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Research report

Doxorubicin and cyclophosphamide lead to long-lasting impairment of spatial memory in female, but not male mice

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HIGHLIGHTS

- Female mice exhibit deficits in spatial memory when exposed to doxorubicin and cyclophosphamide.
- The spatial memory of male mice is not affected by exposure to doxorubicin and cyclophosphamide.
- Spatial memory deficits in females persist following exposure to chemotherapeutic agents.

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ABSTRACT

Self-reports of chemotherapy-related cognitive deficits (CRCDs) are more prevalent among women than men, suggesting that women may be more vulnerable to the cognitive-impairing effects of chemotherapy. However, there have been no direct comparisons of females and males using objective measures of cognitive function either during or following exposure to the same chemotherapeutic regimen. The present study used an animal model, and a prospective longitudinal design, to assess sex differences in the manifestation and persistence of spatial memory deficits resulting from exposure to doxorubicin (DOX) and cyclophosphamide (CYP), commonly used anticancer drugs. The spatial memory of female and male BALB/C mice was assessed using the Morris water maze prior to, during and following 4 weekly intravenous injections of DOX (2.5 mg/kg) and CYP (25 mg/kg) or vehicle. Females receiving DOX + CYP experienced significant deficits in spatial memory during and following injections when compared to baseline or females receiving vehicle. These deficits persisted for at least 34 days following the final injection. In contrast, males receiving DOX + CYP injections did not exhibit alterations in spatial memory relative to baseline or males receiving vehicle. These findings indicate that females may be more vulnerable than males to the cognitive-impairing effects of DOX + CYP and demonstrate that deficits in females persist for at least several weeks following drug exposure. Preclinical studies of CRCDs should parallel clinical work by including females and examine sex specific factors as potential mechanisms.

1. Introduction

Advances in cancer treatment over the past several decades have led to increased 5-year survival rates for patients, from 48.9% in 1975, to 68.7% in 2011 [1], creating a large population of survivors and a corresponding need for studies on long term quality of life for these individuals. Cognitive deficits are a common experience for cancer patients receiving treatment with chemotherapeutic agents, with as many as half of cancer survivors reporting some cognitive decline after treatment [2–5]. Reports of cognitive decline have been characterized as "fuzzy-headness" or "mental slowness," giving rise to the terms "chemo-brain" and "chemo-fog" [6]. Although self-reports of cognitive deficits generally exhibit poor concordance with performance on neuropsychological tests [2,3,7,8], objective studies of cognitive function have demonstrated the presence of cognitive deficits in cancer patients and survivors that receive chemotherapy; for review see [9,10]. These chemotherapy-related cognitive deficits (CRCDs) most commonly involve deficits in attention, learning, memory and information processing speed. While generally mild, CRCDs can persist for as many as 20 years following treatment [3] and are sufficient to impair day-to-day functioning and adversely impact quality of life [3].

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Although many quantitative clinical studies have demonstrated cognitive decline following chemotherapy and deficits relative to healthy controls, not all studies agree that these deficits are present following exposure to chemotherapeutic agents [11–13]. Further, not all studies that report cognitive deficits agree that these deficits are a consequence of exposure to chemotherapeutic agents specifically [14–17]. Rather, these studies suggest that the deficits are the result of biological processes involved in the cancer itself (e.g., inflammation). To this point, a recent meta-analysis of 27 clinical studies of breast cancer survivors indicated that cognitive deficits may be present among cancer patients irrespective of chemotherapy [18]. These inconsistencies likely reflect the inherent difficulty of identifying and controlling extraneous and confounding factors in human studies, making it difficult to clearly identify the effects of specific therapeutic agents.

Preclinical studies, which have greater control than clinical counterparts, have consistently demonstrated the presence of cognitive deficits during and/or following exposure to chemotherapeutic agents, providing strong evidence that cognitive deficits specifically result from several different chemotherapeutic agents; for review see [19]. Interestingly, although doxorubicin (DOX) and cyclophosphamide (CYP) are frequently administered in combination for breast cancer and these drugs are commonly present in clinical studies of CRCDs, few preclinical studies to date have examined whether this chemotherapeutic combination leads to cognitive deficits. Macleod et al. [20] demonstrated DOX + CYP-induced deficits in contextual fear conditioning in young (8 weeks) ovariectomized (OVX) Sprague-Dawley rats. A similar study reported that DOX + CYP reduced locomotor activity, working memory and spatial memory in both intact and OVX females [21], while another study determined that DOX + CYP produced deficits in passive avoidance learning in 10 month-old female breeder dams [22]. In addition, a recent study demonstrated that DOX + CYP impairs spatial memory in male Sprague-Dawley rats using a novel object location task [23]. To date, only one study of cognitive function following exposure to DOX + CYP has included both females and males; however, groups were assessed in two separate, methodologically distinct experiments, preventing comparison across sex, and no cognitive deficits were detected [24]. These studies demonstrated that the administration of DOX + CYP can produce cognitive deficits in animals when assessed using tasks dependent on hippocampal function. However, these studies did not determine if the deficits were persistent or whether the observed impairments were sex-specific.

Because chemotherapy-related cognitive deficits can persist for years after treatment [3], it is important for translational preclinical studies to demonstrate that deficits continue after chemotherapy has been completed. However, few preclinical studies to date have extended the assessment of cognitive function for more than a few weeks following the final administration of the chemotherapeutic agent. Winocur et al. [25] reported deficits in spatial memory, conditional associative learning and discrimination learning that lasted for 3 months following exposure to methotrexate (MTX) and 5-fluorouracil (5-FU), and Fardell et al. [26] demonstrated deficits in novel object and place recognition 4–11 months following exposure to oxaliplatin. Interestingly, deficits in novel object recognition were not present prior to 4 months post-treatment, while deficits in novel place recognition were apparent 2 weeks post-treatment, suggesting that deficits emerge at different rates depending on the cognitive domain assessed. Additionally, this finding points to the importance of assessing cognitive function at multiple time points following chemotherapy because deficits may emerge days to weeks following exposure to chemotherapeutic agents. Indeed, Li et al. [27] reported impaired spatial memory in 30 days, but not 1 day, following exposure to cytosine arabinoside (Ara-C). In addition, Mondie et al. [28] demonstrated a deficit in object recognition memory that did not emerge until 8–12 weeks following exposure to thioTEPA, and a deficit in place recognition that did not emerge until 20 weeks following exposure to thioTEPA.

Determining whether chemotherapy-induced cognitive impairments are sex specific is critical because many chemotherapeutic agents decrease circulating estrogen levels secondary to ovarian suppression [29–34], a factor which may be directly or indirectly related to the manifestation of cognitive deficits following chemotherapy. Between 20–70% of pre-menopausal women undergoing breast cancer treatment experience chemotherapy-induced amenorrhea, with less than 50% experiencing a resumption of menses within 2 years of treatment [31–34]. Fluctuations in circulating estrogen levels affect the cognitive performance of female animals on a variety of behavioral tasks [35–45], suggesting that the impact of chemotherapy on estrogen may mediate the occurrence of CRCDs. While some studies indicate that males are also vulnerable to CRCDs, research in male laboratory animals has demonstrated that the administration of the estrogen receptor antagonist tamoxifen produces cognitive deficits similar to those seen following the administration of MTX or 5-FU [46]. Thus, alterations in estrogen signaling can affect the cognitive performance of males. Interestingly, men undergoing androgen ablation therapy for prostate cancer exhibit a significant decline in cognitive ability [47–50], an effect that may be due to the loss of estrogen, since estrogens are a product of androgen aromatization [51].

Sex specific vulnerability to CRCDs has not been adequately addressed in either the clinical or preclinical literature. Approximately 90% of clinical CRCD studies have been conducted exclusively in women, which is not surprising given that women report cognitive deficits with far greater frequency than men both during and following chemotherapy [52]. Although the frequency of self-reported cognitive difficulties suggests that women may be more vulnerable to CRCDs, there have been no direct comparisons of females and males, exposed to the same chemotherapeutic regimen, using objective measures. This is critical given evidence that self-reported cognitive difficulties may not be a valid indicator of cognitive deficits. A few studies of the sex-specific effects of chemotherapy in survivors of childhood cancer has provided evidence of greater cognitive impairment in females than in males [53]; However, it is unclear whether outcomes from childhood treatment are relevant to CRCDs resulting from treatment in adulthood. Of particular importance, clinical studies that identify and characterize CRCDs, and preclinical studies that model these deficits to identify causes and potential treatments, differ considerably in their inclusion of females and males. Although the vast majority of clinical CRCD studies have been conducted in women, only about 30% of preclinical CRCD studies have included females. Therefore, preclinical studies may not be effectively modeling the condition. Thus, research is needed to assess and compare cognitive deficits in females and males in controlled experiments to establish whether sex confers a vulnerability to CRCDs, evidence that may help isolate the mechanisms underlying these adverse effects.

To determine if chemotherapeutic agents produce similar deficits in hippocampal function of females and males, and whether the observed deficits are present following the completion of the chemotherapeutic regimen, the present study used the Morris water maze (MWM) to assess sex-related changes in the spatial memory of BALB/C mice during and following exposure to the chemotherapeutic agents DOX and CYP. The MWM was selected because it is sensitive to hippocampal deficits [54] and studies have demonstrated DOX + CYP-induced deficits on hippocampal-related tasks [20,22]. Results indicate that the administration of DOX + CYP produces deficits in spatial memory in females, but not males, that persist for several weeks following completion of the