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Edaravone alleviates cisplatin-induced neurobehavioral deficits via modulation of oxidative stress and inflammatory mediators in the rat hippocampus

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ABSTRACT

Cisplatin is a chemotherapeutic agent used in the treatment of malignant tumors. A major clinical limitation of cisplatin is its potential toxic effects, including neurotoxicity. Edaravone, a potent free radical scavenger, has been reported to have the neuroprotective effect against neurological deficits. The aim of the present study was to determine the neuroprotective effect of edaravone against cisplatin-induced behavioral and biochemical anomalies in male Wistar rats. Our results showed that cisplatin (5 mg/kg/week, i.p.) administration for seven weeks caused marked cognitive deficits and motor incoordination in rats. This was accompanied by oxido-nitrosative stress, neuroinflammation, NF-κB activation and down-regulation of Nrf2/HO-1 gene expression level in the hippocampus. Edaravone (10 mg/kg/week, i.p.) treatment for seven weeks inhibited the aforementioned neurobehavioral and neurochemical deficits. Furthermore, edaravone was found to up-regulate the gene expression level of Nrf2/HO-1 and prevented the cisplatin-induced NF-κB activation. These findings demonstrated that oxido-nitrosative stress and inflammatory signaling mediators play a key role in the development of cisplatin-induced neurobehavioral deficits which were prevented by edaravone treatment.

1. Introduction

Cisplatin (cis-diamminedichloridoplatinum(II)-CDDP), is a well-known platinum-based chemotherapeutic agent that is extensively used for the treatment of various malignant tumors in the breast, bladder, head, neck, ovary, and testicles (McWhinney et al., 2009; Stathopoulos, 2010). The mechanism of action of cisplatin has been associated with its ability to interfere with DNA replication and/or transcription process and DNA repair mechanism that eventually leads to oxidative stress and mitochondria-dependent apoptosis (Podratz et al., 2011). Moreover, the cisplatin-induced neurotoxicity occurs due to increased oxido-nitrosative stress, proinflammatory cytokines, mitochondrial dysfunction, DNA damage and apoptotic cell death resulting in various morphological changes in the neurons such as axonal shrinkage and demyelination (Sayre et al., 2008; Tuncer et al., 2010). Cisplatin also affects the function of neural progenitor cells in the hippocampus region by repressing cell proliferation and neurogenesis (Piccolini et al., 2012; Hinduja et al., 2015).

Increased level of oxidants causes the imbalance between inflammatory and anti-inflammatory mediators. The cisplatin-induced toxicity which is mediated through activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) which in turn increases inflammatory mediators that further responsible for altering the long-term potentiation (LTP) in the hippocampus region (So et al., 2007; Kim et al., 2014). Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that plays a key role in the protection against free radical scavenger by regulating the expression of several antioxidant/detoxification genes such as heme oxygenase-1 (HO-1) and glutathione S-transferases. Therefore, oxidative stress-mediated neurotoxicity can be alleviated by activating the Nrf2/HO-1 pathway (Ye et al., 2016). Previous experimental studies in animals demonstrated that cisplatin treatment-induced cognitive dysfunction and motor impairment due to dysregulation of hippocampal and cerebellar functions (Shabani et al., 2012a,b). Moreover, cisplatin-induced

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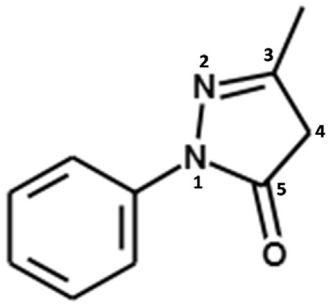

Fig. 1. Structure of Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one).

oxidative stress raises the level of acetylcholinesterase (AChE) in the hippocampus (Chtourou et al., 2015). The exact mechanism underlying the cisplatin-induced behavioral deficits still remains elusive.

Several antioxidant molecules such as coenzyme Q10 (Da Silva et al., 2013), cyanidin (Li et al., 2015), walnut (Shabani et al., 2012b), D-Methionine (Gopal et al., 2012), alpha lipoic acid, melatonin (Tuncer et al., 2010) and curcumin (Mendonça et al., 2013) have been reported to prevent cisplatin and its derivatives-induced neurotoxicity. Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one) is a novel antioxidant with the potent free radical scavenging property (Fig. 1). Previous reports suggested its protection in various animal models such as diabetic stroke (Srinivasan and Sharma, 2012), ischemic reperfusion injuries (Yoshida et al., 2006), restraint stress (Jangra et al., 2016d), lipopolysaccharide-induced behavioral anomalies (Sriram et al., 2016), Alzheimer's disease (Jiao et al., 2015), Parkinson's disease (Xiong et al., 2011), and amyotrophic lateral sclerosis (Nagase et al., 2015). Based on significant protective profiles of edaravone, it is worthwhile to evaluate its neuroprotective potential against cisplatin-induced neurotoxicity. Therefore, in present study we have investigated the possible effect of edaravone in cisplatin induced-neurotoxicity model of learning and memory deficits by carrying out certain behavioral studies and measured oxidative stress markers, proinflammatory cytokines, acetylcholinesterase (AChE) activity, brain-derived neurotrophic factor (BDNF) and gene expression levels of NF-κB, Nrf2, HO-1 in the hippocampus.

2. Material and methods

2.1. Chemicals

Edaravone, cisplatin, Griess reagent, thiobarbituric acid, 5,5′-dithiobis-(2-nitrobenzoic acid) SOD assay kit, catalase assay kit were purchased from Sigma-Aldrich, St. Louis, MO, USA. Interleukin-1β, tumor necrosis factor-α (Thermo Fisher Scientific, India) ELISA kits, BDNF Emax® ImmunoAssay kit (Promega, Madison, WI, USA) were used. Total RNA extraction kit (Hi-Media India), RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific, India), and primers (Imperial life sciences (P) Limited India) were purchased. All other chemicals used in the experimental study were of analytical grade.

2.2. Animals

Male Wistar rats (weight 150–200 g; 5–6 weeks old) were used in the present study. Animals were procured from the Gauhati Medical College, Guwahati, and acclimatized to laboratory conditions for 7 days before commencement of the experiment. Rats were housed in groups of four per cage and were given food and water ad libitum. The study was approved (approval no. MC/05/2015/58) by the Institutional Animal Ethics Committee (IAEC), Gauhati Medical College and Hospital (CPCSEA Registration No. 351, 3/1/2001). All the experiments were performed according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. The animals were housed at room temperature (24 ± 1 °C), with 65 ± 5% humidity, and 12 h light and dark cycles.

2.3. Experimental design

Cisplatin (5 mg/kg) and edaravone (10 mg/kg) were prepared freshly in saline (0.9% NaCl) and were administered intraperitoneally (i.p.) once in a week for 7 weeks. The animals were randomly divided into four experimental groups: Group 1 as Control group: normal saline (0.9%) was administered per week for 7 weeks. Group 2 as Cisplatin group: Cisplatin-5 mg/kg was administered per week for 7 weeks. Group 3 as edaravone and cisplatin treated group: Edaravone and cisplatin at the dose of 10 and 5 mg/kg respectively were administered per week for 7 weeks. Group 4 as only Edaravone treated group: Edaravone at the dose of 10 mg/kg was administered per week for 7 weeks. The doses of cisplatin and edaravone were selected based on previous experimental reports (Sriram et al., 2016; Oz et al., 2015). Behavioral studies were performed from 46th to 50th day of the study (Fig. 2.). The experimental groups consisted of 18 animals each. Due to animal's mortality in certain groups, 12 animals were randomly taken further to carry out the different behavioral assessment, biochemical evaluation and gene expression studies of NF-κB, Nrf2, and HO-1 by reverse transcriptase PCR. Moreover, a gap of 3–4 h was maintained for different behavioral assessments in different sets of animals. At the end of the experiment, animals were killed by cervical dislocation. The hippocampus was quickly dissected out from the isolated brain and homogenized in ice-cold phosphate buffer saline (pH 7.4). Homogenates samples were preserved at -80 °C for further biochemical estimation.

2.4. Behavioral studies

2.4.1. Morris water maze (MWM) test

MWM test was performed to assess the learning and memory function after cisplatin administration (Vorhees and Williams, 2006). In brief, circular tank (145 cm diameter and 50 cm height) consisted of four equal quadrants containing opaque water (26 ± 1 °C) was used. The platform (10 cm diameter) was placed 2 cm below the water level in the acquisition phase. The animal was placed in the pool facing towards the tank wall and allowed to search platform for 120 s during the acquisition phase. If the animal failed to locate the platform in 120 s, then the animal was guided towards the way to platform and remain on the platform for further 30 s. Each animal was trained through four different quadrants to locate the platform for four consecutive days. On the fifth day, the single probe trial was performed for 120 s by removing the platform. The retention memory of trained animals was recorded in terms of time spent in the target quadrant.

2.4.2. Novel object recognition test (NORT)

NORT was performed to investigate the recognition memory according to the method described by Bevins and Besheer (2006). NORT consisted of three phases: habituation, familiarization, and test phase. A black-colored open field box ($50 \times 50 \times 36$ cm^3) was used in this test. In the habituation phase, each animal was habituated to an empty open field by placing it in the open field arena and allowed to explore for 5 min twice in a day. After habituation phase, familiarization phase was performed by placing the two objects (a rectangular wooden block and a small rubber ball) at the left and right position in the open field apparatus. The rat was