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Radiolabeled annexin V imaging: A useful technique for determining apoptosis in multiple sclerosis

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ABSTRACT

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) that involves myelin, oligodendrocytes and axons and culminates in consecutive neuronal death and progressive neurologic disability. Based on magnetic resonance imaging (MRI), neuroaxonal loss in MS results in brain atrophy and has a strong correlation with neurological disability.

The newer MR imaging tools seem to be sensitive biomarkers for measuring the pathogenetic processes associated with disease activity and progression. However, they are unable to detect apoptosis in neurodegenerative diseases.

Annexin V has a high affinity for phosphatidylserine (PS) that presents on the outer surface of the plasma membrane early on during the onset of apoptosis. Radiolabeled annexin V imaging may reveal the initiation and degree of neuronal apoptosis.

We propose that radiolabeled annexin V imaging is a useful modality in determining apoptosis in MS and can assess and monitor the effectiveness of neuroprotective and immunomodulatory therapies on the clinical course of MS.

Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) that involves myelin, oligodendrocytes and axons and culminates in consecutive neuronal death and progressive neurologic disability [1,2]. Histopathological work-ups have demonstrated apoptotic neuronal cell death in demyelinated cerebral cortex lesions of MS patients [3] that cannot be identified by magnetic resonance imaging (MRI). It is difficult to monitor apoptotic neurons in the human brain in real time because of the rapid kinetics of apoptosis-related intracellular signaling cascades. Therefore, much of the current knowledge about apoptotic neuronal cell death in MS comes from studies involving experimental autoimmune encephalomyelitis (EAE) models [4,5].

Annexin V, a marker of phosphatidylserine (PS) expression, is a useful means of detecting early apoptosis. In positron emission tomography (PET) or single photon emission computed tomography (SPECT), radiolabeled annexin V can be used to investigate the apoptosis process in both animal and human models [6]. This article reviews the pathology of neuronal loss in MS, the role of apoptosis in this process and its importance in neurological disability. We propose that radiolabeled annexin V imaging is a useful technique in determining apoptosis and monitoring the effect of neuroprotective and immunomodulatory therapies on the clinical course of MS.

Atrophy and disability

Neurologic disability in patients with MS has been associated with atrophy in the brain and spinal cord [7,8]. Brain atrophy is observed when clinical symptoms appear and is noticeable in secondary progressive (SP) MS compared to relapsing remitting (RR) MS [9,10]. Gray matter (GM) atrophy is an important indicator in irreversible disability and is observed predominantly in progressive and chronic forms of MS [11]. On the other hand, early cortical involvement observed in imaging modalities raises the intriguing possibility that the GM may represent the primary and initial targets of the disease process, leading to axonal degeneration and subsequent demyelination [12].

Cause of atrophy

The amount of tissue loss in MS may represent a balance between a number of pathological processes: irreversible axonal and myelin loss on one hand, with partial compensation by inflammation-associated cellular infiltrates, and cellular (including axonal) and interstitial edema on the other hand [11].

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Brain atrophy may derive from antrograde or retrograde neuroaxonal tract degeneration associated with focal white matter (WM) inflammatory lesions, as well as a process directly targeting neurons and myelin, including cortical demyelination and glia [11]. Axonal loss is a possible contributor to atrophy in MS, although demyelination and reduced axon diameter may also shrink tissue volume [13].

Neuroaxonal loss

Axonal damage in MS could result from an immunologic attack, inflammatory mediators or the secondary effects of chronic demyelination [14]. It appears that axonal loss begins at the onset of the disease and remains subclinical during RRMS. The switch from RRMS to progressive MS could therefore occur when a threshold of neuronal or axonal loss is achieved or when adaptive responses of the CNS are exhausted [15]. Peterson et al. identified transected axons, transected dendrites and neuronal cell death in cortical MS lesions in patients aged two weeks to 27 years with clinical disease [3]. In addition, Lovas et al. described about a 57% reduction in axonal density of normal-appearing white matter (NAWM) from cervical spinal cords in SPMS patients [16].

Diminished *N*-acetylaspartate [17] and progressive CNS atrophy [8] seen on MRIs are noninvasive indicators of axonal loss in MS patients. Axonal damage is probably caused by proteolytic enzymes, cytokines and free radicals mainly generated by activated immune and glial cells in an inflammatory response [18]. It has been suggested that in the course of EAE, a lymphocyte attack may play a role in both oligodendrocyte and neuron degeneration, as happens with motoneurons in the spinal cord [19]. Alternatively, an inflammatory axonal transection in WM and the cortex might initiate a retrograde degeneration of neurons and apoptosis and a Wallerian degeneration distal to the site of the transaction [15].

Apoptosis and evidence

Apoptosis is an important feature of nervous system development and appears to play a role in several neurodegenerative diseases, such as amyotrophic lateral sclerosis, Parkinson's disease and Alzheimer's disease [20]. Neuronal apoptosis occurs in the EAE model and in MS plaques that involve both spinal cord and cerebral cortical GM [3,21]. Peterson et al. combined a terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay and neurofilament immunocytochemistry (ICC) to investigate neuronal apoptosis in 22 MS lesions from seven patients. They found apoptotic neurons in 56% of the chronic active lesions and in 45% of the chronic inactive lesions, but not in the active lesions. Most of the apoptotic neurons were large and pyramidal and were located in cortical layers 3 and 5. Since apoptotic neurons are cleared within 24 h, neuronal loss by apoptosis could be very significant and cumulate over time [3].

Apoptosis source

Cerebrospinal fluid (CSF) from MS patients provokes apoptotic neuronal death in cell cultures, indicating that apoptosis results from immunomodulatory and inflammatory mediators during demyelination [22,23]. Axonotmesis as a result of inflammation in MS lesions can also lead to retrograde neuronal degeneration and apoptosis [15].

After demyelination in the WM, alterations in the distribution of sodium channels occur along the demyelinated axons that are positive for amyloid precursor protein (a marker for axonal damage). Although this redistribution of ion channels improves the functionality of injured axons, it demands higher cellular adenosine triphosphate (ATP); therefore, it increases the energy expenditure of neuronal conduction, with concurrent abnormalities found in mitochondrial functioning. Higher energy demands and hampered mitochondrial functions make demyelinated axons more susceptible to hypoxic damage, which ultimately results in neuroaxonal degeneration [2].

Apoptosis detection

Apoptosis is preceded by the selective exposure of PS on cell surfaces. All cells, including neurons, rapidly redistribute PS, an anionic constitutive membrane lipid, from the inner to outer leaflets of the plasma membrane shortly after the onset of apoptosis [22]. In contrast to the other lipids, PS is restricted to the inner leaflet of the cell membrane as a result of the action of translocase and floppase enzymes. When the caspase cascade is activated in an apoptotic cell, the above-mentioned enzymes are inactivated (Fig. 1) [22,24,25].

Annexin V is normally found in the cytoplasm of a variety of cells that have a high affinity for cell surface-exposed PS [26]. Fluorescent or biotin-labeled annexin V are the most widely used nonnuclear modalities in in vitro detection of apoptosis. An alternative approach uses radiolabeled forms of annexin V for PET or SPECT to

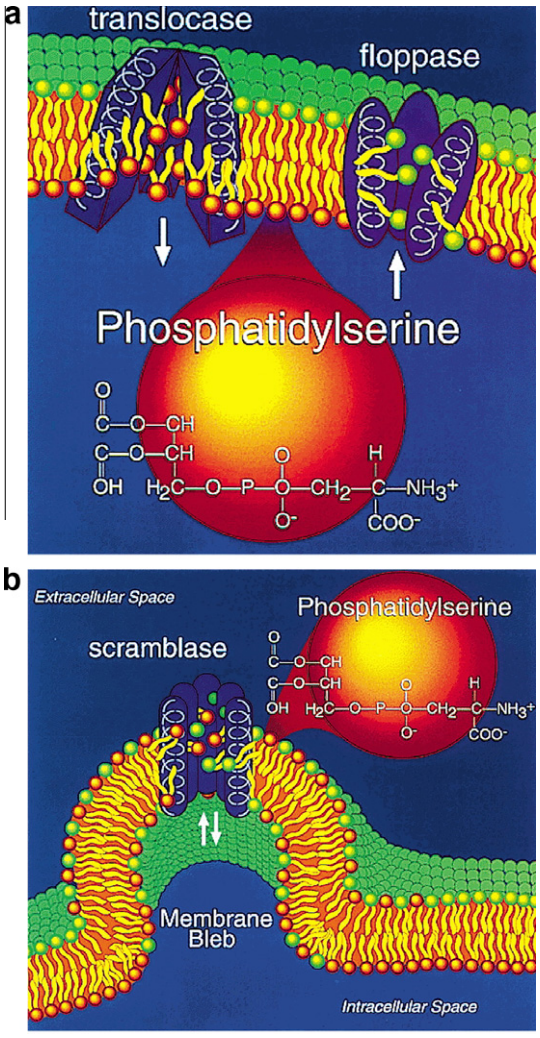

Fig. 1. Maintaining normal membrane polarity by translocase and floppase activity and expression of phosphatidylserine due to scramblase activation (Nucl Med Commun. 2000; 21(3):241–250 with permission).[22].

identify apoptosis in vivo [22]. Organ uptake values indicate that Tc-99m-annexin is accumulated mainly in the kidneys, liver and spleen [27]. Since uptake in the normal brain is negligible, it is an ideal tracer for apoptosis detection in the brain [28]. Recent investigations have shown that neurons in regions of augmented annexin V uptake with low to moderate levels of PS expression were under stress and experienced reversible apoptosis, whereas regions with high levels of PS expression have irreversible apoptosis and dying neurons [29].

In comparison of PET using fluorine-18 and 124 I labeling of annexin V with SPECT using 99mTc-annexin V, the uptake of PET radiotracers in the liver, spleen and kidney is lower than 99mTc-annexin V [30,31]. Therefore, radiolabeled annexin V with PET radiotracers facilitates better apoptosis imaging in the abdominal region relative to most forms of 99mTc-annexin V [30,31]. In addition, because of higher contrast and spatial resolution of PET system rather than SPECT imaging, radiolabeled annexin V with PET radiotracers results to better detection and quantitation of the apoptotic lesions [22].

Blankenberg et al. investigated if technetium-99m (99mTc) conjugated with annexin V could identify hypoxic/ischemic cerebral reperfusion injury (HII) in animal model imaging. As early as 2 h following reperfusion, radiolabelled annexin V images revealed a two to threefold increase in annexin V uptake in the involved hemisphere compared with the normal contralateral hemisphere. There was no evidence of blood brain barrier (BBB) breakdown following reperfusion, as demonstrated by contemporaneous 99Tcm-DTPA imaging [22]. D'Arceuil et al. also showed that 99mTc-HYNIC-annexin V imaging could identify neuronal apoptosis within 2 h after the reversal of hypoxia in an animal model of global cerebral ischemia [32]. Lampl et al. also evaluated the feasibility of PS imaging in the brains of patients with Alzheimer's disease using 99mTc-HYNIC-annexin V SPECT [33].

In dosimetric assessment, use of 99mTc-annexin V to patients develops an effective dose of 7.6 ± 0.5 microsievert/megabecquerel (μSv/MBq), congruent with a total effective dose of 4.6 ± 0.3 millisievert (mSv) for a typical patient dose of 600 MBq [27] which are in the lower range of values reported for commonly used 99mTc compounds [34]. It reflects that it is a safe radiopharmaceutical with an acceptable radiation dose [35]. Therefore, this point is an important value when serial monitoring of apoptosis in the same patient is needed.

Hypothesis. Radiolabeled annexin V imaging can be a determinate of apoptosis in MS.

Radiolabeled-annexin V imaging may also determine apoptosis in MS patients. As discussed above, MS pathology involves inflammatory demyelination and neurodegeneration. The latter, arising from apoptosis, is considered the main cause of disability in MS patients. These newer tools seem to be sensitive biomarkers for measuring the pathogenetic processes associated with disease activity and progression, but they are not capable of identifying programmed neuronal death. However, radiolabeled annexin V imaging may be a determinate of the beginning and degree of neuronal apoptosis.

Potential implications

Although immunomodulatory therapies such as beta-interferon have shown efficacy in reducing new WM lesion formations in MS patients, the degenerative phase of the disease may not be fully interrupted and their effect in reducing atrophy, as a marker of neurodegeneration, has been modest [10,11]. Research now focuses on a new class of treatments, such as Epo [36] or ciliary neurotrophic factor [37] and even glatiramer acetate [38], that target neuroprotection rather than a reduction of inflammation. Brain atrophy is frequently used as an outcome measure in treatment trials and is estimated using conventional MRI metrics [39]. However, this technique is unable to directly identify the apoptosis process in this neurodegenerative disorder. Radiolabeled-annexin V seems to be helpful in identifying disease activity and the efficacy of the neuroprotective agents.

Conflict of interest statement

The authors declare that they have no conflict of interests.

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