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DOX and MET treatment induces cognitive impairment through downregulation of IL-1-alpha and IRS-1 in the rat brain

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Abstract. – OBJECTIVE: Chemotherapeutic drugs are effective in the treatment of various types of cancers. However, the secondary side effects of chemotherapy, such as cardiotoxicity, hepatotoxicity, and cognitive impairment, limit its clinical effectiveness in cancer treatment. The present study was aimed at investigating the effects of doxorubicin (DOX) on cognitive impairment through its effects on interleukin (IL)-1, insulin receptor substrate 1 (IRS-1), IL-6, Akt, and tumor necrosis factor (TNF)-alpha expression.

MATERIALS AND METHODS: Rats were treated with DOX, metformin (MET), and DOX+MET, and IL-1, IRS-1, IL-6, Akt, and TNF-alpha expression levels were assessed using Enzyme-Linked Immunosorbent Assay kits.

RESULTS: The DOX-treated rats showed significantly decreased IL-1 and IRS-1 expression in the brain, and the expression of these proteins was rescued on MET administration. On the other hand, IL-6, protein kinase B (PKB/Akt), and TNF-alpha expression was unaltered in the brain of DOX- and MET-treated rats.

CONCLUSIONS: Our findings showed that DOX induces cognitive impairment by modulating IL-1-alpha and IRS-1 expression and that MET administration failed to rescue the DOX-mediated memory impairment.

Key Words:
Doxorubicin, Cognitive impairment, Interleukin-1, Interleukin-6, Insulin receptor substrate 1, TNF-alpha.

Introduction

Many anticancer medications have several side effects, which may be temporary or persistent and affect all body functions or specific organs in the form of issues, such as cardiotoxicity, hepatotoxicity, nephrotoxicity, or impaired brain function. Hence, these side effects affect the quality of life of cancer survivors. Chemobrain or chemofog, which is a change in cognitive function due to the toxic effects of systemic chemotherapy on the brain, is one such side effect[1]. The symptoms of chemobrain include memory dysfunction, a lack of concentration, slow processing speed, and language difficulties[2]. Chemobrain has been reported to affect up to 75% of chemotherapy-treated cancer survivors[3] and may persist in approximately 33% of chemotherapy-treated cancer patients[4].

Doxorubicin (DOX) is a chemotherapeutic medication that belongs to the anthracycline group; it is used to treat various types of cancers[5]. Its major side effect is cardiotoxicity[6]. It has been reported to cause chemobrain despite not passing the blood-brain barrier (BBB)[7]. DOX exerts its effects on cancer cells *via* multiple mechanisms, including DNA intercalation and impairment of RNA synthesis, inhibition of topoisomerase II resulting in DNA damage, and increased generation of reactive oxygen species (ROS), which oxidize phospholipids and lead to apoptosis[8,9]. The underlying mechanism of DOX-induced chemobrain is not fully understood. However, DOX has been reported to potentially induce chemobrain by mediating oxidative stress, inflammation, decreased neurogenesis, or altered neurotransmitter levels[9-13].

Metformin (MET) is an antiglycemic drug used for the treatment of type 2 diabetes mellitus[14]. Its mechanism involves the enhancement of AMPK

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protein activity and thus stimulation of the Akt pathway. Akt is known to mediate the trafficking of glucose transporters to reduce blood glucose levels. MET has been shown to have other effects in patients with other diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), and cancer[15-17]. For instance, it inhibits AD progression. It has also been reported to have a synergistic effect with chemotherapeutic agents, such as DOX and cyclophosphamide, methotrexate, and fluorouracil (CMF) and cause the inhibition of mTOR protein expression, which is important for cell survival[18]. MET-induced mTOR inhibition could limit the cellular repair system and thus increase cell death, ultimately leading to an increase in the mortality rate associated with some chemotherapeutics agents[19]. However, MET has also been reported to exert protective effects against the side effects of some chemotherapy agents, such as cyclophosphamide used singly[20].

DOX poorly crosses the BBB. However, a few studies[21-23] conducted in animal models have revealed the potential mechanisms by which DOX induces cognitive impairment. The current study is a follow-up study aimed at investigating MET's effect against DOX-induced cognitive dysfunction by evaluating some parameters that play important roles in learning and memory processes, such as tumor necrosis factor (TNF)-alpha, interleukin (IL)6, IL-1a, Akt, and insulin receptor substrate 1 (IRS-1).

Materials and Methods

Chemicals

DOX (ADRIM®) was obtained from Fresenius Kabi Oncology Ltd. (India), and MET hydrochloride (Metfor®) was obtained from Tabuk Pharmaceuticals (Saudi Arabia).

Animals and Treatments

Forty male rats were individually housed in a pathogen-free room with a 12-h/12-h light-dark cycle (lights were turned on at 6:00 am). The rats were allowed access to food and water at all times. The animals were divided into four groups (n = 10 in each group). The control group received five doses of saline. The DOX group received five doses of 4 mg/kg DOX i.p. The MET group received only MET in the drinking water daily at a concentration of 2.5 mg/ml. The DOX+MET group received MET in the drinking water continuously and five doses of 4 mg/kg DOX i.p. weekly.

Enzyme-Linked Immunosorbent Assay

Hippocampi from 12- to 13-week-old rats from the control, MET, DOX, and DOX+MET groups were collected after the animals were euthanized using CO_2 and lysed with lysis buffer. The samples were sonicated Q-sonica homogenizer 30 Hz pulses for 20 second (Qsonica, Newtown, CT, USA) and then centrifuged at 12,000 × *g* for 10 min. The supernatant was collected, aliquoted into 200-µL vials, and stored at -80°C. Protein in the samples was quantified by bicinchoninic acid (BCA) assay [(Pierce, Oakland, CA, USA)]. The samples were tested using Enzyme-Linked Immunosorbent Assay (ELISA) kits for TNF-alpha, Akt, IL-1a, IL-6, and IRS-1 antibodies as described in the manufacturer's (Mybiosource, San Diego, CA, USA) protocol. Measurements were performed at 450 nm by using an ELx800 Absorbance Microplate Reader (BioTek Instruments, Inc. Winooski, VT, USA). The ethics and protocol of this research were approved by the Research Unit at the College of Pharmacy at Qassim University. There is no informed consent required for this study.

Statistical Analysis

All results are presented as the mean ± standard error of the mean (S.E.M.) and analyzed using GraphPad Prism 5 software (San Diego, CA, USA). ELISA results for each group were analyzed by one-way analysis of variance, followed by Dunnett analysis. A p-value < 0.05 was considered statistically significant.

Results

Memories are formed because of alterations in protein expression, and some proteins are directly or indirectly involved in memory formation. Changes in memory function are caused by alterations in some proteins that affect the pathway circuits in the brain. Rat brains were collected after DOX, MET, and DOX+MET treatments to evaluate the effects of these drugs. The expression of some proteins involved in the memory function was investigated following the DOX, MET and DOX+MET treatments by using ELISA kits (Figures 1-5). ELISA revealed that DOX reduced IL-1-a and IRS-1 expression levels in the treatment groups significantly compared to the corresponding levels in the control group. Furthermore, ELISA performed to quantify IL-6, TNF-alpha, and

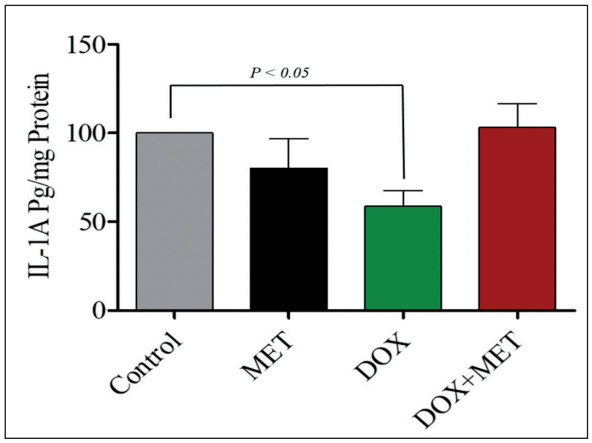

Figure 1. Effect of DOX and MET treatments on IL-1a regulation in rat brains. ELISA showed that IL-alpha expression levels were significantly lower in the DOX-treated group than in the control group. However, the IL-alpha expression levels in the MET- and DOX+MET-treated groups did not differ compared to the levels in the control group. Protein levels were normalized to the total protein content and stated as a percentage of the value for the control group, which was set as 100%.

Akt-a levels revealed no significant changes in these levels in the treatment groups compared to the corresponding levels in the control group (Figures 3-5).

Discussion

The present work attempted to elucidate the mechanism underlying DOX-induced memory dysfunction. Male rats were used to establish a model of DOX-induced cognitive deficits, and it was assumed that MET could protect against the DOX-induced impairments in memory function. A previous study[24] revealed that DOX induced deficits in spatial memory in Y-maze tasks, while NOR and EPM did not. MET was co-administered for 5 weeks orally along with five doses of intraperitoneally injected DOX in the induced rat chemobrain models, and the results showed that MET did not prevent the adverse effects of doxorubicin.

Recent investigations[25-27] have increasingly documented the beneficial effects of MET on multiple diseases other than diabetes. For instance, long-term use of MET is associated with anticancer effects and extended lifespan. However, research on its effect on cognitive function has produced controversial findings. Some studies have reported the beneficial effects of MET in improving memory function in rodents[20,28]. Other studies, however, have demonstrated that MET could result in memory impairment, particularly at high doses. MET has been also shown to decrease the risk for Alzheimer disease and potential adverse effects on the cognitive performance of diabetic patients, who are commonly prescribed this drug[29,30]. In this study, we demonstrated the effects of MET on DOX-induced cognitive performance deficit by using rat models of chemobrain. This result is consistent with the findings of other preclinical and clinical studies in which DOX appeared to alter cognitive function[31,32].

The proinflammatory cytokine interleukin-1 (IL-1) is produced by various types of cells, such as immune cells in the peripheral tissues and neurons in the brain[33,34]. IL-1 binds to the interleukin-1 receptor (IL-1R), which is a cytokine receptor that belongs to the tyrosine kinase receptor superfamily. The type I receptor (IL-1r1) is mainly responsible for mediating the inflammatory effects of IL-1, while the type II receptor functions as a suppressor of IL-1 activity[35]. IL-1r1 mediates several physiological functions by binding to either IL-1α or IL-1β, which play essential roles in health and disease[36]. For instance, IL-1β and IL-1α activation is associated with hippocampus-dependent memory tasks and long-term potentiation (LTP) and acellular memory level measurements[37,38]. In addition, the expression of hippocampal IL-1α and

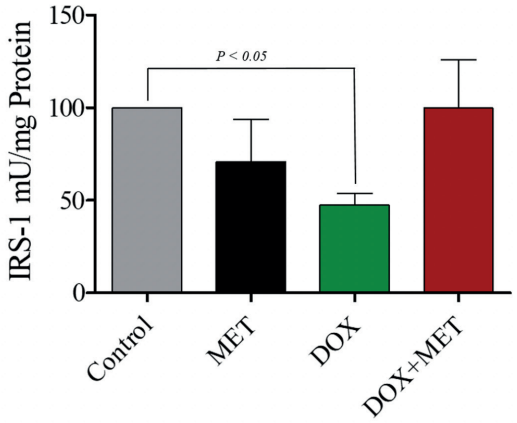

Figure 2. Effect of DOX and MET treatments on IRS-1 regulation in rat brains. ELISA showed that IRS-1 expression levels significantly reduced in the DOX-treated group compared to that in the control group. However, the IRS-1 expression levels in the MET- and DOX+MET-treated groups did not differ compared to the levels in the control group. Protein levels were normalized to the total protein content and stated as a percentage of the value for the control group, which was set as 100%.

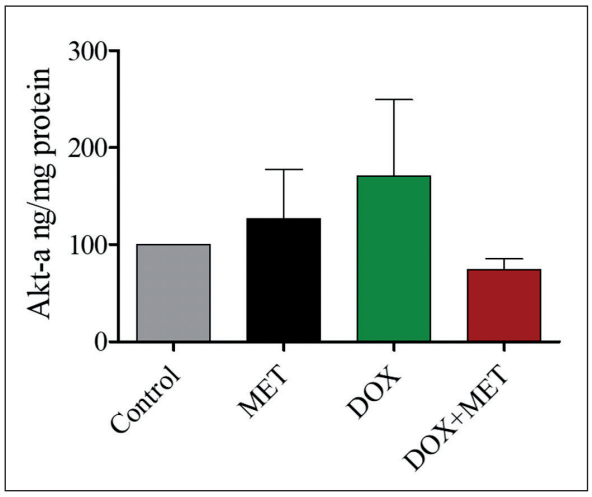

Figure 3. Effect of DOX and MET treatments on Akt-a regulation in rat brains. ELISA showed that Akt-a expression levels did not significantly change in any of the treated groups. Nevertheless, the DOX- and MET-treated groups tended to show greater Akt-a expression levels than that shown by the DOX+MET and control groups. Protein levels were normalized to the total protein content and stated as a percentage of the value for the control group, which was set as 100%.

IL-1β has been reported to significantly increase in adult mice during learning and memory processes[39]. However, increased levels of IL-1β in the hippocampus as a result of inflammation induced by lipopolysaccharide (LPS) via peripheral stimulation or intracerebral injection have been shown to cause memory impairment[40]. Thus, in the present study, the significantly reduced IL-1α expression in DOX-treated rats explains the potential mechanism of memory dysfunction in these rats.

Insulin signaling plays an important role in regulating learning and memory processes through its downstream signaling pathway. When insulin binds to the insulin receptor, it induces phosphorylation of the insulin receptor substrates-1 (IRS-1), particularly on Tyr-608, Tyr-628, and some serine residues, leading to activation of the protein. Activation of IRS-1 leads to phosphorylation of phosphatidylinositol 3-kinase (PI3K), and protein kinase B (PkB, also known as Akt)[41]. Phosphorylation of Akt inactivates glycogen synthase kinase-3 beta (GSK3β) by phosphorylating the serine-9 residue, which leads to GSK3β inhibition and increases learning and memory formation as well as synaptic plasticity[42]. However, other studies have identified some serine residues, such as serine-302 and/or serine-307, that can inhibit IRS-1 activity, and ultimately cause insulin resistance[43] and a blockade of the signaling cascade, resulting in memory impairment.

Doxorubicin is known to increase the expression of proinflammatory cytokines, such as TNF-alpha and IL-6, and elevated levels of both of these cytokines in peripheral tissues can result in their crossing the BBB and inducing central inflammation in the brain, resulting in memory impairment[44,45]. TNF-alpha is proposed to inhibit mitochondrial function and increase oxidative stress, thereby causing memory deficits[46,47]. In this study, MET was hypothesized to rescue this memory deficit by reducing inflammation. However, the results surprisingly showed that DOX treatment significantly reduced TNF-alpha with the presence of memory impairment, as we reported in a previous study[24]. The interpretation of this result is that the DOX-induced increase in TNF-alpha levels peaks after 72 hours, whereas this study lasted for 5 weeks. In addition, a previous study reported that DOX can lead to reduced neurogenesis in the brain[13], and the reduction in TNF-alpha levels could be a result of the reduced total neurons in the brain. Moreover, scholars[48] revealed that the role of IL-6 in learning and memory is very important, with IL-6 KO mice showing deficits in spatial learning tasks. In addition, while blockade of IL-6 by the injection of anti-IL-6 antibodies in rat models induced memory impairment[49], overexpression of IL-6 caused

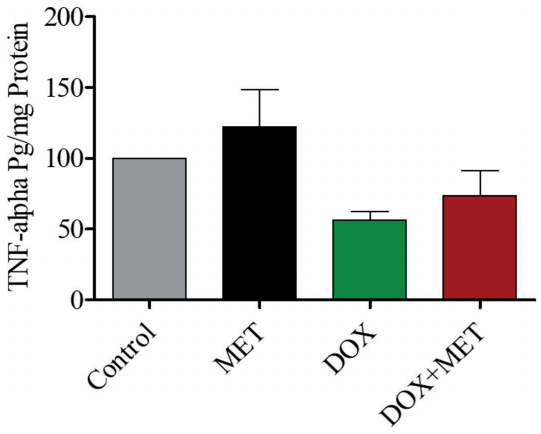

Figure 4. Effect of DOX and MET treatments on TNF-alpha regulation in rat brains. ELISA showed that TNF-alpha expression levels did not significantly differ among the treatment groups. However, the DOX- and DOX+MET-treated groups tended to show decrease TNF-alpha expression levels compared to those in the MET-alone and control groups. Protein levels were normalized to the total protein content and stated as a percentage of the value for the control group, which was set as 100%.

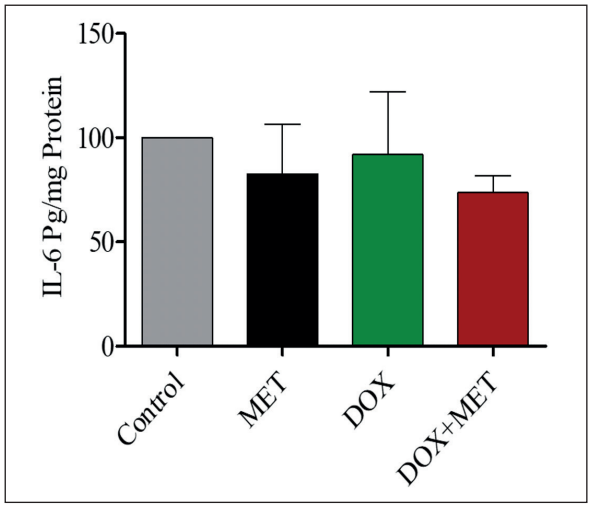

Figure 5. Effect of DOX and MET treatments on IL-6 regulation in rat brains. ELISA showed that IL-6 expression levels did not significantly differ among the treatment groups compared to the levels in the MET-alone and control groups. Protein levels were normalized to the total protein content and stated as a percentage of the value for the control group, which was set as 100%.

by inflammation in AD also induced memory dysfunction[45,50]. However, in this study, the expression of IL-6 was not altered, indicating that DOX-induced impairments in memory function follow a pathway different from the IL-6 pathway

Conclusions

Summarily, this study used rat chemobrain models to identify several key proteins that may be altered following DOX and MET treatment. The findings can thus shed light on the molecular mechanisms underlying DOX-induced cognitive impairment and the protective effects of MET against these deficits. These results indicate that DOX may induce cognitive dysfunction by altering IL-1-alpha and IRS-1 protein expressions and elucidate the chronic effects of DOX and MET on memory function. Additional investigations are required to determine the effects on specific cognitive deficits such as attention or working memory.

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