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Translational potential of long-term decreases in mitochondrial lipids in a mouse model of Gulf War Illness

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ABSTRACT

Gulf War Illness (GWI) affects 25% of veterans from the 1990–1991 Gulf War (GW) and is accompanied by damage to the brain regions involved in memory processing. After twenty-five years, the chronic pathobiology of GWI is still unexplained. To address this problem, we examined the long-term consequences of GW exposures in an established GWI mouse model to identify biological processes that are relevant to the chronic symptoms of GWI. Three-month old male C57BL6 mice were exposed for 10 days to GW agents (pyridostigmine bromide and permethrin). Barnes Maze testing conducted at 15- and 16-months post-exposure revealed learning and memory impairment. Immunohistochemical analyses showed astroglia and microglia activation in the hippocampi of exposed mice. Proteomic studies identified perturbation of mitochondria function and metabolomics data showed decreases in the Krebs cycle compounds, lactate, β-hydroxybutyrate and glycerol-3 phosphate in the brains of exposed mice. Lipidomics data showed decreases in fatty acids, acylcarnitines and phospholipids, including cardiolipins in the brains of exposed mice. Pilot biomarker studies showed that plasma from exposed mice and veterans with GWI had increases in odd-chain, and decreases in long-chain, acylcarnitines compared to their respective controls. Very long-chain acylcarnitines were decreased in veterans with GWI compared to controls. These studies suggest that mitochondrial lipid disturbances might be associated with GWI and that further investigation is required to determine its role in the pathophysiology of this illness. Targeting mitochondrial function may provide effective therapies for GWI, and that lipid abnormalities could serve as biomarkers of GWI.

1. Introduction

Nearly 250,000 veterans from the 1990–1991 Gulf War (GW) continue to suffer from Gulf War Illness (GWI), which is a chronic and debilitating condition with a central nervous system (CNS) component (White et al., 2016; Binns et al., 2008) that is characterized by reduced volume and hypometabolism in the hippocampi of veterans with GWI (White et al., 2016). A functional imaging study showed that deficits in working memory are associated with an abnormal activation of the prefrontal cortex of ill GW veterans (Hubbard et al., 2014). A diffusion tensor imaging study suggested that compared to controls, veterans with GWI have atrophy of the axonal tracts that link the cortical gray matter regions involved in fatigue, pain and cognition (Rayhan et al., 2013b). Current treatments for GWI are based on symptom

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management due to a lack of understanding of the complex pathophysiology of GWI. Many GW veterans have not been able to receive an appropriate diagnosis of their conditions due to the unavailability of objective biological markers that can quantify the underlying pathology. Hence, investigations into chronic pathophysiology of GWI is required for developing treatments and diagnostic biomarkers of this illness.

Reports compiled by the Research Advisory Committee (RAC) on Gulf War Veterans' Illnesses implicate exposure to GW agents, such as the acetylcholinesterase (AChE) inhibitor pyridostigmine bromide (PB) and pesticides permethrin (PER), in the pathogenesis of GWI (White et al., 2016; Binns et al., 2008). Evidence for a causal role of GW agents in GWI comes from studies showing elevated rates and severity of illness among veterans who consumed a large quantity of PB tablets and also applied pesticides (White et al., 2016; Binns et al., 2008; Steele et al., 2012). A case-control study of 300 GW veterans showed that compared to controls, veterans with GWI had a higher prevalence of exposure to PB and pesticides (White et al., 2016; Steele et al., 2012). Given the CNS dysfunction in GWI, identifying molecular changes in the brain necessitates the use of well-established animal models of GWI. We developed a GWI mouse model using combined exposure to PB and PER that exhibits anxiety and cognitive problems that are similar to the symptoms reported by veterans with GWI (White et al., 2016; Abdullah et al., 2011; Zakirova et al., 2015; Sullivan et al., 2003). Hence, this mouse model captures one of the key clinical features of GWI and is suitable for examining long-term changes that are relevant to the current clinical condition of veterans with GWI.

Histopathological analyses of this PB + PER mouse model revealed an increase in astroglia activation in exposed mice (Abdullah et al., 2011; Zakirova et al., 2015), which is also observed in several other rodent models of GWI (Abdullah et al., 2012, 2011; Zakirova et al., 2015; Parihar et al., 2013; Ojo et al., 2014). In the brain, astroglia play a fundamental role in providing support to neurons by maintaining the extracellular environment and delivering nutrients to neurons that are acquired from the peripheral circulation. For instance, astroglia take up glucose from the brain capillaries via their end-feet and deliver to neuronal axons (Baltan, 2015). While glucose is the primary energy source which fuels the tricarboxylic acid (TCA) cycle, other nutrients, such as ketone bodies and lactate, are used for neuroenergetics when the glucose supply runs low or during high neuronal demand (Logica et al., 2016). In the brain, β-oxidation of free fatty acids (FFA) yields acetyl-CoA molecules for the TCA cycle to generate ATP (Guzmán and Blázquez, 2004). Importantly, astroglia are the primary cell type in the brain that can store glucose as glycogen which can be converted to lactate and transported to neurons via mono-carboxylate transporters (MCT) (Newington et al., 2013). In this regard, a recent imaging study showed impaired lactate utilization in the brains of veterans with GWI (Rayhan et al., 2013a), which could suggest potential disruption of the astroglia-neuron shuttle in relation to GWI.

The goal of this current work is to elucidate long-term biological disturbances associated with GW agent exposure. Several decades have now elapsed since veterans were exposed to GW agents, and, therefore, an interaction between exposure to these chemicals and aging is also a concern for this vulnerable population (Institute of Medicine, 2013). Given the complexity of biological response to GW agents, the heterogeneity of the clinical presentation of GWI and the chronicity of the illness (White et al., 2016), we used omic approaches (proteomics, lipidomics and metabolomics) to identify biological pathways associated with GW agent exposure. These analyses pointed to mitochondrial lipid and energetic disruptions in our mouse model. In a pilot study, we examined the translational value of mitochondria-specific lipids (acylcarnitines) in plasma and showed that these lipids have the potential to differentiate veterans with GWI from controls. Targeting mitochondrial lipid metabolism may be an avenue for developing treatments for the chronic and persistent CNS problems currently experienced by veterans with GWI. These lipids may also be useful for identifying blood biomarkers of GWI.

2. Materials and methods

2.1. Animals

All animal experiments were approved by the Institutional Animal Care and Use Committee and performed as previously described (Abdullah et al., 2011; Zakirova et al., 2015). Male C57BL6 mice (3-months of age, weight 25 g ± 0.7 SD) were co-administered 0.7 mg/kg of PB and 200 mg/kg of PER in a single intraperitoneal injection volume of 50 μl in dimethyl sulfoxide (DMSO) or the same volume of DMSO alone (control) daily for 10 consecutive days (n = 10 for controls, n = 9 for GW agent exposed mice). The doses of PB and PER are less than 1/5 and 1/2 of the known LD_{50} values in rodents, respectively (Chaney et al., 2002; Williamson et al., 1989). Given the paucity of information on doses and routes of PER exposure, we used 200 mg/kg of PER to mimic a high-level exposure that is similar to that used in mice by previous studies showing adverse behavioral or pathological outcomes (Abdullah et al., 2011; Zakirova et al., 2015; Dodd and Klein, 2009; Pittman et al., 2003). The cis/trans ratio of PER were based on the recommended by the World Health Organization (25% cis and 75% trans) (WHO, 2003). The dose of 0.7 mg/kg of PB has been shown to elicit neurobehavioral deficits relevant to GWI symptom presentation (Zakirova et al., 2015). Experimenters for neurobehavioral, neuropathological, lipidomics, metabolomics and proteomic analyses were blinded to the exposure assignment. For these studies, post-exposure time-points of 15- and 16-months were chosen at which point these mice were 18- and 19-months of age, respectively.

2.2. Human subjects

Plasma samples from 12 veterans with GWI and 8 healthy controls who were deployed to the GW were assessed in this pilot study. Plasma samples from GW veterans were provided by our collaborators at the Boston University and NOVA Southeastern University sites from an established biorepository of samples from GW veterans who agreed to share their blood samples from prior studies including the Boston Gulf War Illness Consortium (GWIC) and the Dynamic Modeling of GWI study from NOVA Southeastern University and the South Florida Veterans Affairs Foundation for Research and Education, Inc. The GWI biorepository is approved by the institutional review board (IRB) at the Boston University, the NOVA Southeastern University and the Miami Veterans Administration Medical Center (VAMC). Samples from the GWI biorepository were all collected from the Boston and Miami sites using the same written standard operating procedures for performing phlebotomy, plasma separation and aliquoting. All samples were stored at −80 °C and were not previously thawed and refrozen. Gulf War veteran participants were consented into their respective studies using the International Conference on Harmonization Good Clinical Practice guidelines. The Kansas GWI criteria (Steele, 2000) were used to determine GWI case and control status. The Kansas GWI criteria require that 1990–1991 GW veterans endorse symptoms in at least 3 out of 6 symptom domains (fatigue/sleep problems, pain, cognitive, mood symptoms, gastrointestinal symptoms, respiratory symptoms and skin abnormalities). Control veterans were also from the 1990–1991 GW who did not endorse 3 out of 6 symptom domains associated with the Kansas criteria. Subjects also completed demographics and health symptom