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Research report

5-Fluorouracil chemotherapy upregulates cytokines and alters hippocampal dendritic complexity in aged mice

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HIGHLIGHTS

- 5-Fu significantly compromised the dendritic architecture.
- 5-Fu decreased spine density and modulated spine morphology throughout Hippocampus.
- 5-Fu upregulated pro-inflammatory and anti-inflammatory cytokines.

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ABSTRACT

5-Fluorouracil (5-Fu) is commonly used chemotherapy drug, but it can lead to the impairment of cognitive function. The pathogenesis of this injury is unknown but may involve modifications to dendritic structure and/or alterations in dendritic spine density and morphology. Dendritic spines are sites of excitatory synaptic transmission and changes in spine structure and dendrite morphology are thought to represent a morphological correlate of altered brain functions associated with hippocampal dependent learning and memory. A total of 28 one-year-old C57BL6/J male mice were used in this study; 14 mice received 5-Fu treatment and 14 were given saline injections. One month post treatment, 14 cytokines were measured at the same time Golgi samples were taken. 8 analytes were significantly elevated in mice treated with 5-Fu. 5-Fu significantly compromised the dendritic architecture and reduced spine density throughout the hippocampal tri-synaptic network. The present data provide the evidence that 5-Fu has deleterious effects on mature neurons associated with hippocampal learning and memory.

1. Introduction

Approximately 1 in 2 adults will be diagnosed with cancer during their lifetime with a median age at diagnosis of 66 years. Improvements in cancer therapy have helped a growing number of patients to survive [1]. It is estimated that by year 2020, there will be approximately 70 million cancer survivors worldwide [2]. Due to this survivorship, there has been a greater focus on researching the long-term adverse effects of treatment and the impact this treatment can have on the daily lives of patients [3]. Chemotherapy is the most commonly implemented treatment, often used in conjunction with local therapies such as surgery and radiotherapy [4]. However, neurotoxicity is a frequent accompaniment of cancer chemotherapy [5]. Chemobrain, also known as "chemofog," is a cognitive impairment consisting of deficits in attention, executive and motor function, memory, and speed of information processing, following chemotherapy for a multitude of cancers [6,7]. These deficits, while usually temporary, can persist for several years after completion of drug treatment in a subgroup (17–34%) of patients and can dramatically affect many aspects of daily living, such as employment, social functioning, and community integration [8].

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5-FU, an anti-metabolite and pyrimidine analogue, was developed in 1957 and has been used to treat breast, bowel, prostate, gastrointestinal, vaginal, and cervical cancer [6,9,10]. 5-FU interacts with nucleic acids within the DNA sequence and interferes with RNA and DNA synthesis. Intracellular activation of 5-FU requires it to be metabolized to one of the following nucleotides: 5-fluorodeoxyuridylate (5-FdUMP), 5-fluorodeoxyuridine triphosphate (5-FdUTP), or fluorouridine triphosphate (5-FUTP). The major cytotoxic effect of 5-FU seen in humans is thought to be from the binding of 5-FdUMP to thymidylate synthase (TS), which results in DNA synthesis inhibition and programmed cell death. The formation of 5-FUTP causes abnormal RNA processing by incorporating itself into the RNA, while converting 5-FU to 5-FdUTP results in DNA strand breaks due to 5-FdUTP being incorporated into the DNA. This incorporation of 5-FdUTP into the DNA causes apoptosis in further rounds of cell division [11].

Animal studies that have examined intraperitoneal injections of 5-FU have shown a disruption of learning and memory across multiple tasks, including object recognition, avoidance conditioning, cue-specific and contextual fear condition tasks, and Morris water maze [7]. 5-FU passes through the blood brain barrier (BBB) by simple diffusion and can affect proliferating cells in neurogenic zones, like the subgranular zone (SGZ) of the hippocampus [7]. 5-FU treatment has been shown to reduce the number of SGZ cells dividing, which are necessary for hippocampal neurogenesis. The SGZ of the dentate gyrus (DG) is one of a few areas where neurogenesis occurs during adulthood. Development of new neurons from proliferating precursor and stem cells within the SGZ is required for spatial memory formation [9,12]. It is proposed that these altered neurogenic zones may contribute heavily to the cognitive deficits seen in those with chemobrain. However, these cognitive deficits are not solely based on altered neurogenic zones; thiamine (vitamin B1) deficiency and/or 5-FU metabolites may also contribute to neuronal toxicity [6].

Cytokines and chemokines (chemoattractant cytokines) are secreted signaling proteins derived from many different types of cells that help determine and regulate immune responses [13]. There are two basic immune responses for cytokine production: (1) antigen presenting cells (APCs) take up antigens, process them, and then present them to T-lymphocytes to produce cytokines and (2) APCs, such as monocytes, are activated to produce cytokines through pattern recognition receptors that recognize a foreign pathogen [14]. Cytokines predominantly produced by APCs include multiple interleukin (IL) and tumor necrosis factor (TNF) molecules [15]. Within the brain, cytokines are mostly produced by microglia, with astrocytes, oligodendrocytes, and neurons contributing smaller amounts. Several clinical studies have demonstrated that administering a standard dose of chemotherapeutic drugs causes an increase in cytokine levels for a variety of cytokines (TNF-a, IL-6, IL-8, IL-10, and monocyte chemotactic protein-1 (MCP-1)).

Acharya et al., found that cyclophosphamide chemotherapy caused significant reductions of certain dendritic spine types in two different areas: (1) stubby and mushroom spines in the DG and (2) all three spine morphologies in cornus ammonis (CA1). Furthermore, these alterations lead to an overall significant decrease in spine density [16]. Cisplatin has also been found to affect dendritic morphology of pyramidal neurons as soon as three days following treatment. Cisplatin reduces dendritic branching and spines in CA1 as well as CA 3 neurons [17].

The present investigation was designed to assess how a relatively low dose of 5-Fu would affect dendritic spine density and morphology. Such data might provide critical information regarding the mechanism of disruption of neural circuitry following chemotherapeutic treatment.

2. Methods

2.1. Animals and study design

Six-month-old male C57Bl6/J wild-type mice (n = 28) purchased from the Jackson Laboratory (Bar Harbor, ME) were used for the experiments. The mice were housed and aged under a constant 12 h light: 12 h dark cycle. Food and water were provided *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee at UAMS.

2.2. Chemotherapeutic paradigm

All chemotherapeutic agents were purchased from the UAMS inpatient Pharmacy then stored according to manufacturer's instructions until use. All chemotherapeutic agents were diluted in sterile saline. Mice received weekly intraperitoneal injections of saline (0.9% sterile saline; n = 14) or 5-fluorouracil (5-FU) (60 mg/kg; n = 14). The injections occurred over 3 weeks for a total of three injections (days 1, 8, and 15). All animals were injected between 0900 h–1200 h. Doses of drug were chosen based on previously published 5-FU studies in mouse models [18].

2.3. Golgi staining and tissue preparation

The brains were carefully extracted and cut in half along the midsagittal plane. The right hemisphere of the brain sham (N = 7) and drug treated mice (N = 7) was immediately subjected to the Bioenno Tech superGolgi kit for Golgi-Cox method of staining. The samples were impregnated with the potassium dichromate and mercuric chloride solution at room temperature for 11 days. After immersion in the post-impregnation buffer for 2 days, brains were serially sectioned at 150 μm using a vibrating microtome and sections were collected in chilled mounting buffer. The sections were mounted on 0.3% gelatin-coated slides, washed in 0.01 M PBS buffer (pH 7.4) with Triton X-100 (0.3%), and stained with ammonium hydroxide solution (provided in the kit). After staining and washing, slides were gradually dehydrated through a series of increasing alcohol concentrations, then with xylene and finally mounted in Permount™ (Fisher) for analysis.

2.4. Spine density and spine morphology

Spine analyses were conducted blind to the experimental conditions on coded Golgi impregnated brain sections containing the dorsal hippocampus. Spines were examined on dendrites of DG granule neurons as well as apical (stratum radiatum) and basal (stratum oriens) dendrites of CA1 and CA3 pyramidal neurons. The neurons that satisfied the following criteria were chosen for analysis in each of the experimental groups: (1) presence of untruncated dendrites; (2) consistent and dark Golgi staining along the entire extent of the dendrites; and (3) relative isolation from neighboring neurons to avoid interference with analysis [19]. Three-5 dendritic segments, each at least 20 mm in length [20], were analyzed per neuron, and 6–7 neurons were analyzed per brain. Neurons that met staining criteria were traced using a 60 X oil objective, a computerized stage, and Neurolucida software (Ver. 11, Microbrightfield, Inc., Williston, VT).

To acquire images for spine analysis, the dendritic segments were imaged under brightfield illumination on a Zeiss Axioimager microscope with a 100× oil immersion objective. Spine analyses were based on the method of Margarinos et al. [21]. This method does not assess spine density in a 3 dimensional manner but focuses on spines that are parallel to the plane of section. Although the method may underestimate the total number of spines, it facilitates a direct comparison of treatment groups when they are analyzed