Imaging of Neuro-Inflammation

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Neuro-inflammation is a pathologic hallmark of many brain disorders. Inflammation is the primary feature in inflammatory disorders (multiple sclerosis (MS), acute disseminated encephalomyelitis (ADEM), Devic’s disease) or infectious disorders (encephalitis). Secondary inflammatory reactions can also be found after a wide variety of brain insults such as stroke, brain trauma or even brain tumors.

Histologically, a common feature of all inflammatory reaction subtypes is the modulation/alteration of the blood brain barrier (BBB). Many agents released during inflammation increase the permeability of the brain endothelium to serum proteins and water, leading to vasogenic edema. BBB breakdown further facilitates an influx of white blood cells into the surrounding brain parenchyma [1, 2].

1. Brain edema

Brain edema is defined as an increase in brain volume resulting from a localized or diffuse abnormal accumulation of fluid within the brain parenchyma [3]. It is classically subdivided into vasogenic and cytotoxic types (first described by Igor Klatzo) [4].

Vasogenic edema is associated with BBB dysfunction, which allows an increase in the passage of plasma proteins and water into the extracellular compartment [3, 5]. This is the main type of edema associated with inflammation and will be the main focus of this thesis. In hydrocephalus, a rise in intraventricular pressure causes the cerebro-spinal fluid (CSF) to migrate through the ependyma into the periventricular white matter. The resulting increase of fluid into the periventricular white matter is quite similar to vasogenic edema but differs because it has the same composition as CSF without serum proteins. It is known as hydrocephalic edema [3, 5].

Cytotoxic edema, which is also known as cellular edema, results from abnormal water uptake by injured cells [3, 5]. Astrocytes, which outnumber neurons 20:1 and can swell up to five times their normal size, are the main cells involved in cytotoxic edema [6]. The most common cytotoxic edema occurs in cerebral ischemia, in which a failure of the ATP-dependent Na⁺ pumps results in intracellular Na⁺ accumulation with a shift of water from the extracellular to the intracellular compartment to maintain osmotic equilibrium [6].
This edema subtype is beyond the scope of this thesis. In many clinical situations, there is a combination of different types of edema depending on the course of the disease.

Magnetic resonance imaging (MRI) can provide quantitative maps (at a millimetric or sub-millimetric resolution) of water molecular diffusion \textit{in vivo} through the diffusion-weighted imaging (DWI) technique [7, 8]. A global parameter, the apparent diffusion coefficient (ADC), is obtained from the integration of all the microscopic displacement distributions of the water molecules present in a voxel and allows inference to be made about the microstructure of the tissue [7]. While the precise underlying mechanisms explaining ADC variations are still debated, it is known that changes in the volume fractions of the intra and extracellular spaces (\textit{i.e.}, cytotoxic or vasogenic edema) always lead to variations in the ADC [9]. Consequently, ADC measurements provide \textit{in vivo} quantitative information related to edema severity, including information on the predominant subtype (\textit{i.e.}, either cytotoxic or vasogenic) and its location at a millimetric scale, with the possibility of follow-up by non-invasive repeated exams.

2. Blood Brain Barrier (BBB) and its breakdown

2.1. The normal BBB

The BBB is a selective barrier that lines cerebral microvessels. The barrier is formed by capillary endothelial cells surrounded by basal lamina and astrocytic perivascular endfeet. Astrocytes provide the cellular link to the neurons forming the neurovascular unit. Pericytes are also associated with the outer surface of capillaries at the BBB level [2, 10] (Figure 1).

Structural features limiting the permeability include (i) circumferential tight junctions between adjacent endothelial cells and (ii) a much lower degree of endocytosis/ transcytosis as defined by fewer caveolae or plasmalemmal vesicles than in the peripheral endothelium [11].

\textit{Figure 1:} The cellular constituents of the BBB are shown in a figure from Abbott et al., Nature Reviews Neuroscience [2]. The perivascular endfeet of the astrocytes are closely opposed to the outer surface of the brain microvessels and have specialized functions in inducing and regulating the BBB.
2.2. BBB “breakdown” and edema formation

Following inflammation or many insults, signals and molecular cascades lead to an increase in BBB permeability, which is the main mechanism by which serum proteins and water enter the interstitial space (vasogenic edema formation). This BBB breakdown can be assessed by tracers. In animal experiments, the extravasation of administered Evans blue dye, horseradish peroxidase (HRP) or the immunodetection of endogenous serum protein extravasation can be used to assess BBB permeability [12]. In vivo, gadolinium-diethylene-triamine-pentaacetic-acid (Gd-DTPA) is the current standard for visualizing BBB breakdown by MRI in humans. Alternatively, other MR contrast agents, such as Gadofluorine M, a gadolinium-based contrast agent with high binding affinity to plasma albumin and extracellular matrix proteins, have also been used in animal studies to assess BBB permeability with a higher sensitivity than gadolinium [13, 14]. These tracers are thought to reflect the extravasation of serum proteins and subsequent water [12].

2.3. BBB “breakdown” and leukocyte infiltration

Breakdown of the BBB can also facilitate an influx of white blood cells into the surrounding brain parenchyma [1]. Nevertheless, cellular infiltration into the central nervous system is not simply the consequence of the breakdown of the BBB but requires additional signaling. Most leukocytes can even cross the BBB transendothelially without the disruption of the tight junctions. Leukocytes migrate and cross the endothelial barrier (i.e., diapedesis) by transmigrating through individual endothelial cells via a transcellular pore [15]. In a murine model of MS, most leukocytes crossed the BBB transcellularly while tight junctions were intact [16]. The very early step of endothelial activation prior to leukocyte invasion has been visualized in vivo with an anti-VCAM antibody conjugated to large iron oxide particles [17]. Another strategy is based on ex vivo iron oxide labeling of inflammatory cells [18] for tracking by MRI after cell transfer [19]. An easier approach is based on the systemic administration of superparamagnetic iron oxide particles (see Box 1).

**Box 1: Superparamagnetic iron oxide particles**

Superparamagnetic MR contrast agents are nanoparticles consisting of a core of insoluble iron oxides, which is solubilized by coating with hydrophilic polymers. Superparamagnetic iron oxides (SPIO) contain several iron oxide crystallite cores with an overall particle size of 50-150 nm, while ultra-small superparamagnetic iron oxides (USPIO) are monocrystalline with a smaller hydrodynamic diameter (20 nm on average). These nanoparticles have in common their specific uptake by the monocyte-macrophage system and can be used to track macrophage infiltration by MRI after systemic injection due to the high r1 and r2 relaxivities of the iron-loaded macrophages [19-21].

Approximately 24 hours after their intravenous injection, free SPIO/USPIO are cleared from the circulation, and signal alterations are thought to arise from the capture of particles by phagocytic cells [22], which allows the the cellular component of inflammation to be visualized more specifically than with Gd. Animal models of MS have shown that the signal alterations observed after SPIO/USPIO administration were correlated with the intracellular localization of iron oxide particles in cells with the typical morphologic features of macrophages [23]. Furthermore, a mismatch between Gd- and USPIO-enhanced lesions demonstrated that the BBB leakage shown by Gd enhancement and the cellular infiltration shown by USPIO enhancement can be distinct [24-26]. The first human studies with USPIO in MS patients have also
reported discrepancies between the BBB leakage demonstrated by Gd enhancement and the cellular infiltration shown by USPIO enhancement [27-30]. In this interpretation, it should be noted that, although USPIO-enhancements most probably reflect the entrance into the central nervous system of labeled monocytes that had taken USPIO in the circulating blood, the passage of some free nanoparticles over a damaged BBB followed by macrophage/microglia uptake cannot be excluded [31].

3. Resolution of edema

Edema resolution

Water can be cleared back into (i) the blood or (ii) the CSF in the subarachnoid space or (iii) the ventricles. At these potential routes of exit, water has to cross intact membranes and barriers (restored BBB, glia limitans, ependyma). While water passage through the lipid bilayer by mere diffusion is very limited, these passages were found to be highly facilitated by a specific water channel protein called aquaporin 4 (AQP4) [32].

Additional References from 33 to 37 help in understanding the role of imaging technique to assess and measure brain edema and cellular imaging.

4. Figures

The figure shows regional differences in edema accumulation, which is predominant in the WM (A) (spreading through the WM tract; here the splenium of the corpus callosum, arrows) while sparing the GM (arrowheads). Such WM/GM differences are well-recognized for MS lesions, in which WM lesions can show signs of activity (Gd enhancement and blurry T2/FLAIR hypersignal at the periphery in relation to the vasogenic edema; arrows in B). On the contrary, GM lesions show little inflammation and little edema and require a more sensitive sequence for identification (such as double inversion recovery, C).

The figure shows three MS lesions that were enhanced a few minutes after Gd injection on T1-wi (arrow and dotted arrows). One lesion was also enhanced 24 hours after USPIO injection and was called “USPIO+Gd” (arrow), as opposed to the “Gd-only” lesions (dotted arrows) and the “USPIO-only” lesions (not shown).
REFERENCES