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Research report

Inflammatory mechanisms contribute to microembolism-induced anxiety-like and depressive-like behaviors

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HIGHLIGHTS

- Small cerebral infarcts cause acute ipsilateral neuroinflammation.
- Small cerebral infarcts generate delayed contralateral neuroinflammation.
- Changes in affective-like behavior after small cerebral infarcts can be prevented by anti-inflammatory treatment.

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ABSTRACT

Poor vascular health, atherosclerosis, or cardiac procedures in the elderly result in clinically silent microvascular infarcts that increase susceptibility to larger ischemic episodes and can precipitate changes in mood and cognition. Although the mechanisms that underlie ischemia-induced behavioral changes have not been fully elucidated, chronic inflammation has been implicated in the pathogenesis. Independent of brain injury, elevated levels of inflammatory cytokines can lead to sickness behaviors and symptoms of depression. Furthermore, in the presence of brain injury, inflammatory activation may serve as the linchpin that precipitates dysregulation of biological systems leading to changes to behavior. In the current study, we tested the hypothesis that cerebral inflammation caused by diffuse ischemia is necessary for the expression of post-injury anxiety- and depressive- like behavior. Using a microsphere embolism (ME) rodent model, we demonstrate prolonged elevations in expression of inflammatory genes in the hippocampus ipsilateral to the injury which are reflected in the contralateral hemisphere by two weeks following injury. Prophylactic administration of meloxicam, a preferential inhibitor of COX-2 activity, prevented both central inflammation and deficits in affective-like behaviors. Furthermore, meloxicam was more efficacious than the selective serotonin reuptake inhibitor fluoxetine in prevention of microembolism-induced changes in inflammation and behavior. These data demonstrate that inflammatory activation is necessary for microembolism-induced behavioral changes and suggest that anti-inflammatory treatments may be an effective therapeutic strategy in patients with risk factors for vascular depression or prior to invasive cardiac procedures.

1. Introduction

Cerebromicrovascular disease (CMVD) affects up to 87% of the population over age 65 [1] and contributes to changes in mood, cognitive deficits, and the worsening of comorbid disease states [2–4]. In humans, the presence of small cerebral infarcts (SCI), or microvascular lesions, within CMVD is associated with depression, more severe cognitive decline, and poor overall prognosis [5,6]. SCI formation is detected following coronary artery bypass grafting (CABG) procedures [7–10], and detection of SCI prior to cardiac procedures predicts worsened postoperative outcome [11]. Evidence for the relationship between vascular disruption and behavioral change is longstanding and wide spread, as patients with vascular damage are at an increased risk for depression for up to two years following the event [12–14]. Along these lines, depression predicts a worsened outcome for CABG patients and behavioral changes following SCIs are frequently reported [12,15,16]. Activation of inflammatory cytokines occurs

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immediately following ischemic events (including CABG) and is linked to the expression of behavioral disruptions [10,17,18]. The role of inflammation in sickness- and affective behaviors are not novel [19–21], nor are the beneficial effects of anti-inflammatory therapeutics on depressive disorders [22]; however, addressing the functional consequences of SCIs from an inflammatory standpoint has not been well explored. In the presence of SCI, depression is associated with increased morbidity and mortality and rates of depression among patients with cardiovascular disease are two-fold that of the general population [23]. Further compounding the implications of SCI and depression is the finding that anti-depressant therapeutics offer poor rates of remission in patients with vascular depression [24] and following CABG [23,25–27]. In contrast, therapeutics targeted at controlling inflammation have been shown to reduce age-associated brain volume loss in humans following CABG and improve cognition in aged rats [10]. Similarly, recent studies indicate that anti-inflammatory therapeutics show promise in patients with high baseline cytokine activity [28] and when anti-inflammatories are administered as an adjunct to standard anti-depressant therapies [29]. Given the relationship between inflammatory activation and altered mood in the presence of acute ischemic injury, chronic inflammation may underlie the delayed manifestation of depression and anxiety following cerebral microvascular damage. Furthermore, given the silent nature of microvascular injury, post-injury control of inflammation, and timing of such therapy, may be difficult to optimize. Previous studies have established that microsphere embolism (ME)-induced microvascular damage in rats leads to increased expression of inflammatory cytokines [30]. The current study builds upon these findings to test the hypothesis that wide spread increases in cerebral inflammation are necessary for the manifestation of post-ME anxiety- and depressive-like behaviors. Furthermore, to test the efficacy of preoperative control of inflammation on outcome, we compared prophylactic treatment with meloxicam, a preferential COX-2 inhibitor, with the anti-depressant, fluoxetine. The data presented indicate that inflammation was necessary for the manifestation of post-ME behavioral changes and that anti-inflammatory treatment outperformed anti-depressant treatment in preventing the manifestation of depressive- and anxiety-like behaviors following ME.

2. Methods and materials

2.1. Inflammatory assessment

2.1.1. Animals

Adult male Wistar rats (3 mos, Charles River) were pair-housed until surgery. An AAALAC-approved facility maintained the rats on a reverse 14:10 light–dark cycle in a temperature- and humidity-controlled vivarium. *Ad libitum* food and water were available throughout the duration of the study. All experiments were performed in accordance with the Institutional Animal Care and Use Committee of Emory University and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals.*

2.1.2. Surgical procedure

Following a one week acclimation, rats were randomly assigned to sham or ME surgical groups. The ME procedure has been described in detail previously [31], but is briefly summarized here. Rats were anesthetized with isoflurane and secured in a supine position. Throughout the procedure, core temperature was maintained at 37 °C with a homeothermic blanket regulated via a rectal temperature probe. An incision was made and the common carotid artery was isolated and ligated with suture followed by ligation of the external carotid artery at the bifurcation with the internal carotid artery. Microspheres (New England Nuclear Inc., Boston, MA; 50 μm in diameter; suspended in 10% Dextran and 0.01% Tween in isotonic saline; PerkinElmer Instruments; Shelton, CT; ≈2500 spheres in 50 μL) were injected with a 30 G needle inserted into the left internal carotid artery. Following injection, direct pressure applied to the injection site facilitated the cessation of bleeding, ligatures were released, and blood flow returned. Sham rats underwent an identical procedure without the infusion of microspheres. Previous studies have characterized the physiological [32] and histopathological [31,33] effects of microsphere embolism procedures; therefore, these endpoints were not repeated within the current set of experiments.

2.1.3. Gene expression

For all rats, brains were frozen and dissected under RNAse-free conditions with regions bilaterally isolated for gene expression analysis. Due to the involvement of the hippocampus in mood disorders [34,35] and its sensitivity to cerebral [36] and cardiac [37] ischemia, the hippocampus was the focus of all gene expression analyses. Brain tissue was homogenized, and RNA was extracted using the Qiagen RNeasy Mini Kit (Valencia, CA) according to manufacturer's instructions. RNA samples were reverse transcribed using Applied Biosystems High Capacity cDNA Reverse Transcription Kit. Resulting cDNA was quantified and normalized using the PicoGreen method (Invitrogen, Grand Island, NY). The Rat Inflammatory Cytokines and Receptors PCR Array (SABiosciences, Valencia, CA) was used to assess inflammatory activation 14 days following sham or ME surgery. Samples were processed according to manufacturer's instructions on an Applied Biosystems HT7900 Fast Real-Time PCR system (Carlsbad, CA). Candidate inflammatory cytokines and receptors that were among the most active (14 days post ME) as detected by the SABiosciences gene array plate and relevant to mood disruption [21,38,39] were isolated and measured over an expanded time-course of 1, 2, 3, 7, 14, 28, and 42 days following sham ($n = 6$) or ME ($n = 6$) surgery. All samples were prepared in triplicate using 1 μg of sample and carried out on the same Applied Biosystems HT7900 Fast Real-Time PCR system. Genes of interest were limited to include tumor necrosis factor α (*Tnf*; Rn01525859_g1), monocyte chemotactic protein-1 (*Mcp1*; Rn00580555_m1), interleukin 1β (*Il-1β*; Rn00580432_m1), secreted phosphoprotein 1 (*Spp1*; Rn01449972_m1), nuclear factor kappa-light-chain-enhancer of activated B cells inhibitor-alpha (*IκB*; Rn01473657_g1), and complement receptor 3 (*Cd11b*; Rn00709342_m1). To confirm results from the PCR array, cDNA from Day 14 samples was run against each individual primer listed above in addition to the detailed expanded timeline. Furthermore, gene expression of infiltrating T and B cells were measured in ipsilateral Day 14 samples via hippocampal detection of *Cd3* (forward primer CAGAACTGTGTGGAGCTGGA and reverse TGCTCGTTCTTCAACAGGAC) and *Cd19* (forward primer CAGTGTGGCTCTGGCTGTT and reverse CCTAGCAGGGTCGGTCATT), respectively. Although broader inflammatory assessments were limited to the injured hemisphere, gene expression of *Mcp1*, *Spp1*, and *Tnf* were measured in the opposite (contralateral to damage) hemisphere to determine the extent to which inflammatory activation spreads beyond the immediate area of injury.

2.2. Pharmacological efficacy

2.2.1. Meloxicam administration

Meloxicam efficacy was tested in a separate cohort of rats. Following a one week facility acclimation period, rats were habituated to 5-min daily handlings for 3 days prior to meloxicam or vehicle administration. Rats were randomly assigned to vehicle (sham $n = 12$; ME $n = 13$) or meloxicam (sham $n = 12$; ME $n = 13$; 1 mg/kg) groups, and intraperitoneal dosing began 4 days prior to surgery