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Review

Analyzing the blood–brain barrier: The benefits of medical imaging in research and clinical practice

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ABSTRACT

A dysfunctional BBB is a common feature in a variety of brain disorders, a fact stressing the need for diagnostic tools designed to assess brain vessels' permeability in space and time. Biological research has benefited over the years various means to analyze BBB integrity. The use of biomarkers for improper BBB functionality is abundant. Systemic administration of BBB impermeable tracers can both visualize brain regions characterized by BBB impairment, as well as lead to its quantification. Additionally, locating molecular, physiological content in regions from which it is restricted under normal BBB functionality undoubtedly indicates brain pathology-related BBB disruption. However, in-depth research into the BBB's phenotype demands higher analytical complexity than functional vs. pathological BBB; criteria which biomarker based BBB permeability analyses do not meet. The involvement of accurate and engineering sciences in recent brain research, has led to improvements in the field, in the form of more accurate, sensitive imaging-based methods. Improvements in the spatiotemporal resolution of many imaging modalities and in image processing techniques, make up for the inadequacies of biomarker based analyses. In pre-clinical research, imaging approaches involving invasive procedures, enable microscopic evaluation of BBB integrity, and benefit high levels of sensitivity and accuracy. However, invasive techniques may alter normal physiological function, thus generating a modality-based impact on vessel's permeability, which needs to be corrected for. Non-invasive approaches do not affect proper functionality of the inspected system, but lack in spatiotemporal resolution. Nevertheless, the benefit of medical imaging, even in pre-clinical phases, outweighs its disadvantages. The innovations in pre-clinical imaging and the development of novel processing techniques, have led to their implementation in clinical use as well. Specialized analyses of vessels' permeability add valuable information to standard anatomical inspections which do not take the latter into consideration.

Contents

1. Introduction 44
2. In vivo biomarkers for enhanced permeability 44
3. In vivo imaging and assessment of brain vessels' permeability 45
   3.1. Pre-clinical imaging modalities 45
      3.1.1. Direct optical imaging-BBB permeability assessment using fluorescence imaging 45
      3.1.2. Direct optical imaging – laser-speckle imaging for BBB permeability assessment 47
      3.1.3. Non-invasive techniques in pre-clinical imaging 47

Abbreviations: BBB, blood–brain barrier; EB, Evans-blue; PET, positron emission tomography; SPECT, single photon emission computerized tomography; MRI, magnetic resonance imaging; DCE, dynamic contrast enhanced; SNR, signal to noise ratio; Gd, gadolinium.

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3.2. Clinical imaging modalities 48
3.2.1. Analytical methods 48
3.2.2. BBB imaging examples 49
4. Summary 50
References 50

1. Introduction

The central nervous system (CNS) is highly affected by changes in its environment. To insure normal function of the neural system, transport of ions and molecules between the CNS and its supporting vascular system must be tightly regulated; keeping homeostasis within the neuropil. The concept of an anatomical separation between the blood and the brain arose in the late 19th century. A German bacteriologist named Paul Ehrlich observed that the intravenous administration of aniline dyes to small animals stained all organs but the brain. Ehrlich's student, Goldman, continued these experiments and injected Trypan blue to the cerebrospinal fluid (CSF) of rabbits and dogs and demonstrated staining of the entire brain without a trace of the dye in the blood stream [1]. These experiments brought about the recognition of a tight barrier between blood and brain environments, known as the blood–brain barrier (BBB). The BBB is a complex structure and functional mechanism underlying the specialized isolation of the CNS from its supporting vascular system. It is formed at the level of endothelial cells comprising the lumen of blood vessels in the CNS [2,3]. Endothelial cells of brain blood vessels are connected by tight junction protein complexes and junction adhesion molecules; these protein structures restrict para-cellular passage of molecules and force most molecular traffic to take place in a trans-cellular manner. Lipophilic or small gaseous molecules can diffuse freely through the cellular membrane; otherwise molecular passage requires specific transport mechanisms within the membrane. Hence the term: "selective isolation". Transport mechanisms include solute carriers for specific molecules, ATP binding cassette transporters, receptor-mediated and adsorptive-mediated transcytosis and cellular migration mechanisms [2]. All of these processes are tightly regulated by endothelial intra-cellular processes (e.g. gene translation). Additionally, regulation of the BBB phenotype extends beyond the endothelial cell. Brain blood vessels are innervated by neurons, astrocytes, pericytes/smooth muscle cells and microglia. These cell–cell interactions form the neurovascular unit and induce cellular processes that determine specific features of the BBB phenotype.

A dysfunctional BBB is a feature of a variety of neurological disorders Such as traumatic brain injury, stroke, cancer, epilepsy and neurodegenerative diseases [4–7]. In stroke for instance, the damage caused to endothelial cells due to ischemia is suggested to result in formation of reactive oxygen species (ROS) leading to an abnormal ion flux, extravasation of proteins and subsequent brain edema [8]. Studies have also shown disruption of tight junction complexes in human gliomas and metastatic adenocarcinomas [5]. Additionally BBB breakdown has been shown to be associated with epilepsy either as a cause or as a consequence [6,9–11]. Seizures are observed in cases of brain insult such as traumatic brain injury and central nervous system infections, conditions known to result in compromised BBB [6,12,13]. Additionally, it has been shown that seizure activity results in BBB impairment [9].

The major role of BBB dysfunction in brain disease has raised the need for accurate and sensitive diagnostic tools that would assess the level of BBB permeability and provide information regarding degradation of brain tissue in pathology.

This review aims to survey past and present diagnostic modalities, emphasizing present day imaging techniques employed in pre-clinical research and in clinical use as well. We present here methods in optical, magnetic resonance and nuclear imaging, previously published and validated, both in-house as well as by others. These imaging platforms provide highly sensitive and reliable tools for BBB permeability assessment.

2. In vivo biomarkers for enhanced permeability

A simple approach for detecting BBB disruptions in vivo is by post mortem visualization of BBB impermeable tracers within brain tissue. The tracers may be substances systemically administered to the anesthetized animal or normal plasma/brain content that is restricted from brain parenchyma/plasma respectively, when the BBB is intact. Methods, in clinical and pre-clinical use, range between those enabling qualitative assessment alone and those allowing quantitative analysis as well.

Systemic administrations of non-BBB permeable tracers, possessing physical properties such as fluorescence, radioactivity, etc., that enable their detection, are commonly used in pre-clinical studies to asses BBB leakage through accumulation of tracer residues in extra vascular tissue. A qualitative macroscopic analysis exemplifying this approach can be found in Fig. 1A/B; following intravenous injection of Evans-blue (EB, 2% in 0.9% NaCl, 2.4 ml/kg), the animal is sacrificed in an open-heart surgical procedure, in which paraformaldehyde (PFA, 4% in phosphate buffered saline) is administered to the cardiovascular system. PFA fixates the brain, which is then extracted for further analysis. EB binds to the serum protein albumin and therefore is BBB impermeable [14]. Thus, blue stains in fixated brains and brain slices indicate local disruption to the BBB (Fig. 1A/B). For more sensitive evaluations, quantitative analysis is required. Given that EB-albumin is fluorescent, the use of spectrophotometers can be applied in order to measure fluorescence intensity and subsequent tracer concentration within brain parenchyma [15]; as was performed by Asahi et al. in attempt to measure BBB permeability in mice following induction of cerebral ischemia [16]. Briefly, extracted brain samples are frozen, homogenized in buffer solution and finally centrifuged. The supernatant is then excited at the appropriate wavelength and the emission is read. The ratio of emission to excitation light intensities can be correlated to substance concentration [17] and therefore the level of extra-vascular EB, reflecting BBB permeability level, can be evaluated. However, this simple calculation is not informative when the dynamic features of molecular passage through the BBB are to be assessed. For that end, there are approaches employing multi-compartmental mathematical models in which unidirectional or bidirectional passage between compartments (representing vascular and extra-vascular regions) is applied under restricting factors [18]. Intra and extra-vascular tracer concentrations are evaluated using physical techniques as the one previously mentioned, and are placed in the model as input. Resolving the model benefits numerical constants reflective of tracer passage.

Physiological markers for BBB damage are abundant. The focus of this approach has been primarily on proteins given the variety of mechanisms by which they penetrate the BBB.

Detection of plasma proteins, normally restricted from the brain by the BBB, in brain parenchyma is a valuable tool for BBB dysfunction diagnosis. An example of such an approach is immunostaining against serum proteins done post mortem. Van Vliet et al. applied