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Targeting demyelination *via* α-secretases promoting sAPPα release to enhance remyelination in central nervous system

Gemma Llufriu-Dabén[a,1], Alex Carrete[a,1], Elena Chierto[a], Jo Mailleux[b], Emeline Camand[a], Anne Simon[a], Tim Vanmierlo[b], Christiane Rose[c], Bernadette Allinquant[c], Jerome J.A. Hendriks[b], Charbel Massaad[a], Delphine Meffre[a,2], Mehrnaz Jafarian-Tehrani[a,*,2]

[a] INSERM UMR-S 1124, Université Paris Descartes, Sorbonne Paris Cité, Faculté des Sciences Fondamentales et Biomédicales, 45 rue des Saints-Pères, 75006 Paris, France
[b] Biomedisch Onderzoeksinstituut, Universiteit Hasselt, Campus Diepenbeek, Agoralaan Gebouw C, B-3590 Diepenbeek, Belgium
[c] INSERM UMR-S 894, Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, 102-108 rue de la santé, 75014 Paris, France

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ABSTRACT

Remyelination is an endogenous regenerative process of myelin repair in the central nervous system (CNS) with limited efficacy in demyelinating disorders. As strategies enhancing endogenous remyelination become a therapeutic challenge, we have focused our study on α-secretase-induced sAPPα release, a soluble endogenous protein with neuroprotective and neurotrophic properties. However, the role of sAPPα in remyelination is not known. Therefore, we investigated the remyelination potential of α-secretase-induced sAPPα release following CNS demyelination in mice. Acute demyelination was induced by feeding mice with cuprizone (CPZ) for 5 weeks. To test the protective effect and the remyelination potential of etazolate, an α-secretase activator, we designed two treatment protocols. Etazolate was administrated either during the last two weeks or at the end of the CPZ intoxication. In both protocols, etazolate restored the number of myelinated axons in corpus callosum with a corresponding increase in the amount of MBP, one of the major myelin proteins in the brain. We also performed *ex vivo* studies to decipher etazolate's mechanism of action in a lysolecithin-induced demyelination model using organotypic culture of cerebellar slices. Etazolate treatment was able to i) enhance the release of sAPPα in the culture media of demyelinated slices, ii) protect myelinated axons from demyelination, iii) increase the number of mature oligodendrocytes, iv) promote the reappearance of the paired Caspr^{+} adjacent to the nodes of Ranvier and v) increase the percentage of myelinated axons with short internodes, an indicator of remyelination. Etazolate failed to promote all the aforementioned effects in the presence of GI254023X, an α-secretase inhibitor. Moreover, the protective effects of etazolate in demyelinated slices were mimicked by sAPPα treatment in a dose-dependent manner. In conclusion, etazolate-induced sAPPα release protects myelinated axons from demyelination while also promoting remyelination. This work, thus, highlights the therapeutic potential of strategies that enhance sAPPα release in demyelinating disorders.

1. Introduction

Demyelination in the central nervous system (CNS) is characterized by myelin sheath loss and oligodendrocyte cell death. It contributes to progressive axonal injury leading to neurological disability as observed in demyelinating diseases or in acute CNS injuries such as multiple sclerosis (MS) (Compston and Coles, 2008; Trapp and Nave, 2008) and traumatic brain injury (TBI) (Armstrong et al., 2015), respectively. In contrast, remyelination is an endogenous spontaneous regenerative process occurring in response to demyelination and is essential for the restoration of saltatory conduction and subsequent prevention of further axonal damage (Franklin and Ffrench-Constant, 2008).

However, remyelination is limited or inadequate in demyelinating diseases leading to progressive axonal loss and irreversible neurological dysfunction in long-term (Dutta and Trapp, 2011). Oligodendrocyte precursor cells (OPCs), the main source of mature myelinating cells in the adult brain are not considered to be a limiting factor for remyelination since they are abundant within demyelinated regions

* Corresponding author at: INSERM UMR-S 1124, Paris Descartes University, Sorbonne Paris Cité, Faculté des Sciences Fondamentales et Biomédicales, 45 rue des Saints-Pères, 75006 Paris, France.
E-mail address: mehrnaz.jafarian@parisdescartes.fr (M. Jafarian-Tehrani).
[1] Equally contributing authors.
[2] Equally contributing authors.

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(Chang et al., 2002). In fact, the main cause of failure in remyelination has been attributed to the inability of OPCs to differentiate into mature myelinating oligodendrocytes (Dulamea, 2017; Fancy et al., 2010; Franklin, 2002). Therefore, therapeutic strategies promoting OPCs differentiation, myelin sheath protection and remyelination may contribute to better axonal preservation and long-term functional recovery (Franklin, 2015; Kremer et al., 2015). It is noteworthy that pharmacological therapies enhancing remyelination in demyelinating diseases such as MS are still to be explored and exploited (Gajofatto and Benedetti, 2015). Therefore, our interest is focused on such strategies enhancing endogenous remyelination to promote myelin repair following demyelination.

In our previous report, we have shown that enhancing the release of the endogenous soluble protein sAPPα, has a therapeutic potential resulting in histological and functional improvements following TBI in mice (Siopi et al., 2013). sAPPα is released by α-secretase processing of β amyloid precursor protein (βAPP) and is known to be neurotrophic and neuroprotective (Chasseigneaux et al., 2011; Hefter and Draguhn, 2017; Mockett et al., 2017). However its effect on white matter protection or repair remains unknown. The α-secretases include different members of ADAM (A Disintegrin And Metalloproteinase) family such as ADAM9, ADAM10 and ADAM17 (Allinson et al., 2003). Within the brain, ADAM10 is the most relevant α-secretase since it leads to a constitutive and regulated α-secretase cleavage of βAPP under physiological conditions (Kuhn et al., 2010; Prox et al., 2013). Although the role of α-secretases in remyelination has not been fully investigated, it has been shown that i) neuronal ADAM10 activity is required for the migration of neural precursor cells into demyelinated lesions (Klingener et al., 2014), and that ii) the modulation of oligodendroglial ADAM17 promotes oligodendrogenesis and myelin repair *in vivo* (Palazuelos et al., 2015; Palazuelos et al., 2014).

Therefore, the purpose of this study was to investigate the protective and remyelination potential of a pharmacological strategy able to promote sAPPα release following demyelination. Etazolate, a pyrazolopyridine derivative, is known to enhance the α-secretase processing of βAPP to sAPPα and also promotes neuroprotection from Aβ peptide neurotoxicity *in vitro* (Marcade et al., 2008) and post-TBI complications *in vivo* (Siopi et al., 2013). Nevertheless, the therapeutic potential of etazolate in myelin sheath protection and remyelination remains unknown.

Hence, we investigated the protective and remyelinating effects of etazolate as an α-secretase activator in C57BL/6 mice. We studied the effect of etazolate treatment at the dosage known to enhance sAPPα release *in vivo* (Marcade et al., 2008) in the cuprizone (CPZ)-induced acute demyelination model. We designed two treatment protocols to test i) the protective effect and ii) the remyelination potential of etazolate. We also used the model of lysolecithin-induced demyelination in organotypic culture of cerebellar slices, a well-known *ex vivo* model to study de(re)myelination processes. The latter was used to study the mechanism of action of etazolate, at the dosage known to increase sAPPα release (Marcade et al., 2008) and also in the presence of GI254023X, an α-secretase inhibitor (Jangouk et al., 2009; Ludwig et al., 2005). Our results showed for the first time that etazolate promotes white matter protection and repair and acts on α-secretases to enhance sAPPα release following demyelination.

2. Materials and methods

2.1. Animals

For the organotypic culture of cerebellar slices and primary mixed glial cultures, we used either C57BL/6 mouse pups (Janvier, Le Genet St Isle, France), or transgenic PLP-eGFP mice (generated by Dr. W.B. Macklin, Cleveland Clinic Foundation, Ohio, USA). The latter overexpress the enhanced green fluorescent protein (eGFP) in the oligodendrocyte lineage under the control of the proteolipid protein (PLP) promoter. Animals were housed in a controlled temperature environment (22 ± 2 °C), under a 12 h light/dark cycle. Animal care and experiments were approved by the Paris Descartes University Animal Ethics Committee (CEEA34.MJT.075.12; APAFIS #3768), adhering to the French regulations and the European Communities Council Directive on the protection of animals used for scientific purposes.

2.2. Cuprizone-induced acute demyelination in vivo

Eight-week-old C57BL/6 male mice (n = 50/protocol) were randomly assigned to different experimental groups after 1 week of habituation. Acute demyelination was induced by feeding mice with 0.2% (w/w) cuprizone (bis(cyclohexanone)oxaldihydrazone) (Sigma-Aldrich) mixed in rodent chow for 5 weeks (Skripuletz et al., 2011). Control group received a normal diet without cuprizone. After 3 weeks of cuprizone intoxication (Protocol n° 1), animals were treated intraperitoneally (i.p.) for 2 weeks either with a vehicle (PBS) or etazolate (10 mg/kg/day), a dose known to increase sAPPα levels *in vivo* (Marcade et al., 2008) (n = 10 per group). In the second protocol (Protocol n° 2), mice were intoxicated with cuprizone for 5 weeks and then allowed to recover for 2 weeks by feeding on normal chow. During this period, animals were treated either with a vehicle (PBS) or etazolate (10 mg/kg/day, i.p.). For histological studies, mice were deeply anesthetized with pentobarbital (60 mg/kg, i.p.), and perfused through the heart with phosphate buffer 0.1 M (pH 7.4) before brain removal. The caudal part of brain hemispheres were immersed in a cold fixative solution (4% PFA, 2.5% glutaraldehyde and 0.1 M phosphate buffer, pH 7.4) and stored at 4 °C until use. For biochemical studies, mice were sacrificed by cervical dislocation, and the brains were removed, instantly frozen in liquid nitrogen and stored at − 80 °C until use.

2.3. Transmission electron microscopy

For EM analysis, caudal part of the brain hemispheres was removed at the given coordinates (− 2.0 to − 3.2 mm from Bregma, Franklin and Paxinos, 2008) and then post-fixed in 4% PFA, 2.5% glutaraldehyde and 0.1 M phosphate buffer, pH 7.4 for 1 week. On the day of the impregnation, caudal-lateral brain slices (− 2.0 to − 3.2 mm from Bregma, and 3.25 to 3.60 mm lateral) were prepared and post-fixed in 2% osmium tetroxide, dehydrated in graded ethanol series and embedded in epoxy resin. We chose to analyze the caudal-lateral region since it is one of the most demyelinated area of the corpus callosum after CPZ intake (Irvine and Blakemore, 2006; Steelman et al., 2012; Stidworthy et al., 2003; personal unpublished data). Semi-thin cross-sections of the epoxy resin bloc were obtained with a glass knife at 0.5–1 μm and stained with methylene blue/azur II for quality control of the appropriate area (cross-sections of lateral corpus callosum) analyzed across different experimental groups. The same resin bloc was further used to obtain ultrathin cross-sections of the lateral corpus callosum for electron microscopy analysis. Ultrathin cross-sections (50–90 nm) were performed at the level of the lateral part of the corpus callosum on an ultramicrotome (8800 Ultratome III, LKB Bromma) and collected on 300-mesh nickel grids. Staining was performed on drops of 4% aqueous uranyl acetate, followed by Reynolds's lead citrate (Reynolds, 1963). Ultrastructural analyses were performed in a JEOL jem-1011 electron microscope and digitalized with DigitalMicrograph software. Electron microscopy images were used for calculating the *g*-ratio (ratio of the axon diameter to the fiber diameter) and the percentage of myelinated axons using ImageJ (10.2, NIH, USA). Three animals per group and an average of ten images (magnification G × 20,000) per animal were analyzed. A total number of at least 2000 axons were counted per group (at least 600 axons per animal).

2.4. Western blot

Samples taken from either brain hemispheres, cerebella or the