and Pharmacology of the Blood–Brain Barrier*, M. W. B. Bradbury Ed. Springer-Verlag, Heidelberg, pp. 371–396 (1992).
14. N. J. Abbott, "Astrocyte-endothelial interactions at the blood–brain barrier," *Nat. Rev. Neurosci.* **7**, 41–53 (2006).
15. P. Balabh, "The blood-brain barrier: an overview structure, regulation, and clinical implication," *Neurobiol. Dis.* **16**, 1–13 (2004).
16. H. Wolburg, "Tight junctions of the blood–brain barrier: development, composition and regulation," *Vascul. Pharmacol.* **38**, 323–337 (2002).
17. K. Matter, "Signalling to and from tight junctions," *Nat. Rev. Mol. Cell. Biol.* **4**, 225–236 (2003).
18. H. Wolburg, "Brain endothelial cells and the glio–vascular complex," *Cell Tissue Res.* **335**, 75–96 (2009).
19. B. T. Hawkins, "The blood–brain barrier/ neurovascular unit in health and disease," *Pharmacol Rev.* **57**, 173–185 (2005).
20. M. Furuse, "Claudin-1 and -2: Novel integral membrane proteins localizing attight junctions with no sequence similarity to occludin," *J. Cell Biol.* **141**, 1539–1550 (1998).
21. K. Morita, "Claudin-11/OSP-based tight junctions of myelin sheaths in brain and Sertoli cells in testis," *J. Cell Biol.* **145**, 579–588 (1999).
22. L. L. Mitic, "Molecular physiology and pathophysiology of tight junctions. I. Tight junction structure and function: Lessons from mutant animals and proteins," *Am. J. Physiol. Gastrointest. Liver. Physiol.* **297**, G250–G254 (2000).
23. M. Furuse, Occludin: A novel integral membrane protein localizing at tightjunctions," *J. Cell Biol.* **123**, 1777–1788 (1993).
24. Y. Ando-Akatsuka, "Interspecies diversity of the occludin sequence: cDNA cloning of human, mouse, dog, andrat–kangaroo homologues," *J. Cell Biol.* **133**, 43–47 (1996).
25. T. Hirase, "Occludin as a possible determinant of tight junction permeability in endothelial cells," *J. Cell.Sci.* **110**, 1603–1613 (1997).
26. M. C. Papadopoulos, "Occludin expression in microvessels of neoplastic and non-neoplastic human brain," *Neuropathol. Appl. Neurobiol.* **27**, 384–395 (2001).
27. M. Furuse, "Manner of interaction of heterogeneous claudin species within and between tight junction strands," *J. Cell Biol.* **147**, 891–903 (1999).
28. I. Martin-Padura, "Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration," *J. Cell Biol.* **142**, 117–127 (1998).
29. M. Aurrand-Lions, "Heterogeneity of endothelial junctions is reflected by differentialexpression and specific subcellular localization of the three JAM family members," *Blood* **98**, 3699–3707 (2001).
30. G. Bazzoni, "Homophilic interaction of junctional adhesion molecule," *J. Biol. Chem.* **275**, 30970–30976 (2000).
31. J. Haskins, "ZO-3, a novel member of the MAGUK protein family found at the tight junction, interacts with ZO-1 and occludin," *J. Cell Biol.* **141**, 199–208 (1998).
32. R. Moretti, "Blood-brain barrier dysfunction in disorders of the developing brain," *Front. Neurosci.* **9**, 15–30 (2015).
33. C. J. Ek, "Barriers in the developing brain and neurotoxicology," *Neurotoxicology* **33**, 586–604 (2012).
34. N. J. Abbott, "Astrocyte–endothelial interactions and blood–brain barrier permeability," *J. Anat.* **200**, 629–638 (2002).
35. D. J. Begley, "Structural and functional aspects of the blood–brain barrier," *Progr. Drug Res.* **61**, 39–78 (2003).
36. M. Dean, "The human ATP-binding cassette (ABC) transporter superfamily," *Genome Res.* **11**, 1156–1166 (2001).
37. D. J. Begley, "ABC transporters and the blood–brain barrier," *Curr. Pharm. Des.* **10**, 1295–1312 (2004).
38. S. Dauchy, "ABC transporters, cytochromesP450 and their main transcription factors: Expression at the human blood–brain barrier," *J. Neurochem.* **107**, 1518–1528 (2008).
39. J. Kamiie, "Quantitative atlas of membrane transporter proteins: development and application of a highly sensitive simultaneous LC/MS/MS method combined with novel in-silico peptide selection criteria," *Pharm. Res.* **25**, 69–83 (2008).
40. C. C. Visser, "Characterization of the transferrin receptor on brain capillary endothelial cells," *Pharm Res.* **21**, 761–769 (2004).
41. B. V. Zlokovic, "Asaturable mechanism for transport of immunoglobulin G across the blood–brain barrier of the guinea pig," *Exp. Neurol.* **107**, 263–270 (1990).
42. W. A. Banks, "The source of cerebral insulin," *Eur. J. Pharmacol.* **490**, 5–12 (2004).
43. I. Glezer, "Neuroprotective role of the innate immune system by microglia," *Neuroscience* **147**, 867–883 (2007).
44. W. F. Hickey, "Leukocyte traffic in the central nervous system: The participants and their roles," *SeminImmunol.* **11**, 125–137 (1999).

45. E. G. Perdiguero, "The development and maintenance of resident macrophages," *Nat. Immunol.* **17**, 2–8 (2016).
46. A. Louveau, "Structural and functional features of central nervous system lymphatic vessels," *Nat.* **523**, 337–341 (2015).
47. H. Wolburg, "Diapedesis of mononuclearcells across cerebral venules during experimental autoimmune encephalomyelitisleaves the tight junctions intact," *Acta Neuropathol.* **109**, 181–190 (2005).
48. A. Mullard, "FDA drug approvals," *Nat. Rev. Drug Discov.* **12**, 87–90 (2013).
49. W. M. Pardridge, "Blood-brain barrier delivery," *Drug Discov. Today.* **12**, 54–61 (2007).
50. W. M. Pardridge, *Brain Drug Targeting: The Future of Brain Drug Development*, Vol. 111, (Cambridge University Press, UK, 2001).
51. A. K. Ghose, "A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases," *J. Comb. Chem.* **1**, 55–68 (1999).
52. C. M. de Lange, *Drug Delivery to the Brain: Physiological Concepts, Methodologies and Approaches* (Springer, New York, 2014).
53. S. Mitragotri, "Devies for overcoming biological barriers: The use of physical forces to disrupt the barriers," *Adv. Drug. Deliv. Rev.* **65**, 100–103 (2013).
54. P. K. Pandey, "Blood brain barrier: An overview on strategies in drug delivery, realistic in vitro modeling and in vivo live tracking," *Tissue Barriers* **4**, e1129476 (2016).
55. V. Kiviniemi, "Real-time monitoring of human blood-brain barrier disruption," *PLoS ONE* **12**, e0174072 (2017).
56. S. Wu, "Intranasal delivery of neural stem cells: A CNS-specific, non-invasive cell-based therapy for experimental autoimmune encephalomyelitis," *J. Clin. Cell. Immunol.* **4**, 42–48 (2013).
57. P. C. Chu, "Focused ultrasound-induced blood-brain barrier opening: Association with mechanical index and cavitation index analyzed by dynamic contrast-enhanced magnetic-resonance imaging," *Sci. Rep.* **6**, 33264 (2016).
58. M. C. Tetard, "Experimental use of photodynamic therapy in high grade gliomas: A review focused on 5-aminolevulinic acid," *Photodiagn. Photodyn. Ther.* **11**, 319–330 (2014).
59. S. A. Friesen, "5-Aminolevulinic acid-based photodynamic detection and therapy of brain tumors (review)," *Int. J. Oncol.* **21**, 577–582 (2002).
60. M. J. Colditz, "Aminolevulinic acid (ALA)-protoporphyrin IX fluorescence guided tumour resection. Part 1: Clinical, radiological and pathological studies," *J. Clin. Neurosci.* **19**, 1471–1474 (2012).
61. T. Kuroiwa, "Photodynamic diagnosis and photodynamic therapy for the brain tumors," *Prog. Neuro-Oncol.* **21**, 14–21 (2014).
62. B. Chen, "Vascular and cellular targeting for photodynamic therapy," *Crit. Rev. Eukaryot. Gene Expr.* **16**, 279–305 (2006).
63. B. Krammer, "Vascular effects of photodynamic therapy," *Anticancer Res.* **21**, 4271–4277 (2001).
64. T. S. Zavadskaya, "Photodynamic therapy in the treatment of glioma," *Exp. Oncol.* **37**, 234–241 (2015).
65. M. S. Mathews, "Cerebral edema following photodynamic therapy using endogenous and exogenous photosensitizers in normal brain," *Lasers Surg. Med.* **43**, 892–900 (2011).
66. S. S. Stylli, "Photodynamic therapy of cerebral glioma – A review Part I – A biological basis," *J. Clin. Neurosci.* **13**, 615–625 (2006).
67. H. Hirschberg, "Disruption of the blood–brain barrier following ALA-mediated photodynamic therapy," *Lasers Surg. Med.* **40**, 535–542 (2008).
68. S. J. Madsen, "Site-specific opening of the blood-brain barrier," *J. Biophoton.* **3**, 356–367 (2010).
69. S. J. Madsen, "Increased nanoparticle-loaded exogenous macrophage migration into the brain following PDT-induced blood-brain barrier disruption," *Lasers Surg. Med.* **45**, 524–532 (2013).
70. S. J. Madsen, "Nanoparticle-loaded macrophage-mediated photothermal therapy: Potential for glioma treatment," *Lasers Med. Sci.* **4**, 1357–1365 (2015).
71. E. Angell-Petersen, "Influence of light fluence rate on the effects of photodynamic therapy in an orthotopic rat glioma model," *J. Neurosurg.* **104**, 109–117 (2006).
72. V. H. Fingar, "Vascular effects of photodynamic therapy," *J. Clin. Laser Med. Surg.* **14**, 323–328 (1996).
73. L. A. Sporn, "Photofrin and light induces microtubule depolymerization in cultured human endothelial cells," *Cancer Res.* **52**, 3443–3448 (1992).
74. S. S. Hu, "Effects of photodynamic therapy on the ultrastructure of glioma cells," *Biomed. Environ. Sci.* **20**, 269–273 (2007).
75. F. Celine, "Innovations of photodynamic therapy for brain tumors: Potential of multifunctional nanoparticles," *J. Carcinog. Mutag.* **8**, (2012).
76. A. J. Trinidad, "Combined concurrent photodynamic and gold nanoshell loaded macrophage-mediated photothermal therapies: An in vitro study on squamous cell head and neck carcinoma," *Lasers Surg. Med.* **4**, 310–318 (2014).

77. A. Aspelund, "A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules," *J. Exp. Med.* **212**, 991–999 (2015).
78. J. J. Iliff, "A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta," *Sci. Transl. Med.* **24**, 147ra111 (2012).
79. N. A. Jessen, "The glymphatic system: A Beginner's guide," *Neurochem. Res.* **40**, 2583–2599 (2016).
80. C. J. Lin, "Reactive macular edema and acute visual loss after photodynamic therapy on the same day of fluorescein angiography," *Oman. J. Ophthalmol.* **4**, 84–86 (2011).
81. M. Jarvi, "Insights into photodynamic therapy dosimetry: Simultaneous singlet oxygen luminescence and photosensitizer photobleaching measurement," *Biophys. J.* **102**, 661–671 (2012).
82. S. A. De Visscher, "mTHPC mediated photodynamic therapy (PDT) of squamous cell carcinoma in the head and neck: A systematic review," *Oral. Oncol.* **49**, 192–210 (2013).
83. O. V. Semyachkina-Glushkovskaya, "Laser-induced generation of singlet oxygen and its role in the cerebrovascular physiology," *Prog. Quantum Electron.* **7**, 1–17 (2017).
84. O. Semyachkina-Glushkovskaya *et al.*, "Photodynamic opening of blood-brain barrier," *Biomed. Opt. Express* (2017) (accepted).
85. O. Semyachkina-Glushkovskaya *et al.*, "Application of optical coherent tomography for in vivo monitoring of cerebral lymphatics during opening of blood-brain barrier," *J. Biomed. Opt.* (2017) (accepted).