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Experimental Research Article

Targeting of demyelinated lesions in experimental models of multiple sclerosis using a fibrin binding peptide

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ABSTRACT

Introduction: Multiple sclerosis (MS) is an autoimmune and inflammatory disease of the central nervous system characterized by demyelination, astrogliosis, blood-brain barrier (BBB) disruption, and axonal damage. Currently available clinical and experimental approaches for MS are not completely effective because of the inability to pass the BBB, off-target distribution, and the need to increase the dosage. It has been shown that BBB disruption and fibrin deposition occur in the region of lesions in MS. Coagulation factor XIII binds to fibrin through the heptapeptide NQEQVSP in its structure. Here, NQEQVSP was used to target the lesions in animal models of MS.

Methods: Microglia, astrocytes, and oligodendrocytes were immuno- stained using antibodies against Iba1, GFAP, and MBP, respectively.

Results: We showed that peptide NQEQVSP binding was specific to the lesion sites induced by lysophosphatidylcholine (LPC) injection into the corpora callosa of mice. The peptide was FAM labeled, and its co-localization with different glial cells was evaluated. Co-localization was mainly detected with the microglia in the damaged area. A lower level of co-localization was also observed for oligodendrocytes.

Conclusion: Our results promise the possibility of using NQEQVSP peptide to target the lesions for specific drug delivery in patients suffering from multiple sclerosis.

Keywords:
Targeting Peptide
Fibrin
Targeted delivery
Glial cells
Multiple sclerosis

Introduction

Multiple sclerosis (MS) is a chronic autoimmune and inflammatory demyelinating disease of the central nervous system (CNS). MS usually occurs in young adults between 20-40 years of age, with a higher prevalence in females (Ford 2020). There is no cure for MS. However,

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immunosuppressive drugs and symptomatic treatments are used to improve the quality of life of patients (Goldenberg 2012). Systemic administration of therapeutics leads to off-target distribution, resulting in unfavorable side effects, reduced efficacy, and the need to increase the dosage for beneficial therapeutic effects (Aguilar 2012). Targeted delivery systems may overcome these limitations by accumulating therapeutics in diseased tissues through overexpressed molecules at the site of damage (Jiang et al., 2019). Hence, peptides are promising because of their lack of immunogenicity, low cost, small size, and easy synthesis (Aguilar 2012).

It has been shown that blood-brain barrier (BBB) disruption leads to fibrin deposition, a major component of the blood clot, in the CNS (Absinta et al., 2020). Fibrin deposition is abundant in many disorders, including stroke, Alzheimer's disease, glioblastoma, spinal cord injury, and bacterial meningitis (Ryu et al., 2015). In addition, fibrin deposition has been reported in all MS lesions (Ryu et al., 2015) and in animal models of MS, such as EAE (Davalos et al., 2012), LPC (Seyedsadr et al., 2019), and Theiler's murine encephalomyelitis virus (Inoue et al., 1997). Coagulation factor XIII binds to fibrin through seven peptides with NQEQVSP sequence in its structure (Schense et al., 2000). Therefore, this peptide was selected for targeting the lesion area in this study.

Recently, we conducted a study on the use of gold nanoparticles for targeted therapy in MS. The research focused on relevant therapeutic properties of gold nanoparticles and delivering them to the MS lesions, aiming to enhance treatment efficacy and reduce side effects associated with systemic administration. The study demonstrated the potential of gold nanoparticles as a therapy in MS by functionalizing the nanoparticles with a specific peptide that can bind to nidogen molecules overloaded in MS lesions. We were able to achieve precise delivery of therapeutic agents to the site of damage and improve the treatment outcomes (Farhangi et al., 2023).

Here, we showed that the heptapeptide NQEQVSP could target glial cells at injured sites in lysophosphatidylcholine (LPC)-induced demyelinated brain tissues with remarkable inflammation. However, the peptide did not show a noteworthy binding to the demyelinated area in cuprizone (CPZ)-treated mice.

Materials and Methods

Animals

Adult male C57BL/6J mice (8-weeks old) were purchased from Pasteur Institute (Karaj, Iran). All mice were kept under a 12 h light/dark cycle with ad libitum access to water and food. All experimental procedures were performed in accordance with the international guidelines for animal studies and approved by the Ethical Committee for Animal Research, Tarbiat Modares University, Tehran, Iran (approval number: IR.MODARES.REC.1397.018).

Induction of demyelination

Cuprizone model: Cuprizone produces toxic demyelination that resembles the demyelination aspect of MS disease. To induce demyelination, mice received 0.2% cuprizone (Sigma-Aldrich, St Louis, MO) in their chow for 12 weeks (Farhangi et al., 2019). This procedure led to extensive demyelination with rare consequent remyelination.

Lysophosphatidylcholine (LPC) model: Mice were anesthetized by intraperitoneal injection of ketamine (70 mg/kg; Alfasan, Woerden, The Netherlands) and xylazine hydrochloride (10 mg/kg; Alfasan), and placed in a stereotaxic frame (Stoelting, Wood Dale, IL, USA). Using a Hamilton syringe, 1 µl of LPC 1% (Sigma, St. Louis, USA) was slowly injected into the corpus callosum (CC) at the following coordinates: Anterior/Posterior (AP): -1 mm from bregma, medial/lateral: +0.7 mm, dorsal/ventral: -1.6 mm from the dura surface. Injection was performed within 5 min, and the needle was kept in place for an additional 5 min to prevent the possible solution reflux through the needle track. Mice were sacrificed at 3, 7, and 14 days post-LPC injection (dpi, 3 mice/group).

Brain sectioning

At the time of brain sampling, mice were deeply anesthetized and perfused with 0.1 M PBS followed by 4% paraformaldehyde (PFA) in 0.1 M PBS. Tissues were immediately harvested and post-fixed overnight in 4% PFA at 4 °C for an additional 24-48 h, then cryopreserved in 30% sucrose solution (in PBS) for 48 h at 4 °C. The brains were embedded in optimal cutting temperature (OCT) compound (Bio Optica, Italy) and frozen. Coronal brain sections (8 µm-thick) were prepared using a cryostat microtome (Histo-line, Italy) and stored

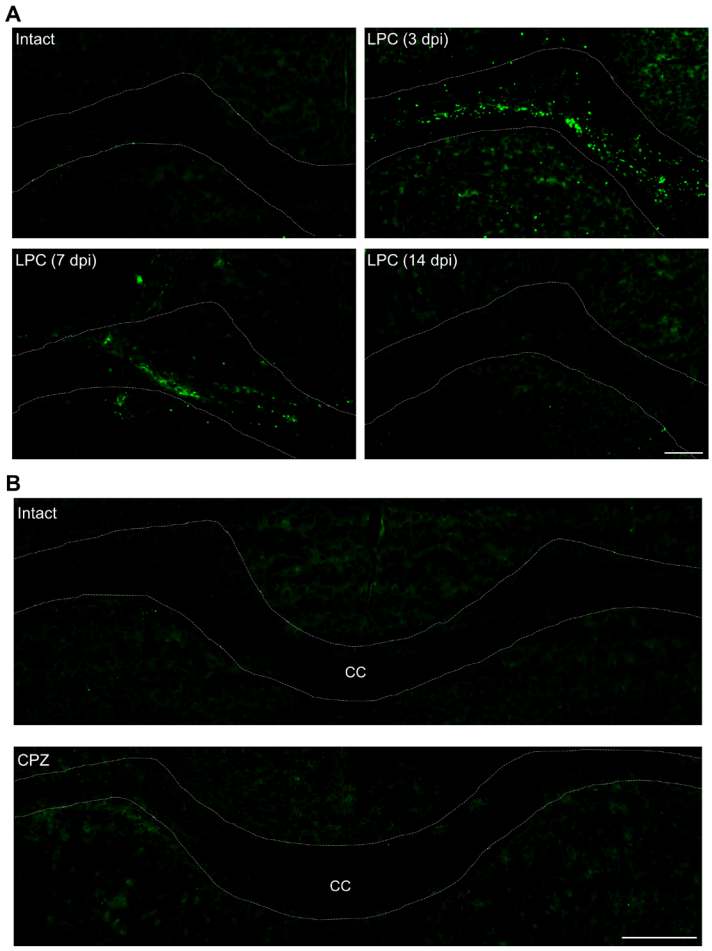

FIGURE 1. The NQEQVSP peptide showed specific binding to the damaged area following toxic-induced demyelination. (a) Fluorescence brain images of intact and LPC-injected mice at 3, 7, and 14 dpi following LPC-induced demyelination. (b) Fluorescence brain images of intact and CPZ-treated mice using the green channel showed no peptide binding. CC: Corpus callosum, LPC: Lysophosphatidylcholine, CPZ: Cuprizone, Scale bar, 100 µm.

at -20 °C until later analysis.

Peptide binding on brain sections

The peptide with the sequence of CGGGNQEQVSP and 6-FAM label was synthesized by ChinaPeptides Co., Ltd (99.07 % purity). The cryosections of the brains were pre-incubated with PBS for 1h, then incubated with 100 nM peptide solution at 4 °C overnight. After washing with PBS, sections were evaluated under an Olympus BX51 fluorescence microscope with a DP72 digital camera by observing the intrinsic emission from the FAM tag on the peptide.

Immunohistofluorescence analysis

For immunostaining, the cryosections of brain were washed with PBS, permeabilized using 0.2% Triton X-100 (Sigma Aldrich, T8532) in PBS for 20 min at room temperature (RT), blocked using 10% normal goat serum for 1 h at RT, and then incubated with primary antibodies against myelin basic protein (MBP, Aveslabs, RRID: AB_2313550), ionized calcium-binding adapter molecule 1 (Iba1, Wako, RRID: 019-19741) and glial fibrillary acidic protein (GFAP, Dako, RRID: Z0334) at 4 °C for overnight. The next day, the sections were washed three times and subsequently incubated with the appropriate secondary fluorescent labeled antibodies (goat anti-rabbit IgG; A-11008 or rabbit anti-chicken IgY; ab6751) for 1 h at room temperature. The sections were washed, counterstained with DAPI (D9542, Sigma-Aldrich, Germany), and photographed using fluorescence microscopy.

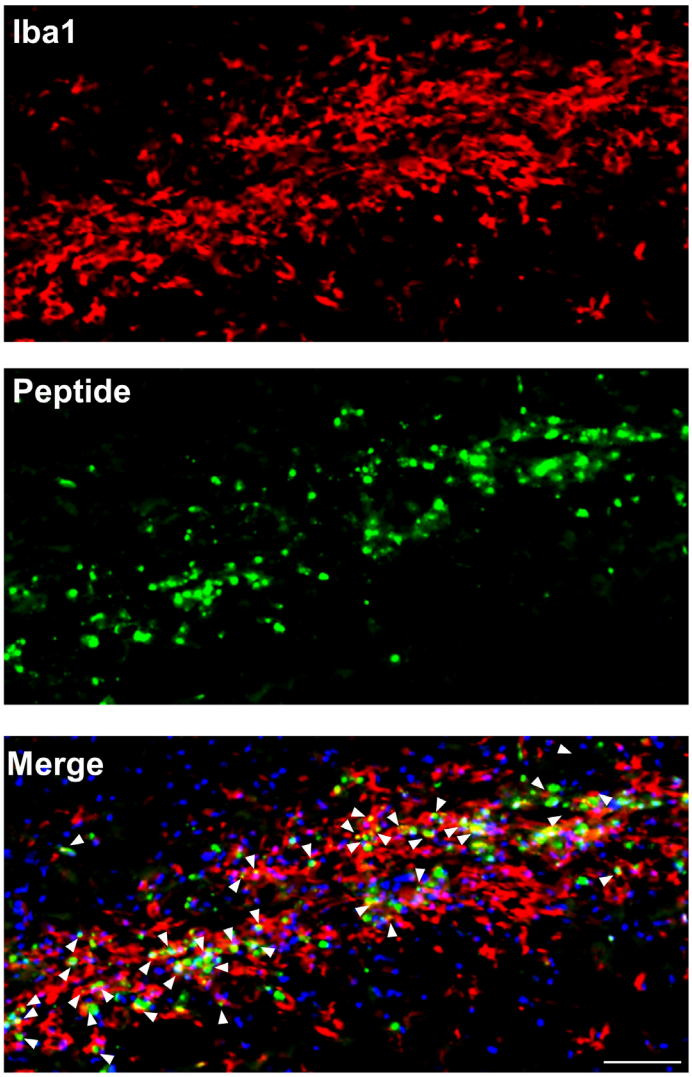

FIGURE 2. NQEQVSP peptide co-localization with microglia in the lesion area at 3 days post LPC-induced demyelination. The sections were evaluated using anti-Iba1 (red), FAM-labelled bounded peptides (green), and DAPI (blue). Arrowheads indicate the co-localization sites. CC: Corpus callosum, LPC: Lysophosphatidylcholine, scale bar, 100 µm.

Results and discussion

Peptide-specific binding to the LPC induced demyelinating lesions

Feeding mice with 0.2% CPZ for 12 weeks has been shown to result in chronic demyelination of brain white and gray matter, as happens in the progressive phase of MS (Zhan et al., 2020). In addition, direct injection of LPC into the white matter leads to severe inflammation at 3 dpi, the highest demyelination level at 7 dpi, and a significant remyelination at 14 dpi (Pourabdolhossein et al., 2014; Seyedsadr et al., 2019). Therefore, we induced irreversible demyelination by a 12-week CPZ feeding period in mice and a focal lesion in the CC using LPC. Peptide binding on brain sections prepared from intact, CPZ-treated, and LPC-injected mice at 3, 7, and 14 dpi was evaluated. The peptide showed strong binding to LPC-demyelinated brain sections at 3 and 7 dpi, with higher binding at 3 dpi (Fig. 1a). However, it showed no binding to demyelinated sections obtained from CPZ-treated mice (Fig. 1b).

Studies have shown that there is no fibrin deposition in the normal CNS or during effective remyelination; however, it is deposited at the site of BBB damage (Adams et al., 2004; Seyedsadr et al., 2019). No peptide binding was detected in the control, CPZ-treated mice, or LPC-injected 14 dpi (remyelination phase) conditions

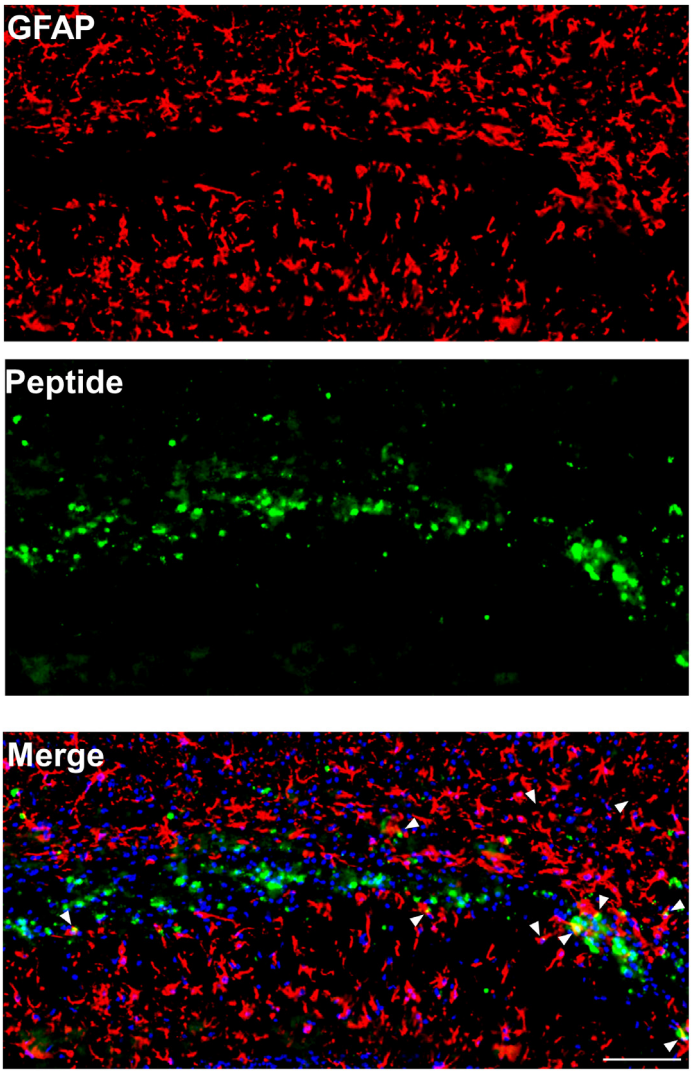

FIGURE 3. NQEQVSP peptide co-localization with astrocytes in the lesion area at 3 days post LPC-induced demyelination. The sections were evaluated using anti-GFAP (red), FAM-labelled bounded peptides (green), and DAPI (blue). Arrowheads indicate the co-localization sites. CC: Corpus callosum, LPC: Lysophosphatidylcholine, Scale bar, 100 µm.

with the intact BBB. As the highest BBB disruption occurs in the LPC model at 3 dpi (Seyedsadr et al., 2019) the highest binding efficacy of the peptide to the damaged site was observed on the same day.

Interaction of the peptide with glial cells

Glial cells play an essential role in MS pathogenesis (Schirmer et al., 2021). Reactive astrocytes are present in MS lesions that initially restrict the damage area, but subsequently reside at the lesion site, contribute to the glial scar formation, and prevent the repair process (Hammond et al., 2014). Reactive astrocytes exacerbate inflammation and demyelination through mechanisms such as recruitment of immune cells into the lesion, release of chemokines, inflammatory cytokines, reactive oxygen species (ROS), as well as the increased BBB leakage (Ponath et al., 2018). Furthermore, myelin repair is decreased by the expression of bone morphogenetic protein and other mediators in astrocytes (Wang et al., 2011). Reactive microglial cells contribute to progressive neurodegeneration and demyelination by producing glutamate, proteases, ROS, inflammatory cytokines, and mitochondrial damage (Kamma et al., 2022). Accordingly, to identify potential targets of the peptide in the injured area at the cellular level, immunohistofluorescence against astrocytes marker (GFAP), oligo-

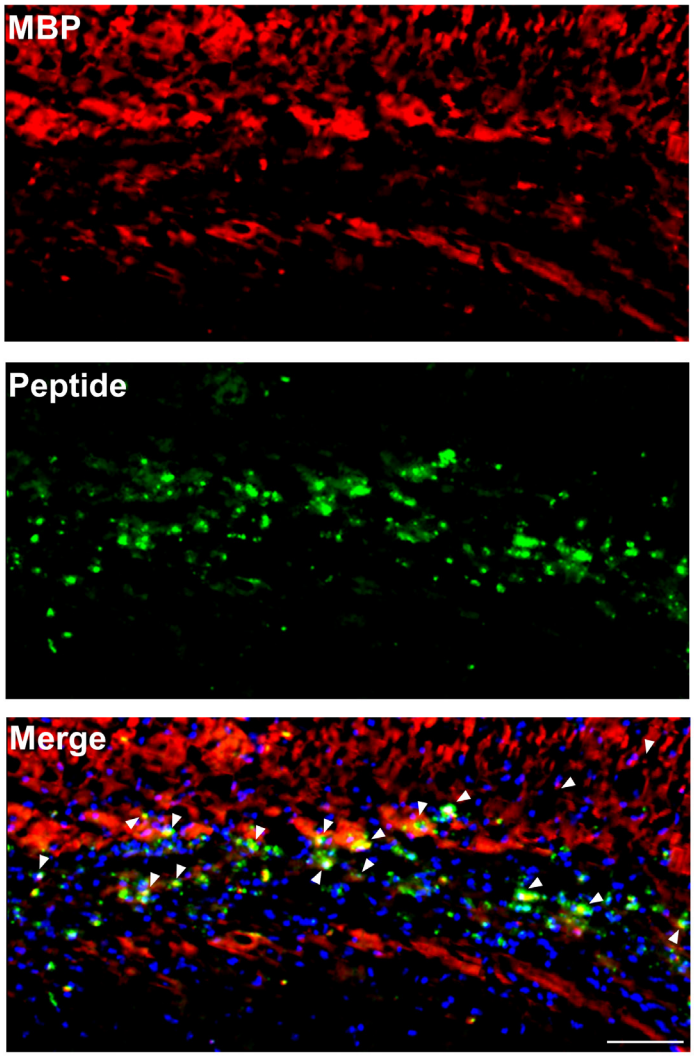

FIGURE 4. NQEQVSP peptide co-localization with oligodendrocytes in the lesion area at 3 days post LPC-induced demyelination. The sections were evaluated using anti-MBP (red), FAM-labelled bound peptides (green), and DAPI (blue). Arrowheads indicate the co-localization sites. CC: Corpus callosum, LPC: Lysophosphatidylcholine, Scale bar, 100 µm.

dendrocytes marker (MBP), and microglial cells marker (Iba1) on the brain sections prepared from tissues obtained from 3 dpi LPC-injected mice was performed. Our data demonstrated that the peptide prominently bound to the microglial cells at the lesion site (Fig. 2). Only a few astrocytes were co-localized with the FAM-labeled peptide (Fig. 3). Oligodendrocytes were colocalized with the peptide at the site of injury to some extent (Fig. 4). Collectively, these findings suggest that the target molecules (fibrin) of the peptide were mainly present on glial cells' surface or their vicinity. This co-staining happened mainly for the microglia cells, the main player of the inflammatory phase of the LPC-demyelination model.

Conclusion

Fibrin, a major component of the blood clot, does not penetrate the BBB and accordingly is not distributed within the normal CNS, but in response to BBB disruption, it passes the BBB and accumulates at the lesion site. Studies have reported fibrin deposition in different types of MS lesions. In this study, we selected the heptapeptide NQEQVSP, known to bind to fibrin, to target the lesion sites in the CPZ and LPC models. The results showed that the peptide was bound to the damaged sites in the LPC model with inflammation generated by mi-

croglia and peripheral immune cells. However, there was no binding of the peptide to the lesion in the CPZ model, which is known for less peripheral cell inflammation. The peptide was evidently co-localized with microglial cells at the lesion site following the LPC induced demyelination. Our data suggest the efficacy of this peptide for targeted delivery in MS and disorders with fibrin deposition. This targeted delivery seems to be more promising in the acute inflammatory phase of MS lesion development.

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Conflict of Interest

The authors declare no conflict of interest.

References

Absinta M, Lassmann H, Trapp B. Mechanisms underlying progression in multiple sclerosis. Current Opinion in Neurology 2020; 33: 277-277. https://doi.org/10.1097/WCO.0000000000000818

Adams R A, Passino M, Sachs B D, Nuriel T, Akassoglou K. Fibrin mechanisms and functions in nervous system pathology. Molecular Interventions 2004; 4: 163-163.

Aguilar Z. Nanomaterials for medical applications: Newnes, 2012.

Davalos D, Kyu Ryu J, Merlini M, Baeten K M, Le Moan N, Petersen M A, et al. Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. Nature Communications 2012; 3: 1-15. https://doi.org/10.1038/ncomms2230

Farhangi S, Dehghan S, Totonchi M, Javan M. In vivo conversion of astrocytes to oligodendrocyte lineage cells in adult mice demyelinated brains by Sox2. Multiple Sclerosis and Related Disorders 2019; 28: 263-272. https://doi.org/10.1016/j.msard.2018.12.041

Farhangi S, Karimi E, Khajeh K, Hosseinkhani S, Javan M. Peptide mediated targeted delivery of gold nanoparticles into the demyelination site ameliorates myelin impairment and gliosis. Nanomedicine: Nanotechnology, Biology and Medicine 2023; 47: 102609-102609. https://doi.org/10.1016/j.nano.2022.102609

Ford H. Clinical presentation and diagnosis of multiple sclerosis. Clinical Medicine 2020; 20: 380-380. https://doi.org/10.7861/clinmed.2020-0292

Goldenberg M M. Multiple sclerosis review. Pharmacy and Therapeutics 2012; 37: 175-175.

Hammond T R, Gadea A, Dupree J, Kerninon C, Nait-Oumesmar B, Aguirre A, et al. Astrocyte-derived endothelin-1 inhibits remyelination through notch activation. Neuron 2014; 81: 588-602. https://doi.org/10.1016/j.neuron.2014.03.007

Inoue A, Koh C-S, Yamazaki M, Yanagisawa N, Ishihara Y, Kim B S. Fibrin deposition in the central nervous system correlates with the degree of Theiler's murine encephalomyelitis virus-induced demyelinating disease. Journal of Neuroimmunology 1997; 77: 185-194. https://doi.org/10.1016/S0165-5728(97)00072-6

Jiang Z, Guan J, Qian J, Zhan C. Peptide ligand-mediated targeted drug delivery of nanomedicines. Biomaterials Science 2019; 7: 461-471. https://doi.org/10.1039/C8BM01340C

Kamma E, Lasisi W, Libner C, Ng H S, Plemel J R. Central nervous system macrophages in progressive multiple sclerosis: relationship to neurodegeneration and therapeutics. Journal of Neuroinflammation 2022; 19: 1-27. https://doi.org/10.1186/s12974-022-02408-y

Ponath G, Park C, Pitt D. The role of astrocytes in multiple sclerosis. Frontiers in Immunology 2018; 9: 217-217. https://doi.org/10.3389/fimmu.2018.00217

Pourabdolhossein F, Mozafari S, Morvan-Dubois G, Mirnajafi-Zadeh J, Lopez-Juarez A, Pierre-Simons J, et al. Nogo receptor inhibition enhances functional recovery following lysolecithin-induced demyelination in mouse optic chiasm. PloS One 2014; 9: e106378-e106378. https://doi.org/10.1371/journal.pone.0106378

Ryu J K, Petersen M A, Murray S G, Baeten K M, Meyer-Franke A, Chan J P, et al. Blood coagulation protein fibrinogen promotes autoimmunity and demyelination via chemokine release and antigen presentation. Nature Communications 2015; 6: 1-15. https://doi.org/10.1038/ncomms9164

Schense J C, Bloch J, Aebischer P, Hubbell J A. Enzymatic incorporation of bioactive peptides into fibrin matrices enhances neurite extension. Nature Biotechnology 2000; 18: 415-419. https://doi.org/10.1038/74473

Schirmer L, Schafer D P, Bartels T, Rowitch D H, Calabresi P A. Diversity and function of glial cell types in multiple sclerosis. Trends in Immunology 2021; 42: 228-247. https://doi.org/10.1016/j.it.2021.01.005

Seyedsadr M S, Weinmann O, Amorim A, Ineichen B V, Egger M, Mirnajafi-Zadeh J, et al. Inactivation of sphingosine-1-phosphate receptor 2 (S1PR2) decreases demyelination and enhances remyelination in animal models of multiple sclerosis. Neurobiology of Disease 2019; 124:

189-201. https://doi.org/10.1016/j.nbd.2018.11.018

Wang Y, Cheng X, He Q, Zheng Y, Kim D H, Whittemore S R, et al. Astrocytes from the contused spinal cord inhibit oligodendrocyte differentiation of adult oligodendrocyte precursor cells by increasing the expression of bone morphogenetic proteins. Journal of Neuroscience 2011; 31: 6053-6058. https://doi.org/10.1523/JNEUROSCI.5524-09.2011

Zhan J, Mann T, Joost S, Behrangi N, Frank M, Kipp M. The cuprizone model: dos and do nots. Cells 2020; 9: 843-843. https://doi.org/10.3390/cells9040843