EVALUATION OF OXIDATIVE STRESS INDUCED HEPATOTOXICITY PRODUCED BY CISPLATIN IN MALE SPRAGUE DAWLEY RATS

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ABSTRACT

Objective: To study the effect of cisplatin administration on oxidative stress induced hepatotoxicity in male Sprague Dawley rats.

Study Design: Randomized controlled trial.

Place and Duration of Study: Study was conducted at department of Physiology, Army Medical College, Rawalpindi. Duration of study was 18 months from Oct 2014 to Apr 2016.

Material and Methods: The trial was performed on sixty male Sprague Dawley rats which were distributed randomly into two groups of 30 rats each. Group I received placebo whereas group II received intraperitoneal cisplatin 2mg/kg body weight two times a week for the period of 4 weeks. After successful treatment, animals were sacrificed and terminal blood sample was collected and used for estimation of serum AST, ALT, albumin and 8-isoprostane. Dissection of rats were done and liver tissue sampled. Tissue homogenate was prepared from liver sample which was used for estimation of total glutathione levels.

Results: In group I, 8-isoprostane was 18.31 ± 3.35 pg/ml, total glutathione was 4.29 ± 0.42 µmol/L, ALT was 36.93 ± 4.72 IU/L, AST was 124.2 ± 12.75 IU/L and Albumin was 4.11 ± 0.26 g/dl whereas in group II, 8-isoprostane was 67.9 ± 8.14 pg/ml, total glutathione was 1.92 ± 0.28 µmol/L, ALT was 87.17 ± 6.47 IU/L, AST was 357.7 ± 19.37 IU/L and Albumin was 2.12 ± 0.25 g/dl. Levels of serum 8-isoprostane, ALT and AST were significantly raised (p<0.001) whereas serum albumin and total glutathione in liver tissue were found significantly low (p<0.001) in group II as compared to group I.

Conclusion: Cisplatin treatment causes hepatotoxicity by increased production of reactive oxygen species in male Sprague Dawley rats.

Keywords: Cisplatin, Hepatotoxicity, Oxidative stress.

INTRODUCTION

Life of aerobic creatures on earth is possible only because of the presence of molecular oxygen in the air. However, due to the significant oxidation potential and usage as a final electron acceptor, the molecular oxygen produces oxygen derived free radicals and non-radicals that exert number of pathophysiological effects to the body[1]. Oxidative stress reflects the imbalance of equilibrium between prooxidants and antioxidants in favor of the former[2]. An altered redox state of a cell influences various aspects of cell functioning such as proliferation, differentiation and cell survival[3]. Cisplatin is an effective antineoplastic drug used to treat varied range of human malignancies. Apart from its potent antineoplastic action, the drug is known to produce toxic damage to the normal tissue[4]. The underlying mechanism involves accumulation of drug in the mitochondria where it persistently binds with mitochondrial DNA (mDNA) to interfere transcription which leads to decreased synthesis of protein components of electron transport chain (ETC). Due to deficient ETC proteins, electron leaks prematurely to the oxygen and results in excess production of reactive oxygen species (ROS)[5].

Liver is one of the vital organ of the body responsible for digestive and excretory functions, storage of vitamins, minerals and nutrients, and the synthesis of the new substances. Being a metabolic factory of the body, liver is highly

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Received: 21 Jan 2019; revised received: 10 May 2018; accepted: 15 May 2019

vulnerable to oxidative stress induced damage[6]. Oxidative stress mediated hepatotoxicity occurs either by direct attack of ROS on essential biomolecules and mDNA of the hepatocytes or by activation of redox sensitive transcription factors such as nuclear factor κB, thus generating cytotoxic and pro-inflammatory mediators by hepatic macrophages[7]. Inflammatory cytokines and chemokines cause recruitment of monocytes and neutrophils in the liver which augment ROS production to accelerate hepatic injury[8]. Deleterious effects of ROS on hepatocytes include lipid peroxidation of membranes, protein oxidation, glutathione depletion, increased membrane permeability and damage to the mDNA that result in necrosis of hepatocytes[9]. Lipid peroxidation products such as malondialdehyde, 4-hydroxy-2-nonenal and 8-isoprostane are useful plasma/tissue markers to detect oxidative stress[10]. Whereas oxidative damage to the liver is characterized by elevated serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyl transpeptidase (γGT) levels[11]. Decreased serum albumin, total glutathione content and glutathione containing antioxidant enzymes have also been documented to be associated with hepatotoxicity[12].

The aim of current study was to determine the development of cisplatin induced oxidative stress followed by evaluation of hepatotoxicity in laboratory animals.

MATERIAL AND METHODS

The present project design was randomized controlled trial. It was conducted at Physiology department, Army Medical College, Rawalpindi in cooperation with National Institute of Health (NIH) Islamabad. The study period comprises of 18 months from October 2014 to April 2016. Experiment was performed on 60 male Sprague Dawley rats which were obtained from NIH, Islamabad. The age and weight of animals ranged between 80-90 days and 200-300 grams respectively. Rats were housed in a separate ventilated room with controlled environment of 12 hours light/dark cycle and 22 ± 3°C temperature. They had free access to standard pallet feed and water ad libitum. Animals were randomly assigned into two groups with thirty (n=30) rats each. Group I (control) did not receive any treatment however to balance the stress level with group II, they were given an intraperitoneal injection of normal saline 10ml/kg body weight twice a week for the period of 4 weeks whereas group II (cisplatin) were administered an intraperitoneal injection of cisplatin 2mg/kg body weight twice a week for the period of 4 weeks.

After completion of 4 weeks treatment period, the animals were anaesthetized using pentobarbital and then euthanized by cervical dislocation. Three ml blood sample was obtained by direct piercing of the heart and stored in EDTA containing test tubes at -80°C. Cold centrifugation of blood sample was done at 4°C and 4000 rpm for 15 minutes to obtain the serum. Serum was used for estimation of 8-isoprostane by ELISA using Cayman 8-isoprostane essay kit on Stat Fax® 2100 Microplate Reader, and for estimation of ALT, AST and albumin levels by using commercial kits on automated clinical chemistry analyzer (Vitalab Selectra E). After blood sample collection, dissection of animals were carried out by giving midline incision in the abdomen. Tissues were incised in layers and liver was identified as a four lobed organ suspended just under the diaphragm. It was carefully dissected out and was washed thoroughly with ice cold 1 x PBS containing 0.16 mg/ml of heparin to prevent coagulation. After drying, part of liver tissue was sliced, weighed and homogenized by using a glass pestle in ice-cold medium containing 50 mmol Tris-HCl and pH 7.4 to give rise 10% (w/v) homogenate. The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was pipetted out and used for quantitative measurement of total glutathione by ELISA using OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit on Stat Fax® 2100 Microplate Reader.

Data were analyzed using IBM (International Business Machine) SPSS (Statistical Package for the Social Sciences) version 23. Numerical

variables were presented as mean ± standard deviation (SD). To determine the difference among two groups, Independent-Samples t-test was applied. A *p*-value of <0.05 was considered statistically significant.

RESULTS

The mean serum 8-isoprostane level in group I was 18.31 ± 3.35 pg/ml while in group II it was 67.9 ± 8.14 pg/ml. The levels were significantly raised (p<0.001) in group II with comparison to the group I (table). The mean total glutathione levels in liver tissue homogenate of group I was 4.29 ± 0.42 µmol/L while in group II it was 1.92 ± 0.28 µmol/L. The values were significantly lower (p<0.001) in group II as compared to group I (table). The mean serum albumin in group I was 4.11 ± 0.26 g/dl while in group II it was 2.12 ± 0.25 g/dl. The values were significantly lower (p<0.001) in group II as compared to group I (table). The mean serum ALT and AST in group I were 36.93 ± 4.72 IU/L and 124.2 ± 12.75 IU/L respectively whereas in group II values were 87.17 ± 6.47 IU/L and 357.7 ± 19.37 IU/L respectively. The levels were significantly raised (p<0.001) in group II with comparison to the group I (figure).

Table: Comparison of 8-isoprostane, total glutathione and albumin between group I and II.

Variables	Study Groups		*p*-value
	Group-I	Group-II	
8-isoprostane (pg/ml)	18.31 ± 3.35	67.9 ± 8.14	<0.001*
Total glutathione (µmol/L)	4.29 ± 0.42	1.92 ± 0.28	<0.001*
Albumin (g/dl)	4.11 ± 0.26	2.12 ± 0.25	<0.001*

*p-value significant (<0.05)

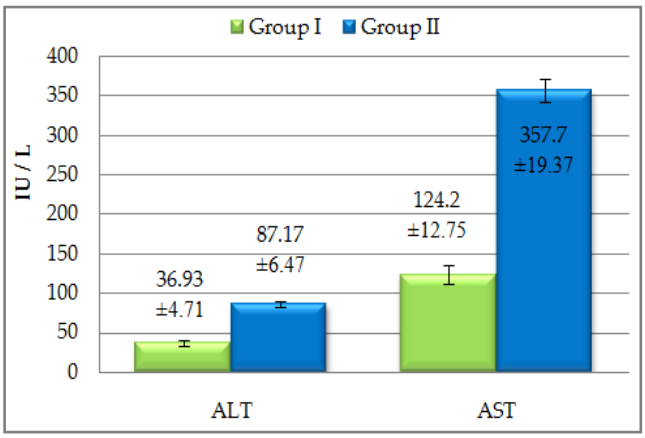

Figure: Comparison of serum ALT and AST between group I and II.

DISCUSSION

The current study was conducted to investigate the toxic effects of cisplatin treatment on healthy liver tissue. There are evidences in the literature conclusive of harmful effects of cisplatin chemotherapy to the various normal tissues of the body including kidney, pancreas, bone marrow, liver and blood vessels. These secondary injuries are attributed to the development of oxidative stress, a detrimental consequence of cisplatin chemotherapy[13,14]. After entering the normal cells, cisplatin preferentially accumulates in the mitochondria where it targets mDNA to hamper cellular respiration which results in excess production of ROS. Polyunsaturated fatty acids are the primary targets for ROS and lipid peroxidation of membranes of cellular and subcellular organelle is the fundamental mechanism that results in severe impairment of normal cellular functioning[13]. The current study revealed significantly increased (p<0.001) 8-isoprostane (a byproduct of lipid peroxidation) levels in cisplatin group as compared to the control which is suggestive of development of oxidative stress in group II. In 2016, Zimet *et al* investigated the effect of elevated concentrations of mine gases (CO_2, CO) and dust on oxidative stress among individuals working in coal mines. They found significantly increased (p<0.001) 8-isoprostane levels in exhaled breath condensate of coal mine workers as compared to control[15]. In 2006, Konishi *et al* evaluated the effect of oxidative stress on plasma 8-isoprostane levels among the patients of non-alcoholic fatty liver disease. Their results revealed significantly increased (p<0.001) 8-isoprostane levels in the subjects of non-alcoholic fatty liver

disease as compared to the controls[16]. The results of above mentioned studies are comparable to our study whereby we also found significantly raised 8-isoprostane levels in cisplatin group.

Under physiological conditions, hepatic metabolism is a major source of ROS production which is balanced by a similar rate of their consumption by various antioxidants in the body. Cisplatin chemotherapy also destabilizes liver's antioxidant defense mechanism by reducing the levels of total glutathione content and glutathione containing antioxidant enzyme (glutathione peroxidase) in the hepatocytes[17]. Glutathione can directly scavenge hydroxyl radical and superoxide anion and indirectly serves as a cofactor for the enzyme glutathione peroxidase that is involved in the metabolism of hydrogen peroxide and lipid peroxides. Decreased hepatocellular glutathione content shifts redox balance towards oxidative stress[18]. In 2006, Mansour *et al* investigated the effect of cisplatin treatment on various parameters of hepatotoxicity in rats. They found significantly decreased (p<0.001) glutathione peroxidase enzymes and total glutathione content in liver tissue of cisplatin treated group as compared to the control which is comparable to the present study[19].

The levels of serum ALT, AST and albumin recorded in the control group of current study were consistent with the levels in healthy Sprague Dawley rats as documented in the literature[20]. Because ALT and AST are normally located in the cytoplasm, clinical evidence of oxidative stress induced hepatic injury has been demonstrated by elevated levels of these enzymes in the serum. The injury is attributed to the wrecked structural integrity of the hepatocytes membrane due to lipid peroxidation[21]. In 2017, Karale *et al* evaluated the effect of cisplatin on oxidative stress and hepatotoxicity in rats. They documented significantly raised (p<0.001) serum ALT and AST levels in the cisplatin treated group of rats as compared to the control. These results are in accordance to our study however, their study differs in the treatment protocol as they administered single high dose intraperitoneal injection of cisplatin (7.5 mg/kg body weight) to produce hepatotoxicity[22]. In 2018, Byun *et al* conducted a study to investigate the effect of oxidative stress on liver tissue. The treatment protocol of their study was different from current study in that they induced oxidative stress by injecting single dose intraperitoneal carbon tetrachloride to the rats. They found significantly raised (p<0.001) serum ALT and AST levels in carbon tetrachloride treated group as compared to control which is analogous to the present study[20]. In 2002, Zicca *et al* evaluated the effect of cisplatin administration on hepatotoxicity. The author and coworkers documented similar findings of significantly decreased (p<0.001) serum albumin levels in cisplatin treated group as compared to the control group as found in current study[23].

Based upon findings of current study, it is recommended that potent antioxidant substances may be co-administered along with cisplatin which would serve as scavenger of ROS, hence prevent undesirable oxidative stress induced damage to the liver and improve quality of life among survivors of cisplatin chemotherapy.

CONCLUSION

Cisplatin treatment has caused hepatotoxicity by increased production of reactive oxygen species in male Sprague Dawley rats.

ACKNOWLEDGEMENT

Authors express gratitude to Dr Yasir Farooq and Dr Shoaib Bin Aleem, Department of Physiology, Army Medical College, Rawalpindi for their unconditional assistance & feedback during all phases of research and to Veterinarian Dr Hussain Ali, National Institute of Health, Islamabad for his cooperation and help in procurement and handling of animals.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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