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Reduced hippocampal volume and verbal memory performance associated with interleukin-6 and tumor necrosis factor-alpha levels in chemotherapy-treated breast cancer survivors

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ABSTRACT

Many survivors of breast cancer show significant cognitive impairments, including memory deficits. Inflammation induced by chemotherapy may contribute to hippocampal changes that underlie these deficits. In this cross-sectional study, we measured bilateral hippocampal volumes from high-resolution magnetic resonance images in 42 chemotherapy-treated breast cancer survivors and 35 healthy female controls. Patients with breast cancer were, on average, 4.8 ± 3.4 years off-therapy. In a subset of these participants (20 breast cancer, 23 controls), we quantified serum cytokine levels. Left hippocampal volumes and memory performance were significantly reduced and interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNFα) concentrations were significantly elevated in the breast cancer group compared to controls. In the breast cancer group, lower left hippocampal volume was associated with higher levels of TNFα and lower levels of IL-6 with a significant interaction between these two cytokines suggesting a potential modulatory effect of IL-6 on TNFα. Verbal memory performance was associated with cytokine levels and left hippocampal volume in both groups. These findings provide evidence of altered hippocampal volume and verbal memory difficulties following breast cancer chemotherapy that may be mediated by TNFα and IL-6.

1. Introduction

Cognitive impairments are a common late effect of breast cancer and its treatments affecting as many as 75% of survivors (Janelsins et al., 2011; Wefel et al., 2011). Memory impairments are among the most consistently observed difficulties following breast cancer treatment (Jansen et al., 2005; Correa and Ahles, 2008; Janelsins et al., 2011; Wefel et al., 2011) and may involve abnormality of the hippocampus, a region critical for memory function (Squire et al., 2010; Squire and Wixted, 2011). Animal models suggest that chemotherapy-related and/or pro-inflammatory cytokine neurotoxicity may specifically cause damage to the hippocampus (Tangpong et al., 2006; Winocur et al., 2006; Fardell et al., 2010; Joshi et al., 2010; Aluise et al., 2011; Seigers and Fardell, 2011).

Although chemotherapeutic agents typically have restricted direct access to brain tissue due to the blood–brain barrier (BBB), disease states such as cancer, radiation treatment, genetic variations and other factors may make the BBB more permeable to chemotherapy, or may induce alterations in endothelial cells that trigger microenvironment changes within the brain (Ahles and Saykin, 2007; Deeken and Loscher, 2007; Wefel et al., 2008). The hippocampus is unique in that it is one of the only brain regions of continued neural stem cell proliferation throughout the lifespan (Eriksson et al., 1998; Gage, 2000). Neural stem cell proliferation is very tightly regulated and highly sensitive to microenvironment changes. Although chemotherapeutic agents such as doxorubicin (one of the most common breast cancer treatments) are not known to readily cross the BBB, these drugs are associated with reduced hippocampal neurogenesis (Janelsins et al., 2010). Specifically, many chemotherapeutic agents cause neural progenitor cells to lose their capacity for self-renewal even after an initial dose, particularly in the hippocampus (Dietrich et al., 2006, 2010; Winocur et al., 2006; Seigers et al., 2008, 2009). Repetitive dosing, as most patients with breast cancer experience, causes persistent suppression of cell division in the hippocampus (Dietrich et al., 2006; Dietrich, 2010; Hyrien et al., 2010). Both dividing and non-dividing neural and glial cells are significantly vulnerable to chemotherapies (Dietrich et al., 2006, 2010; Winocur et al., 2006; Seigers

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et al., 2008, 2009) and even small amounts of chemotherapy in the brain may cause long-term damage by disrupting cellular plasticity (Dietrich, 2010).

Chemotherapy also may cause indirect neurotoxic brain injury via pro-inflammatory cytokine pathways. Breast cancer chemotherapy and radiation treatments have been shown to elevate peripheral cytokine levels (Vardy et al., 2007; Seruga et al., 2008; Bower et al., 2009; Vardy, 2009; Janelsins et al., 2012). Cytokines readily cross the BBB via active transport mechanisms as well as through circumventricular regions where the BBB is less rigid (Wilson et al., 2002). Binding of cytokines to endothelial receptors in the brain vasculature with subsequent release of other mediators (e.g. chemokines, prostaglandins) leads to impairment of BBB integrity (Wong et al., 1996; Anthony et al., 1997; Konsman et al., 2004). Cytokines activate microglia and astrocytes, stimulate local inflammation and induce oxidative and nitrosative brain damage, particularly in the hippocampus (Tangpong et al., 2006, 2007; Joshi et al., 2007, 2010; Lynch, 2010; Aluise et al., 2011). Cytokine receptors are abundantly expressed in the hippocampus and increased peripheral cytokine levels have been associated with disrupted hippocampal stem cell function, reduced hippocampal volume and reduced memory performance (Das and Basu, 2008; Marsland et al., 2008; McAfoose and Baune, 2009).

Women with breast cancer are at increased risk for psychiatric distress and dysfunction of the hypothalamic–pituitary-adrenal (HPA) axis (Giese-Davis et al., 2004, 2006; Spiegel et al., 2006). HPA dysfunction in patients with breast cancer is associated with immunosuppression and impairment in cytokine regulation (Sephton et al., 2009). Abnormal diurnal cortisol patterns (indicative of HPA dysfunction) have been consistently observed among women with breast cancer (Abercrombie et al., 2004; Spiegel et al., 2006). Glucocorticoids such as cortisol tend to mediate the effects of cytokines on hippocampal memory and plasticity (Yirmiya and Goshen, 2011). Chronic stress and exposure to endogenous cortisol elevations can result in decreased hippocampal volumes (Erickson et al., 2003; Brown et al., 2004). Cortisol can also impair memory performance at low or high concentrations (Erickson et al., 2003; Lupien et al., 2005).

Although abnormal hippocampal structure and function have been noted in previous studies of breast cancer chemotherapy (McDonald et al., 2010; Bergouignan et al., 2011; de Ruiter et al., 2011), to date, there have been no investigations of the relationships between hippocampal structure and peripheral cytokine levels in breast cancer survivors. We therefore measured several serum cytokines, hippocampal volume and verbal memory performance in chemotherapy-treated breast cancer survivors and matched healthy female controls. We hypothesized that increased pro-inflammatory cytokine levels would be associated with decreased hippocampal volumes and verbal memory scores in the breast cancer group compared to the control group.

2. Methods

2.1. Participants

This study included 44 female survivors of primary (stage I-IIIA) breast cancer who all underwent surgery and adjuvant chemotherapy treatment (doxorubicin + cyclophosphamide or paclitaxel = 36; cyclophosphamide + 5-fluorouracil and paclitaxel or methotrexate = 6) who were, on average, 4.8 ± 3.4 years off-therapy (range = 1–12 years). Survivors were excluded for history of relapse or prior chemotherapy treatment and were all disease and relapse free at the time of evaluation. Also included were 38 healthy female controls. There was no significant difference between groups in terms of age (range = 41–73 years), education, global intelligence and minority status but the breast cancer group had significantly more postmenopausal women (Table 1). BC survivors were recruited via the Army of Women (http://www.armyofwomen.org/), community-based BC support groups and local media advertisements. Healthy controls were recruited via the Army of Women and local media advertisements. The Army of Women advertisement indicated that researchers at Stanford were seeking to better understand "problems related to attention, memory, depression, and anxiety" following breast cancer and its treatments. Participants were excluded for previous chemotherapy treatment, neurological, psychiatric, or medical conditions known to affect cognitive function as well as any magnetic resonance imaging (MRI) contraindications. This study was approved by the Stanford University Institutional Review Board and all participants provided informed consent.

2.2. Cognitive assessment

Memory function was measured using the Multifactorial Memory Questionnaire Ability Scale (MMQ) (Troyer and Rich, 2002) and the Hopkins Verbal Learning Test Revised (HVLT) (Wefel et al., 2011). The MMQ provides subjective assessment of one's memory abilities. The HVLT is a measure of verbal memory and learning. The HVLT Total score represents the total number of words recalled across three list learning trials and HVLT Delayed score indicates the number of words recalled following a 20 min delay. Global intelligence (IQ) was measured using a composite of the Matrix Reasoning and Information subtests of the Wechsler Adult Intelligence Scale 4th Edition (Wechsler, 2008). Participants were also administered other cognitive measures including the Wisconsin Card Sorting, Categories, Behavioral Rating Inventory of Executive Function and Verbal Fluency tests which are not reported here given that the focus of this study was on memory.

2.3. Psychiatric function

Although participants were excluded for diagnosed psychiatric disorders, the Clinical Assessment of Depression (CAD), which measures depression, anxiety and cognitive/physical fatigue (Aghakhani and Chan, 2007), was included given the known effect of these symptoms on cognitive status, hippocampal physiology and cytokine levels (Seguin et al., 2009; You et al., 2011).

2.4. MRI Acquisition

MRI scanning was performed on a research-dedicated, GE Discovery MR750 3.0 Tesla whole body scanner (GE Medical Systems, Milwaukee, WI). High-resolution T1-weighted images were acquired with 3D spoiled gradient echo pulse sequence using the following parameters: TR = 8.5 ms, TE = 3.396, TI = 400 ms, flip angle = 15°, FOV = 220 mm, number of excitation = 1, acquisition matrix = 256 × 192, 124 contiguous coronal slices, in-plane

Table 1
Participant characteristics. Data are shown as mean (standard deviation) unless otherwise indicated.

	Breast cancer	Controls	t/c^2	p
N	42	35		
Age	54.6 (6.5)	55.5 (9.3)	0.474	0.64
Education (years)	16.3 (2.6)	17.0 (2.7)	1.09	0.28
Minority status	*N* = 3 (7%)	*N* = 4 (11%)	2.63	0.62
Postmenopause	*N* = 33 (79%)	*N* = 18 (51%)	9.11	0.004
Tamoxifen	*N* = 22 (52%)			
Radiation	*N* = 29 (69%)			
Time off-therapy (years)	4.8 (3.4)			
Stage	2.12 (0.72)			