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REVIEW

The Role of Microglial Exosomes in Clozapine Treatment: Effect on Cognition in Schizophrenia

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Abstract

Schizophrenia is a complex neuropsychiatric disorder characterized by a spectrum of symptoms including cognitive impairments and psychotic episodes. Clozapine, an atypical antipsychotic drug, is a widely recognised treatment option for patients with drug-resistant schizophrenia, due to it having the highest efficacy out of all the antipsychotic drugs. Despite its efficacy, clozapine's impact on cognition and brain structure in schizophrenia patients remains a subject of ongoing research and debate, with accumulating evidence indicating negative impacts on cognitive performance and changes in brain volume. Changes in the immune system are linked to variations in cognitive functioning in schizophrenia. Previous research has indicated that microglia, the primary innate immune cells of the brain, have been associated with decreased cognitive performance when dysfunctional. Evidence suggests that brain structure may mediate the observed relationship between microglia and cognition. Microglial exosomes, integral to neuroinflammation and cellular communication, could provide insight into the neurobiological mechanisms underpinning the effects of clozapine treatment. This review focuses on the proposition that alterations in microglial exosome composition, particularly miRNAs, are involved in mediating clozapine's diverse effects on cognition by influencing brain macrostructure. This review aims to highlight new directions for future research that could lead to more effective and targeted therapeutic approaches in the management of schizophrenia.

Key points

• Clozapine is the gold standard treatment for treatment-resistant schizophrenia patients, due to it having the highest efficacy out of all the antipsychotic drugs.
• While clozapine is effective in treatment-resistant schizophrenia, it has been linked to some structural brain changes and negative effects in certain cognitive domains.
• Recent findings highlight the significant role of microglial exosomes in modulating cognitive function, relevant in schizophrenia. The cognitive effects observed with clozapine treatment may relate to miRNA within microglia-derived exosomes, known to affect brain structure and function.
• Future investigations should determine if clozapine's cognitive side effects are linked to induced changes in miRNAs within microglial exosomes, aiming to elucidate its impact on brain and cognitive function in schizophrenia. This could open new avenues to optimize clozapine's benefits while mitigating its potential side-effects.

Keywords Schizophrenia · Exosome · miRNA · Clozapine · Microglia · Cognition

Background

Schizophrenia

Schizophrenia, a severe neuropsychiatric disorder, presents with a mix of psychotic, motivational, and cognitive dysfunctions, affecting approximately 1% of the global population (Kahn et al. 2015; Li et al. 2016). It is marked by symptom categories: positive symptoms like hallucinations,

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negative symptoms such as avolition, and cognitive disruptions in areas like attention, working memory, and executive function (Bowie and Harvey 2006; Joseph et al. 2015; Correll and Schooler 2020). Sex differences also exist; while males typically experience onset between 21 and 25 years, females often see two peaks: 25–30 years and another after 45 (Li et al. 2016).

Clozapine

Clozapine is an atypical antipsychotic drug which is used to treat schizophrenia and bipolar disorder (Forte et al. 2021). Clozapine is a widely recognised treatment option for patients with drug-resistant conditions, due to it having the highest efficacy out of all the antipsychotic drugs (Wagner et al. 2021). However, its use is typically reserved as a last line treatment due to increased risk of immune effects such as agranulocytosis, as well as substantial weight gain, metabolic syndrome, diabetes, severe constipation, and bowel obstruction. The effects of clozapine on CNS (central nervous system) immune cells is less clear.

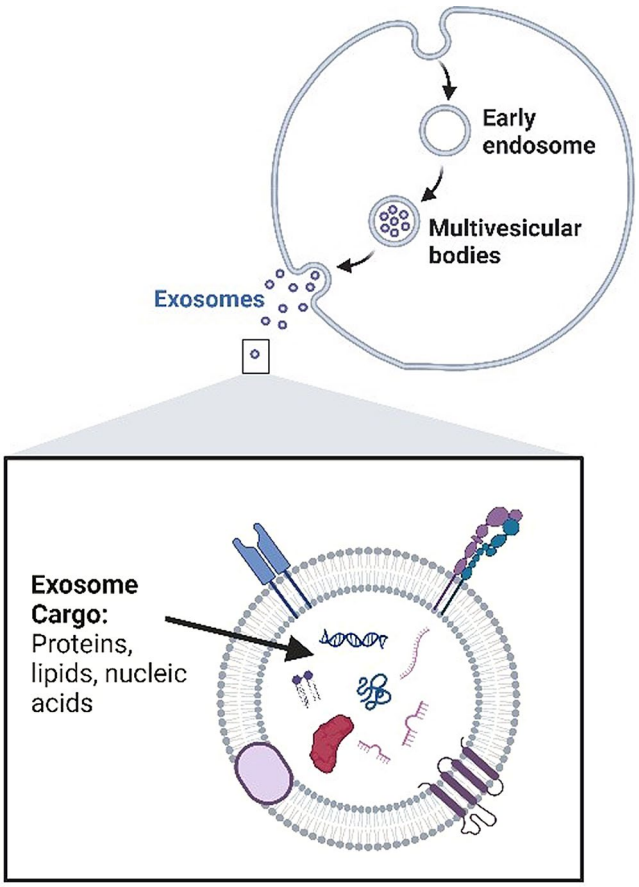

Fig. 1 Diagram illustrating exosome generation through the endocytic pathway and the basic structure of exosomes. Created with biorender.com

Microglia

Microglia, the primary innate immune cells of the brain, account for approximately 15% of all CNS cells in humans and are the principle component of neuroinflammation (Müller et al. 2016). These cells originate from myeloid progenitors in the yolk sac and migrate to the brain during early embryogenesis (Ginhoux et al. 2010; Brawek and Garaschuk 2013).

Under healthy physiological conditions, microglia exhibit a ramified morphology, characterized by a small soma with elaborate ramifications, enabling them to perform surveillance of the brain microenvironment, continually surveying for pathogens and debris with motile processes and protrusions (Nimmerjahn et al. 2005; Davis et al. 2017). Also known as 'M0', resting microglia can activate into proinflammatory (M1) or anti-inflammatory (M2) states (Tanaka et al. 2020). However, this binary classification has become less favoured due to the recognition of microglia's ability to adopt various activation states beyond the M1 and M2 categories (Ransohoff 2016).

Exosomes

Exosomes are extracellular vesicles (EVs), approximately 30 to 150 nm in diameter, secreted by all cell types. These vesicles originate from the endocytic pathway (Fig. 1), starting as early endosomes that mature into multivesicular bodies (MVBs). MVBs contain intraluminal vesicles formed by inward budding, which then release into the extracellular space as exosomes (Zhang et al. 2019; Gurung et al. 2021).

Rich in diverse biological materials, exosomes carry membrane, cytosolic, nuclear, and extracellular matrix proteins, metabolites, lipids, as well as various nucleic acids including mRNA, DNA, and notably, miRNAs (Valadi et al. 2007; Saeedi et al. 2019; Kalluri and LeBleu 2020; Amiri et al. 2022). MiRNAs within exosomes can modulate gene expression in recipient cells, impacting their phenotype (Zhang et al. 2015; Wang et al. 2022).

Exosomes facilitate intercellular communication, especially notable in the CNS. They play a significant role in physiological changes within recipient cells upon uptake (Bang and Thum 2012). Their involvement is increasingly recognized in neurodegenerative diseases, including schizophrenia, Alzheimer's disease (AD), and Parkinson's disease (Shi et al. 2014; Asai et al. 2015; Cerri et al. 2018).

Clozapine and the Immune System

Clozapine's role in modulating the immune system presents a complex and fascinating aspect of its pharmacological profile. Its interactions with various components of the immune system, including microglia and other immune cells, as well as its influence on cytokine profiles and exosome dynamics, have significant implications for understanding its therapeutic effects in psychiatric disorders. Exploring these mechanisms offers valuable insights into the broader implications of clozapine's use, particularly in conditions characterized by immune dysregulation. The effects of clozapine on immune cells is summarised in Table 1.

The body of research on the effects of clozapine on immune cells largely agrees on its anti-inflammatory role, consistently indicating the suppression of proinflammatory cytokines (such as TNF-α, IL-1β, IL-6, IL-8, IL-1α, IL-2, IL-17, and IFN-γ) and the upregulation of anti-inflammatory cytokines (such as IL-10) (Sugino et al. 2009; Chen et al. 2012; Hu et al. 2012; Giridharan et al. 2020; Yuhas et al. 2022). Moreover, clozapine has been shown to inhibit Th1 cell differentiation and suppress the production of IFN-γ in peripheral blood mononuclear cells, further supporting its role in immune modulation (Chen et al. 2012).

In addition to the suppression of proinflammatory cytokines, in vitro studies highlight clozapine's ability to modulate key signaling pathways, including Akt phosphorylation, in diverse cell types such as human-derived astrocytes and rat microglia (Jeon et al. 2018; Yuhas et al. 2022). The potential of clozapine to influence the neuroinflammatory pathway is further demonstrated by its ability to inhibit the Ca2+/CaM/Akt-mediated NF-KB pathway in microglial cells, a pathway known for its role in inflammatory responses (Jeon et al. 2018). This action was evident in both in vitro and in vivo experiments, where clozapine effectively reduced the expression of MHC class II on microglia in the brain, indicating an inhibitory effect on lipopolysaccharide (LPS)-induced microglial activation (Jeon et al. 2018). Additionally, clozapine's protective role in inflammation-induced neuronal damage has been documented, particularly in its ability to mitigate over-activation of microglia, thus protecting dopaminergic neurons from neurotoxicity (Hu et al. 2012).

In vivo studies have consistently shown reductions in immune cell activation and infiltration in a range of models, notably those pertaining to multiple sclerosis (MS) and neuroinflammation (Templeton et al. 2019; Robichon et al. 2020; Ceylan et al. 2021). Moreover, the administration of clozapine has been demonstrated to reduce the infiltration of peripheral immune cells into the CNS (Robichon et al. 2020). This finding is supported by the observed decrease in the expression of chemokines CCL2 and CCL5 in the CNS, suggesting that clozapine may reduce immune cell migration into the CNS by modulating chemokine expression (Robichon et al. 2020).

In the context of neurological disorders, clozapine has been shown to enhance functional recovery following demyelination, attributed to its capacity to decrease levels of astrocyte and microglial activation (Templeton et al. 2019). Other studies on progressive MS have attributed clozapine's efficacy to its modulating effects on microglial function, particularly under conditions of iron-induced disruptions (Ceylan et al. 2021). Not only does clozapine counteract the negative impact of iron on microglial phagocytosis, but it also significantly reduces IL-6 release in iron-treated microglia, further underscoring its immunomodulatory capacity. Additionally, an inverse relationship has been observed between norclozapine (clozapine's major metabolite) plasma concentrations and neutrophil counts in treated individuals, pointing to a potential impact on neutrophil dynamics (Willcocks et al. 2021).

While chronic treatment with some antipsychotics, such as haloperidol and olanzapine, has been associated with an activated microglia morphology in the brain, clozapine's impact appears distinct (Cotel et al. 2015). Notably, clozapine treatment, particularly under inflammatory conditions, has demonstrated a significant reduction in proinflammatory cytokines in a primary murine microglia cell model (Giridharan et al. 2020). This suggests that unlike some other antipsychotics, clozapine may exert a more obvious anti-inflammatory effect on microglia. These findings highlight the nuanced role of clozapine in modulating microglial activity, distinguishing it from other antipsychotic treatments.

Clozapine's effects on the immune system are extensive, impacting various aspects of the immune response, including reducing neuroinflammation, altering cytokine profiles, and influencing microglial function. While there are some variations in clozapine's effects, particularly across different disease models, cell types, and species, the overarching evidence highlights its anti-inflammatory impact on immune cells. These findings not only enhance our understanding of clozapine's mechanisms of action, but also open new avenues for research into its potential roles in treating conditions involving immune system dysregulation, and in elucidating the causes of side effects associated with its treatment.

Clozapine and Cognition in Schizophrenia

Few studies have investigated clozapine's impact on cognition in schizophrenia, yielding varied findings including decreased, increased, mixed, or no effects on cognitive

Table 1 Studies investigating the effect of clozapine on the immune system

Author (Year)	Study type	Model	Key Findings	Additional notes
Robichon et al. (2020)	1. In vitro 2. In vivo	1. Primary murine microglia, Primary bone marrow-derived macrophages 2. EAE model of MS in mice	1. Decreased expression of CCL2 and CCL5 2. Decreased expression of CCL2 and CCL5 in the CNS to healthy control levels 2. Reduced CNS infiltration of monocytes, neutrophils, and T cells. No change in microglia numbers in CNS. 2. Reduced counts of monocytes and neutrophils in the spleen and blood in clozapine-treated EAE mice. However, macrophage and T cell numbers in spleen and blood were unaltered.	
Sugino et al. (2009)	In vivo murine	1. LPS-treated mice 2. Poly)I: C)-treated mice	1. Suppression of proinflammatory cytokines TNF-α and IL-6, and upregulation of the anti-inflammatory cytokine IL-10. 2. Suppression of TNF-α and IL-6, and upregulation of IL-10.	
Templeton et al. (2019)	In vivo murine	Cuprizone mouse model of demyelination	Clozapine did not prevent demyelination. Reduced microglia and astrocyte activation after cessation of cuprizone treatment. Enhanced functional recovery post demyelination.	Reduced glial activation correlated with observed functional improvements.
Willcocks et al. (2021)	Human cohort study	NA	Plasma concentrations of norclozapine were inversely associated with absolute neutrophil counts	
Yuhas et al. (2022)	In vitro	Human-derived astrocytes stimulated with cytokine mix	Suppression of mRNA expression of proinflammatory cytokines TNFα, IL-1β, and IL-8 Upregulation of cyclooxygenase 2 mRNA expression Inhibition of Akt phosphorylation	
Chen et al. (2012)	In vitro	1. Peripheral blood mononuclear cells (PBMCs) treated with phorbol myristate acetate and ionomycin 2. PMBCs stimulated with anti-CD3/CD28 antibodies	1. Suppression of interferon-γ (IFN-γ) 2. Suppression of IFN-γ 2. Reduction in T-bet mRNA and protein. Enhanced expression of Signal Transducer and Activator of Transcription 6 (STAT6) and GATA-binding protein 3 (GATA3)	1. Th1 cell differentiation, is associated with IFN-γ production. 2. T-bet mRNA and protein, are critical for Th1 differentiation. 2. STAT6 and GATA3, are important for Th2 differentiation.
Giridharan et al. (2020)	In vitro	Primary rat microglia stimulated by poly (I: C)	Reduction of proinflammatory cytokines IL-1α, IL-1β, IL-2, and IL-17. Reduction of the level of poly (I: C)-activated NLRP3 expression by 57%.	Clozapine exhibited greater inhibition than the NLRP3 inflammasome inhibitor, CRID3 which was had a 45% reduction

Table 1 (continued)

Author (Year)	Study type	Model	Key Findings	Additional notes
Ceylan et al. (2021)	1. In vitro 2. In vivo	1. Human microglia treated with iron as an EAE model. 2. EAE mouse model	1. Normalisation of phagocytosis and reduction in IL-6 release. 2. Reduction in clinical signs of EAE, reduction in demyelination 2. Reduced frequency of CD4 + T cells in blood, spleen, and lymph nodes, with the strongest effects observed in lymph nodes. Th17 cells were reduced in the spleen. Clozapine induced a reduction of regulatory (CD4 + CD25 + FoxP3+) T cells in the blood and lymph nodes. CD86 + antigen-presenting cells were reduced in the spleen.	
Jeon et al. (2018)	1. In vitro 2. In vivo	1. Primary rat microglia 2. LPS-treated mice	1. Suppression of LPS-induced activation of the NF-κB pathway, via inhibition of phosphorylation of IκBα and p65/RelA, and downregulated Akt phosphorylation. 1. Reduction in TLR4-mediated NF-κB activation 2. Decreased the number of MHC class II-positive cells in the mouse brain after LPS injection, indicating reduced microglial activation.	
Hu et al. (2012)	In vitro	1. 1-methyl-4-phenylpyridinium+ (MPP+)-treated neuron-microglia co-culture 2. LPS-treated neuron-microglia co-culture	1. Inhibition of MPP+-mediated neurotoxicity a microglia-dependent mechanism 2. Inhibition of microglial activation evidenced by decreased Iba1 expression and morphology. In addition, clozapine significantly reduced the percentage of MHC-II OX-6-positive cells in HAPI microglial cell line cultures, suggesting a reduction in microglial activation. 2. Attenuation of extracellular superoxide and intracellular reactive oxygen species (ROS) production in microglia-enriched cultures. It also decreased the release of nitric oxide (NO) and TNF-α after LPS stimulation.	

function. This discrepancy highlights the need for more research in this area, and may reflect the complexity of clozapine's interaction with cognitive processes in schizophrenia.

Hoff et al. (1996) reported a mixed effect of clozapine on cognition. Their analysis of neuropsychological test data from a subset of schizophrenic human patients suggested improvement on measures of verbal fluency and graphomotor speed, but deterioration on measures of visual memory and executive/frontal ability (Hoff et al. 1996). This duality in cognitive outcomes highlights the potentially selective impact of clozapine on different cognitive domains.

Regarding animal models, Addy et al. (2005) reported differential effects of clozapine on working memory, depending on the physiological state of the subjects. In their study, fimbria-fornix-lesioned rats, exhibited improvements in working memory following clozapine treatment, whereas intact rats experienced working memory impairments (Addy et al. 2005). This finding suggests that the underlying brain state or pathology might influence clozapine's cognitive effects.

In a retrospective cross-sectional analysis, Rajji et al. (2010) linked clozapine plasma concentration with cognitive outcomes in hospitalized schizophrenia patients. Their study found a positive correlation between clozapine plasma concentration and severe cognitive impairment, as measured by a clinically validated global cognition scale (Rajji et al. 2010). Notably, the ratio of clozapine to N-desmethylclozapine was more strongly associated with cognitive deficits (Rajji et al. 2010). This specific association could be important in understanding the pharmacological basis of clozapine's cognitive effects.

Despite these results, the variable findings across different studies point to a need for more comprehensive research. Understanding the factors contributing to these divergent outcomes, including study design, patient characteristics, and drug dosage, is essential for a clear understanding of clozapine's impact on cognition in schizophrenia.

Cognitive Impairment in Untreated Schizophrenia

Schizophrenia is well-established as a disorder characterized by cognitive impairments that are independent of antipsychotic treatment (Fatouros-Bergman et al. 2014). Underscoring this, schizophrenia was historically referred to as "dementia praecox," highlighting cognitive deficits as a core feature of the disorder (Adityanjee et al., 1999). These cognitive impairments are persistent and often remain even when positive symptoms, such as hallucinations, are managed (McCutcheon et al. 2023).

Given that cognitive deficits are intrinsic to the pathology of schizophrenia, it is essential to consider these baseline

impairments when evaluating the cognitive effects of pharmacological treatments like clozapine. Since schizophrenia is rarely left untreated, distinguishing the cognitive effects of the drug from those of the underlying pathology can be challenging. Therefore, a clear understanding of the inherent cognitive dysfunction associated with schizophrenia, alongside studies focused on clozapine's effects alone, is crucial for distinguishing between impairments caused by the disease itself and those potentially exacerbated or induced by antipsychotic intervention.

As previously noted, isolating the cognitive effects of clozapine from those of schizophrenia pathology is challenging. However, recent MRI studies provide stronger evidence by tracking brain structural changes in schizophrenia patients both before and after clozapine treatment over periods of 3 to 6 months (Tronchin et al. 2020; Krajner et al. 2022). This approach may provide better insight into clozapine's effects, as it better isolates drug-induced changes by using a before-and-after design, where clozapine is the only newly introduced variable. These studies report volume reductions in key brain regions, including the caudate nucleus, putamen, and hippocampus, alongside lateral ventricle enlargement and cortical thinning in areas such as the left inferior temporal cortex and right temporal pole (Tronchin et al. 2020; Krajner et al. 2022). These regions are critical to cognitive functioning. The caudate nucleus contributes to learning and memory, particularly through the excitation of correct action schemas and the selection of appropriate subgoals based on an evaluation of action-outcomes, affecting the ability to plan and execute tasks (Grahn et al. 2009). The right temporal pole has been strongly associated with various high-level cognitive functions, particularly socio-emotional processes such as empathy, theory of mind, and emotion recognition (Herlin et al. 2021). It is activated during tasks involving the understanding of others' emotions or perspectives, as well as in social behavior regulation (Herlin et al. 2021). Additionally, the right temporal pole plays a role in visual processing for complex objects and face recognition, autobiographical memory, and semantic processing (Herlin et al. 2021). Atrophy of the right temporal pole has been associated with deficits in emotion recognition, social cognition, and semantic memory (Herlin et al. 2021). The hippocampus, central to episodic memory and spatial navigation, shows strong links between volume reductions in subfields like CA1 and subiculum and global cognitive decline (Lisman et al. 2017; Doran et al. 2023). These structural volume losses in regions essential for cognitive functioning implies that clozapine may contribute to cognitive impairment.

The evidence for clozapine's direct impact on cognition, independent of schizophrenia pathology, is clearer in animal studies that eliminate confounding disease factors. In healthy rats, clozapine has been shown to impair working memory, suggesting the drug may independently contribute to cognitive deficits (Addy et al. 2005). Similarly, in mice, clozapine disrupted spatial memory acquisition, impaired memory consolidation, and affected memory retrieval (Mutlu et al. 2011). More relevant to human application, studies in macaque monkeys treated chronically with olanzapine revealed significant brain volume reductions (8–11%) and structural changes in both gray and white matter, particularly in the frontal and parietal lobes, after 1.5 to 2.3 years of exposure (Dorph-Petersen et al. 2005). These findings, with drug levels comparable to those in human treatment, raise important questions about the potential long-term effects of clozapine on brain structure, though to the best of our knowledge, similar primate studies for clozapine have not been conducted. Given that clozapine and olanzapine are both atypical antipsychotics, it is plausible that clozapine could result in similar brain volume reductions, with implications for cognitive outcomes in patients with schizophrenia.

Clozapine and Brain Structure

The neurobiological effects of clozapine on brain structure may provide insights into its complex influence on cognition in schizophrenia patients.

The prevailing hypothesis is that these brain macrostructural deficits, common in schizophrenia and potentially exacerbated by antipsychotic use, arise from synaptic and dendritic loss due to glutamate excitotoxicity or neuroinflammation and microglial activation, rather than direct neuronal loss (Krajner et al. 2022).

Longitudinal studies, albeit limited, consistently report a reduction in caudate nucleus volume within 6 months to 2 years of initiating clozapine treatment (Chakos et al. 1995; Frazier et al. 1996; Scheepers et al. 2001b; Tronchin et al. 2020; Krajner et al. 2022). This is particularly salient as the caudate nucleus is implicated in a number of cognitive processes (Grahn et al. 2008).

Krajner et al. (2022) examined 24 patients with treatment-resistant schizophrenia, finding that 12 weeks of clozapine treatment led to reductions in caudate volume by 3.5% and putamen volume by 4%, along with a 7.5% enlargement of the lateral ventricles. Additionally, cortical thinning was observed in regions such as the left inferior temporal cortex and right temporal pole (Krajner et al. 2022). These structural changes, were linked to reductions in N-acetylaspartate levels, suggesting potential decreases in neuronal or metabolic integrity.

Scheepers et al. (2001a) reported that treatment with clozapine in patients with treatment-resistant schizophrenia

resulted in a decrease in left caudate nucleus volume. This structural change was correlated with improvements in positive and general symptoms of schizophrenia (Scheepers et al. 2001a). The study did not evaluate the effects of clozapine on cognitive symptoms.

Tronchin et al. (2020) investigated the impact of clozapine treatment on brain morphology in treatment resistant schizophrenia patients over a 6 month period. The study revealed significant morphometric changes in patients after 6 months of clozapine treatment, including a 14.96% enlargement of the lateral ventricles and reductions in the volumes of the thalamus (−3.21%), caudate (−4.83%), putamen (−6.07%), hippocampus (−2.63%), pallidus (−1.74%), amygdala (−2.16%), and nucleus accumbens (−3.50%) (Tronchin et al. 2020). Notably, reductions in the volumes of the thalamus and putamen were associated with an improvement in Positive and Negative Syndrome Scale for Schizophrenia score (Tronchin et al. 2020). The investigation, however, did not address whether these brain volume changes also correspond to alterations in cognitive functions.

The current body of evidence suggests a link between clozapine use and brain structural changes. Future research should focus on elucidating the mechanisms behind this and determining whether these structural brain changes correspond to cognitive deficits or improvements. Longitudinal studies, advanced imaging methodologies, and in vitro work could be particularly valuable in this area. Understanding the relationship between clozapine-induced structural changes and cognitive impairment in schizophrenia is essential in developing more targeted therapeutic strategies.

Exosomes and Cognition

Recent research has begun to elucidate the potential role of exosomes in mediating cognitive effects. Exosomes, as vehicles for the transfer of functional proteins and RNA between cells, have been shown to significantly influence cellular functions, including those related to cognition (Koh et al. 2020). This leads to the consideration that clozapine-induced changes in exosome profiles could be a key factor in the drug's impact on cognitive processes.

In broader neurological contexts, the significance of exosomes is increasingly recognised. For instance, in MS, a correlation has been observed between gray matter demyelination and memory decline and cognitive dysfunction, with evidence demonstrating that serum exosomes from young or environmentally enriched animals can promote oligodendrocyte precursor cell differentiation and remyelination, potentially improving cognitive function (Pusic and Kraig 2014). This study by Pusic and Kraig (2014) showed that environmental enrichment (EE) of aging animals produces exosomes that mimic the promyelination effect, and that exosomes derived from EE animals are enriched in miR-219, which is essential for the production of myelinating oligodendrocytes. In addition, the study found that the nasal administration of exosomes to aging rats also improved myelination, suggesting that exosomes may be a useful therapy for remyelination and improvement of cognitive function in MS patients (Pusic and Kraig 2014). These results demonstrate the potential for exosomes to play a crucial role in remyelination and the improvement of cognitive function in patients with MS and other neurological disorders.

A recent study by Liu et al. (2022) investigated the potential of bone-marrow mesenchymal stem cell-derived exosomes (BMSC-exos) as a therapeutic avenue for Alzheimer's disease, a neurodegenerative disorder marked by cognitive decline. The researchers isolated BMSC-exos from mice and administered them via lateral ventricle injection to an AD mouse model induced by intracerebroventricular injection of streptozotocin (Liu et al. 2022). Remarkably, this intervention improved AD-like behaviors in the mice, as evidenced by enhanced performance in various behavioral tests like the open field and novel object recognition tests (Liu et al. 2022). The treatment also modulated the hyperactivation of microglia and astrocytes in the hippocampus, reducing the expression of inflammatory markers (IL-1β, IL-6, TNF-α) and AD-related neuropathological features such as Aβ1–42 and phosphorylated Tau (Liu et al. 2022). Additionally, an upregulation of synapse-related proteins and brain-derived neurotrophic factor was observed, correlating positively with cognitive performance improvements (Liu et al. 2022). These findings suggest that BMSC-exos, particularly when administered directly to the brain, could modulate glial activation and associated neuroinflammation, thereby impacting AD-related neuropathology and cognitive functions (Liu et al. 2022).

Similarly, in the sphere of cancer-related cognitive impairment, exosomes released by CNS cancers such as glioblastoma and medulloblastoma, have been linked to chemotherapy resistance and loss of endothelial barrier integrity, leading to debilitating cognitive deficits (Treps et al. 2016; Koh et al. 2020).

Overall, the emerging evidence suggests that exosomes play an important role in modulating cognitive functions, a finding particularly pertinent in schizophrenia research. The exact mechanisms by which clozapine affects cognition remain elusive, prompting research into alterations in exosomal profiles as a potential key factor. This idea, supported by observations in various neurological conditions, opens a promising avenue for investigation. Investigating the interaction between clozapine and exosomes could shed light on its cognitive effects in schizophrenia and guide the development of more targeted therapeutic approaches.

Microglial Exosomes on Cognition

Recent studies have explored the role of microglial exosomes in influencing cognitive functions, particularly in neurodegenerative contexts, suggesting a potential avenue for understanding clozapine's impact on cognition.

Chen et al. (2023) demonstrated that near-infrared photobiomodulation induces a phenotypic shift in microglia, leading to the secretion of exosomes that enhance cognitive function in AD models. This phenomenon, mediated by miR-7670-3p in the exosomes, suggests a pathway through which microglial exosomes can influence cognition.

Similarly, Ge et al. (2020) found that microglial exosomes, enriched with miR-124-3p following repetitive mild traumatic brain injury, could mitigate neurodegeneration and improve cognitive outcomes. This study highlights the potential of microglial exosomes in reversing cognitive deficits associated with brain injuries, an aspect that might be relevant in the context of clozapine-induced changes in brain structure and function.

Further supporting this, Li et al. (2022) observed that M2 microglia-derived exosomes exert neuroprotective effects in AD models, enhancing neuronal health and cognitive function. This effect was mediated by facilitating PINK1/Parkin-mediated mitophagy, a key process in cellular homeostasis (Li et al. 2022).

Moreover, Zhang et al. (2023) explored the role of microglial exosomes in cognitive impairment due to intermittent hypoxia. They found that microglial exosomes containing miR-146a-5p could modulate neuroinflammation and improve cognitive dysfunction, suggesting a therapeutic strategy that could intersect with clozapine's pharmacological profile (Zhang et al. 2023).

Additionally, Zhao et al. (2021) emphasized the negative impact of activated microglia-derived exosomes on neurite outgrowth and synapse recovery following traumatic brain injury, a process that could be reversed by manipulating miR-5121 levels in these exosomes.

A recent study utilising the UK biobank database found that increased microglial polygenic risk scores are associated with lower cognitive performance in both schizophrenia patients and healthy controls (Corley et al. 2021). Furthermore, the study found that this association is likely mediated by total grey matter volume, indicating that microglial dysfunction impacts cognitive function through its effects on brain structure (Corley et al. 2021). Unlike neuronal genes, the microglial gene-set did not show significant enrichment for schizophrenia risk, indicating that microglial variations might be more relevant to general neurodevelopmental processes affecting cognition rather than being specific to schizophrenia (Corley et al. 2021). Although this study did not explore microglial exosomes, the results lend support to microglia involvement in cognitive processes.

Collectively, these studies indicate that microglial exosomes play a significant role in modulating cognitive functions, particularly under neurodegenerative or injury conditions. Given clozapine's emerging effects on brain structure and exosome production, these findings open up possibilities for investigating microglial exosomes as a target to understand and potentially mitigate clozapine's adverse cognitive effects.

Clozapine and its Impact on Exosomes in Schizophrenia

Recent research has highlighted the significant role of exosomes in the therapeutic effects of antipsychotic drugs, particularly in psychiatric disorders like schizophrenia. Exosomes, as carriers of various biomolecules including RNA, proteins, and lipids, have the capability to influence the behaviour of recipient cells. This is particularly evident in the context of how antipsychotic drugs, such as clozapine, modify exosomal composition, thereby potentially impacting disease pathology and treatment outcomes.

A recent study by Funahashi et al. (2023) specifically underscores clozapine's influence in treatment-resistant schizophrenia. Their findings demonstrate that clozapine treatment induces substantial changes in miRNA expression within plasma exosomes, with 13 miRNAs upregulated and 18 downregulated (Funahashi et al. 2023). These miRNAs are associated with neuronal and brain development, suggesting that clozapine's effects on cognition may be mediated through these exosomal alterations. Such changes in exosomal content provide a possible mechanism into clozapine's unique impact on cognitive functions in schizophrenia. However, it is important to note that a cautious approach is needed when interpreting these findings, as the lack of a direct control group of untreated treatment-resistant schizophrenia patients makes it difficult to conclusively attribute these changes solely to clozapine treatment (Hewitt and Huang 2024). While the evidence strongly points in the direction of clozapine's influence, further studies are required to confirm these results (Hewitt and Huang 2024).

Significantly, Funahashi et al. (2023) discovered that miR-675-3p was upregulated in the plasma-derived exosomes of clozapine-treated patients. This effect was demonstrated in exosomes derived from SH-SY5Y cells (Funahashi et al. 2023). One of the predicted targets of miR-675-3p, is Myocyte Enhancer Factor 2 C (MEF2C), a protein that plays essential roles in cognitive function and neurodevelopment (Funahashi et al. 2023). Analysis of human data from clinical and neuro-transcriptomic repositories shows that higher

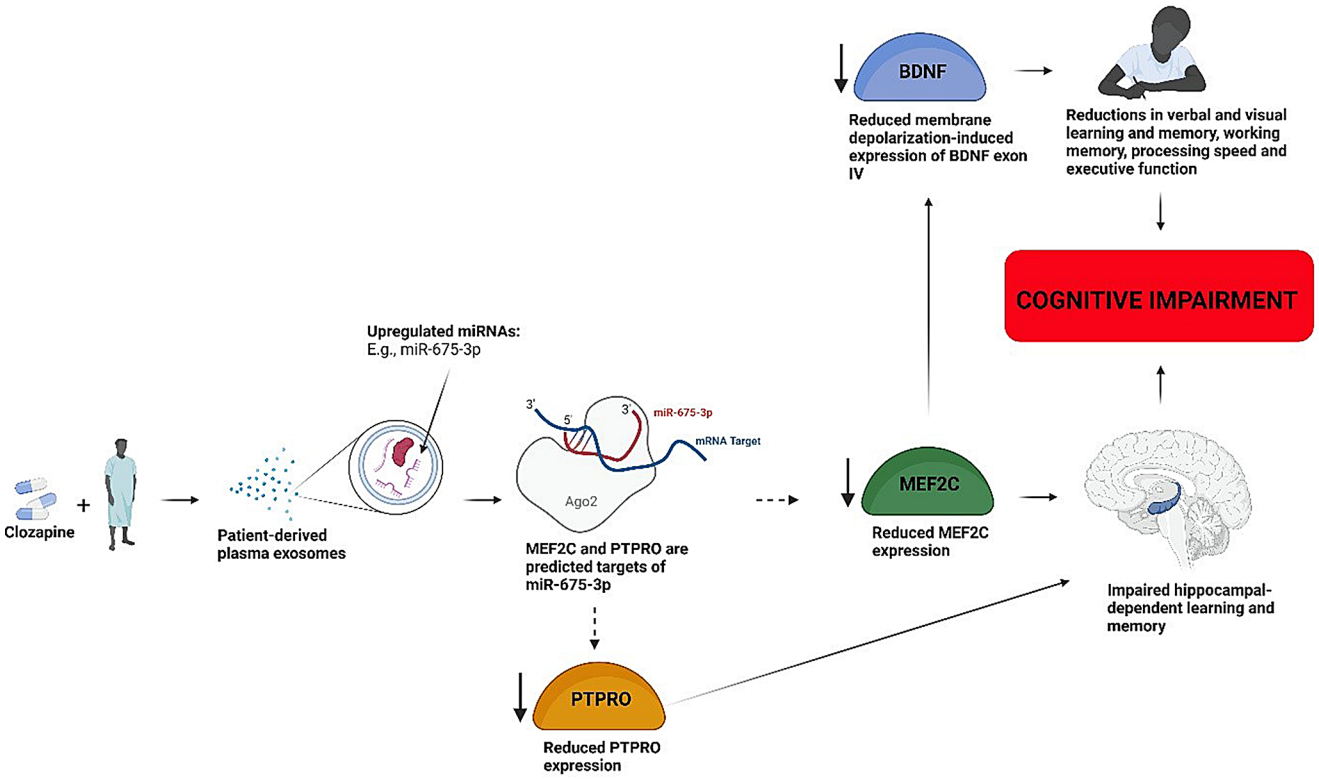

Fig. 2 Diagram illustrating pathway linking clozapine treatment to cognitive effects in schizophrenia patients. The diagram highlights the upregulation of miR-675-3p in exosomes from clozapine-treated patients and its predicted targets, MEF2C and PTPRO. It illustrates the roles of these targets in cognitive processes and suggests how miR-675-3p-mediated inhibition of MEF2C and PTPRO might contribute to clozapine's effects on cognitive function. Created with BioRender.com

levels of MEF2C and its target genes are associated with better cognitive function and resilience (Barker et al. 2021). Interestingly, increased expression of MEF2C in cognitively resilient individuals was observed specifically in a subset of excitatory neurons, suggesting an important role of MEF2C in maintaining cognitive function in the human brain (Barker et al. 2021). This is further supported by research indicating that MEF2C is involved in the molecular response to cognitive stimulation, with its transcriptional activity being a key factor in cognitive resilience (Barker et al. 2021). Additionally, in a mouse model of tauopathy and neurodegeneration, overexpression of MEF2A/C, improved cognitive function and mitigated disease-induced hyperexcitability (Barker et al. 2021). Similarly, it has been demonstrated that deletion of MEF2C in the CNS of mice impairs hippocampal-dependent learning and memory (Barbosa et al. 2008). Furthermore, MEF2C knockdown has been found to significantly impair membrane-depolarization-induced expression of BDNF exon IV, which could in turn leads to decreases in learning and memory associated with BDNF reduction (Lyons et al. 2012; Ferrer et al. 2019). Another predicted target of miR-675-3p is protein tyrosine phosphatase receptor type O (PTPRO) (Funahashi et al. 2023). Similar to MEF2C, PTPRO deletion is associated with hippocampus-associated cognitive dysfunction (Yao et al. 2023). The inhibition of MEF2C and PTPRO through miR-675-3p-containing exosomes may elucidate the observed negative effects of clozapine on cognitive function. This is summarised in Fig. 2.

This emerging evidence builds on the broader understanding of antipsychotics' influence on exosomes. For instance, a recent study found in drug-naïve first-episode psychosis patients with schizophrenia an upregulation of miRNA203a-3p and suppression of its target protein DJ-1 in whole blood and blood-derived exosomes, changes implicated in the pathology of the disorder (Tsoporis et al. 2022). Notably, olanzapine treatment in these patients attenuated miR203a3p expression and increased DJ-1 levels, effectively normalizing these markers in blood-derived exosomes to levels observed in healthy controls (Tsoporis et al. 2022). This reinforces the notion that antipsychotic drugs, including clozapine, might exert a part of their therapeutic effect through modulating exosomal content.

Given these findings, the proposal that clozapine's cognitive effects in schizophrenia are exerted through its impact on exosomes opens a promising avenue for research. This area offers potential for developing more effective and targeted interventions in schizophrenia treatment, though

further investigation is needed to fully understand the mechanisms and therapeutic implications of targeting exosomes.

Exploring the Potential Mechanisms of Differential miRNA Sorting in Microglial Exosomes Induced by Clozapine

Although the mechanisms behind miRNA loading into exosomes are not fully understood, some progress has been made in understanding this complex process. For instance, the binding of miRNA to a sumoylated version of the heterogenous nuclear ribonucleoprotein A2B1 (hnRNPA2B1), a protein present in exosomal membranes, is one identified mechanism (Villarroya-Beltri et al. 2013). In the context of KRAS colorectal cancer cells, there is evidence of a cooperative role between KRAS and Ago2 in the targeted delivery of miRNAs into exosomes (Cha et al. 2015; McKenzie et al. 2016). Furthermore, a variety of other proteins have been implicated in the process of RNA loading into exosomes. For example, the major vault protein has been associated with the transport of miR-193a in colon cancer cells and mRNAs in neurons (Teng et al. 2017; Pastuzyn et al. 2018). Similarly, the HuR protein has been identified as a key regulator in the extracellular export of miR-122 through exosomes in human hepatic cells (Mukherjee et al. 2016). These proteins, integral to RNA-binding, play significant roles in RNA sorting within exosomes.

Given the complexity of miRNA sorting into exosomes, coupled with clozapine's diverse receptor interactions, the impact of clozapine on exosome miRNA content could be multifaceted. A key area of potential influence is clozapine's effect on intracellular calcium levels. Studies, including those by Savina et al. (2003) and Yamaguchi et al. (2023), have highlighted the critical role of intracellular calcium in the formation and release of exosomes. Furthermore, Hagiwara et al. (2015) have shown how intracellular calcium levels can influence the process of miRNA packaging into these vesicles, demonstrating the complex mechanisms governing exosomal content and function. Specifically, in their study, Hagiwara et al. (2015) uncovered a pivotal role of the ANXA2 protein in the incorporation of miRNAs into extracellular vesicles. They focused on a group of miRNAs (including miR-16, miR-21, and miR-24, among others) known to be present in EVs derived from PC3 cells (Hagiwara et al. 2015). Their findings revealed a direct correlation between the intracellular levels of ANXA2 and the presence of these miRNAs in EVs (Hagiwara et al. 2015). Notably, overexpression of ANXA2 led to its increased presence in both cells and EVs, alongside a notable enrichment of miR-16 in the EVs (Hagiwara et al. 2015). This observation did not significantly alter the miR-16 transcript levels in the cells, thereby supporting the regulatory influence of ANXA2 on miRNA packaging into EVs (Hagiwara et al. 2015). Importantly, their research unveiled that the enrichment of miRNAs in EVs is $Ca2+$dependent (Hagiwara et al. 2015). Thus, Hagiwara et al. (2015) propose a hypothesis that $Ca2+$enrichment in EVs could enhance the ANXA2-mediated loading of miRNAs into these vesicles, highlighting the significance of calcium in this mechanism.

Moreover, recent research, such as the study by Al Abadey et al. (2022), shows that clozapine significantly reduces intracellular calcium levels in splenocytes. Complementing this, Park et al. (2001) reported that clozapine treatment inhibited norepinephrine secretion and decreased cytosolic $Ca2+$levels. Pochet et al. (2003) also observed that clozapine inhibits the rise in $Ca2+$levels induced by norepinephrine and epinephrine in rat submandibular acinar cells.

This reduction in calcium, induced by clozapine, could influence the dynamics of the ESCRT machinery, or other proteins such as ANXA2, critical for miRNA packaging during exosome formation. Altered calcium levels might impact the efficiency and specificity of miRNA packaging into exosomes, potentially leading to a unique miRNA profile within the exosomes. This change could involve the preferential inclusion or exclusion of specific miRNAs, altering their composition. Such shifts could significantly influence cell-to-cell communication and microglial functions, given the pivotal role of miRNAs in gene regulation and signaling pathways.

Clozapine's ability to modulate intracellular calcium levels may lead to alterations in the function of the ESCRT machinery or other proteins, subsequently affecting the miRNA content of exosomes. This potential mechanism, derived from an integration of various studies, offers a perspective on how clozapine could influence miRNA-mediated cellular communication.

Future Directions: Exploring Clozapine's Impact on Microglial Exosome Composition and Cognition

As research continues to uncover the interaction between antipsychotic drugs, brain structure, and cognition in schizophrenia, particular attention should be focussed on the role of microglial exosomes under the influence of clozapine. Investigating how clozapine may alter the composition of these exosomes, particularly miRNA content, could clarify the mechanisms behind its cognitive effects and impact on brain macrostructure.

While some evidence suggests that clozapine-induced microglial exosomes could negatively affect cognition, it

is important to consider that these exosomes might also contribute to the therapeutic benefits of clozapine. These exosomes could even prove to have a beneficial effect on cognition. Further research is needed to determine the overall impact of these exosomal changes on brain structure and cognition.

Understanding the connection between alterations in microglial exosome profiles and specific cognitive outcomes in schizophrenia is an important research avenue. To further understand clozapine's effects on cognition, future research should focus on determining the miRNA cargo of exosomes from clozapine-treated microglia, studying their effects on neurons in vitro, and examining their direct cognitive effects in in vivo models. Integrating neuroimaging techniques and cognitive behavioural tests with exosomal analysis could further enhance our understanding, enabling a more comprehensive view of the relationships between clozapine treatment, brain structural changes, and cognitive outcomes. This multidisciplinary approach could advance our understanding of schizophrenia's treatment and pathophysiology, leading to improved patient care and outcomes.

Moreover, parallel investigations into how clozapine's modulation of microglial exosomes compares with other antipsychotics could reveal unique pathways of drug action and offer broader insights into schizophrenia's pathology and treatment. Such comparative analyses might also identify novel therapeutic targets and strategies, potentially enhancing clozapine's therapeutic efficacy or mitigating its potential side effects.

The potential impact of clozapine on microglial exosome composition and its subsequent mediation of effects on brain structure and cognition presents a promising and innovative research trajectory. This line of inquiry not only aligns with current scientific understanding but also holds significant potential for clinical impact in the treatment of schizophrenia.

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Declarations

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Consent to Participate Not applicable.

Consent for Publication Not applicable.

Review Methodology This manuscript is structured as a narrative review, synthesizing current research on the role of microglial exosomes in clozapine treatment and their potential impact on cognition in schizophrenia. Relevant literature was sourced from several databases, including PubMed, Web of Science, and Google Scholar. Search terms such as "schizophrenia," "clozapine," "microglial exosomes," "miRNA," and "cognition" were used.

Competing Interests The authors declare no competing interests.

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