Biochemistry and Biophysics Reports 4 (2015) 202–206

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

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

Fluvoxamine alleviates paclitaxel-induced neurotoxicity

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ARTICLE INFO

Article history:
Received 4 May 2015
Received in revised form
15 September 2015
Accepted 21 September 2015
Available online 25 September 2015

Keywords:
Chemobrain
Paclitaxel
Endoplasmic reticulum stress
Fluvoxamine
Sigma 1 receptor
Selective serotonin reuptake inhibitor

ABSTRACT

Paclitaxel (Px) is an effective chemotherapeutic agent for the treatment of various cancers. However, it is often associated with neurological side effects, including chemotherapy-associated cognitive impairment (CACI), such as “chemobrain”. Previously, we reported that endoplasmic reticulum (ER) stress is involved in Px-induced neurotoxicity, and immunoglobulin heavy chain binding protein (BiP) inducer X (BIX) alleviates Px-induced neurotoxicity. However, BIX has not been used in clinical practice yet. We recently reported that fluvoxamine (Flv) alleviates ER stress via induction of sigma-1 receptor (Sig-1R). The purpose of this study was to investigate whether Flv could alleviate Px-induced neurotoxicity *in vitro*. SK-N-SH cells were pre-treated for 12 h with or without 10 μg/ml Flv followed by treatment with 1 μM Px with or without co-existence of 10 μg/ml Flv for 24 h. To investigate the involvement of Sig-1R in alleviation effect on Px-induced neurotoxicity,1 μM NE100, an antagonist of Sig-1R, was added for 24 h. Neurotoxicity was assessed using the MTS viability assay and ER stress-mediated neurotoxicity was assessed by evaluating the expression of C/EBP homologous protein (CHOP), cleaved caspase 4, and cleaved caspase 3.

Pre-treatment with Flv significantly alleviated the induction of CHOP, cleaved caspase 4, and cleaved caspase 3 in SK-N-SH cells. At the same time, pre-treatment with Flv significantly induced Sig-1R in SK-N-SH cells. In addition, viability was significantly higher in Flv-treated cells than in untreated cells, which was reversed by treatment with NE100.

Our results suggest that Flv alleviates Px-induced neurotoxicity in part through the induction of Sig-1R. Our findings should contribute to one of the novel approaches for the alleviation of Px-induced neurotoxicity, including chemobrain.

1. Introduction

Cancer treatments, including chemotherapy, radiation therapy, and targeted biological therapies, have made great advances in the last century and have led to improved survival. However, their administration is often associated with several side effects and may reduce patient quality of life (QOL). Cognitive impairment is among the most frequently reported problems by patients during the treatment, especially in the context of chemotherapy. This cognitive impairment related to chemotherapy is known as “chemofog” or “chemobrain” and is well studied in breast cancer patients. Reports demonstrate that 15–75% of breast cancer survivors have cognitive impairment in the domain of memory, processing speed, attention, and executive function [1–4]. Multiple hypotheses exist regarding chemobrain, including disruption of hippocampal cell proliferation and neurogenesis [5], chronic increases in inflammation [6,7], increased oxidative stress [6], white matter disruption [8,9], and long-term changes in cerebral blood flow and metabolism [10]. However, a detailed mechanism and intervention for CACI have not been established.

Paclitaxel (Px) is a taxane agent that binds microtubules, stabilizes microtubule dynamics, and arrests the cell at the mitotic phase [11]. However, Px often induces side effects such as arthralgia, myalgia, and ataxia. In addition, pronounced emotional distress, including depression, and reduced mental QOL through adjuvant treatment has recently been reported [12]. Though, it is

Abbreviations: BiP, immunoglobulin heavy-chain binding protein; BIX, BiP inducer X; CACI, chemotherapy-associated cognitive impairments; CHOP, C/EBP homologous protein; CYP, cytochrome P450; ER, endoplasmic reticulum; Flv, fluvoxamine; JNK, c-Jun NH2-terminal kinase; Px, paclitaxel; QOL, quality of life; Sig-1R, sigma 1 receptor; SSRI, selective serotonin reuptake inhibitor; UPR, unfolded protein response

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http://dx.doi.org/10.1016/j.bbrep.2015.09.014

believed that Px is prevented from penetrating into the brain, a positron emission tomography study demonstrated detectable levels of radiolabeled Px in the brain after intravenous administration [13], indicating that Px may directly influence the central nervous system.

The endoplasmic reticulum (ER) stress response, also called the unfolded protein response (UPR), is a defense system that deals with the accumulation of unfolded proteins in the ER lumen. However, when ER stress is very severe, cells induce and/or activate C/EBP homologous protein (CHOP), the c-Jun NH2-terminal kinase (JNK) pathway, and caspase 4, which lead to apoptosis. Accumulating evidence demonstrates the importance of ER stress and of UPR in the pathophysiology of human neurological diseases, such as Parkinson's disease [14,15], Alzheimer's disease [15–18], and causes of cognitive dysfunction. Recently, we reported that ER stress is involved in Px-induced neurotoxicity [19]. In addition, we have also reported that immunoglobulin heavy-chain binding protein (BiP) inducer X (BIX) attenuates Px-induced neurotoxicity through alleviation of ER stress [19]. The effect of BIX in the alleviation of ER stress has been reported in many other situations [20–25]. However, BIX has not been approved for clinical practice use yet and is only permitted for experimental use. Thus, the exploration of agents like BIX in drugs that have already been licensed for the clinical setting should be fast way to practical use.

Recently, we reported that fluvoxamine (Flv), a selective serotonin reuptake inhibitor (SSRI) that is widely used in clinical practice as an antidepressant, alleviates ER stress *in vitro* and in animal experiments [26].

In the present study, we investigated the effect of Flv on Px-induced neurotoxicity using SK-N-SH cells *in vitro*.

2. Materials and methods

2.1. Chemicals

Flv (Sigma-Aldrich, St. Louis, MO, USA) and NE100 (Santa Cruz Biotechnology, Dallas, Texas, USA) were dissolved in double-distilled water (DDW). Px (Sigma) was dissolved in dimethyl sulfoxide (DMSO).

2.2. Cell culture

SK-N-SH neuroblastoma cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; GIBCO/Invitrogen Life Technologies, Paisley, UK) with 10% fetal bovine serum (FBS; JRH, Woodland, CA, USA). Cells were maintained at 37 °C in an incubator within an atmosphere of 5% carbon dioxide (CO_2). Cells were routinely passaged using trypsin (0.25%)–EDTA (0.1%) solution in Hank's Balanced Salt Solution (HBSS; Himedia Laboratories Pvt. Ltd., Mumbai, India).

2.3. MTS cell viability assays

Cellular viability was assessed using CellTiter 96 Aqueous One Solution Cell Proliferation Assays (Promega, Madison, WI, USA). Briefly, SK-N-SH cells were seeded in 96-well plates. Cells were allowed to attach for 24 h. For evaluation of the toxicity of Flv on SK-N-SH cells, cells were treated with 10, 25, 50, 75, or 100 μg/ml Flv for 24 h at 37 °C. For evaluation of the alleviation effect of Flv on Px-induced neurotoxicity, SK-N-SH cells were pre-treated with or without 10 μg/ml Flv for 12 h followed by 1 μM Px treatment with or without 10 μg/ml Flv for 24 h. To confirm the involvement of Sig-1 R in alleviation effect on Px- induced neurotoxicity, SK-N-SH cells were incubated with 1 μM Px, 10 μg/ml Flv and 1 μM NE100 for 24 h. Next, 20 μl of MTS reagent was added to each well and cells were incubated for 2 h. Optical density was measured at 490 nm using a Micro Plate Reader (Bio-Rad, Hercules, CA, USA).

2.4. Western blots

SK-N-SH cells were pre-treated with or without 10 μg/ml Flv for 12 h followed by 1 μM Px treatment with or without 10 μg/ml Flv for 24 h at 37 °C. Cells were washed in Tris-buffered saline (TBS), harvested, and lysed in RIPA buffer (Thermo Fisher Scientific, Inc., Rockford, IL, USA) with a protease inhibitor cocktail (Roche, Mannheim, Germany), and a phosphatase inhibitor cocktail (Roche). Lysates were sonicated on ice three times for five seconds each, and then incubated for 15 min. After centrifugation for 20 min at 13,000 g, supernatants were retained and boiled in SDS sample buffer. Lysates (10 μg) were separated on SDS-polyacrylamide gels and transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad, Hercules, CA, USA). Non-specific protein binding was blocked by incubating membranes for 1 h at room temperature in 5% w/v non-fat milk powder in TBS-T [50 mM Tris–HCl (pH 7.6), 150 mM NaCl, and 0.1% v/v Tween-20]. The membranes were incubated overnight at 4 °C with the following primary antibodies: anti-CHOP (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-caspase 4 (1:500; Medical and Biological laboratories, Nagoya, Japan), anti-caspase 3 (1:1000; Cell Signaling Technology, Boston, MA, USA), anti-sigma 1 receptor (Sig-1R) (1:250; Abcom, Cambridge, UK) and anti-GAPDH (1:1000; Thermo Fisher Scientific, Waltham, MA, USA). The membranes were then washed three times in TBS-T for 5 min. Finally, the membranes were incubated for 60 min at room temperature with HRP-conjugated anti-rabbit or anti-mouse antibodies (Promega, Madison, WI, USA). Protein bands were detected using the ECL Plus kit (GE Healthcare, Buckinghamshire, UK). The intensity of each band was quantified using NIH image J software.

2.5. Statistical analyses

Data are presented as mean values ± standard deviation (SD). Unpaired student's *t*-test or one-way analysis of variance (ANOVA) followed by Tukey–Kramer test were used to determine the levels of significance between sample means. All results are representative of at least three independent experiments.

3. Results

3.1. Flv toxicity on SK-N-SH cells

The toxicity of Flv on SK-N-SH cells was examined using an MTS assay. We used 10, 25, 50, 75, or 100 μg/ml Flv or a vehicle control to treat SK-N-SH cells. SK-N-SH cells treated with Flv showed 80% (25 μg/ml), 29% (50 μg/ml), 19% (75 μg/ml), and 18% (100 μg/ml) viability compared to the vehicle control cells ([$p < 0.001$] at all doses) (Fig. 1). However, SK-N-SH cells treated with 10 μg/ml Flv did not show reduced viability (102%) compared to the vehicle control (Fig. 1). Based on these data, we used 10 μg/ml Flv in all subsequent experiments.

3.2. Flv alleviates Px-induced ER stress mediated apoptosis

Next we investigated whether Flv could alleviate Px-induced ER stress-mediated apoptosis in SK-N-SH cells by monitoring CHOP, cleaved caspase 4, and cleaved caspase 3, an active form of each caspase. CHOP, cleaved caspase 4, and cleaved caspase 3 were induced in cells treated with Px compared to control cells (Fig. 2a–c, [$p < 0.01$] at each comparison), which is consistent with our previous report [19], On the other hand, when cells were pre-