The effects of blood–brain barrier disruption on glial cell function in multiple sclerosis

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Abstract

Dysfunction of the BBB (blood–brain barrier) is a major hallmark of MS (multiple sclerosis). Studies in our laboratories over the last decade have shown that increased BBB permeability is associated with decreased expression of TJ (tight junction) proteins in brain capillary endothelial cells. Results have revealed that TJ abnormalities were most common in active lesions (42% of vessels affected), but were also present in inactive lesions (23%) and in MS normal-appearing white matter (13%). Importantly, TJ abnormality was also positively associated with leakage of the serum protein fibrinogen which has recently been shown to be an activator of microglia. TJ abnormality and the resultant vascular permeability in both lesional and non-lesional white matter may impair tissue homeostasis, which may have effects on disease progression, repair mechanisms and drug delivery.

Introduction

MS (multiple sclerosis) is an autoimmune demyelinating disease of the central nervous system associated with the development of focal lesions throughout the neural axis. BBB (blood–brain barrier) leakage has been recognized at the histological level in MS tissue since the early 1960s [1] and clinically since the advent of CT (computed tomography) and MRI (magnetic resonance imaging) in the 1980s [2]. Increasing evidence shows that BBB impairment and leakage in MS extends well beyond the CELs (contrast-enhancing lesions) which are associated with acute inflammation and demyelination. Resolution of CELs is often considered a surrogate for BBB restoration either naturally or as a result of drug therapy. Lesions are invariably centred on blood vessels, and these become inflamed and leaky from an early stage [3]. Moreover, both MRI and confocal microscopic studies point to BBB disturbances in NAWM (normal-appearing white matter) in MS tissue that precede the formation of demyelinating lesions and also in inflammatory silent lesions [4–8].

Key words: blood–brain barrier, fibrinogen, glia, imaging, multiple sclerosis (MS), tight junction.

The main function of TJs is to restrict the paracellular passage of molecules through the BBB and to maintain endothelial polarity [9]. Compromised BBB TJs are recognized as hallmarks of neuroinflammatory and other CNS (central nervous system) diseases. Dysfunctional TJs and subsequently impaired endothelial function allow more cells and immunologically active molecules access to the privileged CNS.

Microscopical data

In our laboratories, we have completed extensive human tissue-based studies using significant numbers of snap-frozen MS and control tissue samples [9–12]. These studies have revealed aspects of the pathological status of the BBB in active and inactive white matter lesions, in NAWM and in grey matter in the two most prevalent clinical presentations of MS: PPMS (primary progressive MS) and SPMS (secondary progressive MS). To achieve this, we have developed robust protocols for the immunocytochemical demonstration and assessment of proteins [ZO-1 (zonula occludens 1), occludin, junctional adhesion molecule-A] that are expressed in the TJs of the vascular endothelium. We also used dual-labelling to systematically assess the relationship between the expression of TJ proteins and leaked plasma protein (fibrinogen) in the different categories of MS tissue.

Studies of histologically well-characterized white and grey matter samples in acute, PPMS and SPMS revealed endothelial abnormalities associated predominantly with active lesions (affecting 40% of vessels) and, to a lesser extent, inactive lesions (affecting 23% of vessels in SPMS and 37% of vessels in PPMS) and MS NAWM (13%). TJ abnormalities were more frequent in the normal-appearing grey matter in SPMS (23%) than in PPMS (10%). Vessels of all sizes were affected equally, supporting a causal role for diffusible inflammatory mediators. In active lesions, dual-labelling showed that 41% of vessels with severe TJ alterations showed pronounced fibrinogen leakage. In both NAWM and inactive lesions, the trend towards vessels with

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increased TJ abnormality showing increased fibrinogen leakage, although less pronounced, was also present.

Discussion

The findings of our studies point to a loss of BBB phenotype and to mechanisms of leakage. We propose that TJ abnormality, which leads to BBB leakage, is a significant form of tissue injury in MS, alongside demyelination and axonopathy. Furthermore, our studies indicate that, apart from the high levels of TJ abnormalities present in active MS lesions, the increased level of TJ abnormality in inactive lesions may be a key factor in impeding BBB repair which, in turn, can have effects on glial cell functions, axonal conduction and the efficiency of remyelination, even when inflammation has abated.

The association of TJ abnormalities with increased fibrinogen leakage may have important implications for lesion initiation through interactions with microglia. Previous publications, including studies that correlate MRI with histology, find fibrinogen deposition to be one of the earliest events associated with MS lesion formation [13,14]. Experiments using fibrinogen-knockin mice have shown fibrinogen to be an activator of microglia, the resident immune cells of the CNS [15]. Activation of microglia appears to be sufficient to induce inflammatory demyelination, even in the absence of T-cells [16], and it has been suggested that focal areas of microglial activation represent an early stage of tissue injury in MS which precedes the formation of the hypoxia-like demyelinated plaques [14]. In our studies, the trend in MS NAWM for vessels with increasing TJ abnormality to show fibrinogen leakage could be suggested to be more important than first realized, as fibrinogen could function to mediate the initial activation of resting microglia leading to increased phagocytosis [17].

Glia and endothelial cells are known, from in vitro studies, to cross-talk with each other and to regulate each other’s function [18]. It would therefore be expected that barrier disruptions such as we have demonstrated to occur in MS could have effects on glial cell function. In this respect, the high levels of TJ disruption that we have found in inactive lesions in PPMS can be implicated in impeding remyelination of surviving demyelinating axons [19]. It is known that remyelination is not limited by the absence of oligodendrocyte progenitor cells that are present in abundance in late-stage lesions [20]. However, persistent TJ alterations on cerebral vessels with associated leakage in inactive lesions would adversely affect CNS homoeostasis and promote astrocytic gliosis. Such a process can include ensheathment and sequestration of demyelinating axons, rendering them inaccessible to oligodendrocyte progenitors [21]. Other in vivo and in vitro studies support the possibility that leakage of basement membrane proteins, such as fibronectin, across a disrupted BBB may influence myelin formation on oligodendrocyte progenitors by interference with fundamental cellular events such as polarized membrane-directed transport [22,23].

It is important to establish whether drugs that have effects on CELs also restore BBB function in inactive lesions and NAWM where inflammation is diminished, but where subtle leakage may affect glial cell functions and, consequently, the demyelinating or remyelinating processes which are so important in MS. The persistent endothelial abnormality with leakage that we and others have described may have a different pathogenesis with adverse affects on CNS homoeostasis (Figure 1). Moreover, the repair of persistent TJ abnormalities may require a different therapeutic approach from that adopted in acute lesions. Behind a restored BBB, the goals of neuroprotection and remyelination may be more effectively pursued.

References


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