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Research report

Long-lasting impairments in adult neurogenesis, spatial learning and memory from a standard chemotherapy regimen used to treat breast cancer

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HIGHLIGHTS

- Cognitive impairments persist 3 months after chemotherapy for breast cancer.
- Neuronal survival and differentiation are reduced during chemotherapy.
- Long-term decline in the proliferative ability of the hippocampus is observed.
- No impact of fish-oil rich diet on chemotherapy-induced cognitive impairments.

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ABSTRACT

The negative impact of chemotherapy on cognitive function in cancer patients has gained increasing attention in the last decade. Whilst the short-term acute effects on cognition are expected following chemotherapy, the persistence of such impairments in the long-term is still in question. This is despite clinical evidence indicating cognitive difficulties may persist well beyond treatment and affect quality of life. In the present study, we assessed the long-term (3 months) cognitive impact of chemotherapy in a mouse model intended to mimic the human female post-menopausal population receiving chemotherapy for breast cancer. Ovariectomized, female, C57BL/6J mice received two doses of Doxorubicin, Cyclophosphamide, and 5-Fluorouracil or saline vehicle (control), separated by one week. During this interval, mice received BrdU injections to label dividing cells. Results indicate a persistent impairment in learning and recall (1 h, 24 h and 48 h) on the Morris water maze, reduced survival and differentiation of new neurons (BrdU+/NeuN+), and a persistent decline in proliferation of new cells (Ki67$^+$) in the dentate gyrus. Locomotor activity, motor performance, and anxiety-like behavior were unaffected. We further evaluated the efficacy of a diet enriched in omega-3-fatty acids (DHA + EPA + DPA), in reversing long-term chemotherapy deficits but no rescue was observed. The model described produces long-term cognitive and cellular impairments from chemotherapy that mimic those observed in humans. It could be useful for identifying mechanisms of action and to test further the ability of lifestyle interventions (e.g., diet) for ameliorating chemotherapy-induced cognitive impairments.

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1. Introduction

In the last two decades, a substantial body of evidence prominently from breast cancer survivors suggests that chemotherapy treatments can have a detrimental impact on human cognitive function. Specifically, studies suggest chemotherapy can affect working memory, visual and verbal memory, attention, processing speed and executive function [1–4]. Although, it is generally accepted that chemotherapy can negatively impact cognitive function during and shortly after treatment [5], the extent to which such impairments persist long after chemotherapy has been completed is still a matter of debate [6–8]. Many studies in cancer patients report cognitive changes lasting from 1 year [9] up to 10 years [10–12] after cessation of chemotherapy, while others find no evidence of long-term impairments [13–15]. Factors such as heterogeneity of the patient populations, different combination(s) of drugs utilized, duration of chemotherapy treatment, pre-treatment cognitive status and in particular, the ethical barrier to performing truly randomized-controlled trials in this research area are major intrinsic confounders in the human clinical data [16]. These and other confounders likely explain the conflict in the literature, and make it difficult to draw definitive conclusions regarding the time-course and persistence of cognitive deficits due to chemotherapy.

Animal studies, which allow for a more strict control of the aforementioned confounding factors, have been consistent with the human literature in reporting short-term cognitive deficits [17,18], more specifically ranging from minutes [19], to days [20] or weeks [21,22] after chemotherapy. Animal models have further allowed for a more systematic study of the underlying mechanisms that might be driving chemotherapy-induced cognitive deficits (reviewed in Ref. [17]). Mechanistic studies show consistent and reproducible decreases in proliferation and survival of new cells in the hippocampus during chemotherapy treatments thought to contribute to cognitive impairments [23,24]. However, animal models addressing the long–term persistence of cognitive and neurogenic deficits following chemotherapy are scarce and overall inconsistent. The few studies that tested animals several months post chemotherapy have been inconsistent, with some studies reporting long-lasting impairments [25–27], while others describe no impairments [28,29] and one study even reports cognitive improvements [30]. In the present work, our first goal was to assess the long-term impact (3 months) of a chemotherapy treatment commonly prescribed in humans to treat breast cancer on both learning and memory as well as hippocampal neurogenesis.

To our knowledge, there are no currently established treatments or preventive measures for the cognitive deficits induced by chemotherapy (reviewed in Ref. [31]). Data from pre-clinical and clinical studies report the potential of drugs, such as donepezil [32], fluoxetine [33,34] or modafinil [35,36] to prevent and/or treat these deficits. However, there are reservations in regards to introducing additional medication during chemotherapy due to potential interactions and/or side effects [37]. On the other hand, low-risk lifestyle interventions such as exercise and fairly modest dietary changes shown to play important roles on cognitive status in other at-risk populations [38,39] have hardly been explored in cancer patients. In that regard, dietary omega-3 polyunsaturated fatty acids (PUFAs) and in particular docosahexaenoic acid (DHA), have been reported to positively affect cognitive function in both rodent models of development and aging [40–44] and in humans [45–50]. Important to this work, such protective actions have been attributed partly to omega-3 PUFAs' anti-inflammatory properties [51,52] associated with their ability to modulate synaptic plasticity [53–55] and neuronal survival and neurogenesis [44,56–58]. As such, we hypothesize that a diet rich in omega-3 PUFAs might help ameliorate the cognitive and/or neurogenic deficits induced from chemotherapy. The second goal of the present study was to test the efficacy of one potential dietary intervention containing omega-3 PUFAs (vitamin C and all natural form of vitamin E) in reversing chemotherapy-induced cognitive impairments.

2. Materials and methods

2.1. Materials

Primary antibodies used were anti-BrdU (Bio-Rad, 1:100), anti-doublecortin (Santa Cruz, 1:1000), anti-NeuN (Millipore, 1:50), anti-Ki67 (Abcam, 1:500). Secondary antibodies used were Goat anti-rat (Vector Laboratories, 1:200), Goat anti-rabbit (Vector Laboratories, 1:250), Donkey anti-goat (Santa Cruiz, 1:200). Other reagents used were 3,3′-diaminobenzidine (DAB) (Sigma), BrdU (Sigma) pentobarbital (Sigma), paraformaldehyde (Fisher). Chemotherapy drugs cyclophosphamide (Baxter Health Care), doxorubicin (Pfizer Labs), and 5-flourouracil (APP Pharmaceuticals), were obtained from the University of Illinois Veterinary Medicine Pharmacy.

2.2. Subjects and husbandry

A total of 90 12 week-old, ovariectomized, female, C57BL/6J mice (n = 22/23 per group over two cohorts) were obtained from Jackson Laboratories (Bar Harbor, ME, USA). Ovariectomized females were used to mimic the human female post-menopausal population receiving chemotherapy for breast cancer. Mice were housed in groups of three upon arrival (cages 29 × 19 × 13 cm) and maintained on a reversed 12 h light/dark cycle (lights on at 8 PM and off at 8 AM) with ad libitum access to food and water. Prior to all experiments animals were acclimated to the room for two weeks during which time they were fed standard Teklad 22/5 rodent diet (Harlan Teklad, Indianapolis, IN). All measures were taken to minimize the number of mice used as well as pain and suffering of the animals. All experimental procedures involving animals were conducted in accordance to the regulations and guidelines set forth by the United States Department of Agriculture (USDA) and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All the experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois.

2.3. Experimental design, chemotherapy treatment and intervention diets

Two weeks after arrival, animals were singly housed and randomized into four experimental groups: i) Control (C, n = 22), ii) Fish oil supplemented diet (FO, n = 22), iii) Chemo treated (Chemo, n = 23), and iv) Chemo + Fish oil diet (Chemo + FO, n = 23) groups. These sample sizes were obtained across two temporally separated cohorts, with all groups represented in both cohorts. About half of the mice (n = 46) received two bouts (1 week apart, postnatal days 99 and 106) of a cocktail of chemotherapeutic agents commonly used to treat breast cancer patient [59]. Doxorubicin was administered intravenously through the tail vein (4 mg/kg BW), followed by a cocktail of cyclophosphamide (80 mg/kg BW) and 5-fluorouracil (40 mg/kg BW) administered intraperitoneally (see Fig. 1). The other half of the animals were sham injected both intravenously (i.v.) and intraperitoneally (i.p.) with a saline solution. During this period (days 1–5 after first chemotherapy session), all mice received daily (i.p.) injections of 5-bromo-deoxyuridine (BrdU; 50 mg/kg BW) to label dividing cells. Following recovery from chemotherapy (approx. 1 week after last chemotherapy session), half of the mice were introduced into an AIN-93G modified diet containing fish oil (FO: 2.1 g/ kg diet) enriched in omega-3