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Circulating endocannabinoids during hematopoietic stem cell transplantation: A pilot study

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ABSTRACT

Objective: Hematopoietic stem cell transplantation (HCT) is a stressful and rigorous medical procedure involving significant emotional and immune challenges. The endocannabinoid (eCB) signaling system is involved in regulation of both the immune system and emotional reactivity, yet little is known about its function during HCT. We investigated the role of the eCB signaling system in a group of HCT recipients.
Methods: A total of 19 HCT recipients were enrolled and provided psychosocial data and blood samples at three peri-transplant time points: prior to transplant, hospital discharge, and approximately 100 days post-transplant. Psychosocial factors, inflammatory molecules, and the eCBs were determined and assessed for changes over this period and association with each other.
Results: HCT recipients demonstrated significant changes over the peri-transplant period in inflammatory molecules and psychosocial functioning, but not in circulating concentrations of the eCBs. Associations among these variables were most likely to be present pre-transplant and least likely to be present immediately post-transplant, with depressive symptoms and inflammation most significantly associated. The eCB 2-arachidonoylglycerol (2-AG) was significantly, positively associated with both interleukin (IL)-6 and C-reactive protein (CRP) and negatively associated with depressive symptoms.
Conclusions: The eCB signaling system may have alternative sources and regulatory mechanisms in addition to the immune system. Given the significant associations with inflammatory molecules and depressive symptoms in the peri-transplant period, it is important to better understand this system and its potential implications in the setting of complex and stressful medical procedures such as HCT.

1. Introduction

Hematopoietic stem cell transplantation (HCT) is an intensive medical procedure used to treat a variety of hematologic malignancies and disorders whereby stem cells are infused into the recipient to replace a damaged immune system. This infusion follows complete or near-complete ablation of the recipient's immune system with chemotherapy and/or radiation. HCT involves significant emotional and immune challenges, with the highest level of psychosocial stress usually occurring during the early post-transplant phase (first 30 days) (McQuellon et al., 1998). This period of time is also marked by significant immune suppression,

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high risk of infection, and other immunologic complications. The endocannabinoid (eCB) system is uniquely positioned to play a significant role in the peri-HCT period as it is a modulator of emotional reactivity and immune and inflammatory responses (Hillard et al., 2012). There are no reports of studies of the eCB system in the transplant setting.

Multiple psychosocial factors can affect outcomes after HCT (Hoodin et al., 2006); in particular, pre-transplant depression and anxiety are associated with worse post-transplant survival (Andrykowski et al., 1994; Loberiza et al., 2002). Previous studies demonstrate that pre-transplant depression and anxiety are also associated with altered immune function during the post-transplant period (Gregurek et al., 1996; Knight et al., 2014; McGregor et al., 2013; Pereira et al., 2010; Pulgar et al., 2012), with pro-inflammatory factors increasing in the immediate post-transplant period before gradually returning to baseline (Wang et al., 2008, 2014). Further, pro-inflammatory pathways can mediate the relationship between psychosocial stress and poor outcomes (Knight et al., 2013; Wang et al., 2008, 2014). These data suggest that the effects of pre-transplant psychosocial pathology negatively influence outcomes as a result of promoting a pro-inflammatory milieu.

The two most well-studied eCBs are 2-arachidonoylglycerol (2-AG) and *N*-arachidonylethanolamine or anandamide (AEA); both of these lipids are present in the circulation (Hillard et al., 2012). The immune system - specifically macrophages and platelets - is a significant source of eCB synthesis and release in response to endotoxins and cytokines (Varga et al., 1998). In support of these data, previous studies demonstrate that circulating concentrations of 2-AG are increased during inflammation (Bluher et al., 2006; Cote et al., 2007; Di Marzo et al., 2009; Weis et al., 2010). Other known sources include adipose tissue (Matias et al., 2008; Spoto et al., 2006) and reproductive organs (El-Talatini et al., 2010). Since the eCBs are lipophilic, it is likely that their concentrations in the circulation are also affected by overflow from tissues with high eCB contents, including the brain (Caille et al., 2007).

Previous studies of circulating eCB concentrations have demonstrated significant correlations with both depression and inflammation. Concentrations of 2-AG are significantly lower in individuals diagnosed with major depression (Hill et al., 2009, 2008). Other studies demonstrate that circulating concentrations of 2-AG are increased during pro-inflammatory states (Bluher et al., 2006; Cote et al., 2007; Di Marzo et al., 2009) and are positively correlated with concentrations of interleukin 6 (IL-6) (Weis et al., 2010). These data led us to hypothesize that the profound suppression of the immune system that occurs during the process of HCT results in loss of eCBs in the circulation. We further hypothesized that depression in this population would be associated with lower circulating 2-AG concentrations as has been seen in other populations.

In this pilot study, we were able to test these specific hypotheses: 1) concentrations of the eCBs are altered over time in HCT recipients; and 2) circulating concentrations of the eCBs are positively correlated with pro-inflammatory molecules and negatively correlated with depression in the peri-transplant period. We further explored the association between the eCBs and anxiety as well as changes in inflammatory markers, depression, and anxiety over time.

2. Methods

2.1. Patients

A total of 19 men and women who underwent HCT at the University of Rochester Medical Center (URMC) between May 2010 and May 2011 for any reason participated in this study. To be eligible for the study, participants had to be at least 18 years of age, English speaking, and able to complete self-report inventories. Exclusion criteria included conditions preventing meaningful participation in the research interview, for example, major, uncorrected sensory impairments, severe communication limitations such as aphasia, active psychosis, or acute substance intoxication at the time of the initial interview as assessed by the study PI (JMK). Participants were recruited through the study PI after being identified by their transplant physician at their pre-transplant visit in the URMC outpatient HCT clinic. Written informed consent was obtained prior to study participation.

2.2. Procedures

First, participants completed a packet of demographic information in addition to the clinical surveys. At the same time, a 30 ml blood sample was drawn by venipuncture of the antecubital vein or from central venous access, if available, by a trained nurse or phlebotomist (time point 1, T1). T1 ranged from 1 to 18 days prior to transplant (median = 7.5), and always preceded pre-conditioning regimens. Surveys were administered and blood collected at two additional time points: at the time of hospital discharge following initial transplant admission (T2) and as close to day 100 following transplant as possible (T3). T2 collection time ranged from day 9 to day 17 post-transplant (median = 12.5) and T3 ranged from day 91 to day 175 post-transplant (median = 104). Blood specimens were obtained between 0900 h and 1400 h and were centrifuged for 10 min at 1000 × g. The plasma was then aspirated, divided into aliquots, and stored at −80 °C. Plasma analyses were performed at the Medical College of Wisconsin (MCW). Both the URMC and MCW Institutional Review Boards approved these procedures.

2.3. Plasma endocannabinoid extraction and measurement

Concentrations of AEA and 2-AG were determined simultaneously in the same plasma sample. Plasma samples (0.5 ml each) were thawed and made up to 15% ethanol, to which the internal standards [$^2H_8$]-AEA (16.9 pmol) and [$^2H_8$]-2-AG (46.5 pmol) (Cayman Chemicals, Ann Arbor, MI) were added. Samples were vortexed and centrifuged at 1000 × g for 4 min. The supernatant was loaded onto Bond Elut C18 solid-phase extraction columns (1 ml; Varian Inc, Lake Forest, CA) which had been conditioned with 1 ml redistilled ethanol and 3 ml of double distilled water (ddH2O). The remaining pellet was washed with 100 μl of 15% ethanol and centrifuged again for 3 min. The resulting supernatant was also loaded onto the C18 column. Columns were washed with 5 ml ddH_2O and eluted with 1 ml of ethyl acetate. The ethyl acetate layer in the resulting eluate was removed and dried under N_2. Lipids in the residual ddH_2O phase were extracted by mixing with an additional 1 ml of ethyl acetate, which was added to the original ethyl acetate solution. Once dried, samples were resuspended in 20 μl of methanol and stored at −80 °C. AEA and 2-AG were quantified using liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry (LC-APCI-MS); selective ion monitoring was used to quantify the biogenic lipids as described previously (Hill et al., 2013).

2.4. Quantitative determination of cytokines in human plasma

Cytokines were quantified in human plasma using Fluorokine MAP multiplex kits (R&D Systems, Inc., Minneapolis, MN) according to the manufacturer's protocol. The Human High Sensitivity Cytokine Base Kit was used with corresponding Fluorokine MAP