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Unfolding the mechanism of cisplatin induced pathophysiology in spleen and its amelioration by carnosine

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ABSTRACT

cis-Diamminedichloroplatinum (cisplatin) is an effective chemotherapeutic and is widely used for the treatment of various types of solid tumors. Bio-distribution of cisplatin to other organs due to poor targeting towards only cancer cells constitutes the backbone of cisplatin-induced toxicity. The adverse effect of this drug on spleen is not well characterized so far. Therefore, we have set our goal to explore the mechanism of the cisplatin-induced pathophysiology of the spleen and would also like to evaluate whether carnosine, an endogenous neurotransmitter and antioxidant, can ameliorate this pathophysiological response. We found a dose and time-dependent increase of the pro-inflammatory cytokine, TNF-α, in the spleen tissue of the experimental mice exposed to 10 and 20 mg/kg body weight of cisplatin. The increase in inflammatory cytokine can be attributed to the activation of the transcription factor, NF-κB. This also aids in the transcription of other pro-inflammatory cytokines and cellular adhesion molecules. Exposure of animals to cisplatin at both the doses resulted in ROS and NO production leading to oxidative stress. The MAP Kinase pathway, especially JNK activation, was also triggered by cisplatin. Eventually, the persistence of inflammatory response and oxidative stress lead to apoptosis through extrinsic pathway. Carnosine has been found to restore the expression of inflammatory molecules and catalase to normal levels through inhibition of pro-inflammatory cytokines, oxidative stress, NF-κB and JNK. Carnosine also protected the splenic cells from apoptosis. Our study elucidated the detailed mechanism of cisplatin-induced spleen toxicity and use of carnosine as a protective agent against this cytotoxic response.

1. Introduction

Cisplatin [*cis*-diamminedichloroplatinum(II)] is a widely used anticancer drug [1]. This molecule has been proved to be a successful chemotherapeutic agent not only in the treatment of lung, bladder, head and neck carcinoma but also in the case of metastatic cancer [2,3]. Despite its wide application as a successful chemotherapeutic agent, severe nephrotoxicity caused by cisplatin limits its use to a great extent and pose a big question regarding its efficacy [4,5]. Cisplatin exerts its toxic effect by binding to DNA and forming intraand inter-strand crosslinks ultimately leading to impaired DNA synthesis, cell cycle arrest and cell death in cancer cells [6]. Oxidative stress and inflammation play apivotal role in cisplatin-induced pathophysiology [7]. Apart from its well-established nephrotoxicity, a side effect of cisplatin also include neurotoxicity and hemolytic uremic syndrome [8,9] Till date, little attention has been paid to the toxicity of cisplatin in the spleen which constitute a major part of the immune system in vertebrates. The immune system helps the body to adapt and defend itself from the attack of harmful agents and disease causing pathogens. Milicevicet al reported the histo-quantitative effect of normal spleen as well as the spleen of cisplatin exposed animals and found that the volume density of red pulp, white pulp and the marginal zone was markedly reduced in the cisplatin exposed organ. The number of follicles per mm^2 of spleen section area and numeric density of follicles were also greatly reduced [10]. A low dose of cisplatin causes damage to erythrocytes and the damaged erythrocytes accumulates in the red pulp of spleen with defective recycling of FPN1 and ferritin protein. This causes iron overload in the spleenocytes and leads to the deposition of hemosiderin in thespleen tissue [11]. Crăciun and Paşca et al. demonstrated that carboplatin (an analog of cisplatin) also affects thymocytes, epithelio-reticular cells and vessels of the thymus [12]. Wen et al. recently reported that cisplatin/etoposide or carboplatin/paclitaxel could significantly

Abbreviations: DHE, dihydroethidium; DTT, Dithiothreitol; FPN1, Ferroprotein 1; iNOS, Inosinducible nitric oxide synthase; ICAM-1, Intracellular adhesion molecule 1; IL-6, interleukin 6; IL-1β, Interleukin 1beta; IL-10, interleukin 10; JNK, c-jun N-terminal kinase; MCP-1, monocyte chemoattractant protein; NF-κ B, Nuclear factor kappa B; ROS, Reactive oxygen species; TNF-α, Tumour necrosis factor

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reduce the spleen volume in patients with Locally Advanced Non-Small Cell Lung Cancer (NSCLC) [13] and that change may be correlated with the alterations in the number and function of different effector cells in the spleen.

Many naturally occurring phytochemicals possess antioxidant and anti-inflammatory properties that can be useful in ameliorating the situation. Mitochondrial antioxidants like mito CP and mitoQ ameliorated cisplatin-induced renal dysfunction [14]. Polyphenols like curcumin [15–18], morin [19], arjunolic acid [20], etc. havebeen shown to ameliorate testicular damage in diabetes, NSAID-induced gastropathy and doxorubicin-induced cardiotoxicity. Moreover, taurine, a sulphonic acid, has been shown to ameliorate doxorubicin-induced cardiac and testicular damage [21,22] and copper nanoparticle-induced hepatic tissue damage [23]. Carnosine (β-alanyl-L-histidine) is a dipeptide containing alanine and histidine and is synthesized by carnosine synthetase. This dipeptide is enriched in muscle and brain tissues. Recent studies from various laboratories havepointed out its anti-inflammatory [24], anti-oxidant [25] and anti-senescence [26] properties. Carnosine also acts as a neurotransmitter [27]. Moreover, carnosine has been proved to be effective against cisplatin induced nephrotoxicity [28,29].

Considering the pleiotropic nature of this molecule, we have examined the beneficial role of this molecule against cisplatin-induced pathophysiology of spleen in a murine model. Our results suggest that cisplatin induces spleen tissue damage via activation of NF-κB, JNK and extrinsic apoptotic pathway and administration of carnosine abates this cisplatin triggered pathophysiological response in the spleen.

2. Materials and methods

2.1. Materials and reagents

Cisplatin and carnosine were purchased from Sigma Aldrich (USA), bovine serum albumin(BSA) were purchased from Sisco Research Laboratory(Mumbai, India). Protease and phosphatase inhibitor cocktail were obtained from Thermo Fisher Scientific Inc., USA. DMSO and formalin were obtained from Merck (Worli, Mumbai). Primary antibodies against caspase-3(#9662), phosphor JNK(#9251)and Lamin b1(#12586) were purchased from Cell Signaling (Cell Signaling TechnologyInc., Danver, MA). TNF-α (ab66579), phospho-NF-κB p65 (ab7970), iNOS(ab15323), caspase-8(ab25901) and HRP (ab97051) antibodies were obtained from Abcam (Cambridge, UK) and GAPDH was purchased from Biobharti, India.

2.2. Animals

Eight weeks old male Swiss albino mice weighing approximately 22–25 g were acclimatized under laboratory conditions for two weeks before starting the experiment. The animals were maintainedon thestandard diet. Animals subjected to 12 h light and dark cycles under standard conditions of temperature and humidity.

2.3. Experimental procedure

Dose and time-dependent toxicity of cisplatin were carried out in this particular study. Animals were administered by intraperitoneal injection of cisplatin once at a dose of 10 mg/kg and 20 mg/kg body weight and were sacrificed after 24, 48, 72 and 96 h and serum was collected. The optimum time for further study was based on survival, expression of TNF-α in spleen and serum NO level. After this, the animals were divided into six groups.

Group 1: animals were treated with vehicle (0.9%NaCl) and served as control.

Group 2: animals were administered cisplatin intraperitoneally(i.p.) once at a dose of 10 mg/kg body weight and sacrificed after 96 h.

Group 3: animals were administered cisplatinin traperitoneally(i.p.) once at a dose of 20 mg/kg body weight and sacrificed after 72 h [30].

Table 1
Primer details.

Gene	Primer sequence (5′ to 3′)	Annealing temperature (°C)	Amplicon Size (bp)
Catalase	Fp:CACACTCACACACACTCATACA Rp:CGCACAGCACAGGAATAAAC	50	178
IL-1β	Fp:GAGTGTGGATCCCAAGCAATA Rp:TCCTGACCACTGTTGTTTCC	45	174
IL-6	Fp:GATAAGCTGGAGTCACAGAAGG Rp:TTCTGACCACAGTGAGGAATG	58.7	163
MCP-1	Fp:GAAGGAATGGGTCCAGACATAC Rp:CACTCCTACAGAAGTGCTTGAG	55	190
ICAM-1	Fp:GTAGATCAGTGAGGAGGTGAATG Rp:TGCCAGTCCACATAGTGTTTAT	58.7	170
TNF-α	Fp:TCTCAGAATGAGGCTGGATAAG Rp:CCCGGCCTTCCAAATAAATAC	55	188
GAPDH	Fp:GGAGAAACCTGCCAAGTATGA Rp:CCAGGAAATGAGCTTGACAAAG	50	193

Group 4: animals were treated with carnosine at a dose of 75 mg/kg body weight i.p 30 min before administration of 10 mg/kg body weight cisplatin and treatment with carnosine was continued after every 24 h up to 96 h [31].

Group 5: animals were treated with carnosine at a dose of 75 mg/kg body weight i.p 30 min before administration of cisplatin at 20 mg/kg body weight and treatment with carnosine were continued every day for 72 h.

Group 6: animals were treated with carnosine75 mg/kg body weight i.p. every day for four days (96 h).

There were six animals in each group (n = 6). All the animals were supplied with food and water and were sacrificed by cervical dislocation at respective time points. Blood samples were collected from each group of animals Spleen from all animals were washed with phosphate buffer saline (PBS), photographed and then stored at −80°C. Serum was isolated for quantification of NO production, Blood urea nitrogen (BUN) and creatinine.

2.4. Histological analysis

The fresh spleen tissues were fixed in 10% buffered formalin at room temperature for 24 h and then embedded in paraffin. Six μm thin tissue sections were then stained with hematoxylin and eosin and pathophysiological changes were observed under bright field microscope (Leica 269Microsystem DN1000; camera: DFC450 C).

2.5. Measurement of reactive oxygen species and nitric oxide (NO) level

Intracellular ROS production was quantified with the help of oxidative fluorescent dye dihydroethidium (DHE), extensively used to monitor superoxide radical production [32]. Cryosections (10 μm) from spleen tissue sample was stained with DHE having a concentration of 10 μmol/L in a light-protected and humidified chamber for 30 min at 37 °C.The analysis of the images for each section was performed using a confocal microscope. Nitric oxide level was measured from serum using Griess reagent [33].

2.6. Measurement of BUN and creatinine

BUN and creatinine are markers related to renal function. These two parameters were measured from serum using standard kits.

2.7. RNA extraction and reverse transcriptase PCR

RNA was extracted from the spleen tissue from all the six groups using TRIZOL reagent by following manufacturer's protocol (Invitrogen,