

Inhibition of CXCR4/CXCL12 signaling: a translational perspective for Alzheimer's disease treatment

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The mechanism of AD remains uncovered:

The current mainstream doctrine in Alzheimer's disease (AD) is the amyloid cascade hypothesis. According to this hypothesis, amyloid- β ($A\beta$) deposition is the main reason for neurofibrillary tangles formation and synaptic dysfunction. However, treatments with antibodies against such targets did not manage to improve cognitive decline and leave some questions regarding the theory of amyloid plaques. Extensive evidence indicates that several pathology changes occur before the appearance of $A\beta$ and tau aggregates. One of the changes is the assault in oligodendrocytes and as a consequence a breakdown of the myelin sheath which is associated with early AD (Dong et al., 2018). Notably, chronic neuroinflammation with sustained activation of microglia and astrocytes may lead to lesion in white matter tracts and disrupt the communication between neurons. Microglia activation and their associated chronic release of inflammatory cytokines in AD subjects attenuate its capacity to clear toxic and harmful substances from the brain which may also underlie the myelin damage. In addition, it was suggested that microglia and immune-related pathways can act as early mediators of synapse loss and dysfunction that occur in AD models before plaques formation. Microglia can exhibit a classically activated phenotype (M1) which exerts toxic effects by secreting proinflammatory cytokines or an alternative activated phenotype (M2) involved in the maintenance of central nervous system homeostasis, phagocytosis of apoptotic bodies or cells, releasing neurotrophic factors, and reducing proinflammatory cytokines. Studies demonstrated that M2 phenotype markers, in contrast to M1 markers, could be modulated in adult microglia, depending on the microenvironment and therefore, have a potential therapeutic effect in neuroinflammation for review see (Tang and Le, 2016.) Apart from resident microglia, another type of microglia has been identified in the brain that originates from monocyte precursor cells from the bone marrow referred as 'microglia-like' cells. The bone marrow-derived microglia-like cells may cross the blood-brain barrier and migrate into the brain in a chemokine-dependent manner (Kawanishi et al., 2018). In AD, bone marrow-derived cells can access the $A\beta$ -laden brain in higher numbers, as demonstrated in APP23 transgenic mice versus age-matched non-transgenic control mice. Microglia, especially bone marrow derived microglia, has been recently thought to play important roles in internalizing and phagocytizing $A\beta$ oligomers. The invading

cells exhibit hematopoietic phenotypes heterogeneously scattered throughout the brain. Hematopoietic stem cells that enter the brain affect brain homeostasis through the secretion of hematopoietic growth factors and cytokines and promote brain repair by increasing neurogenesis

The CXCR4/CXCL12 (SDF1) axis: The CXCR4/CXCL12 (SDF1) axis is one of the major signal transduction cascades involved in the inflammation process and regulation of homing of hematopoietic stem cells (HSCs) within the bone marrow niche. CXCR4/CXCL12 based mechanism suggests that $A\beta$ plaques attract microglia to activate the inflammatory cascade by which CXCL12 stimulates CXCR4-dependent signaling both in microglia and in astrocytes to release pro-inflammatory cytokines such as tumor necrosis factor α . The Involvement of Ca^{2+} cascade is also suggested to be involved based on this signaling mechanism which ends in activation of kinases, phosphorylation and further excitotoxicity cascade triggered by excessive stimulation by glutamate (Bezzi et al., 2001).

CXCR4, a chemokine receptor protein with broad regulatory functions in the immune system is involved in cell cycle regulation through p53 (Boidot et al., 2012). The expression of CXCR4 and functionally associated genes, were altered in multiple neurodegenerative diseases.

The role of CXCR4 in stimulating p53-dependent pathways in neurons responsive pro-apoptotic genes is likely involved in CXCR4-mediated cell death. Stabilization/up-regulation of the p53 protein is an important first step that takes place in neurons under various kinds of toxic insults. Thus, modulation of specific p53 responsive genes may determine the final outcome of CXCR4 activation on neuronal survival in neuroinflammatory disorders.

CXCL12 promotes neuronal survival under various conditions by stimulating phosphorylation and triggering nuclear translocation of anti-apoptotic proteins regulated by (Proctor and Gray, 2010).

In tissues, the cytokine CXCL12 is secreted as a consequent to an injury or a degenerative condition, however, in the bone marrow, CXCL12, is constitutively produced, released, and its binding to its receptor CXCR4 leads to the adherence of stem cells to the bone marrow matrix.

Notably, the damaged brain exhibits high levels of chemokines like CXCL12, which attract circulating HSCs due to their surface expression of CXCR4. We demonstrate that inhibition of CXCR4 signaling leads to

increase of monocarboxylate transporters 1 (MCT1) involved in the myelin maintenance and lactate transport) (Gabriel et al., 2020)

MCT1: In animal models, disruption of lactate transporter MCT1 results in widespread axonal damage and neuronal death. Reduced expression of these transporters has been identified in multiple sclerosis and other neurodegenerative disease, including, amyotrophic lateral sclerosis and AD as well as in the SOD1G93A mouse model of amyotrophic lateral sclerosis and in APP/PS1, mouse model of AD (Rabinovich-Nikitin et al., 2016, Zhang et al., 2018) MCTs are a family of proton-linked plasma membrane transporters that allow the passage of monocarboxylates, including lactate, pyruvate, and ketone bodies. Though there are 14 members of this family, only the first four (MCT1-4) have been recognized experimentally each one with distinct substrate and inhibitor affinities. MCT1 is the primary and most abundantly expressed lactate transporter in peripheral nerves, MCT1 has been found to be crucial for Schwann cell biology and it contributes. MCT1 has also an essential role in axonal regeneration and it is associated with oligodendrocytes, which may be a novel therapeutic intervention for the prevention and treatment of AD Lactate which is transported exclusively by mono-carboxylate transporters plays a key role in the metabolic support of neurons and remyelination (Sun et al., 2017). Increase in MCT1 transporters level enables a better utilization of exogenous and endogenous lactate to be utilized as an energy substrate by neurons and myelin (Domènech-Estévez et al., 2015).

L-lactate is an active metabolite capable of moving into or out of cells, acting as a signaling molecule, and regulating diverse physiological and pathophysiological cascades. Therefore, maintaining homeostatic circulating levels of lactate may mitigate or reverse neurodegenerative disease progression

AMD3100, a reversible antagonist of CXCR4:

We recently reported that inhibition of CXCR4/CXCL12 axis with AMD3100, a reversible antagonist of CXCR4. AMD3100 was found to attenuate the neuroinflammation and demyelination and upregulate MCT1 as well as mobilize endogenous HSCs from the bone marrow to the periphery and into the brain. Furthermore, a direct link between p53 and MCT1 expression was identified under hypoxic conditions, showing that loss of p53 promotes MCT1 expression and lactate export, both *in vitro* and *in vivo* (Boidot et al., 2012). Notably, upregulation of MCT1 by inhibition of signaling of CXCR4/CXCL12 axis led us to test the feasibility of the combined treatment of AMD3100 and L-lactate in attenuation of neuroinflammation and remyelination in a ($A\beta$)-induced AD mouse model.

Beneficial treatment of a combination of AMD3100-L-lactate in mouse model of AD:

We investigated the short-term (twice a week for two weeks) combined treatment

with 5 mg/kg (5 mg) AMD3100 and 900 mg/kg (900 mg) lactate in acute AD mouse model (Gavriel et al., 2020). Acute AD mouse model was obtained by injecting A β _{25–35} fibrils (10 μ g) directly to wild-type mice' hippocampi (bilateral), leading to detected pathologies that are directly correlated with A β deposits and neuroinflammation. A acute model is well established and has a wealth amount of evidence to characterize various AD pathologies in rodents: long term learning and working memory impairment, high oxidative and endocrine stress, astrocytes activation to neuroinflammation process. We found that the cognitive deficit associated with this model is correlated with elevated microglia activity in addition to A β deposition, neurofibrillary tangles formation and synaptic dysfunction amyloid precursor protein (APP) and p53 levels were markedly elevated following intrahippocampal injection of A β _{25–35}. Previous studies showed that p53 can affect glycogen synthase kinase-3 β (GSK3 β) activity by inducing tau phosphorylation, and under stress, p53 forms a complex with GSK3 β resulting in increased GSK3 β activity and consequent phosphorylation of tau (Proctor and Gray, 2010).

The combination of AMD3100 L-lactate was found to have a beneficial effect by improving cognitive deficit, reducing tau and APP pathologies and most importantly, led to shift in microglia to anti-inflammatory M2 profile. The observed downregulation of interleukin 6 (IL-6), tumor necrosis factor α , and monocyte chemoattractant protein-1 together with upregulation of IL-4 and IL-10 may explain the anti-inflammatory effect that we achieved by changing the lineage of the microglia. Another possible explanation for the shift in microglial profile may be explained by recruitment of new microglia-like cells from the bone marrow that may either change the resident microglial cell population and/or the release factors that may recover the resident cells profile. The recruited microglia, as well as neuronal cells, may be supported by the energy supply of lactate, which is more efficiently transported into the brain via elevated MCT1 expression. Other possible mechanism suggests that AMD3100 acts as a CXCR4 antagonist and therefore inhibits the toxic inflammation process and consequently the general phosphorylation process that eventually leads to attenuation in cognitive impairment. AMD3100 ameliorates AD's main pathology by increasing both in brain derived neurotrophic factor (BDNF) production and synaptophysin expression. The increase in BDNF and synaptophysin suggests that activation of the synaptogenesis process may act as a compensating mechanism for plasticity during neurodegeneration. The observed increase in MCT1 levels is the suggested mechanism by which lactate was transported into the brain in order to enable this cascade of events. Moreover, lactate was shown to be involved in several cellular processes that include interference with the CXCL12/CXCR4 signaling axis, increase BDNF levels, reduced intracellular Ca²⁺ influx, and protection of neurons from glutamate toxicity. It has been found that

downregulation of myelin is correlated with downregulation of MCT1 in the APP/PS1 mouse model, as both are associated with oligodendrocytes. Some evidence suggests that lactate transport by astrocytes may be utilized as an energy substrate by neurons and myelin formation. Accordingly, our study shows that combining AMD3100 with L-lactate may improve cognitive function, as was shown by two distinct behavioral tests, better than each component alone.

It is noteworthy that the combined treatment did not demonstrate any adverse effects on the mice and resulted in a significant improvement in cognitive/memory functions, improved re-myelination and alleviated AD pathologies and neuroinflammation. Update studies on clinical applications of AMD3100 were recently described (De Clercq, 2019).

Summary: Taken together, the multifaceted role of CXCR/CXCL12, suggests that inhibition approaches towards the CXCR4/CXCL12 signaling may result not only in preventing the toxic cascade of glutamate release from glial cells and neuronal apoptosis but also in rapid mobilization of hematopoietic stem/progenitor cells into the circulating blood. Though the contribution of MCTs to human neurological diseases still requires further study, the published studies are provocative and suggest that MCTs are critical for the maintenance of neuronal integrity and function in the neurologic disease. Since AMD3100 mobilizes HSCs within hours rather than days it can be considered as an alternative approach for the multistep procedures of transplantation of stem cells in the treatment of AD. In addition, AMD3100 is known to be well tolerated, with no significant side effects, as supported by various clinical trials conducted to date (De Clercq, 2019).

Combining anti-inflammatory and remyelinating therapeutics may decrease neurological disability and possibly restore neurological function to AD patients. The therapeutic approach that we presented herein not only opens up an innovative path to intervene in AD, but may also drive clinical trials in shorter time frames, as we focus on repurposing an existing drug whose pharmacokinetics and pharmacodynamics have already been established. Though the exact mechanism has not been elucidated in these paradigms, these results suggest that MCTs play a role in the development of AD and that targeting MCTs may provide an avenue for the development of novel therapies.

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