Cytokine Mediated Chemotherapy Induced Cognitive Impairment in Cisplatin and Methotrexate Treated Sprague-Dawley Rats

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Abstract

As the survival rates of cancer patients gradually increase, patients often report chemotherapy-induced cognitive impairment (CICI). These cognitive impairments are diverse but are most often reported to affect memory and learning, attention, concentration, processing speeds and executive function. It is well known that cytokines mediate neuronal and glial cell function to facilitate neuronal regeneration or neurodegeneration, and cytokine dysregulation is linked to microglial activation, neuroinflammation, neuronal damage, and cognitive deficits. The specific role of cytokines in CICI was examined in this study as they may be biomarkers of CICI and allow investigation of therapeutic targets against CICI.

In this study, juvenile male and female rats were administered three intraperitoneal injections, with one injection per week for three weeks on postnatal day (PND 23, 30, and 37) of either cisplatin (2 or 4 mg/kg), methotrexate (20 or 40 mg/kg), or saline. The frontal cortex, hippocampus, cerebellum, and plasma were then collected on either PND 43 or 57 for cytokine analysis. In PND 43 rat frontal cortex, both high dose cisplatin (4 mg/kg) and high dose methotrexate (40mg/kg) resulted in significant increases in proinflammatory cytokines interleukin 6 (IL-6), interleukin 12 (IL-12), and interleukin 1α (IL-1α). In PND 43 and 57 rat plasma, high (40 mg/kg) and low (20 mg/kg) dose methotrexate had significant increases in IL- 1α, IL-6 and IL-12. These results indicate that an enduring increase in systemic proinflammatory cytokines may occur after treatment with chemotherapeutics. This cytokine dysregulation may be a contributing factor to CICI.

Background

Advances in cancer treatments have increased survival rates in several cancer types. However, the cytotoxic effects of chemotherapeutic drugs for cancer treatment may cause unintended damage to normal central nervous system neuronal structure or function. This might lead to adverse effects on cognitive function. Recent studies have shown that chemotherapy induced cytokine dysregulation is associated with drugs that generate ROS/RNS in the periphery. This can lead to brain structural and functional damage and cognitive impairments. Clinical studies have shown an association between levels of inflammatory cytokines and chemotherapeutic drugs. As an increasing number of patients are surviving cancer, some are reporting residual and lingering effects from treatment. New evidence suggests that CICI may be caused by the release of excess cytokines. Cytokine dysregulation is linked to microglial activation, neuroinflammation, neuronal damage, and cognitive deficits Control of the elevated levels of the blood-brain-barrier (BBB) permeable pro-inflammatory cytokines, may help mediate CICI.

Methods

Animals:

Sprague Dawley rats (Charles River) were used for this work. Animals were housed 2 or 3-per cage (12-hour light dark cycle) and given full access to food and water. Animals were received on PND 14 and weened and tattooed on PND 21.

Chemotherapeutic drug treatment:

Animals were administered 1 intraperitoneal injection per week in 3 consecutive weeks on PND 23, 30, and 37. Animals received either cisplatin (2 or 4 mg/kg), methotrexate (20 or 40 mg/kg), or 200 µL of saline. All animals were sacrificed at either PND 43 or 57 with Euthasol, and brains and plasma were collected for cytokine analysis. There is an n=8 for each of the 5 groups.

Tissue processing:

Animals were perfused with heparinized saline on either PND 43 or 57. The frontal cortex, hippocampus and cerebellum were removed and flash frozen. The samples were then stored at -80°C. 3x3mm samples were cut and lysed and a protein concentration of 500µg/mL was determined for use during cytokine analysis.

Plasma sample preparation:

Blood was drawn via cardiac puncture prior to perfusion into a collection tube containing EDTA. Samples were then centrifuged at 1000 x g for 15 min at 4°C and the plasma was transferred to a clean tube for analysis.

Cytokine analysis:

The Bio-Plex Rat Cytokine 23-plex assay from Bio-rad was used for the cytokine analysis.

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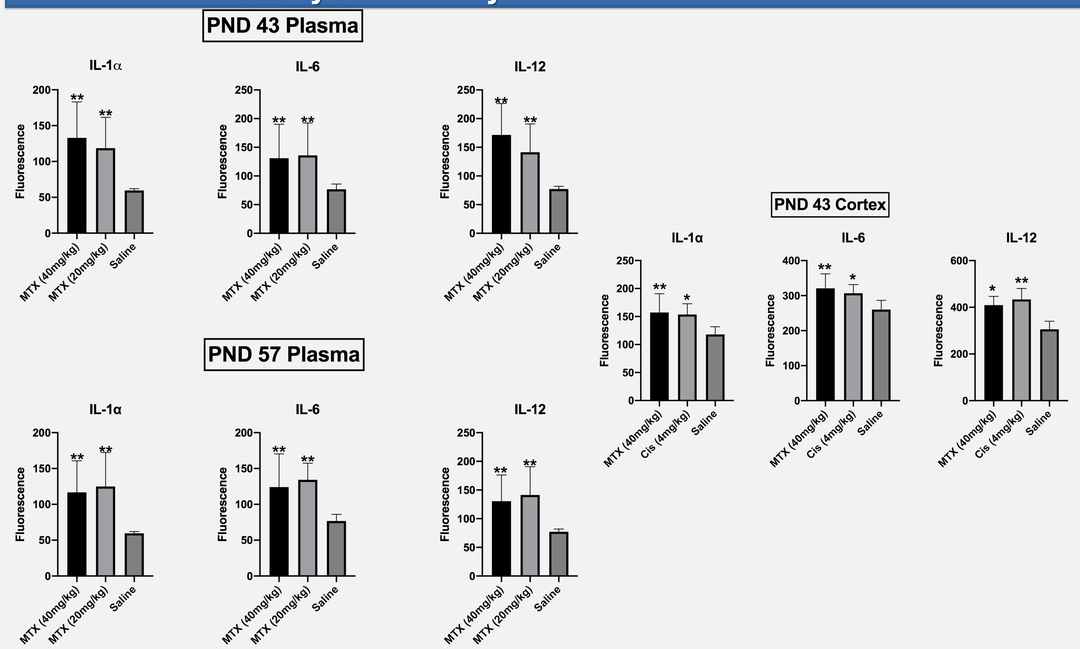
Cytokine Analysis of Plasma and Cortex



Results and Conclusions

In plasma samples taken from juvenile male and female rats on PND 43 that received high MTX (40mg/kg) or low MTX (20mg/kg), IL-1α, IL-6, and IL-12 were elevated significantly from the control rats that were given saline. Plasma samples taken from rats on PND 57 also showed significantly elevated IL-1α, IL-6, and IL-12 in both the low and high MTX treatment groups. Samples taken on PND 43 from the cortex resulted in elevate levels of IL-1α, IL-6, and IL-12 in both the high dose MTX (40mg/kg) group and the high dose Cisplatin (4mg/kg) group.

The BBB is made of tightly joined endothelial cells that prevent toxins from entering the brain. Many current chemotherapeutic drugs cannot penetrate the BBB, but chemotherapy induced pro-inflammatory cytokines such as IL-1 and IL-6 are able to cross the BBB and induce local inflammatory responses in the brain. Our study has shown that rats treated with the chemotherapeutic drugs methotrexate and cisplatin demonstrate an increase in proinflammatory cytokines.

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