Behavioural Brain Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Changes in cognition and dendritic complexity following intrathecal methotrexate and cytarabine treatment in a juvenile murine model

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ARTICLE INFO

Keywords:
Chemotherapy
Cognitive
Dendritic
Hippocampus
Impairment
Morphology

ABSTRACT

Acute lymphoblastic leukemia (ALL) is the most prevalent childhood cancer and accounts for 26.8% of cancer diagnoses among children, worldwide—approximately 3000 children each year. While advancements in treating ALL have led to a remission rate of more than 90%, many survivors experience adverse neurocognitive and/or neurobehavioral effects as a result of intrathecal chemotherapy. Methotrexate (MTX) is commonly administered with cytosine arabinoside (AraC, cytarabine) during intrathecal chemotherapy for ALL. To date, few studies exist that test the cognitive effects of intrathecal injections of MTX/AraC on juvenile populations. The purpose of our study was to investigate the combined effects of MTX/AraC on cognition and dendritic structure in the hippocampus in juvenile male mice. Twenty, 21-day-old male C57BL/6 mice were used in this study; 10 mice received intrathecal MTX/AraC treatment, and 10 were given intrathecal saline injections. Five weeks after injections, we tested the animals' hippocampus-dependent cognitive performance in the Morris water maze. After the first day of hidden-platform training, we observed that the mice that received MTX/AraC treatment showed signs of significant impairment in spatial memory retention. MTX/AraC treatment significantly compromised the dendritic architecture and reduced mushroom spine density in the dorsal ganglion (DG), CA1, and CA3 areas of the hippocampus. The present data provided evidence that MTX/AraC compromised the dendritic architecture and impaired hippocampal dependent cognition. This could provide insight into chemotherapy-induced cognitive decline in juvenile patients treated for ALL.

1. Introduction

In the past 50 years, the survival rate for acute lymphoblastic leukemia (ALL) has risen from 10%–90%, stemming from the use of chemotherapy, radiation, and central nervous system (CNS)-directed therapies [1–4]. In addition, antibiotic and blood product support introduced in the '60s and '70s greatly improved outcomes [5]. Though ALL treatment protocols vary greatly among clinicians, most therapies consist of a three-part paradigm, which includes induction, consolidation, and maintenance [6]. The goal of induction is to induce remission and restore hematopoiesis, while consolidation is meant to reduce the tumor burden further. Finally, maintenance aims to prevent relapse, and may last for two years, post consolidation [7]. Protocols typically involve some systemic chemotherapy and central nervous system (CNS) directed therapy that consists of intrathecal methotrexate (MTX), cytosine Arabinoside (AraC), and sometimes cranial irradiation [7].

Methotrexate (MTX) is commonly used in the maintenance phase of the ALL treatment protocol. The main mechanism of action of MTX is the inhibition of the enzyme dihydrofolate reductase (DHFR). Since DHFR is necessary for the formation of intracellular folates, reduced DHFR activity leads to decreased production of the purines and pyrimidines that are necessary for DNA replication and cell division [8]. Unfortunately, MTX is associated with intense neurotoxicity, especially in CNS-directed therapies; this can result in seizures, occlusive vascular-like events. These MTX-induced toxicities happen acutely and do resolve over time [9]. Damage to the CNS can result in long-term deficits

Abbreviations: ALL, acute lymphoblastic leukemia; AraC, cytosine Arabinoside; BrdU, bromodeoxyuridine; CNS, central nervous system; DG, Dorsal Ganglion; DHFR, dihydrofolate reductase; IP, intraperitoneal; LSD, Fisher's least significant difference; MTX, methotrexate; MWM, Morris water maze; NOR, novel object recognition; SEM, Standard Error of the Mean

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https://doi.org/10.1016/j.bbr.2017.12.008
Received 22 August 2017; Received in revised form 4 December 2017; Accepted 7 December 2017

in cognition [10].

The chemotherapy drug cytosine arabinoside (AraC) is commonly used in ALL treatment, and is often given through an intrathecal injection in conjunction with MTX. Highly effective at preventing DNA synthesis, AraC is a viable treatment for cancer [11], and more specifically the treatment of tumors, as it effectively reduces tumor size [12]. Cytosine arabinoside (AraC) prevents cells from moving beyond the S-phase of mitosis, making it a potent cell-cycle arrest molecule [13]. With a well-defined mechanism of action, AraC is incorporated into the cellular DNA as a cystine analogue, but because it contains an extra hydroxyl group at the 2′ position of the β configuration, it does not allow for proper DNA or RNA synthesis. This leads to cell-cycle arrest and ultimately apoptosis [14]. Unfortunately, AraC's functional inhibition of cell-cycle arrest affects all dividing cells, not just cancerous ones. AraC can prevent the formation of new neurons, as neurogenesis continues into adulthood, leading to neurotoxicity and deficits in cognition. In the CNS, AraC has been associated with encephalopathy, seizures, cerebellar syndrome, and cranial neuropathy [14]. These toxicities, especially cranial neuropathy, may lead to post-treatment cognitive dysfunction.

Often referred to as "chemobrain", chemotherapy-induced cognitive deficits can last for years after treatment [15]. Chemobrain causes impairments in memory, learning, concentration, reasoning, executive function, attention, and visuospatial skills [16]. The cognitive effects of chemotherapy apply to children, as well. Studies of pediatric patients treated for ALL show that many experience cognitive dysfunction after treatment [17,18]. CNS toxicity is inevitable as chemotherapy is injected directly into the spinal fluid, and these therapies are closely associated with a decrease in IQ score that can be rather significant. Pediatric patients affected with chemobrain have IQ scores that are at least one standard deviation below the mean of other children their age [19].

Very few studies have been published that examine the effects of ALL treatment utilizing a juvenile murine model. The most prominent study was conducted in 2013 by Bisen-Hersh et al. In the study, MTX and AraC were given intraperitoneally to pre-weanling pups. The team then conducted a novel object-recognition task to measure short-term memory, and a conditional-discrimination task to measure mental flexibility. The results showed that the mice that had received MTX alone or MTX in conjunction with AraC performed less well on behavioral tasks when compared to the non-treatment cohort [20]. Unfortunately, the study did not investigate physiological changes that could have confirmed the behavioral results, such as molecular assays. Assessment of neurophysiology could be beneficial in understanding mechanistic changes that lead to damage. Since much of the retention and consolidation of memory is localized to the hippocampus, evaluation of damage to this brain region and interruptions in connectivity could provide insight into the detrimental effects of chemotherapy [21–23]. Therefore, more murine models are needed to study possible mechanisms underlying cognitive decline in juvenile ALL treatment, as well as changes in hippocampal physiology. Furthermore, their study used an intraperitoneal injection method to administer the therapy. In humans, the treatment protocol for ALL involves administration of the drug directly into the spinal canal via intrathecal injection. It is logical that administration of a therapy directly into the CNS would illicit different effects than administration of the same therapy into the abdominal cavity. Intrathecal administration of chemotherapy is more clinically relevant for ALL treatment, models of this treatment paradigm should explore this method.

Therefore, we aimed to not only address the gap in knowledge regarding cognitive decline in pediatric patients, but to provide greater insight into the physiological changes and therapy-induced cognitive decline that accompany intrathecal MTX and AraC therapy, through the use of protocols similar to those used in humans. To accomplish our aims, we developed a study to measure the effects of MTX and AraC on cognition and hippocampal physiology in juvenile mice. We further supported our findings with molecular assays that allow a more thorough understanding of the effects of intrathecal chemotherapy.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice (Jackson Laboratory, Bar Harbor, ME), 21 days old (n = 20), were used in this study. The mice were housed under a constant 12-hour light/12-hour dark cycle and were cared for in compliance with the University of Arkansas for Medical Sciences' (UAMS) Institutional Animal Care and Use Committee.

2.2. Chemotherapy injections

We administered either 5 mg/kg AraC combined with 10 mg/kg MTX (n = 10), or 0.1% saline (n = 10) to the mice intrathecally, between the L5 and L6 vertebrae. Once a week for three weeks, each mouse received three injections of either MTX + AraC or saline. Following the chemotherapy injections, we administered five injections of bromodeoxyuridine (BrdU) (10 mg/kg) to each mouse. We then conducted behavioral testing using the Morris water maze (MWM) four weeks after the final BrdU injection.

2.3. Morris water maze (MWM)

We assessed hippocampus-dependent cognitive performance 60 days after initiation of chemotherapy administration. To determine if radiation affected the ability of mice to swim or learn the water maze task, a circular pool (diameter, 140 cm) was filled with opaque water (24 °C) and the mice were trained to locate a visible platform (luminescence, 200 lx). We trained the mice to locate a clearly marked platform (visible-platform, days 1 and 2) using strategically placed visual cues. During visible-platform training, we moved the platform to a different quadrant of the pool for each session. The acquisition phase of the hidden-platform training required the mice to learn the location of the platform based on extra-maze spatial cues. In our study, we trained the mice to locate a platform that remained in the same location, and was hidden beneath opaque water (days 3–5). Mice were excluded from the study if 20% of body mass was lost over one week during treatment.

For both visible- and hidden- platform paradigms, we placed mice in the water, facing the edge of the pool, in one of nine random locations. We conducted two daily sessions, two hours apart, with each mouse. Each session consisted of three trials for which the start location changed (with 10-minute intertrial intervals). A trial ended when the mouse located the platform, and mice that failed to locate the platform within 60 s were led to the platform by the examiner, then forced to stay on it for 10 s. We removed mice that found the platform from the pool after they were on the platform for at least 3 s. We video-recorded the probe trials to determine distance moved using the EthoVision XT video tracking system (Noldus Information Technology), set at 6 samples/second.

To measure spatial memory retention, we conducted probe trials (platform removed) on each day of hidden-platform training, 1 h after the last trial (i.e., three separate probe trials). For the probe trials, we placed mice into the water in the quadrant opposite the target quadrant (i.e., where the platform was previously located during hidden-platform training), and allowed the mice 60 s to search for the platform. We then compared the time spent in the target quadrant with the time spent in the three non-target quadrants. We also used the average velocity and distance to the platform as a measure of performance for the visual- and hidden- platform sessions.