Ketamine – an antidepressant drug

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Ketamine – a new antidepressant drug with anti-inflammatory properties

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Abstract

Ketamine is a new, potent and rapid-acting antidepressant approved for therapy of treatment-resistant depression, which has a different mechanism of action than currently-available antidepressant therapies. It owes its uniquely potent antidepressant properties to a complex mechanism of action, which currently remains unclear. However, it is thought that it acts by modulating the functioning of the glutamatergic system, which plays an important role in the process of neuroplasticity associated with depression. However, preclinical and clinical studies have also found ketamine to reduce inflammation, either directly or indirectly (by activating neuroprotective branches of the kynurenine pathway), among patients exhibiting higher levels of inflammation. Inflammation and immune system activation are believed to play key roles in the development and course of depression. Therefore, the present work examines the role of the antidepressant effect of ketamine and its anti-inflammatory properties in the treatment of depression.

Significance Statement
The present work examines the relationship between the antidepressant effect of ketamine and its anti-inflammatory properties, and the resulting benefits in treatment-resistant depression (TRD). The antidepressant mechanism of ketamine remains unclear, and there is an urgent need to develop new therapeutic strategies for treatment of depression, particularly TRD.

**Abbreviations**

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF, brain-derived neurotrophic factor; DHNK, dehydronorketamine; eEF2, eukaryotic elongation factor 2; ERK1/2, extracellular signal-regulated kinase; GABA, γ-aminobutyric acid; HNK, hydroxynorketamine; IL-6, interleukin 6; IL-1β, interleukin 1β; KYN, kynurenine; KYNA, kynurenic acid; MDD, major depressive disorder; NMDA, N-methyl-D-aspartate glutamate; QIN, quinolinic acid; SSRI, selective serotonin reuptake inhibitor; SNRI, selective serotonin and norepinephrine reuptake inhibitor; RAD, rapid-acting antidepressant; STAT3, transcription factor 3; TNF-α, tumour necrosis factor; TRD, treatment-resistant depression.

**Introduction**

Major depressive disorder (MDD), often referred to as depression, is a common illness affecting approximately 350 million people worldwide. According to the World Health Organisation (WHO), depression is the fourth most serious illness in the world and one of the leading causes of suicide (Shorey et al., 2022). Experts predict that by 2030, it will have become the first most commonly diagnosed disease worldwide. The increasing prevalence of depression and anxiety has been attributed to *inter alia* lifestyle, stress, and spending increasing time in the virtual reality of the Internet (social media, computer games). These numbers were also increased by the SARS-CoV-2 coronavirus pandemic, which lasted more than two years and significantly reduced social contacts. Due to the increasing incidence of MDD, together with the high partial response rate, lack of response to currently-available
antidepressants, low remission rate and delayed onset of antidepressant therapy (more than two weeks), there is a need for new antidepressants with new mechanisms of action.

The poor efficacy of commonly-available antidepressants, whose mechanism of action mainly involves normalisation of biogenic amine levels, may be attributed to the complex pathophysiology of depression (Hurley and Tizabi, 2013). It is becoming increasingly apparent that inflammation is involved in the pathophysiology of MDD. Elevated levels of pro-inflammatory cytokines and immune cell activity have been observed among depressed patients, particularly those refractory to standard treatment and with suicidal thoughts or attempts (Serafini et al., 2013; Pitharouli et al., 2021).

Therefore, an increasing number of clinical trials have examined the potential therapeutic benefits of dual therapy, i.e., antidepressant and anti-inflammatory treatment, in these patients (Fourrier et al., 2018; Miller and Pariante, 2020; Nettis et al., 2021). The studies indicate that some drugs characterised with anti-inflammatory properties, such as selective cyclooxygenase-2 inhibitors, cytokine inhibitors or statins, are effective against MDD when administered together with standard antidepressants. However, these drugs cause side effects such as cardiovascular problems (celecoxib), immunosuppression (infliximab) or an increased risk of rhabdomyolysis (lovastatin), especially when used on a long-term basis (Kohler et al., 2016). Hence, there is an urgent need to develop effective rapid-acting antidepressants (RADs) with a dual mechanism of action, i.e. treating both inflammation and depression. Such drugs would have important clinical significance, especially among depressed patients with suicidal thoughts and who may be refractory to standard treatment due to the presence of inflammation. One such possible candidate is ketamine, which will form the basis of this paper.

Ketamine
Ketamine is a derivative of phencyclidine (PCP), which was introduced in the 1960s as a fast and powerful general anaesthetic characterised with a new mechanism of action. Its mind-body dissociating properties earned it the name “dissociative anaesthetic”; this contrasted with traditional anaesthetics, which completely shut down consciousness. The drug was also known to have an analgesic effect, but no one at that time expected it to demonstrate an antidepressant effect. Several decades later, a new study showed promising results in mice, where N-methyl-D-aspartate glutamate (NMDA) receptor antagonists, such as AP-7 and MK-801 or ketamine, produced antidepressant-like effects, and it was suggested that drugs with such a mechanism of action could be used clinically to treat depression (Hess et al., 2022).

A breakthrough came later when ketamine was administered intravenously at a dose of 0.5 mg/kg for over 40 minutes to people with depression, who then demonstrated a significant improvement in their depressive symptoms on the Hamilton Depression Rating Scale (HDRS) after only three days of administration (Berman et al., 2000). Similar satisfactory results were obtained in patients with treatment-resistant depression who had previously failed at least two adequate antidepressant trials. In these subjects, a significant improvement in depressive symptoms, measured by the HDRS scale, was observed as early as 110 minutes after infusion of ketamine (0.5 mg/kg/40 min). Interestingly, the effect of a single dose of ketamine persisted in these patients for up to one week (Zarate et al., 2006). For a more convenient route of drug administration, (S)-ketamine was introduced in the form of an intranasal spray and the antidepressant efficacy was assessed in patients with treatment-resistant depression (Daly et al., 2018). In this study, intranasal treatment with (S)-ketamine (28 or 84 mg) resulted in a similar rapid reduction (within two hours) of depressive symptoms, which was confirmed by the Montgomery-Asberg Depression Rating Scale (MADRS). This effect persisted even after a week following a single administration of the preparation. Further studies showed that long-term treatment (weekly or bi-weekly) with (S)-ketamine nasal spray
(56-84 mg; 16 weeks) in combination with an oral antidepressant drug (selective serotonin reuptake inhibitor – SSRI – or selective serotonin and norepinephrine reuptake inhibitor - SNRI) was well tolerated by patients and resulted in significant remission of symptoms. Moreover, patients who were in remission were more likely to relapse when switched to a placebo nasal spray in combination with an oral antidepressant (Daly et al., 2019).

Based on the positive results of clinical trials, in March 2019, the FDA registered the (S)-ketamine preparation in the form of a nasal spray for supportive treatment of adult patients with drug-resistant depression or depression with concomitant suicidal thoughts (Cristea and Naudet, 2019). Although ketamine is a highly-effective antidepressant, it still causes side effects such as dissociative and psychotomimetic reactions, and bears the risk of abuse and addiction. Moreover, the drug must be administered under strict medical supervision in hospital environment or a specialist clinic (Liu et al., 2016; Gastaldon et al., 2019; Turner, 2019). Hence, there is a need to identify further substances that would mimic the effects of ketamine, but are free of the side effects of the parent drug. Of particular interest are its active enantiomer, (R)-ketamine, and some ketamine metabolites, which also demonstrate antidepressant effects and are characterised by lower potential for side effects.

(R,S)-ketamine, a racemic mixture of (R)-ketamine and (S)-ketamine, is used as an anaesthetic. Some studies indicate that (R)-ketamine has even stronger and longer-lasting antidepressant effects than (S)-ketamine in several animal models of depression and, most importantly, it is characterised by lower potential for side effects than ketamine racemate or even (S)-ketamine (Yang et al., 2015; Fukumoto et al., 2017; Hashimoto, 2020). Nevertheless, (S)-ketamine was the first to be introduced to the pharmaceutical market, and currently (R)-ketamine is in the first phase of clinical trials (Hashimoto, 2019).

**Pharmacokinetics of ketamine**
Ketamine undergoes rapid and stereoselective metabolism by cytochrome P450 to multiple metabolites, some of which are neuroactive (Zanos et al., 2018; Highland et al., 2021). Ketamine is first metabolized in the liver to (R,S)-norketamine (Figure 1), which is then converted to either (R,S)-dehydro-norketamine (DHNK) or to (R,S)-hydroxy-norketamine (HNK). In particular, 12 different HNKs were detected in human plasma after ketamine treatment. The metabolites are classified according to the location of the hydroxyl group (-OH) on the cyclohexanone ring (4-, 5- or 6-position) and the stereochemical arrangement of the (-OH) group and the amino group (NH2) at two stereochemical centres (2- and 4-position). Norketamine, DHNK and HNK are detected in human plasma during the first 230 minutes after ketamine infusion at the antidepressant dose of 0.5 mg/kg for over 40 minutes, with the most common HNK being (2R,6R)-HNK, (2S,6S)-HNK, (2S,6R)-HNK and (2R,6S)-HNK (Zarate et al., 2012; Zhao et al., 2012). In the tested samples, the concentration of DHNK was unmeasurable; this correlates with studies on murine brain tissue samples, in it was not detected after ketamine administration (Can et al., 2016). DHNK probably does not cross the blood-brain barrier. The half-life of ketamine and its metabolites is between two and four hours, with slight differences depending on the route of administration (Hess et al., 2022).

Antidepressant doses of ketamine administered parenterally or intranasally reach the brain easily. The effect of ketamine is extremely rapid. Indeed, a wide range of symptoms, behaviours and cognitive deficits resembling endogenous psychoses, in particular schizophrenia and dissociative reactions, were observed 10 minutes after administration intravenous infusion of ketamine at 0.5 mg/kg/40 min (Krystal et al., 1994). Importantly, not only ketamine but also its metabolites, norketamine and (2R,6R)-HNK, are detected in murine brain tissue samples within 10 minutes following ketamine administration (at a dose of 10 mg/kg, i.p.), which implies that all three compounds may be responsible for its rapid therapeutic effect (Zanos et al., 2016).
Mechanism of antidepressant action of ketamine

The antidepressant action of ketamine is complex and has not yet been fully elucidated (Zanos et al., 2018; Jóźwiak-Bębenista et al., 2022). However, ketamine is known to be a non-competitive inhibitor of the ionotropic receptor for N-methyl-D-aspartate glutamate (NMDA), and this antagonist property may well influence its antidepressant activities (Musazzi, 2021). Dysfunction of the glutamatergic system plays an important role in the pathophysiology of MDD, TRD and the psychopathology underlying suicidal ideation (Sanacora et al., 2012; Deutschenbaur et al., 2016).

The drug is believed to act by blocking NMDA receptors on inhibitory γ-aminobutyric acid (GABA) interneurons, thus preventing the activation of GABA neurons, and resulting in the disinhibition of glutamate transmission by pyramidal cells. This mechanism of action is also responsible for the dissociative and psychomimetic effects of ketamine. However, no such antidepressant effects are observed for other NMDA receptor antagonist drugs (e.g. memantine); it turns out that the effects require a blockade of NMDA receptors with subunit 2B (NR2B) in their structure (Lang et al., 2018). The NMDA receptor with the NR2B subunit mediates phosphorylation of eukaryotic elongation factor 2 (eEF2) kinase and subsequent translational repression of the brain-derived neurotrophic factor (BDNF), which plays an important role in synaptogenesis (Deutschenbaur et al., 2016). The antidepressant effect of ketamine is believed to be associated with increased expression of BDNF and its receptor, i.e. tropomyosin-related kinase receptor type B (TrKB). BDNF activates the rapamycin (mTOR) signalling pathway in the prefrontal cortex, which is responsible for dendritic spine formation, and synaptic protein synthesis and strengthening (Zagrebelsky et al., 2020).

Preclinical studies have shown that the effects of ketamine on synaptogenesis and antidepressant activity are dependent on the mTOR pathway (Yang et al., 2013; Zhou et al., 2014) and these effects are inhibited in the presence of rapamycin, a selective inhibitor of the
mTOR pathway (Li et al., 2010; Matveychuk et al., 2020). Ketamine affects the expression of BDNF and also, indirectly, its activity (Yao et al., 2022). Excess extracellular glutamate, resulting from the action of ketamine, stimulates \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, which, by activating calcium channels, lead to increased release of BDNF from postsynaptic terminals (Yang et al., 2018; Li et al., 2019; Suzuki et al., 2023). Therefore, BDNF appears to be essential for the ketamine-induced full antidepressant response.

The mechanism of action of ketamine is not restricted to effects on the glutamatergic and GABAergic systems. The drug also affects many other neurotransmitters of the dopaminergic, serotonergic, adrenergic and cholinergic systems involved in affective disorders (Zanos et al., 2018). In addition, the antidepressant effect of ketamine may depend on the pharmacological activity of its metabolites through an NMDA receptor-independent mechanism of action. However, these surprising preclinical findings in animal models need to be confirmed in clinical trials (Zanos et al., 2016). Moreover, ketamine elicited a sustained increase in cellular cyclic adenosine monophosphate following the translocation of G alpha subunits (G\( \alpha \)s) from lipid rafts, in an NMDA receptor-independent manner (Wray et al., 2019). There is post-mortem evidence that in subjects with MDD, lipid rafts are enriched in the heterotrimeric G protein, G\( \alpha \)s, consistent with diminished cAMP signaling (Targum et al., 2022). G\( \alpha \)s stimulates adenylyl cyclase more efficiently when located outside lipid rafts than when it is located within them, and chronic treatment with ketamine facilitates G protein exodus from those rafts (Wray et al., 2019; Schappi and Rasenick, 2022). The complex mechanism of antidepressant action of ketamine has been extensively discussed in many reviews (Kowalczyk et al., 2021; Hess et al., 2022). Recently, preclinical and clinical studies have paid much attention to the anti-inflammatory effect of ketamine, which probably influences its antidepressant effect.
Depression and inflammation

As mentioned in the Introduction, elevated levels of inflammatory biomarkers and immune system cells have been found in some patients with depression, particularly TRD. During depression, inflammation and immune system activation is observed in both the peripheral and central nervous systems (CNS). Studies have shown that people with MDD exhibit significantly higher levels of tumour necrosis factor (TNF-α), interleukin 6 (IL-6) and interleukin 1β (IL-1β), as well as increased levels of the acute-phase protein, i.e., C-reactive protein (CRP) in the blood and cerebrospinal fluid compared with those without MDD. Furthermore, elevated levels of IL-2, IL-4, interferon gamma (INF-γ) and prostaglandin E2 (PGE2) have been reported in patients with MDD (Raison et al., 2006; Miller et al., 2009; Kopschina Feltes et al., 2017; Amodeo et al., 2018).

Activated inflammatory mediators cause depressive symptoms by directly affecting brain tissue, modulating the monoaminergic system and initiating neurotoxic processes in brain areas responsible for emotions and emotional memories (the hippocampus, amygdala, prefrontal cortex) (Figure 2). Activation of the kynurenine pathway following inflammation reduces the level of tryptophan required for serotonin biosynthesis and shifts the response towards the production of kynurenine and further neurotoxic metabolites, thus contributing to neurodegeneration (Nikkheslat et al., 2015; Sforzini et al., 2019). This process will be discussed in detail in a subsequent section of this article. In addition, cytokines activate the glutamatergic system, which results in increased generation of free radicals and decreased production of BDNF. This, in turn, initiates changes in neuronal plasticity (Miller et al., 2009). Cytokines constitute an important element of communication between the peripheral and central immune system, and the neurotransmitter and endocrine systems. It is known that changes in the function of the hypothalamic-pituitary-adrenal (HPA) axis and ineffective

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negative feedback signalling under the influence of cytokines in depression are responsible for glucocorticosteroid (GCS) resistance in the brain, e.g. by reducing the expression of GCS receptors (Miller et al., 2009; Anisman, 2011).

The above-mentioned effects of pro-inflammatory cytokines play an important role in the pathophysiology of depression. In patients who are particularly unresponsive to treatment with standard antidepressants, or those who have suicidal thoughts or attempts, inflammation acts as an important source of cytokines, which resist therapy. Such patients mostly benefit from treatment with a drug demonstrating dual therapeutic properties, i.e. one inducing antidepressant and anti-inflammatory effects (Adzic et al., 2018; Cattaneo et al., 2020).

Anti-inflammatory effects of ketamine

The effect of ketamine on inflammation has been studied since its large-scale application as an anaesthetic. Even then, ketamine was found to act as a unique “homeostatic regulator” of the acute inflammatory response and stress-induced immune dysfunction (De Kock et al., 2013). Given short- and long-term detrimental consequences of inflammation, regulation of the inflammatory process is an important factor contributing to a positive surgical outcome and faster patient recovery. Evidence obtained during clinical trials indicates that intraoperative administration of ketamine during major surgeries, including abdominal or cardiac surgeries, attenuated the inflammatory response, manifested by significant inhibition of the IL-6 level (Dale et al., 2012). Even a subanaesthetic dose of ketamine administered before induction of general anaesthesia affected immune cells in the early postoperative period by attenuating ex vivo production of IL-6 and TNF-α and inhibiting IL-2 secretion (Beilin et al., 2007).

The anti-inflammatory properties of ketamine are thought to play a key role as this drug is able to prevent an increase in systemic inflammation without interfering with local
healing processes. Ketamine appears to exert its anti-inflammatory effects by activating the immune response, rather than acting as an immunosuppressive agent (Loix et al., 2011). This immunomodulatory effect, combined with its antidepressant properties, make ketamine a highly desirable antidepressant, especially for MDD patients with elevated inflammation.

In the last decade, numerous studies have examined the immunomodulatory properties of ketamine in MDD and evaluated the influence of its ability to regulate inflammation on its rapid antidepressant effect. *In vitro* studies indicate that ketamine inhibits the production and release of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α in macrophages (Chang et al., 2010), microglia cells (Chang et al., 2009) and astrocytes (Yuhas et al., 2015). In animal studies, ketamine administered at 10 mg/kg was shown to alleviate depression-like behaviours in a rat model of depression (unpredictable chronic mild stress - UCMS), such as immobility in the forced swim test (FST); in addition, the levels of cytokines IL-1β, IL-6 and TNF-α were found to be elevated in the hippocampus of the tested animals, while they were reduced in those of the group administered ketamine alone (Wang et al., 2015). In a study on a mouse model, analysing a similar chronic restraint stress (CRS) model of depression, ketamine administered at 20 mg/kg resulted in an antidepressant effect, a reduced number of activated microglia cells in the hippocampus, and decreased levels of IL-1β, IL-6 and TNF-α. The anti-inflammatory action of ketamine might be independent of or coexist with NMDA receptor blockade (Figure 4). In the model of depression discussed above, ketamine inhibited cytokine synthesis via the toll-like receptor type 4/p38 mitogen-activated protein kinase (TLR4/p38 MAPK) signalling pathway, and cytokine release from the microglia was down-regulated via the P2X7 receptor in the hippocampus, whose expression is associated with inflammation (Tan et al., 2017).

Results obtained during clinical trials on depressed patients are consistent with preclinical studies, which strongly indicate that ketamine is able to reduce pro-inflammatory
cytokines. Both preclinical and clinical trials reveal a reduction in the level of peripheral inflammatory markers, including IL-1β, IL-6 and TNF-α, following ketamine treatment (Kiraly et al., 2017; Kopra et al., 2021). An interesting study on patients with drug-resistant depression was conducted by two independent research centres. The patients demonstrated high serum levels of the inflammatory marker IL-6 (J-J Yang et al., 2015) as well as changes in plasma levels of adipokines (adiponectin, resistin), i.e. compounds involved in the regulation of inflammatory pathways and neuroplasticity (Machado-Vieira et al., 2017). It is possible that the presence of specific inflammatory markers may indicate whether a patient may possess drug resistance to a standard treatment, and whether they will respond to ketamine treatment. Patients selected in this way would benefit most from the rapid effect of ketamine treatment. The latter study confirmed that the lower anti-inflammatory adiponectin levels are associated with more frequent affective episodes, and a better response to ketamine. Furthermore, ketamine appeared to reduce the levels of resistin, an adipokine with pro-inflammatory properties in these patients, which may also reflect the anti-inflammatory properties of this drug (Machado-Vieira et al., 2017).

In turn, a recent randomised controlled clinical trial found a rapid improvement in mood in patients with drug-resistant depression, measured by the MADRS scale, to be associated with rapid suppression of elevated TNF-α levels. Pro-inflammatory cytokine levels were suppressed within 40 minutes of 0.5 mg/kg ketamine infusion in patients with TRD, which correlated with clinical improvement; this may indicate that the rapid antidepressant effect of the drug is based to some extent on its anti-inflammatory effect (Chen et al., 2018). The rapid onset of antidepressant activity observed with ketamine is still an area of active research and is not fully understood. However, current evidence suggests that multiple factors, such as its anti-inflammatory effects and its pharmacokinetics, i.e. its ability to rapidly cross the blood-brain barrier, may contribute to its rapid effects.
Most studies on immune cell activity and inflammatory factors are based on peripheral blood; relatively few evaluate these parameters in the human CNS. Nevertheless, it is an accepted and confirmed fact that circulating immune cells and their activation products interact and infiltrate into the CNS (Dantzer et al., 2008). Furthermore, immunocompetent CNS cells, such as astrocytes and microglia cells, produce cytokines and present their receptors, confirming that both peripheral and central immune cells play important roles (Kelley et al., 2007). As such, ketamine appears to have central immunomodulatory effects based on its direct effects on glial cells (Zhang et al., 2021). Depressed patients with suicidal thoughts demonstrate microglial activation and release of the cytokine TNF-α and nitric oxide (NO), which are key mediators of acute and chronic inflammation, as well as neurodegenerative processes (Steiner et al., 2008). Indeed, microglia cells can affect the regulation of BDNF synthesis, reducing the expression of BDNF and its receptor TrkB (Jin et al., 2019). A postmortem study of the brains of depressed patients who committed suicide showed high levels of microglia and macrophage accumulation in the dorsal anterior cingulate cortex (dACC), an area involved in cognitive-emotional processes (Torres-Platas et al., 2014).

Ketamine has been found to inhibit TNF-α synthesis stimulated by lipopolysaccharide (LPS), a substance used to induce inflammation in vitro, in both astrocytes and microglia (Shibakawa et al., 2005). In studies conducted on LPS-treated rat primary microglia cultures, ketamine demonstrated anti-inflammatory effects by reducing levels of NO, IL-1β and, to a lesser extent, TNF-α; the observed drug-induced microglia inactivation was likely mediated by inhibition of extracellular signal-regulated kinase (ERK1/2) phosphorylation (Chang et al., 2009). In contrast, studies on human microglia cell line (HMC3 cells), ketamine and its two active metabolites (2R,6R)-HNK and (2S,6S)-HNK were shown to be involved in the regulation of the type I interferon (IFN) pathway through activation of transcription factor 3 (STAT3), which plays a key role in the immune response (Figure 4). As a further
consequence, eEF2 was found to increase and elevate the expression of BDNF, which, as mentioned above, is involved in the antidepressant mechanism of action of ketamine (Ho et al., 2019). Furthermore, in cultured human astroglial cells, ketamine inhibited the gene expression and synthesis of IL-6 and TNF-α within 24 hours of administration, confirming that the immunomodulatory activity of ketamine is related to its rapid antidepressant effect (Yuhas et al., 2015).

Studies show that the anti-inflammatory effect of ketamine, part of its antidepressant effect, acts by both reducing pro-inflammatory cytokines and indirectly influencing the kynurenine pathway, responsible for tryptophan metabolism (Zunszain et al., 2013). A detailed description of the kynurenine pathway and the effect of ketamine is shown in Figure 3. Activation of the kynurenine pathway by inflammation reduces the availability of tryptophan, required for serotonin biosynthesis, and shifts the response towards the production of kynurenine (KYN) and further neurotoxic metabolites such as quinolinic acid (QIN), which, as an NMDA receptor agonist, exhibits neurotoxic effects (Sforzini et al., 2019; Nikkheslat, 2021). Being mediated by passive diffusion, the neurotoxic metabolite QIN poorly penetrates the blood-brain barrier. In contrast, greater permeability is noted in neural tissue disorders, cerebral hypoxia, autoimmune disorders and inflammatory conditions.

In patients with MDD, high levels of cytokines are thought to lead, via activation of the kynurenine pathway, to shift the balance from neuroprotective to neurotoxic tryptophan metabolites, which are responsible for activation of the glutamatergic system (Savitz, 2017; Ogyu et al., 2018). Patients with symptoms of major depression accompanied by suicide attempts show reduced levels of the neuroprotective metabolite kynurenine, or kynurenic acid (KYNA), with increased levels of the neurotoxin QIN in cerebrospinal fluid (CSF) compared to healthy subjects (Bay-Richter et al., 2015). Interestingly, a study on MDD patients with suicidal thoughts showed that 24 hours after ketamine (0.5 mg/kg) administration, a rapid
reduction in depressive symptoms was observed, accompanied by an increase in KYNA levels and KYNA/KYN ratio, which acts as an NMDA receptor antagonist (Zhou et al., 2018). This indicates that the kynurenine pathway may be involved in the rapid antidepressant effect of ketamine, and that early changes in serum KYNA levels and KYNA/KYN ratio could be potential indicators of the efficacy of ketamine in the treatment of depression.

In preclinical studies on mice, ketamine was also found to have a direct effect on quinolinic acid by blocking its effect on the NMDA receptor (Walker et al., 2013). This effect is important as quinolinic acid causes excessive stimulation of NMDA receptors, increased oxidative stress and inflammation in the CNS, and elevated apoptosis, and ultimately neurodegeneration (Lugo-Huitrón et al., 2013). Elevated levels of quinolinic acid and inflammation have been noted in the CSF of suicide attempters (Erhardt et al., 2013). Ketamine, which is characterized with anti-inflammatory properties, relieves the inflammatory process and may hence be effective in improving mental state regarding suicidal thoughts.

Furthermore, scientific observations suggest that ketamine may be particularly effective in patients with drug-resistant depression, with increased inflammation, and in those who have not received anti-inflammatory treatment. Conversely, anti-inflammatory therapy can be used in these patients as a strategy to sustain a positive response to ketamine treatment (Miller, 2013). A paper reviewing the systematic treatment of patients with TRD affected by inflammation process found these patients to respond better to drugs with anti-inflammatory properties, including infliximab (a TNF-α inhibitor) or ketamine, than to treatment with standard antidepressants (Yang et al., 2019).

The site of anti-inflammatory and antidepressant activity by ketamine in the CNS merits some mention. Studies suggest that astrocytes and microglia can be considered target cell types for ketamine, mainly by inhibiting the release of pro-inflammatory cytokines; such
studies have focussed specifically on the role of the kynurenine pathway (Kadriu et al., 2019; Sforzini et al., 2019; Jóźwiak-Bębenista et al., 2022). Suppression of the inflammatory response in reactive astrocytes and microglia has a positive effect on neurons, which are under the constant influence of glial cells. However, it cannot be excluded that the direct effect of ketamine on neurons (prefrontal cortex, hippocampus, amygdala) via NMDA receptor inhibition, diminishes the influence of interleukins (Sforzini et al., 2019). Moreover, the relative contributions of astrocytes, microglia and neurons, as well as other cell types, to the antidepressant and anti-inflammatory effects of ketamine may vary depending on the specific context and conditions being studied; hence, further research is needed to determine the precise site of these effects. The proposed direct anti-inflammatory effect exerted by ketamine, based on its influence on the production and release of cytokines from glial cells, via NMDA receptor-dependent and non-dependent pathways, is shown in Figure 4.

**Anti-inflammatory effect of ketamine metabolites**

Recently, despite lacking psychomimetic side-effects and the risk of abuse typical of the parent drug, the ketamine metabolite (2R,6R)-HNK has shown high efficacy in animal models of depression and has received much attention from researchers (Zanos et al., 2016). In contrast, (S)-ketamine metabolites have been found to offer promise based on studies in a mouse model of depression, which indicate that (2S,6S)-HNK has potent, rapid and long-lasting antidepressant effects in rodents, which interestingly, are more potent even than R-enantiomers (Yokoyama et al., 2020). This raises questions regarding the division of roles between racemic ketamine and its metabolites concerning their antidepressant action, i.e. whether the metabolites, e.g. HNKs, could be responsible for the observed effective effects of ketamine. It is also not clear whether the antidepressant effect of HNKs are better, worse or equal to that of the racemic (R,S)-ketamine mixture.
Consequently, several investigators have sought to understand whether HNKs also exert anti-inflammatory effects as ketamine. One recent work by Ho et al. examined the transcriptome of HMC3 cells after 24-hour exposure to (2S,6S)-HNK and (2R,6R)-HNK (400 nM) (Ho et al., 2019); the results indicate that both HNKs indicated a significant increase in indicators of type I interferon pathway activity. Exposure to both HNKs also increased the expression and nuclear translocation of signal transducer and activation of STAT3, a transcription factor important for interferon pathway regulation and gene expression. Notably, signal transducer and activation of STAT3 binds to eEF2 in the cytoplasm prior to translocating to the nucleus, which may explain, at least in part, the reduction in eEF2 phosphorylation and subsequent increase in BDNF, as well as changes in other synaptic proteins, observed after treatment of mice with (2R,6R)-HNK (10 mg/kg, i.p.) (Zanos et al., 2016; Suzuki et al., 2017).

In contrast to ketamine, (2R,6R)-HNK (10 mg/kg, i.p.) had no significant effect on several systemic markers of inflammation in socially defeated mice (Xiong et al., 2019). Specifically, no changes were observed in the plasma levels of the bone inflammatory markers osteoprotegerin, receptor activator of nuclear factor kB ligand or osteopontin (Xiong et al., 2019). However, a recent proteomics study in mice demonstrated that (2R,6R)-HNK (10 mg/kg, i.p.) decreased hippocampal expression of peptidyl prolyl cis-trans isomerase A, a mediator of inflammation and immunosuppression (Rahman et al., 2020). The potential anti-inflammatory effects of HNKs require further study, including their potential effects on cytokines or other anti-inflammatory measures (Highland et al., 2021).

**Future perspective**

It is noteworthy to highlight that current research is uncovering new directions in the study of potential novel targets for ketamine, contributing to its complex mechanisms of antidepressant and anti-inflammatory action. Converging evidence suggests that the brain and the gut microbiota are in bidirectional communication with each other and also with inflammatory processes (Getachew et al., 2018; Bhatt et al., 2023; Donoso et al., 2023).
There is an increasing number of evidence that the gut microbiota may play a crucial role in the antidepressant effects of ketamine and its metabolites (Wilkowska et al., 2021; Hua et al., 2022). For instance, S-ketamine exerted potent antidepressant-like effects in LPS-induced mice (an inflammation model of depression), and its mechanisms might be associated with regulating the composition of the gut microbiota that modulate immune response and reduce inflammation (Wang et al., 2021). Nevertheless, the exact mechanism of action of ketamine and its metabolites, as well as their potential effects on microbiome, remain unclear. This research signifies the expanding scope of our understanding of ketamine's antidepressant effect and underscores the importance of considering novel avenues of investigation concerning the connections between the gut microbiota, the immune system, and neuroplasticity.

Moreover, the implication of the kynurenine pathway in depression poses a challenge for future studies. This pathway could emerge as a novel target for innovative derivatives of ketamine with anti-inflammatory properties. These compounds may aim to reduce the synthesis of neurotoxic metabolites (KYN, QIN) within the kynurenine pathway. Additionally, similar to ketamine, new drugs could potentially stimulate the neuroprotective and anti-inflammatory branches of this pathway, as seen in ketamine's ability to increase the concentration of KYNA.

**Summary**

Preclinical studies based on various experimental models have shown that low sub-anaesthetic doses of ketamine elicit antidepressant effects. In addition, clinical studies, despite being limited, indicate that in addition to these antidepressant effects, ketamine also reduces inflammation, either directly or indirectly by activating neuroprotective branches of the kynurenine pathway, at least in the subgroup of TRD patients with inflammatory processes. Moreover, the anti-inflammatory properties of ketamine may contribute to its rapid antidepressant effects.

Ketamine represents a breakthrough in treatment of depression, especially TRD, so understanding its therapeutic mechanism is of paramount importance in the context of development of new rapid-acting antidepressants characterised by a higher safety profile and
possibly, anti-inflammatory properties. The immunomodulatory effect of ketamine requires further research, both at the baseline and at clinical levels; such studies should include an evaluation of its effects on cytokines and other markers of the inflammatory process, which change rapidly in patients treated with this drug. Standardised markers are needed to determine the efficacy of ketamine in treating patients with severe depressive symptoms, particularly suicidal thoughts, who are refractory to treatment with standard antidepressants, and who require effective but, most importantly, immediate intervention.

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Data Availability

This article contains no datasets generated or analyzed during the current study.

Authorship contributions

Wrote or contributed to the writing of the manuscript: Jóźwiak-Bębenista, Sokołowska, Wiktorowska-Owczarek, Kowalczyk, Sienkiewicz.

References


Sforzini L, Pariante CM, Palacios JE, Tylee A, Carvalho LA, Viganò CA, and Nikkheslat N (2019)


Shorey S, Ng ED, and Wong CHJ (2022)


Role of the AMPA receptor in antidepressant effects of ketamine and potential of AMPA receptor potentiators as a novel antidepressant. *Neuropharmacology* **222**:109308.


Torres-Platas SG, Cruceanu C, Chen GG, Turecki G, and Mechawar N (2014)

Tóth F, Cseh EK, and Vécsei L (2021)

Turner EH (2019)

NMDA receptor blockade by ketamine abrogates lipopolysaccharide-induced depressive-like behavior in C57BL/6J mice. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol* **38**:1609–1616.


Gut microbiota is involved in the antidepressant-like effect of (S)-norketamine in an inflammation model of depression. *Pharmacol Biochem Behav* **207**:173226.


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Conflicts of Interest

No author has an actual or perceived conflict of interest with the contents of this article.
Figure captions

Figure 1.
   A. Ketamine metabolism
   B. HNKs found in the highest concentrations in humans

Figure 2.

The role of the inflammatory process as a source of cytokines in the pathophysiology of depression. Explanation in the text.

Figure 3.

Anti-inflammatory effect of ketamine. Ketamine achieves its anti-inflammatory effect directly by reducing pro-inflammatory cytokine content, and indirectly through its effect on the kynurenine pathway, responsible for tryptophan metabolism. Kynurenine pathway: tryptophan metabolism via the kynurenine pathway leads to the formation of a number of active metabolites that exhibit antagonistic effects towards each other. Initially, the amino acid is converted to kynurenine by the enzyme indoleamine 2,3-dioxygenase, present inter alia in microglia, astrocytes and neurons, whose activity is increased under the influence of inflammatory cytokines (mainly IFN-γ but also IFN-β, IFN-α, IL-1 and TNF-α) (Guillemin, 2012). The metabolism of kynurenine (KYN) can take place via three pathways and lead to the formation of kynurenic acid (KYNA), 3-hydroxykynurenine (3-HKA) and anthranilic acid (AA). Both 3-HKA and AA can convert to 3-hydroxyanthranilic acid (3-HANA), which
converts to the neurotoxic metabolite quinolinic acid (QIN) via an unstable intermediate product. All these compounds share the common name *kynurenine* (Miller, 2013).

QIN and 3-HKA exhibit neurotoxic effects through multiple mechanisms and may additionally exacerbate inflammation. Quinolinic acid is an NMDA receptor agonist, which enhances glutamate excitotoxicity, leading to reduced BDNF, protein synthesis and synaptogenesis (Lugo-Huitrón *et al.*, 2013). It has been found that 3-HK increases ROS levels in the brain, contributing to oxidative stress and neuronal apoptosis, especially in the hippocampus (Colín-González *et al.*, 2013).

KYNA, on the other hand, is the only known endogenous NMDA receptor antagonist present in the mammalian brain. It attenuates the neurotoxic activity of quinolinic acid and other excitatory amino acids. KYNA also has neuroprotective and anti-inflammatory effects, enhancing synaptic plasticity and removing excess glutamate from the brain (Tóth *et al.*, 2021). The kynurenine pathway is found in both the peripheral and central nervous system. They are not completely independent of each other: the central pathways are strongly influenced by peripheral pathways (Allison and Ditor, 2014).

Figure 4.

The proposed direct anti-inflammatory effect of ketamine; the production and release of cytokines from glial cells is influenced via the NMDA receptor-dependent or receptor-independent pathway. Abbreviations are explained in the text.
Fig. 1A.

(R,S) - ketamine

\[ \text{demethylation} \]

(R,S) - norketamine

\[ \text{dehydroxylation} \quad \text{hydroxylation} \]

(R,S) – dehydronorketamine (DHNK) does not pass to CNS

(R,S) – hydroxynorketamine (HNK)

12 types of HNK depending on:
- the arrangement of the - OH group in the cyclohexanone ring (in position 4, 5 or 6)
- stereochemistry of - OH and -NH2 groups (in positions 2 and 4) \( \Rightarrow (R,R; S,S; R,S; \text{ or } S,R) \)

Fig. 1B.

(R,S) – dehydronorketamine (DHNK)

(R,S) – hydroxynorketamine (HNK)

\[ \text{H}_2\text{N} \quad \text{O} \quad \text{OH} \]

\[ \text{H}_2\text{N} \quad \text{O} \quad \text{OH} \]

\( (2R, 6R) \) - HNK

\( (2S, 6S) \) - HNK

\( (2R, 6S) \) - HNK

\( (2S, 6R) \) - HNK
Inflammation

↑ pro-inflammatory cytokines

- glutamatergic system
- monoaminergic system
- kinurenin pathway
- HPA

- ↑ ROS
- ↓ BDNF

- tryptophan
- serotonin
- neuroprotective metabolites
- IDO
- neurotoxic metabolites
- GCS resistance
- immune cells

Fig. 2.

DEPRESSION
Fig. 3. KETAMINE

- **direct effect**
  - ↓ pro-inflammatory cytokines

- **indirect effect**
  - tryptophan → kynurenine pathway
    - kynurenine (KYN) → 3-hydroxykynurenine (3-HKA)
      - ↑ kynurenic acid (KYNA)
    - 3-hydroxyanthranilic acid (3-HANA)
      - ↓ quinolinic acid (QIN)

tryptophan → indoleamine 2,3-dioxygenase (IDO)
Fig. 4.

Ketamine induces neuroinflammation through various pathways involving TLR2 and TLR4 receptors.

- TLR2 activates ERK1/2 and MAPKK, leading to NF-κB and p38 activation.
- TLR4 activates STAT3 and eEF2.

These pathways involve astrocytes and microglia, with NF-κB and STAT3 activation resulting in the production of pro-inflammatory cytokines such as IL-6, IL-1β, and TNFα.

This process contributes to neuroinflammation.