Brain, Behavior, and Immunity xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Modulating the immune response with the wake-promoting drug modafinil: A potential therapeutic approach for inflammatory disorders

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ARTICLE INFO

Keywords:
Modafinil
Immunity
Inflammation
Dopamine
Microglia
T cells
Macrophages

ABSTRACT

Modafinil is a psychostimulant drug approved by the FDA primarily for the treatment of sleep disorders such as narcolepsy, excessive daytime sleepiness and sleep apnea. Several documented but not yet approved uses for modafinil have been described over the last 30 years, including alleviating fatigue in neurological and neurodegenerative disorders. Recent evidence has suggested that modafinil may have an immunomodulatory effect. Here, we review the different effects of modafinil treatment in animal models of brain inflammation and peripheral immune function. We conclude that there is unequivocal evidence of an anti-inflammatory effect of modafinil in experimental animal models of brain inflammation and neurodegenerative disorders, including systemic inflammation and methamphetamine-induced neuroinflammation, Parkinson's disease, brain ischemia, and multiple sclerosis. Modafinil acts on resident glial cells and infiltrating immune cells, negatively affecting both innate and adaptive immune responses in the brain. We also review the outcomes of modafinil treatment on peripheral immune function. The results of studies on this subject are still controversial and far from conclusive, but point to a new avenue of research in relation to peripheral inflammation. The data reviewed here raise the possibility of modafinil being used as adjuvant treatment for neurological disorders in which inflammation plays an important role.

1. Introduction

Modafinil is a psychostimulant drug first described in France during the 1980s by the group of Michel Jouvet (Bastuji and Jouvet, 1988). Its medical use was approved in the United States in 1998 by the Food and Drug Administration (FDA) for the treatment of narcolepsy, excessive daytime sleepiness and obstructive sleep apnea (Bastuji and Jouvet, 1988; Kuan et al., 2016), because it reduces the need for sleep without the side-effects of caffeine and amphetamine use, such as depression and headache (Boutrel and Koob, 2004).

Among the documented but not yet approved uses for modafinil, it has been reported to have potential therapeutic use in attention deficit hyperactivity disorder (ADHD), depression, cocaine dependence, schizophrenia, obesity and fatigue in several neurological and neurodegenerative diseases (Ballon and Feifel, 2006). Modafinil is also used widely off-prescription for its perceived cognitive enhancing ability (nootropic effect) as a "smart drug" by students, soldiers and shift workers because it allegedly increases mental focus and alertness and reduces sleepiness (for review, see Battleday and Brem, 2015).

In the recent years, different research groups have reported potential immunomodulatory effects of modafinil using several different animal models of immune response and inflammation. The aim of this review is to present research that addresses the immunomodulatory properties of modafinil treatment, and to discuss its possible mechanisms of action in the central nervous system and the peripheral immune response.

2. Pharmacological mechanism

Modafinil is distinct from amphetamine and other psychostimulants in its structure, and its neurochemical and behavioral effects. Although its mechanisms of action are still not entirely understood, modafinil has a number of effects on neurochemistry, as reported in studies using animal models and in clinical trials. The primary action of modafinil is believed to be through catecholamine neurotransmission. Some studies reported a significant affinity of modafinil to the dopamine transporter (DAT) (Madras et al., 2006), while others reported a relatively low to medium affinity to DAT (Loland et al., 2012). The affinity level to DAT seems to be highly dependent on the chemical structure of the compound and its analogues, with the R-enantiomer having higher affinity than the S-enantiomer (Cao et al., 2011; Loland et al., 2012). It was also shown that the deletion of DAT in mice abolishes the wake-promoting effect of modafinil (Wisor et al., 2001).

In vivo imaging studies reported increased extracellular dopamine

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https://doi.org/10.1016/j.bbi.2020.04.038
Received 26 November 2019; Received in revised form 16 March 2020; Accepted 15 April 2020

(DA) in the striatum of rhesus monkeys (Andersen et al., 2010) and the nucleus accumbens of humans (Volkow et al., 2009) following modafinil treatment. A microdialysis study further revealed a significant increase in dopamine levels in the prefrontal cortex (PFC) of rats, which lasted until 200 min after the modafinil injection (de Saint Hilaire et al., 2001). The same findings were reported in rat nucleus accumbens (Murillo-Rodriguez et al., 2007) and in the caudate nucleus of narcoleptic orexin/hypocretin receptor 2 mutant dogs (Wisor et al., 2001). In mice, a microdialysis study reported increased extracellular DA levels in the nucleus accumbens shell and core, with no significant differences between these two regions (Mereu et al., 2017a). When compared to cocaine, modafinil was shown to have a lower potency and efficiency in stimulating extracellular levels of dopamine in the nucleus accumbens shell, although sharing some subjective effects with cocaine (Mereu et al., 2017a).

Interestingly, the magnitude of these changes varied according to the vehicle used for suspending the drug. The increase in extracellular dopamine levels was 3-fold higher in the first hour after injections with DMSO/Tween-80 compared to when modafinil was suspended in Arabic gum, probably due to increased CNS bioavailability (Mereu et al., 2017a). This finding may well explain the discrepancies between the reported effects of modafinil in different studies and should raise the awareness of researchers about the importance of the chosen vehicle.

The dopamine D1 and D2 receptors have been considered to be essential in mediating the wakefulness promoted by modafinil (Chen et al., 2013; Qu et al., 2008), and have been shown to undergo epigenetic changes, particularly the enrichment of acetylated histone 3 and 4 (H3ac and H4ac, respectively), at their promoters in the medial prefrontal cortex (mPFC) following a single modafinil treatment, without affecting their messenger RNA (mRNA) levels (Gonzalez et al., 2019).

Modafinil also affects the central norepinephrine system by binding to the norepinephrine transporter (NET) (Madras et al., 2006) and has been shown to markedly increase norepinephrine levels in the rat prefrontal cortex and hypothalamus (de Saint Hilaire et al., 2001). Additionally, the participation of the α-1 adrenergic receptor seems to be crucial for the wake-promoting effect, since the genetic deletion of these receptors attenuated the behavioral stimulation (Stone et al., 2002), and the pharmacological blockage prevented the EEG desynchronization (Chen et al., 2013) promoted by Modafinil. An *in vitro* study performed in rat brain slices revealed that modafinil potentiates the norepinephrine-induced inhibition of the sleep-promoting neurons of the ventrolateral preoptic nucleus, further supporting the notion that the wake-promoting effect of modafinil is mediated, at least in part, by blockage of norepinephrine reuptake (Gallopin et al., 2004). Curiously, González and colleagues (2018) have recently shown that repeated modafinil treatment in mice induces a specific increase in the enrichment of acetylated histone 3 under α-1 adrenergic receptor promoter and increases the mRNA levels of this receptor in the mPFC of mice, without affecting acetylation and mRNA levels of other dopaminergic and adrenergic receptors.

Although studied to a lesser extent compared to the catecholaminergic system, other neurotransmitters and neuropeptides are also affected by modafinil treatment. In particular, many studies have reported a reduction in extracellular GABA levels following modafinil treatment in several brain regions of rats and guinea pigs. These negative effects on GABAergic transmission were likely correlated to increased brain levels of glutamate and serotonin promoted by modafinil (Kumar, 2008; Minzenberg and Carter, 2008). In addition, although still controversial, orexin/hypocretin as well as histamine transmission also seems to be affected by modafinil, and have a role in its stimulating effects (Kumar, 2008; Minzenberg and Carter, 2008).

Besides acting on neurotransmitter systems, increasing evidence suggests that the wake-promoting activity of modafinil is closely related to its effects on GAP junctions and neuronal interconnectivity in the reticular activating system (RAS) (Garcia-Rill et al., 2007; Urbano et al., 2017). Urbano and colleagues revealed that modafinil increases the connexin (Cx)-mediated electrical coupling between cortical interneurons and, therefore, enhances thalamocortical activity (Urbano et al., 2007). These findings were expanded to show that modafinil injected into the pedunculopontine nucleus (PPN), an essential component of the RAS, increases the amplitude of the sleep state-dependent P13 potential, thus contributing to its stimulating properties, an effect that is blocked by antagonists of GAP junctions (Beck et al., 2008). It was later revealed that modafinil increases Cx30 expression and enhances GAP junction communication of cortical astrocytes (Liu et al., 2013), and that the modafinil-promoted arousal is related to Cx30 signaling (Duchene et al., 2016), indicating that this effect on GAP junctions is not restricted to neurons.

In summary, modafinil interacts with multiple systems to promote wakefulness and stimulation. Its primary stimulatory actions have been demonstrated to be mediated by both dopamine and norepinephrine. However, a few studies have further revealed modafinil's influence on the GABA, glutamate, serotonin, histamine, and orexin/hypocretin systems and on neuro-glial interactions. Although this goes beyond the scope of this review, the collective data indicate how complex the effects of modafinil on neurochemistry and behavior are.

3. Behavioral effects of modafinil

The effects of modafinil on behavior are reported to be rather diverse as it acts on multiple neurotransmitter systems. As a stimulant of the central nervous system, modafinil treatment is classically known to increase locomotor behavior in animal behavioral models in a similar way to other psychostimulant drugs that act on the brain dopaminergic system. Modafinil also produces locomotor sensitization, a phenomenon that is used to study the neuroplasticity of the dopaminergic mesolimbic system after repeated administrations (Gonzalez et al., 2018; Soeiro Ada et al., 2012; Wuo-Silva et al., 2011). Modafinil was found to induce rapid-onset sensitization to locomotor stimulation after a single treatment administered 4 h earlier (Wuo-Silva et al., 2016), which is associated with dopaminergic D1R, but not D2R (Wuo-Silva et al., 2019).

Studies addressing the rewarding effects of modafinil with the conditioned place preference (CPP) paradigm have produced contrasting results. Some studies reported rewarding effects (Shuman et al., 2012; Wuo-Silva et al., 2011), while others revealed negative outcomes (Deroche-Gamonet et al., 2002; Quisenberry et al., 2013), and one study showed CPP in females, but not males, demonstrating a sex-dependent rewarding effect (Bernardi et al., 2015). Despite these inconsistent findings in pre-clinical research, the use of modafinil in clinical practice has shown a low abuse liability, with modafinil even being suggested as a treatment for cocaine addiction, since it was found to block the euphoric effects of cocaine in several studies in humans (Dackis et al., 2003; Hart et al., 2008; Malcolm et al., 2006; Verrico et al., 2014). These differences in reinforcing effects can be attributed to a lack of selectivity of modafinil to specific conformational states of DAT that, in turn, can influence the ability of modafinil to stimulate the extracellular levels of dopamine in the nucleus accumbens shell, which is considerably lower than that of cocaine (Loland et al., 2012; Mereu et al., 2013; Mereu et al., 2017a).

Pre-clinical and clinical research has consistently demonstrated that modafinil improves different aspects of cognitive function. In rodents, modafinil has been shown to improve object recognition memory deficits induced by methamphetamine, which is correlated with the ability of modafinil to restore novelty-induced ERK phosphorylation in the mPFC (Gonzalez et al., 2014). Others reported beneficial effects of modafinil on working memory in different behavioral paradigms,

including sequential and spontaneous alternation tasks, delayed non-matching to position tasks and serial reversal discrimination tasks in a dose and duration-dependent fashion, as well as in attention tasks (for review, see Minzenberg and Carter, 2008). In humans, growing evidence indicates that modafinil is beneficial in improving working and recognition memory, attention and other cognitive functions. It has also proven effective in improving cognition in adolescents with ADHD and adults with schizophrenia (for review, see Battleday and Brem, 2015; Minzenberg and Carter, 2008).

In contrast with the effects of modafinil, mice with genetic hypofunction of DAT (DAT +/-) demonstrated a behavioral phenotype characterized by hyperactivity, increased impulsivity, and attentional deficits, which were ameliorated by low doses of amphetamine, consistent with ADHD symptomatology and therapy (Ciampoli et al., 2017; Mereu et al., 2017b). These cognitive impairments under DAT hypofunction suggest that the improvement of cognition under modafinil treatment is not mediated by dopaminergic modulation, but by other mechanisms yet to be described. They might be related with the ability of modafinil to increase hippocampal neurogenesis, an effect reported following acute treatment in both mice and rats (Brandt et al., 2014; Sahu et al., 2013).

The behavioral data from multiple pre-clinical and clinical experiments suggest that modafinil has stimulating properties related with dopaminergic modulation, although its rewarding properties are still a matter of debate. In addition, modafinil has the ability to improve several aspects of cognition, which seems to be mediated by mechanisms other than dopamine.

4. The effects of modafinil in animal models of neuroinflammation

Besides its effects on neurochemistry and behavior, emerging research has revealed the potential benefits of modafinil on *in vivo* and *in vitro* models of neuroinflammation, decreasing brain inflammation in different animal models of disease. Modafinil has been shown to act by impacting specific aspects of the brain immune response, such as monocyte recruitment and activation (Zager et al., 2018a), T cell recruitment and differentiation, cytokine production (Brandão et al., 2019) and glial activation (Raineri et al., 2012).

Raineri and colleagues (2012) revealed that modafinil decreased the neurotoxic and neuroinflammatory effects of repeated methamphetamine administration in mice. This treatment regimen prevented the activation of microglia and astrocytes in the striatum promoted by methamphetamine, as well as decreased methamphetamine-induced hyperthermia. In addition, modafinil has also prevented the decrease of tyrosine hydroxylases (TH) and DAT expression in the striatum induced by methamphetamine. These findings indicate a link between the modafinil-mediated prevention of glial activation and decreased neurotoxicity of TH-positive neurons (Raineri et al., 2012)

In a recent report, we demonstrated that a single modafinil administration is able to decrease the behavioral symptoms of an acute systemic inflammation induced by high dose of lipopolysaccharide (LPS) (Zager et al., 2018a). Specifically, modafinil reduced the locomotor impairment, as well as the anxiety-like and depressive-like behaviors, induced by systemic inflammation. These preventive effects were correlated with a decreased number of brain-derived $CD11b^{+}$ monocytes and downregulated expression of interleukin (IL)-1β in brain regions. Further analysis revealed that modafinil acts specifically on the $CD11b^{+}CD45^{high}$ subset of monocytes, which are infiltrating monocytes and neutrophils and resident macrophages.

The pharmacological blockage of D1R reverted the preventive effects of modafinil on locomotion and anxiety, but not its effects on depression and activated brain monocytes. Collectively, data show that D1R mediates, at least in part, the effects of modafinil on LPS-induced sickness behaviors and neuroinflammation (Zager et al., 2018a). Curiously, modafinil treatment failed to prevent the motor impairment induced by IL-1β injected i.v. in female mice (Bonsall et al., 2015), in contrast with the findings observed in LPS-induced inflammation.

In addition to the *in vivo* neuroinflammation models promoted by methamphetamine and immune challenges, a recent study addressed the effects of modafinil treatment on the neuroinflammation induced by sleep deprivation (SD). Wadhwa and colleagues (2018) studied glial activation and immune related genes expression in the hippocampus, along with behavioral alterations following 48 h of sleep deprivation in rats. The authors showed that modafinil administered daily during the SD protocol prevented the SD-induced increase in hippocampal pro-inflammatory cytokines tumor necrosis factor (TNF) and IL-1β, as well as reverted the decrease in the anti-inflammatory cytokines IL-4 and IL-10. These changes were correlated with blockage of SD-induced microglial activation in the hippocampus, as demonstrated by ionized calcium-binding adapter molecule 1 (Iba-1) immunoreactivity, cell number and morphological alterations characteristic to the activated phenotype. The reversal of changes in the activated morphology were also present in hippocampal astrocytes. The anti-inflammatory effect of modafinil in this model led to improvements in the motor impairment and anxiety-like behavior promoted by SD (Wadhwa et al., 2018).

So far, the collective *in vivo* data has shown that modafinil is anti-inflammatory and negatively affects the activation of microglia. However, with *in vivo* models, it was not possible to determine whether the effects of modafinil are due to the direct action of the drug on microglia, or are mediated by an indirect and currently unknown pathway. To address this question, Jung and colleagues (Jung et al., 2012) used the immortalized cell line BV-2 to study the *in vitro* anti-inflammatory activity of modafinil in isolated microglia. The authors showed that modafinil derivatives reduced *in vitro* nitrite production, as well as iNOS and COX-2 expression in LPS-stimulated BV-2 microglia cells, indicating that it exerts a direct anti-inflammatory effect on microglia. Additional *in vitro* experiments revealed that modafinil also prevents glutamatergic neurotoxicity in a cell culture of primary cortical neurons, further demonstrating the neuroprotective potential of modafinil and a direct effect in neurons (Antonelli et al., 1998).

Taken together, the data from *in vivo* and *in vitro* models of neuroinflammation consistently show that modafinil is an effective blocker of microglial activation and, consequently, of *in situ* production of pro-inflammatory cytokines and other inflammatory mediators, being a promising therapeutic approach to neurodegenerative disorders, in which neuroinflammation plays a significant role.

5. Use of modafinil in neurological and neurodegenerative disorders

Past research has reported the potential anti-inflammatory activity of modafinil in neuroinflammation models in rodents, mainly due to its negative effects on the infiltration of leukocytes into the brain parenchyma and on microglial activation. Given that cellular infiltration and microglia activation are two of the main markers of a number of neurological and neurodegenerative disorders (Glass et al., 2010), further research is warranted to address the effect of this drug treatment on the progression of neurodegenerative diseases.

In recent years, modafinil has been prescribed to reduce fatigue and increase wakefulness in several neurological and neurodegenerative disorders, including Parkinson's disease, multiple sclerosis, traumatic brain injury, stroke and amyotrophic lateral sclerosis, with an evident improvement in the quality of life of patients. To date, the improvement in fatigue was believed to be due to the psychostimulant effect of modafinil, and the neuroprotective and anti-inflammatory potential of this drug has been elusive.

However, some research groups have demonstrated that modafinil

treatment is neuroprotective in an experimental model of Parkinson's disease, a neurological disorder in which the dopaminergic neurons from the substantia nigra (SN) are selectively degenerated, leading to motor symptoms, such as tremors, bradykinesia and muscle rigidity, as well as non-motor symptoms, such as cognitive disturbance, mood and sleep disorders. Fuxe and colleagues were the first to show that modafinil treatment prevents the loss of dopaminergic neurons in the SN induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice, a model that mimics the pathophysiology of human Parkinson's disease (Fuxe et al., 1992). Later, the same group further demonstrated that the neuroprotective effect of modafinil is not limited to dopaminergic (TH+) neurons, but also extends to GABAergic neurons in the substatia nigra, with a small increase in the number of non-neuronal cells, likely glial cells (Aguirre et al., 1999).

These findings were later expanded to reveal that the decrease in striatal dopamine, norepinephrine and serotonin content promoted by MPTP was prevented by modafinil, which also reduced the impact of MPTP on GABA and glutathione levels in the SN (Xiao et al., 2004). The collective data provide evidence that anti-oxidative action might be related to modafinil-mediated neuroprotection. However, the effects of modafinil on the glial conversion of MPTP into its toxic metabolite MPP^+, which, in turn, upregulates TNF, IL-1β, IL-6 and nitric oxide (NO), remains unexplored to date. Modafinil has also been shown to be neuroprotective against the degeneration of nigrostriatal dopaminergic neurons following a partial mechanical hemitransection of mesencephalic dopaminergic pathways in rats (Ueki et al., 1993a).

As for brain ischemia models, Ueki and colleagues demonstrated in a pioneering study that modafinil pretreatment prevents the ischemic lesion produced by endothelin-1 in the striatum of rats. This protective effect is related to a reduction in the endothelin-1-induced ischemic area, lactate levels and astrogliosis (Ueki et al., 1993b). These findings were later corroborated using a middle cerebral artery occlusion (MCAO) model of focal cerebral ischemia in rats. In this experiment, modafinil reduced the infarct area by up to 40% in a dose-dependent manner, as well as decreased brain water content, apoptosis, astrogliosis and oxidative damage induced by ischemia-reperfusion following the MCAO (Abbasi et al., 2019). Recently, by using a model of bilateral common carotid artery (BCA) occlusion, Yousefi-Manesh and colleagues (2019a) revealed that the preventive effect in ischemic stroke lesions is correlated with decreased levels of phosphorylated NF-κB, IL-1β, TNF and malondialdehyde (MDA, a lipid peroxidation marker) in the brain of modafinil-treated rats.

Modafinil has been shown to be an effective therapy in reducing the fatigue caused by multiple sclerosis (MS), the most common symptom, affecting more than 80% of MS patients (Shangyan et al., 2018). MS is a neurological disorder characterized by the presence of autoreactive T cells that infiltrate the CNS and drive an autoimmune response targeting the myelin, leading to motor and sensory impairments, paralysis and optic neuritis. A study by members of our research group showed for the first time that the therapeutic properties of modafinil in MS-related fatigue are closely linked to its anti-infammatory effect. Using MOG-induced experimental autoimmune encephalomyelitis (EAE), an experimental model of MS, we demonstrated that therapeutic modafinil treatment not only ameliorates the clinical symptoms and motor impairment of EAE in mice, but also negatively affects the autoimmune response in the CNS (Brandão et al., 2019).

By treating mice with modafinil after the onset of EAE symptoms, we revealed that modafinil drastically reduced the inflammatory cellular infiltrate into the CNS, which was correlated to a reduction in the frequency of T helper (Th)-1 cells, one of the main effector cells that drive autoimmunity against myelin. This reduction was accompanied by a drastic decrease in interferon (IFN)-γ mRNA and protein levels in the spinal cord, as well as in T-bet mRNA levels, a critical transcription factor for Th1 cell differentiation (Brandão et al., 2019). Interestingly, the effect of modafinil in reducing the severity of EAE symptoms was also evident when modafinil was given prior to immunization and before the onset of symptoms, indicating a preventive effect, probably through a different mechanism (data not published).

Despite clinical studies failing to report any significant improvements promoted by modafinil treatment in Alzheimer's-related apathy (Frakey et al., 2012), the disease symptomatology is more complex than just its depressive episodes and no studies have yet addressed the potential benefits of this therapy on cognitive impairment and neuroinflammation in patients with mild to moderate Alzheimer's disease.

Taken together, the data point to a promising neuroprotective and anti-inflammatory effect of modafinil in animal models of Parkinson's disease, cerebral ischemia and MS. Although the anti-inflammatory properties have not been explored in depth in the MPTP-induced Parkinsonism and in brain ischemia models, these approaches also indicated an anti-oxidative effect of modafinil. The results suggest that modafinil is a promising adjuvant anti-inflammatory therapy for neurodegenerative disorders and future research is warranted to further explore this pharmacological therapy in other animal models of neurodegeneration, as well as in patients affected by these conditions.

6. The effects of modafinil on peripheral immune response

Given that the main effects of modafinil are restricted to the brain, most studies have so far focused on the immunomodulatory properties of modafinil in animal models of neuroinflammation and neurodegeneration. However, emerging research has revealed that the peripheral immune response is equally affected by modafinil.

In this respect, we demonstrated a dual *in vitro* effect of modafinil in primary peripheral immune cells (Zager et al., 2018b). On one hand, higher concentrations of modafinil inhibited the *in vitro* activation of T cells and macrophages, as well as decreased the secretion of IL-2, IL-6, TNF, IFN-γ and IL-17 in primary cultures of spleen cells from naive mice. On the other hand, lower concentrations of modafinil increased CD25 (or IL-2R) expression by T cells and enhanced the IL-2, IFN- γ and IL-17 secretion by spleen cells stimulated *in vitro*.

We then addressed the effects of *in vivo* treatment with modafinil on the *ex vivo* activation of splenic immune cells and secretion of pro-inflammatory cytokines. In order to compare different treatment regimens, mice were subjected to single or repeated (five consecutive days) administration of modafinil prior to the collection of immune cells. Our data revealed that neither of the treatment regimens tested affected the activation of T cells and macrophages, and did not change the oxidative burst and phagocytosis of neutrophils. However, the levels of cytokines produced by spleen cells from the mice treated with modafinil revealed that both treatment regimens potentiated the secretion of IL-6, TNF and IFN-γ by T cells, as well as IL-6 and TNF by macrophages. Since our results indicated the increased secretion of IFN-γ following *in vitro* and *in vivo* treatment with modafinil, we investigated how the *in vivo* treatment would affect the number of IFN-γ producing cells in mice and in patients with narcolepsy type 1. The data showed that the chronic, but not acute, modafinil treatment drastically increased the number of IFN-γ producing cells in the spleen of mice.

Corroborating our pre-clinical data, the continuous treatment with modafinil also increased the number of circulating IFN-γ producing cells in the peripheral blood mononuclear cells (PBMCs) of patients with narcolepsy type 1, compared to samples from the same patients collected before the treatment started. Narcolepsy type 1 is a neurological disorder characterized by destruction of orexin/hypocretin neurons in the hypothalamus, leading to excessive daytime sleepiness and cataplexy. The most accepted hypothesis as to the etiology of narcolepsy type 1 is that genetic predisposition combined with as yet-unknown environmental triggers leads to an autoimmune response to these neurons (Szabo et al., 2019). Considering the aforementioned

effects on autoimmune models (i.e. multiple sclerosis), the immunomodulatory property of modafinil on IFN-γ producing cells is extremely relevant in the context of narcolepsy and warrants further investigation. Our study was the first to show that modafinil treatment is able to increase peripheral IFN-mediated immunity in mice and humans (Zager et al., 2018b).

In a separate study, Han and colleagues showed that modafinil treatment decreased the development of aortic atherosclerosis in apolipoprotein E (apoE)-deficient mice (Han et al., 2018). In this transgenic mouse model, the apoE-deficient animals developed atherosclerosis after being fed with a high-fat diet. This preventive effect of modafinil was correlated with a decrease in serum levels of pro-inflammatory cytokines, such as IL-6, TNF and IFN-γ, and increased levels of the anti-inflammatory cytokines IL-4 and IL-10. Additionally, modafinil decreased IL-6, TNF and IFN-γ expression, as well as inhibited *in vitro* NF-κB activity, in macrophages isolated from apoE-deficient mice, indicating that the preventive effect on atherosclerosis is mediated by macrophages (Han et al., 2018).

Modafinil has also been shown to be an effective anti-inflammatory treatment in a model of testicular torsion. Yousefi-Manesh and colleagues (2019b) showed that modafinil was able to reduce the levels of pro-inflammatory cytokines TNF and IL-1β, as well as decrease the degeneration in germinal cells and histopathological changes in the testicular tissue of rats following mechanical torsion. This procedure leads to inflammatory and oxidative damage by ischemia/reperfusion of the tissue, similar to that which occurs in brain ischemia models, which are counteracted by continuous modafinil treatment.

In addition, modafinil has been shown to have beneficial effects in a rat model of inflammatory bowel disease induced by acetic acid. In this experiment, the authors found that modafinil decreased colitis-induced macroscopic and microscopic lesions in a dose-dependent manner. This effect was linked with a reduction in TNF and IL-1β levels in the colon tissue and decreased inflammatory cell infiltrate. The injection of nitric oxide synthase (NOS) inhibitors prior to modafinil reversed these beneficial effects, suggesting that NO mediates, at least in part, the protective effects of modafinil in colitis (Dejban et al., 2020).

In summary, although modafinil has been shown to be pro-inflammatory in peripheral immune cells of naive mice, stimulating IFN-mediated immunity, it was demonstrated to be anti-inflammatory in animal models of inflammatory diseases such as atherosclerosis and testicular torsion.

7. Proposed mechanisms of the immunomodulatory properties of modafinil

7.1. Anti-inflammatory effects in the CNS

The data so far indicate an anti-inflammatory effect of modafinil treatment in animal models of neuroinflammation and neurodegenerative disorders. It is possible, therefore, to speculate about the

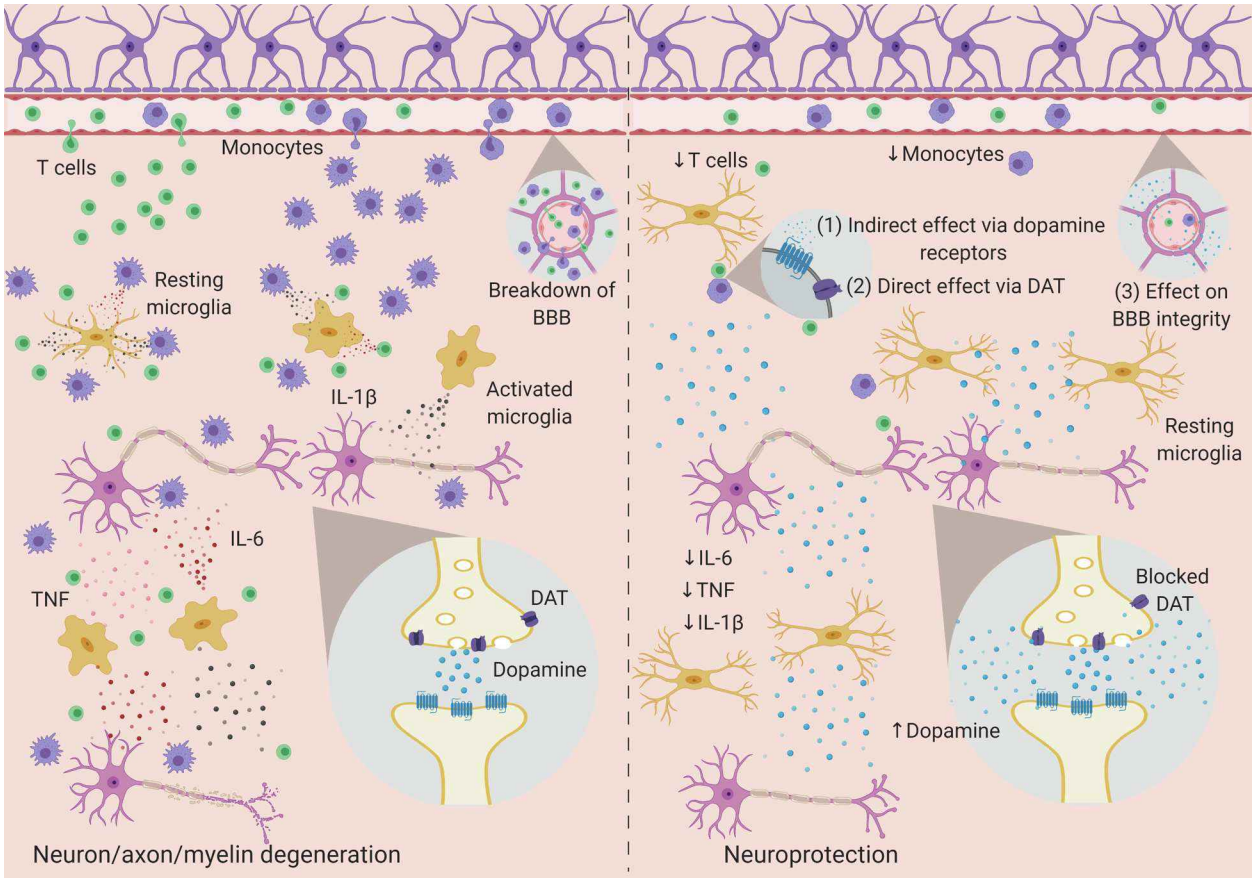

Fig. 1. Illustration of dopamine hypothesis for the anti-inflammatory effects of modafinil in the CNS. In the left panel, inflammatory events without modafinil treatment, such as the infiltration of immune cells (T cells and macrophages) from the periphery that produce pro-inflammatory cytokines *in situ*, which can lead to microglial activation, and further propagate the inflammation and produce axonal, myelin and/or neuronal degeneration. The right panel illustrates our hypotheses on the neuroinflammatory response in an individual under modafinil treatment. (1) The excessive extracellular dopamine negatively influences the infiltration and activation of immune cells and resident microglia, probably by acting on the dopaminergic receptors of these cells. (2) Direct action of modafinil on DAT expressed by immune cells and glia, which is a mechanism independent of brain dopamine levels. (3) Modafinil directly or indirectly influences the function of the BBB or circumventricular organs, such as the choroid plexus, probably by affecting its permeability to immune cells and inflammatory mediators.

mechanism of modafinil-induced anti-inflammatory effects in the brain. Our first hypothesis is that the increased extracellular dopamine in the brain following modafinil treatment negatively affects the infiltration, activation and function of immune cells, such as macrophages (Jiang et al., 2016; Yan et al., 2015) and T cells (Ghosh et al., 2003; Huang et al., 2016; Levite et al., 2017; Saha et al., 2001), as well as activation of resident glial cells (Abbasi et al., 2019; Jung et al., 2012; Ueki et al., 1993b), all of which express dopaminergic receptors. The fact that D1R blockage by SCH-23390 prevented some of the modafinil-mediated effects in LPS-induced systemic inflammation, including recruitment of brain-derived monocytes (Zager et al., 2018a), corroborates this hypothesis.

A second hypothesis is that modafinil might act directly on DAT expressed by immune and glial cells. The fact that immune cells, such as T cells and monocytes/microglia, express DAT constitutively (Buttarelli et al., 2011; Gaskill et al., 2012; Gopinath et al., 2020; Mackie et al., 2018) and that modafinil has direct *in vitro* effects on mouse splenocytes (Zager et al., 2018b) and on BV-2 microglia (Jung et al., 2012) indicates a mechanism independent of brain dopamine levels.

A third hypothesis as to the beneficial effects of modafinil on neuroinflammation is a direct effect on the functionality of the blood-brain-barrier (BBB) and circumventricular organs. A recent study from Castellani and colleagues (2019) showed that genetic DAT hypofunction leads to decreased microglia activation and reduced macrophage recruitment in the brains of mice. These effects were linked with weaker activation of the choroid plexus, as demonstrated by reduced NF-κB activity. Although performed under baseline conditions (i.e. no immune challenge or disease), these experiments demonstrate the closeness of the relationship between the dopaminergic system and BBB function, and how this might influence neuroinflammatory response (Castellani et al., 2019). Fig. 1 summarizes these hypotheses, illustrating how modafinil treatment might lead to decreased infiltration of immune cells and reduced microglial activation.

However, the possibility that the anti-inflammatory effect of modafinil in the brain is mediated by other neurotransmitters and neuropeptides besides dopamine cannot yet be excluded. Immune cells express receptors for many other neurotransmitters that are affected by modafinil treatment, including norepinephrine and acetylcholine, which might account for the immunomodulatory effects of the drug. This might be the case for the effects of modafinil in peripheral immune response, where increased extracellular dopamine does not seem to play a significant role in modafinil effects. Future research is warranted to determine the precise mechanisms of modafinil-mediated effects on brain inflammation. In addition, the neuroprotective potential of modafinil also seems to be mediated by its anti-oxidant effects in the models of MPTP-induced parkinsonism and cerebral ischemia, which, in turn, can also be influenced by decreased glial activation.

7.2. Modafinil-mediated modulation of peripheral immune function

The modulatory effect of modafinil on peripheral immune function is still controversial. On one hand, our research showed that modafinil pre-treatment exerts a direct *in vitro* and *in vivo* effect on peripheral immune cells, potentiating pro-inflammatory cytokine release and increasing IFN-mediated immunity in naive mice (Zager et al., 2018b). On the other hand, *in vivo* modafinil treatment had anti-inflammatory and anti-atherosclerotic effects in apoE-deficient mice fed with a hyperlipidic diet (Han et al., 2018), as well as decreased inflammatory and oxidative damage in a model of mechanical testicular torsion (Yousefi-Manesh et al., 2019b) and inflammatory bowel disease (Dejban et al., 2020).

The effects of modafinil treatment in the peripheral immune response seem, therefore, to be distinct from its effect on brain inflammation, and somehow dependent on whether there is pre-existing inflammation. In addition, given that these effects are peripheral, they are unlikely to be mediated by dopamine released centrally. These discrepancies, however, can be explained by the distinct roles of the autonomic nervous system on peripheral immune response in a naive situation and during a pre-existing inflammatory condition.

Box 1
Dopamine and the reward system as a new pathway between the brain and immunity.

Several lines of evidence have supported the reciprocal interactions between the nervous and immune system. These interactions have been studied in depth over the last decades and provided the basis for psychoneuroimmunology research that has revealed the pathways by which the brain and peripheral immune system influence each other's function.

One of the first studies revealing a close relation between immune response and dopaminergic mesolimbic pathway was that by Lacosta and colleagues (Lacosta et al., 1994). They demonstrated in rats that an immune activation produced by inoculation with sheep red blood cells increased extracellular dopamine levels in the nucleus accumbens, which temporally coincided with the peak of immune response.

However, recent evidence suggest that this response might be part of a compensatory mechanism. Ben-Shaanan and others have shown that activation of the dopaminergic reward system via "designer receptors exclusively activated by designer drugs" (DREADDs) expressed in the ventral tegmental area (VTA) in naive mice boosted the peripheral anti-bacterial activity to an experimental E. coli infection and increased IgG and IFN-mediated immune response (Ben-Shaanan et al., 2016). The same group expanded those findings, revealing that the activation of DREADDs in the VTA also increased the anti-tumor immunity, as demonstrated by decreased tumor size and weight (Ben-Shaanan et al., 2018). This effect is mediated by myeloid-derived suppressor cells, which normally suppress the anti-tumor immune response, and exhibited an attenuated immunosuppressive profile following activation of the reward system. These mechanisms were shown to be highly dependent on sympathetic nervous system (SNS) activation and norepinephrine released peripherally, given that chemical ablation of SNS with 6-OHDA and pharmacological blockage of β-adrenergic receptors abolished those effects (Ben-Shaanan et al., 2016, 2018).

These data reveal a new route of communication between the brain reward system and the peripheral immune function. Although DREADDs is an artificial experimental approach that does not completely represents naturally occurring rewarding conditions, it shed some light on to the brain regions involved in immune regulation. This discovery suggests that activities that physiologically stimulate the reward system, such as engaging in sex, social interactions and even physical activity, might have beneficial effects via this newly proposed mechanism when fighting against an infection or cancer.

In naive animals, the activation of the brain reward system indirectly boosts peripheral anti-bacterial and anti-tumor immunity through sympathetic activation and norepinephrine release (Ben-Shaanan et al., 2016, 2018), as explained in Box 1. During ongoing inflammation, however, the cholinergic anti-inflammatory reflex is previously activated via acetylcholine binding to the α7 nicotinic receptor, which leads to norepinephrine secretion by T cells. The norepinephrine released by T cells binds to the β2 receptor in macrophages, decreasing TNF release and NF-κB activation and limiting chronic inflammation (for review, see Pereira and Leite, 2016; Reardon, 2016). This complex response to chronic inflammation might act synergistically and be potentiated by modafinil, by mechanisms that are, as yet, still unknown.

Given that modafinil acts on multiple neurotransmitter systems, it is difficult to pin point the exact mechanism of these peripheral effects. However, it is plausible that these differences in autonomic nervous system activity in physiological versus inflammatory milieu might

account for these observed differences in the effects of modafinil treatment on peripheral immune response. Therefore, this hypothesis still needs to be tested in mice under chemical ablation of the sympathetic nervous system, as well as in mice subjected to vagus nerve transection/stimulation.

8. Emerging questions

Although recent advances described in the literature have helped to characterize the immunomodulatory properties of modafinil in several animal models of immune-inflammatory response, many questions emerge from this potential new medical use:

- The effects of modafinil treatment on other symptoms elicited by inflammation is yet to be studied, including memory impairment, conditioned aversion, fever and sleep response;
- The specific role of dopamine and other neurotransmitters in the modafinil-mediated effects on immune function, as well as their receptors on immune and glial cells, are of particular interest. This could be investigated by either chemical ablation of these neurotransmitters, by pharmacological blockage of receptors, or even with the use of mice with cell-specific deletions;
- Finally, given the fact that modafinil significantly decreased inflammatory cellular infiltrate into the CNS in LPS-induced systemic inflammation and EAE, it would be of extreme value to understand whether modafinil is able to affect the integrity of the BBB, and whether it has protective potential against inflammation-induced breakdown of BBB.

9. Concluding remarks

Since modafinil was first described more than 30 years ago, there has been accumulating evidence indicating that it has a promising anti-inflammatory effect in animal models of neuroinflammation and/or neurodegeneration, such as inflammation-induced sickness symptoms, methamphetamine-induced glial activation, multiple sclerosis, Parkinson's disease and brain ischemia. It does so not only by acting on infiltrating immune cells in the CNS, but also on resident glial cells, negatively impacting both innate and adaptive immune response in the brain, probably through a number of different mechanisms. This goes far beyond the initial development of modafinil as a treatment to promote wakefulness in patients with sleep disorders and points to the possibility of modafinil being used as adjuvant treatment for many disorders in which neuroinflammation plays a vital role.

Although the effects of modafinil on peripheral immune response are still debatable and results in this area need to be interpreted with caution, they should lay the foundation for future research to study its potential for treating infectious and inflammatory diseases in both animal models and human patients to develop a better understanding of its prospective new medical uses and possible side effects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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