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Full-length Article

Antidepressant imipramine diminishes stress-induced inflammation in the periphery and central nervous system and related anxiety- and depressive- like behaviors ☆

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ABSTRACT

In order to relieve anxiety and depression accompanying stress, physicians resort to tricyclic antidepressants, such as imipramine. We had previously shown that imipramine reversed stress-induced social avoidance behavior, and down-regulated microglial activation 24 days after stress cessation. To further characterize the effects of imipramine on stress induced neuroimmune dysregulation and associated changes in behavior, the aims of this study were to determine if imipramine 1) ameliorated stress-induced inflammation in the periphery and central nervous system, and 2) prevented stress related anxiety- and depressive-like behaviors. C57BL/6 mice were treated with imipramine (15 mg/kg) in their drinking water, and exposed to repeated social defeat (RSD). Imipramine attenuated stress-induced corticosterone and IL-6 responses in plasma. Imipramine decreased the percentage of monocytes and granulocytes in the bone marrow and circulation. However, imipramine did not prevent splenomegaly, stress-related increased percentage of granulocytes in this organ, and the production of pro-inflammatory cytokines in the spleen, following RSD. Moreover, imipramine abrogated the accumulation of macrophages in the brain in mice exposed to RSD. Imipramine blocked neuroinflammatory signaling and prevented stress-related anxiety- and depressive-like behaviors. These data support the notion that pharmacomodulation of the monoaminergic system, besides exerting anxiolytic and antidepressant effects, may have therapeutic effects as a neuroimmunomodulator during stress.

1. Introduction

The prolonged inflammatory state associated with social stress has the potential to contribute to the etiology of anxiety and depression. Analysis of peripheral inflammatory markers in patients with mood disorders reveals constant elevations in interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) (Russo and Nestler, 2013). There is evidence that activation of microglia in animal models of stress, potentiates hypothalamic-pituitary-adrenal (HPA) axis stimulation through the release of IL-1β within the hypothalamus (Goshen and Yirmiya, 2009), augmenting neuroendocrine outflow that may reinforce stress-associated behaviors. Thus, physiological microglia alterations are likely to contribute to the dysfunctional neurobiological stress interpretation in the CNS.

Repeated social defeat (RSD), a model of psychosocial stress in mice, provides a probe to study the mechanisms leading to stress-related alterations in inflammation in both the periphery and CNS, and associated anxiety- and depressive-like behaviors (Kinsey et al., 2007; Wohleb et al., 2013). RSD causes an increase in pro-inflammatory factors and enhances the capacity of immune cells to enter circulation and organs, such as the spleen and brain. Also, RSD augments levels of corticosterone in circulation following the second cycle of RSD, and peaks at the sixth cycle (Engler et al., 2005).

Mice exposed to RSD display an increase in plasma and tissue catecholamines. This is meaningful, since peripheral immune cells express receptors for norepinephrine, and when these receptors are stimulated, functional responses occur that influence the

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development and mobility of these cells, as well as their inflammatory phenotype (Powell et al., 2013). For example, repeated activation of the sympathetic nervous system (SNS) causes an increase of norepinephrine in the bone marrow (BM) (Hanke et al., 2012) that promotes a shift in myelopoiesis after RSD exposure. These inflammatory myeloid progenitor cells (MPCs) were found to be glucocorticoid (GC)-resistant and trafficked throughout the body with an increased production of pro-inflammatory cytokines in response to lipopolysaccharide (LPS) stimulation (Avitsur et al., 2001; Bailey et al., 2009).

Microglia isolated from socially defeated mice have a higher mRNA expression of pro-inflammatory cytokines and chemokines compared to home cage controls (Wohleb et al., 2011). Also, microglia from socially defeated mice and cultured *ex vivo* produced exaggerated levels of IL-6, TNF-α, and CCL-2 following stimulation with LPS (Wohleb et al., 2011), even 24 days after stress cessation (Ramirez et al., 2015). Neuroinflammatory factors such as IL1-β, TNF-α, and IL-6, are associated in the neurobiological changes that reinforce fear/anxiety and threat circuitry (Wohleb et al., 2014), promoting anxiety-like behavior.

Neuronal and microglia activation and production of pro-inflammatory molecules as a result of stress exposure, promote the development of a reactive endothelium (Wohleb et al., 2014). Peripherally derived monocytes differentiate into perivascular and parenchymal macrophages (Wohleb et al., 2014) within the fear, anxiety, and threat appraisal circuitry (Wohleb et al., 2013). The accumulation of macrophages in the CNS elicited by RSD, increases neuroinflammatory signaling.

Clinical and experimental research has demonstrated that antidepressants can also prevent the expression of pro-inflammatory cytokines (Xia et al., 1996; Yirmiya et al., 2001; Castanon et al., 2002; Hashioka et al., 2007; Hwang et al., 2008). In animal models, imipramine and fluoxetine suppressed the production of TNF-α, IL-1β, and IL-6 by glial cells (Lim et al., 2009). Imipramine inhibited interferon (IFN)-γ stimulated microglial production of IL-6 and nitric oxide (Hashioka et al., 2007), and TNF-α production in microglia and astrocyte cultures (Hwang et al., 2008). In patients suffering from acute depression, fluoxetine reduced enhanced plasma levels of IL-6 (Sluzewska et al., 1995).

Recent findings from our laboratory showed RSD promoted long-lasting microglial activation associated with social avoidance behavior, which was maintained for at least 24 days after stress cessation (Wohleb et al., 2013). Imipramine treatment by intraperitoneal (i.p.) injection (20 mg/kg) or in the drinking water (15 mg/kg) reversed stress-associated social avoidance behavior and stress-induced increased neuroinflammatory signaling at this time point (Ramirez et al., 2015). Moreover, microglia from RSD mice produced exaggerated levels of pro-inflammatory molecules following LPS-stimulation, even 24 days after stress termination, and this was prevented with imipramine treatment.

The mechanism of medicinal action of tricyclic antidepressants such as imipramine in relation to the monoaminergic system has been well established. These drugs inhibit the reuptake of serotonin, norepinephrine, and dopamine by directly blocking neurotransmitter transporters (Eshleman et al., 1999; Zhou et al., 2007). Neurotransmitter transporters for serotonin, norepinephrine, and dopamine in the presynaptic membrane restricts neuronal signal transmission (Glowinski and Axelrod, 1964; Iversen, 2006) and drugs used to block these systems have been used successfully for the treatment of depression (Klimek et al., 1997; Zhou et al., 2007). However, more research is needed to establish the influence of antidepressants on stress-related catecholaminergic mechanisms, specifically in the context of HPA axis, SNS activation, and cytokine production in both the periphery and central nervous system (CNS). The objective of this study was to further determine if neuroinflammatory signaling, and behavioral alterations after six cycles of RSD, could be reversed with imipramine treatment. Hence, the effect of imipramine on stress-induced shift in myelopoiesis, and trafficking of MPCs to blood, spleen, and brain, and associated anxiety- and depressive like behaviors were studied.

2. Materials and methods

2.1. Animals

Male C57BL/6 (6–8 weeks old) and CD-1 (12 months old, retired breeders) mice were purchased from Charles River Breeding Laboratories (Wilmington, Massachusetts) and allowed to acclimate to their surroundings for 7–10 days prior to initiation of experiments. C57BL/6 mice were housed in cohorts of three and CD-1 mice were singly housed in 11.5 × 7.5 × 6 in. polypropylene cages. Mice were maintained at 21 °C under a 12:12 h light: dark cycle with *ad libitum* access to water and rodent chow in the animal facility at The Ohio State University. All procedures were in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and approved by The Ohio State University Institutional Laboratory Animal Care and Use Committee.

2.2. RSD

RSD was performed as described previously (Wohleb et al., 2011). In brief, an intruder male CD-1 mouse was introduced into home cages of male C57BL/6 mice (three per cage) for 2 hours (h) on 6 consecutive nights. Behavior was observed to make certain that the intruder was aggressive. If the CD-1 mouse did not initiate an attack within 5–10 min or was attacked by resident mice, a new CD-1 mouse was introduced. At the end of the 2 h the CD-1 mouse was removed and the resident mice were left undisturbed until the next day when the same paradigm was repeated. During RSD, resident mice display submissive behaviors such as upright posture, fleeing, and crouching (Stark et al., 2001; Hanke et al., 2012). Home cage control (HCC) cohorts were left undisturbed in a separate room.

2.3. Pharmacological treatments and administration procedures

C57BL/6 mice were randomly selected for inclusion into different experimental treatment groups. The groups were: RSD/imipramine, RSD/vehicle, HCC/imipramine, and HCC/vehicle. Mice in the RSD/imipramine received daily imipramine (15 mg/kg) treatment in their drinking water as previously described (Ramirez et al., 2015), starting two days before the initiation of social defeat. HCC/imipramine received daily imipramine treatment in their drinking water at the same dose while RSD/vehicle and HCC/vehicle groups drank untreated water (Fig. 1a). Bottles of water were changed daily before and during the experimental protocol. The dose of imipramine was based on a previous study with C57BL/6 mice, in which chronic administration at 15 mg/kg in drinking water effectively reversed social avoidance behavior and neuroinflammatory signaling after 24 days of social defeat cessation (Ramirez et al., 2015).

The amount of water consumed for each cage was registered daily throughout the experiment (from Day 0 to Day 6). The calculation of the concentration of imipramine in drinking water was based on the evaluated mean volume of daily water consumption, assessed by weighing the bottles daily (from Day 0 to Day 6) from our previous imipramine study (Ramirez et al., 2015). An average of 9.0 ml per day/per cage intake was calculated. Water consumption in the four groups of mice was in fact similar. Based on this