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Reversible inhibition of chemokine receptor CXC4 signaling via AMD3100 mitigates neuroinflammation in Alzheimer's disease

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Abstract

The CXCL12/CXCR4 signaling axis plays a pivotal role in various physiological processes and the regulation of multiple signaling pathways. Notably, the CXCR4/CXCL12 [stromal cell-derived factor-1 (SDF-1)] interaction is instrumental in activating microglia and astrocytes, which are central mediators of inflammatory responses in the central nervous system. This activation contributes to neurotoxic stress within the neuroinflammatory cascade by promoting the secretion of proinflammatory factors. Furthermore, this axis influences glutamate release from astrocytes, impacting neuronal function and apoptosis, and is essential for maintaining hematopoietic stem cells (HSCs) within the bone marrow. Consequently, targeting the CXCR4/CXCL12 axis may mitigate neuroinflammation and facilitate the mobilization of HSCs from the bone marrow into the bloodstream and subsequently to the brain. Pharmacological blockade of CXCR4/CXCL12 interactions using AMD3100 (Plerixafor), a reversible CXCR4 antagonist, has been shown to reduce neuronal apoptosis. This effect is attributed to the mobilization of HSCs from the bone marrow into the bloodstream, enabling their migration across the blood-brain barrier (BBB) to sites of neuronal damage. Our findings, alongside those of others, demonstrate that AMD3100 treatment significantly alleviates Alzheimer's disease (AD)-associated pathologies and cognitive deficits. The increased presence of HSC markers in the brains of treated mice suggests that these cells originate from the bone

marrow. Systemic administration of CXCR4/CXCL12 inhibitors such as AMD3100, which mobilize endogenous HSCs, may offer therapeutic benefits and address the challenges associated with stem cell transplantation-based approaches.

Keywords: Neuroinflammation, microglia-like cells, CXCR4, Alzheimer's disease, hematopoietic stem cells

INTRODUCTION

The precise etiology of neurodegeneration remains unclear, but inflammatory processes and the aggregation of endogenous pathological proteins are recognized as key contributors to neurodegenerative progression. Neuroinflammation, a hallmark of many neurodegenerative diseases, is closely linked to the activation of microglia and astrocytes, which are central regulators of inflammatory responses in the central nervous system (CNS). Under normal physiological conditions, the CXCL12/CXCR4 axis is essential for the development of the CNS, heart, and immune system[1]. However, in neurodegenerative disorders such as Alzheimer's disease (AD), this axis plays a pivotal role in glial cell activation, contributing to the neuroinflammatory cascade[2-4]. Dysregulation of this signaling pathway can lead to neurotoxic stress through mechanisms such as the release of glutamate from astrocytes, which modulates neuronal function and apoptosis[5,6]. Understanding the CXCL12/CXCR4 axis's involvement in neuroinflammation is vital for the development of targeted therapies aimed at reducing neuroinflammatory responses and mitigating disease progression.

Therapeutically targeting CXCR4 signaling holds promise for treating a range of diseases, including neurodegenerative conditions. While inhibiting the CXCR4/CXCL12 axis offers potential benefits, it is crucial to preserve the axis's physiological roles to avoid adverse effects. Significant efforts have been directed toward developing small-molecule and peptide-based CXCR4 antagonists for therapeutic use. Among these, only one small-molecule antagonist, AMD3100 (Plerixafor), has received Food and Drug Administration (FDA) approval. AMD3100, a reversible CXCR4 antagonist, has shown the potential to attenuate neuroinflammation and facilitate the mobilization of hematopoietic stem cells (HSCs) from the bone marrow into the brain[7].

Pharmacological mobilization of endogenous bone marrow-derived stem cells (BMSCs) through systemic administration of CXCR4/CXCL12 inhibitors, such as AMD3100, has demonstrated beneficial effects in preclinical models. Specifically, this approach has been shown to alleviate pathological features associated with AD, highlighting its therapeutic potential in mitigating neurodegenerative disease progression.

MAIN TEXT

Bone marrow-derived cells

The CXCL12/CXCR4 signaling axis is a cornerstone of hematopoietic stem and progenitor cell (HSPC) biology, governing their homing and retention within the specialized bone marrow niches[8] [Figure 1A]. Under physiological stress or pathological conditions, such as brain injury or neurodegenerative disease, this axis facilitates the mobilization of HSPCs from the bone marrow into the peripheral circulation, where they are attracted to regions of damage. These remarkable multipotent cells, capable of self-renewal and differentiation into various blood lineages, hold significant therapeutic potential for neurological disorders due to their ability to cross the blood-brain barrier (BBB) and promote neural repair.

The detailed mechanism of action proposed for the CXCR4 partial antagonist, AMD3100, may relate to the mobilization of HSCs. AMD3100 (Plerixafor) disrupts the CXCL12/CXCR4 complex, releasing CXCL12

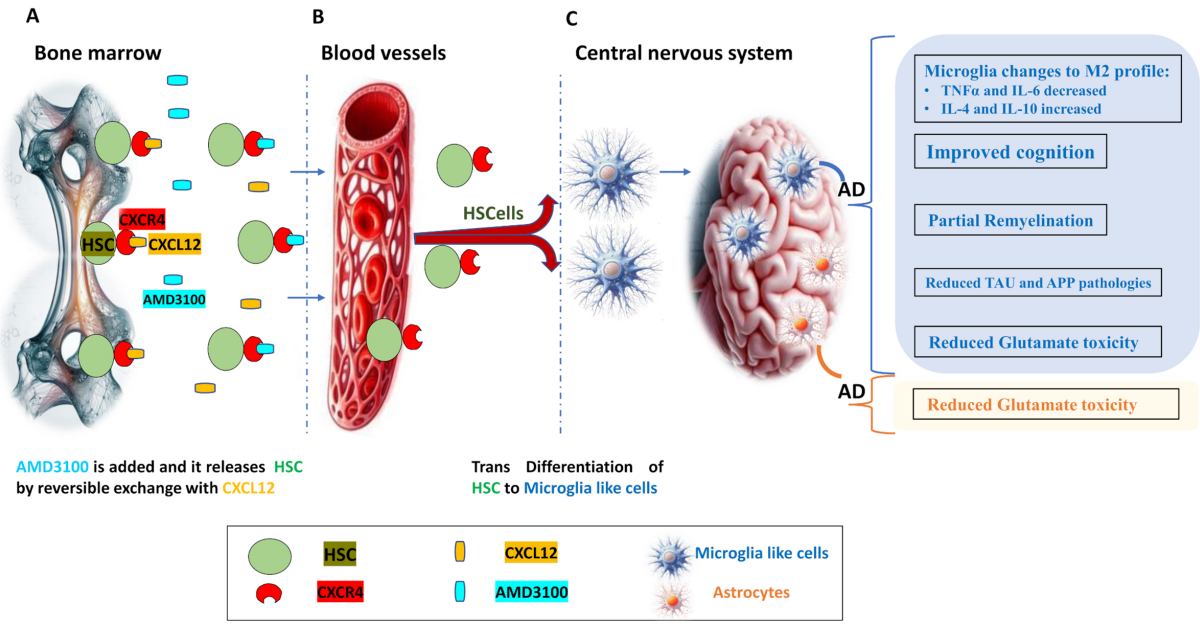

Figure 1. Illustration of the proposed favorable effect of CXCR4/CXCL12 inhibition by AMD3100. (A) Bone marrow: HSCs, colored green, with CXCR4, colored red, are anchored to bone marrow stromal cells by the chemokine CXCL12, colored yellow. Administration of the CXCR4 antagonist (AMD3100), colored cyan, leads to the breakdown of the CXCR4/CXCL12 complex and the release of CXCL12 and HSCs from the stromal cells into the bloodstream; (B) Blood vessels: HSCs from the blood are recruited into the injured brain; (C) CNS: The HSCs undergo possible trans-differentiation into microglia-like cells, colored light blue, that contribute to increased cognition, increased anti-inflammatory cytokines, and reduced TAU and APP pathologies in AD. Astrocytes, colored white-orange, are present inside the brain and contribute to the reduced Glutamate toxicity in AD and to the remyelination of the damaged neurons. The astrocyte-microglia interaction is involved in CXCR4-dependent glutamate release and activated microglia amplify glutamate release from astrocytes. Created with The GIMP team, GIMP 2.8.10, www.gimp.org, 1997-2014, retrieved on 31.07.2014. HSCs: Hematopoietic stem cells; CXCR4: chemokine receptor type 4; CNS: central nervous system; APP: amyloid precursor protein; AD: Alzheimer's disease; Ils: interleukins, TNFα: tumor necrosis factor-alpha.

into circulation [Figure 1A]. This process frees HSCs from their quiescent niches, enabling them to enter the bloodstream [Figure 1B]. These mobilized cells, attracted by chemotactic signals like stromal cell-derived factor-1 (SDF-1) in the injured brain, migrate into the CNS [Figure 1C]. Once there, they differentiate into microglia-like cells, contributing to neuroinflammation resolution and neural repair[9-12] [Figure 1C].

Microglia-like cells are bone marrow-derived cells (BMDCs) and share some immunological functions with native microglia but lack their full characteristics. They bolster the resident microglial population by enhancing phagocytosis and mitigating chronic proinflammatory insults[13-15]. In AD models, repeated administration of AMD3100 has been shown to reduce tau and amyloid precursor protein (APP) pathologies[16]. This is accompanied by cognitive improvements and an anti-inflammatory shift in cytokine profiles, characterized by increased levels of IL-4 and IL-10 and reduced levels of proinflammatory cytokines [Figure 1C]. The mobilized HSCs enhance neurogenesis and release neurotrophic and anti-inflammatory factors that attract monocyte precursors from the bone marrow to the brain, where they differentiate into phagocytically active microglia-like cells. The presented data are summarized in Figure 1.

Notably, bone marrow-derived microglia (BMDM) exhibit a greater capacity for amyloid-beta (Aβ) clearance than resident microglia, making them a promising target for therapeutic interventions in AD. Strategies that promote BMDM recruitment and phagocytosis could offer a novel approach to mitigating AD pathologies and restoring cognitive function.

HSPCs exist in a delicate balance between quiescence and activation[17]. Periodic mobilization, such as that induced by AMD3100, prompts these cells to temporarily exit quiescence for proliferation and repair functions before returning to their niches. This cyclical process supports hematological recovery and tissue repair while minimizing the risks associated with permanent CXCR4 blockade, such as disrupted hematopoiesis.

The ability of AMD3100 to mobilize HSPCs has broad therapeutic implications, extending beyond AD to include neuroinflammation, brain injury, and even cancer. For instance, while AMD3100 promotes HSC mobilization for neural repair, CXCR4-targeting antibodies offer therapeutic benefits in cancer by neutralizing CXCL12/CXCR4 signaling and inhibiting chemotaxis, effectively halting metastasis.

The CXCL12/CXCR4 axis represents a versatile target for therapeutic intervention, with AMD3100 offering a unique mechanism to harness the regenerative potential of HSCs. By balancing mobilization, differentiation, and quiescence, AMD3100 and its derivatives provide a promising platform for addressing neurodegenerative diseases, chronic inflammation, and beyond. However, preserving the physiological functions of the CXCR4/CXCL12 pathway remains critical to maximizing therapeutic benefits while minimizing potential side effects.

Update of CXCR4 antagonists

The development of novel CXCR4 antagonists represents an exciting frontier for therapeutic strategies across a diverse range of diseases and conditions. Notably, AMD3100, discovered in 1994 as a potent inhibitor of HIV entry, later revealed its true target: the CXCR4 receptor. This receptor facilitates HIV entry into T-lymphocytes and plays a pivotal role in numerous physiological and pathological processes. Since then, AMD3100 has become an essential part in the study of CXCR4/CXCL12-mediated pathways.

Efforts to discover additional CXCR4 antagonists have identified promising compounds, including quinoline and quinazoline derivatives, as well as "chemokine neuroligands", which inhibit CXCL12-CXCR4 interactions. Among the leading small-molecule modulators are AMD11070 and IT1t, which exhibit improved potency, affinity, and pharmacokinetic (PK) profiles compared to earlier candidates[18].

However, AMD3100's low oral bioavailability posed a significant hurdle, prompting researchers to modify its structure. These efforts yielded derivatives such as AMD346560 and AMD07061, which boast enhanced oral bioavailability and therapeutic potential. These advancements represent a leap forward in CXCR4-targeted therapies, offering more effective and patient-friendly treatment options[19].

AMD3100, widely known as Plerixafor, gained FDA approval in 2008 for mobilizing HSPCs in patients with non-Hodgkin's lymphoma and multiple myeloma undergoing autologous transplantation. It has demonstrated a robust safety and PK profile across clinical trials, with no significant adverse effects linked to CXCL12/CXCR4 blockade.

PK studies reveal that AMD3100 is rapidly absorbed following subcutaneous administration, reaching maximum plasma concentrations within 0.5 to 1 h[7]. It exhibits a half-life of approximately 3.6 h after intravenous injection and high bioavailability (87%) via subcutaneous routes, though it shows negligible activity with oral administration. The compound has been well-tolerated, with mild and reversible side effects, including injection site discomfort, headache, and nausea.

Exploratory studies, such as those involving patients with WHIM syndrome (a primary immunodeficiency linked to CXCR4 mutations), highlight AMD3100's potential for long-term use. In both human and animal models, AMD3100 effectively mobilizes HSPCs, inducing transient leukocytosis and a dose-dependent increase in circulating CD34+ cells[16].

The success of AMD3100 in mobilizing HSPCs underscores its therapeutic promise in other domains, particularly chronic inflammatory diseases mediated by the CXCR4 pathway. The combination of AMD3100 with agents like G-CSF further enhances its utility, paving the way for innovative treatment approaches in neurodegenerative and inflammatory disorders.

In summary, the evolution of CXCR4 antagonists, spearheaded by AMD3100, exemplifies the potential of targeting chemokine receptor pathways to address complex medical challenges. Future research focusing on optimizing oral bioavailability, enhancing potency, and preserving physiological balance will undoubtedly expand the therapeutic applications of this promising class of compounds.

In vivo AMD3100 treatment applications

AD, a progressive and irreversible neurodegenerative disorder, is characterized by gradual cognitive decline and a complex array of pathological hallmarks. These include extracellular Aβ plaques (senile plaques), neurofibrillary tangles (NFTs), oxidative stress, mitochondrial dysfunction, myelin disruption, and neuroinflammation. Neuroinflammation, a central feature of AD, involves the activation of astrocytes and microglia, alongside the infiltration of peripheral immune components. In both human and rodent brains, CXCR4 is expressed not only by astrocytes but also, at a high density, by microglia[6]. The astrocyte-microglia interaction is involved in CXCR4-dependent glutamate release and activated microglia amplify glutamate release from astrocytes, thus conferring pathological relevance to the process [Figure 1C].

Activated microglia, while central to the inflammatory response, have a dual role in AD pathogenesis. On one hand, they produce and release a variety of inflammatory mediators, including complement factors, chemokines, and cytokines such as interleukins (ILs), tumor necrosis factor-alpha (TNFα), and transforming growth factor-beta (TGFβ). These mediators contribute to the amplification of neuroinflammatory cascades, exacerbating neuronal damage as described before. On the other hand, certain microglial functions are protective; for example, activated microglia can facilitate the clearance of Aβ accumulation through enhanced phagocytosis. Furthermore, Aβ1-40 and Aβ1-42 peptides are continuously degraded by the insulin-degrading enzyme (IDE), a metalloprotease secreted by microglia and other neural cells, supporting their role in mitigating plaque formation[5].

Microglia also secrete trophic factors such as glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF), both of which are well-known for their neuroprotective properties. However, in the context of AD, the CXCR4/CXCL12 axis plays a significant role in neuroinflammation. Aβ plaques are thought to attract microglia through CXCL12 signaling, activating CXCR4-dependent pathways in both microglia and astrocytes. This activation leads to the release of proinflammatory cytokines like TNFα, exacerbating inflammation[6].

Further compounding the damage, this signaling mechanism involves a calcium (Ca^{2+}) cascade, which triggers kinase activation, phosphorylation events, and excitotoxicity driven by excessive glutamate stimulation. Collectively, these pathways underline the intricate balance of microglial functions in AD, oscillating between neuroprotection and neurotoxicity, and highlight potential therapeutic targets for mitigating disease progression[6].

Stromal-derived factor 1 alpha (SDF-1α), a potent chemoattractant for hematopoietic progenitor cells, is believed to play a role in guiding the migration of BMDMs from the peripheral circulation into the brain [Figure 1].

Further investigations have demonstrated that combining AMD3100, a CXCR4 antagonist, with granulocyte-colony-stimulating factor (G-CSF) enhances the mobilization of CD34+ hematopoietic progenitor cells. In a study using APP/PS1 double-transgenic mice, systemic intraperitoneal administration of AMD3100 and G-CSF increased the mobilization of HSPCs. This combination therapy significantly ameliorated memory deficits associated with AD, despite having no observable effect on Aβ depositions[19].

In our study, using the 3 × Tg-AD mouse model, mice were treated with either 5 mg or 500 μg of AMD3100 for 4 months[16]. The 3 × Tg-AD model exhibits age-dependent changes in TNFα mRNA levels that strongly correlate with cognitive deficits.

Our findings revealed that AMD3100 treatment provided multiple benefits: it improved cognitive deficits, reduced tau and APP pathologies, and importantly, shifted microglia toward an anti-inflammatory M2 phenotype [Figure 1C]. This microglial polarization was accompanied by downregulation of proinflammatory mediators such as interleukin-6 (IL-6), TNFα, and monocyte chemoattractant protein-1 (MCP-1), along with upregulation of anti-inflammatory cytokines IL-4 and IL-10, highlighting a robust anti-inflammatory effect [Figure 1C].

One potential explanation for this shift in microglial phenotype is the recruitment of microglia-like cells from the bone marrow. These recruited cells may alter the resident microglial population directly or release factors that restore the profile of resident microglia. Both AMD3100 doses (5 mg and 500 μg) were found to significantly improve tau pathology, underscoring the therapeutic potential of modulating the CXCR4/CXCL12 axis in AD.

Tau pathology in this study was assessed using three distinct antibodies, each targeting different forms of tau associated with AD. Remarkably, a significant reduction - approximately 40% - was observed across all three tau forms at both tested doses of AMD3100. This finding strongly suggests that AMD3100 directly impacts tau formation.

Furthermore, AMD3100 treatment enhanced microglial activation, favoring a shift toward an alternative, neuroprotective microglial phenotype. This reprogramming of microglia underscores the compound's potential in mitigating neuroinflammation and supporting neuronal health.

Beyond its role in mobilizing HSPCs, AMD3100 may influence other physiological pathways, further broadening its therapeutic potential. The recruitment of bone marrow-derived microglia-like cells (BMDMs) into the CNS could play a critical role in modifying the disease course. These cells may help reshape the resident microglial population, supporting the transition to a neuroprotective profile and alleviating neuroinflammation.

Overall, BMDMs represent a promising cell-based therapeutic approach to modulate neuroinflammatory processes and target the underlying mechanisms of neurodegenerative diseases within the CNS.

CONCLUSIONS

The neuroinflammation process was found to be associated with neurodegeneration in a multifaceted process driven mainly by activated microglia and astrocytes involved in the regulation of neuroprotection/neurotoxic balance.

The inhibition approaches toward the CXCR4/CXCL12 signaling prevent the toxic cascade of glutamate release from glial cells and neuronal apoptosis and encourage the mobilization of HSPC from the bone marrow stromal cells to the bloodstream.

When designing CXCR4 antagonists, it is essential to prioritize the development of partial antagonists or modulators that preserve the normal function of the pathway to some extent. This is because the CXCR4/CXCL12 pathway is vital for maintaining normal physiological functions, including the recruitment of HSCs for tissue repair.

DECLARATIONS

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Authors' contributions

Developed and performed the experiments: Gabriel Y
Contributed to the final version and the figure of the manuscript: Voronov-Goldman M
Wrote the manuscript and supervised the project: Solomon B
All authors discussed the results and contributed to the final manuscript.

Availability of data and materials

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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