POSSIBLE AMELIORATIVE ROLE OF LOW DOSE OF RADIATION AGAINST CISPLATIN INDUCED OXIDATIVE STRESS AND TISSUE DAMAGE IN MALE RATS

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ABSTRACT: The present study describes antioxidant effect of low dose of radiation against cisplatin induced toxicity in rats. Oxidative stress was induced in rat by a single dose of cisplatin (10 mg/kg body weight I.P), 24 hr post- cisplatin treatment, the animals exposed to 0.3 Gy single dose of gamma ray. The effect of treatments in influencing the oxidative stress as well as biochemical cisplatin changes on brain, kidney and testis were studied. The data showed significant increase in serum urea, creatinine, creatine kinase isoenzymes (CKBB) and tumor necrosis factor (TNF-α) as well as in tissue MDA levels in animals treated with cisplatin while this effects were attenuated by radiation exposure. Moreover, treatment with cisplatin caused a significant decrease in serum testosterone and tissues SOD activities, which was shown to be reversed by low dose of radiation treatment. Treatment with low dose of radiation significantly reduced the oxidative stress effects induced by cisplatin administration. It can be concluded that exposed animals to a low dose of radiation after cisplatin treatment can speed the recovery of the body during the chemotherapeutic treatment.

KEYWORDS: Oxidative stress, Cisplatin, Brain, Kidneys, Testes, Low dose of radiation.

INTRODUCTION

Cisplatin is quite efficient in the treatment of testicular and ovarian cancers and is also excessively utilized for treating bladder, cervical, head and neck, oesophageal and small cell lung cancer. Despite its success, cisplatin has several disadvantages, which include severe side effects including nephrotoxicity and neurotoxicity (Giaccone, 2000). These toxic effects limit the dose that can be applied to patients. Although cisplatin is widely used in clinic, its application is still limited to a relatively narrow range of tumour types. Some tumours such as colorectal and non-small cell lung cancers have intrinsic resistance to cisplatin, while others such as ovarian or small cell lung cancer increase acquired resistance after the initial treatment (Fuertes et al., 2003). It has emerged from the studies on the molecular mechanisms of antitumor action of cisplatin, that the biochemical mechanisms of cisplatin cytotoxicity involve the binding of the drug to DNA and non-DNA targets and further induction of cell death through apoptosis, necrosis or both within the heterogeneous population of cells that forms a tumour mass (Cvitkovic, 1998). Cisplatin is known to accumulate in mitochondria of renal tubular epithelial cells together with ROS; renal tubular cell
mitochondrial dysfunction is also important in cisplatin-induced nephrotoxicity by decreasing the activity of antioxidant enzymes and by depleting intracellular concentrations of GSH (Kawai & Gemba, 2007). A depletion of GSH can lead to increased lipid peroxidation with concomitant changes in membrane permeability and cellular damage, but an increase in GSH level enhances antioxidant protection and cellular function.

Low doses of ionizing radiation induce various effects, including radio-adaptive response (Ikushima et al., 1996), an increase the antioxidant activity against environmental chemical pollutant (Yamaoka et al., 2004 and Gharib et al., 2012), activation of immune function (Nogami et al., 1993) and enhancement of resistance to high-dose radiation (Yonezawa et al., 1996). Kojima et al. (2004) reported that low dose of radiation significantly increased total glutathione levels in organs such as liver, pancreas and brain. In our previous studies, treatment with low dose of gamma rays (0.5 Gy) ameliorate harmful effects induced by TCE taking in consideration the effect of gamma radiation as a stimulant of radical detoxification (Gharib et al., 2012). In the light of above mentioned antioxidant properties of low dose of radiation, this study was passed out to investigate in how extent low dose of radiation can speed the oxidative stress recovery of the body during the chemotherapeutic treatment.

MATERIAL AND METHODS

Animals
Male albino rats weighing approximately 120-150 g were used for this experiment. They were housed in polypropylene cages in an air conditioned room with temperature maintained at 25 °C ± 3 °C, relative humidity of 50 % ± 5 % and 12 h alternating light and dark cycles. The rats were provided with a nutritionally adequate chow diet and drinking water ad libitum throughout the study. Experiments were begun after four-week acclimatization period.

Chemicals
Cisplatin was purchased from Central Drug House (Egypt). The chemicals used in this experiment were obtained from Sigma Chemical (USA). Kits used in this experiments were purchased from Bio-Diagnostics (UK).

Radiation process
A single dose whole body irradiation (0.3 Gy) was performed with rats, using gamma rays by Cesium 137 irradiation unit, National Center for Radiation Research and Technology (NCRRT), with the dose rate 0.74589 rad/ sec. The gamma cesium cell was calibrated by alanine dosimetry relative to a primary standard. Correction were made daily for humidity, temperature, and barometric pressure.

Experimental design
Adult male albino rats were divided into 4 groups of 6 rats each and treated as follows: Control group, received distilled water. Cisplatin group, received freshly dissolved cisplatin in 1 ml distilled water at a dose of 10 mg/ kg body weight (I.P); low radiation dose group, animals were exposed to a single low gamma radiation dose of 0.3 Gy and Cisplatin+ low radiation dose group, animals were administered with Cisplatin. 24 hr post-cisplatin administration the animals were exposed to a
single low dose of gamma rays (0.3 Gy). The dose of cisplatin was decided on the basis of Máthé et al. (2006). The animals were given one-day rest, killed by decapitation and the blood samples were collected. Brain, kidneys and testes were excised, homogenized in ice-cold salin and utilized for various oxidative stress and biochemical analysis.

**Biochemical analysis**

Blood samples were centrifuged at 3000 rpm for 15 min., clear serum were collected and stored in a refrigerator. The concentrations of urea and creatinine in serum were analyzed. Testosterone hormone in serum were assayed in duplicate using a double antibody kit (Diagnostic system Lab, Webstet, Tx, USA) with a minimum detection of 0.05/ng/ml and a range up to 25ng/ml. In addition CK BB iso-enzyme was measured using immunoassay kit which allows *in vitro* quantitative determination. The microtitr plate provided in this kit has been pre-coated with an antibody specific to CK BB. The concentration of CK BB in CK BB, biotin- conjugated Avidin will exhibit a change in colour. The enzymatic substrate reaction is terminated by the addition of sulphuric acid solution and color change is measured spectrophotometrically at wave length 450nm ± 2nm (eiaab kit). serum tumor necrosis factor (TNF- α) were determined using Elisa (quantikin R & D system, USA) according to the manufactures’ instructions (Maskos et al., 1998) and were carried out at the Central Laboratory, Radioisotope Dept., AEA, Giza, Egypt. Brain, kidneys and testes were minced and homogenized (10 % w/ v) in ice-cold normal saline solution. The homogenate was centrifuged at 3,000 rpm for 15-20 min at 4 °C. The resultant supernatant was used for various biochemical assays. MDA and SOD levels were measured. Urea was estimated by kits according to the method of Halled and Cook (1971). Creatinine level was measured according to the method of Henery (1974). In addition MDA concentration was measured by using Yoshioka, *et al.* (1979). SOD activity was estimated by the method of Marklund and Marklund (1974). All biochemical assays were performed with a Helios Thermo-Spectronic spectrophotometer (Thermo Spectronic, UK).

**Statistical Analysis:**

To assess the significant level of influence caused by low dose of radiation in cisplatine administrated rats, one way analysis of variances (ANOVA) followed by Tukey's multiple comparison test was used. Data are representative of 6 independent experiments carried out in triplicate. Statistical analysis was performed by using Graph-Pad software, San Diago, CA, USA) Differences were considered statistically significant when the P value was less than 0.05.

**RESULTS**

Table (1 to 4) reported the effect of cisplatin alone and the ameliorating effects of low dose of radiation individually on some biochemical variables in brain, kidneys and testes of various groups.

Table (1): Effect of low dose of radiation (0.3 Gy) on some serum biochemical variables in cisplatin exposed rats.
Data in table (1) shows that there are significant increases in serum urea, creatinine, CKBB and TNF-α levels that recorded (P<0.001, 188%), (P<0.0001, 174%), (P<0.0001, 63%) and (P<0.0001, 128%) respectively in cisplatin exposed rats as compared with normal control rats. Treatment with low dose of radiation significantly suppressed the increased levels of urea (P<0.0001, -63%), creatinine (P<0.0001, -1%), CKBB (P<0.0001, -33%) and TNF-α (P<0.0001, -33%) when compared with cisplatin treated animals. On the other hand, cisplatin-induced depletion in testosterone level (P<0.0001, -60%) that was significantly prevented by treatment with low dose of radiation at a dose of 0.3 Gy when compared with cisplatin treated animals.

Table (2): Antioxidant potential of low dose of radiation on lipid peroxidation and SOD activity in brain of cisplatin exposed rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Cisplatin</th>
<th>Low radiation dose</th>
<th>Cisplatin + low radiation dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µM/g tissue)</td>
<td>111.1±17.34</td>
<td>163.3±31.73*</td>
<td>121.9±18.67(°)</td>
<td>134.4±28.55</td>
</tr>
<tr>
<td>SOD (unit/ml of tissue)</td>
<td>4.264±0.7263</td>
<td>2.650±0.5320*</td>
<td>4.033±0.6831(°)</td>
<td>4.783±0.9867(°)</td>
</tr>
</tbody>
</table>

Legend as in table (1).

**Brain oxidative stress and antioxidant defense variables:**
Effect of cisplatin alone and ameliorating effect of low dose of gamma radiation at a dose of 0.3 Gy individually after cisplatin administration on MDA and SOD activities in brain of various groups were assessed and presented in Table 2. The level of malonaldehyde showed non-significant increase in irradiated groups as compared with the normal control level. Cisplatin at a dose of 10 mg/kg body weight caused significant increase in the level of brain MDA. This increase was recorded (p<0.05, 47%) accompanied with a significant decrease in SOD activity (p<0.05, -38%), in comparison to control group. However, treatment with low dose of radiation after cisplatin administration caused a non-significant change in MDA level when compared with both control and cisplatin induced group. A significant increase (p<0.001, 80.5%) in the activity of SOD was observed after the treatment with low dose of gamma radiation (0.3 Gy), as compared with cisplatin exposed group.

Table (3): Antioxidant potential of low dose of radiation on lipid peroxidation and SOD activity in kidneys of cisplatin exposed rats.
Parameters | Control | Cisplatin | Low radiation dose | Cisplatin + low radiation dose |
<table>
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<tbody>
<tr>
<td>MDA (µM/g tissue)</td>
<td>112.8 ± 16.68</td>
<td>145.6 ± 15.91*</td>
<td>107.5 ± 7.933(*)</td>
<td>108.8 ± 25.42(*)</td>
</tr>
<tr>
<td>SOD (unit/ml of tissue)</td>
<td>6.195 ± 1.373</td>
<td>3.030 ± 0.4705*</td>
<td>4.622 ± 0.7176*($)</td>
<td>4.464 ± 0.9212*</td>
</tr>
</tbody>
</table>

Legend as in table (1).

Renal oxidative stress and antioxidant defense variables:
Effect of cisplatin alone and ameliorating effect of low dose of gamma irradiation individually after cisplatin administration on MDA and antioxidant related parameters in kidneys of various groups were assessed and presented in Table 3.
The level of MDA was significantly higher (p<0.05, 29%) in cisplatin-treated animals than that of normal untreated rats. Whereas, significant decrease (p<0.001, -51%) in renal SOD activity, of rats were observed in cisplatin treated animals as compared with control group. After the treatment with low dose of gamma rays at a dose of 0.3 Gy, a significant decrease (p<0.05, -25%) in the level of MDA was observed in comparison to cisplatin-treated group. In addition SOD activity in cisplatin-treated group showed insignificant increase due to radiation exposure.

Table (4): Antioxidant potential of low dose of radiation on lipid peroxidation and SOD activity in testes of cisplatin exposed rats.

Table (4) represent the effect of low dose of radiation on cisplatin induced oxidative stress in testes tissue. Cisplatin at a dose of 10 mg/kg body weight caused significant increase in the level of testes MDA. This increase was recorded (p<0.001, 100%) accompanied with a significant decrease in SOD activity (p<0.05, -46%), in comparison to control group. However, treatment with low dose of radiation after cisplatin administration showed a significant decrease in MDA level when compared with cisplatin induced group. The MDA decrease recorded -64% (p<0.001). However, a significant increase (p<0.001, 105.5%) in the activity of SOD was observed in (Cisplatin + low radiation dose) group, as compared with cisplatin exposed group.

DISCUSSION
Cisplatin, a heavy metal complex, is an effective chemotherapeutic agent for a wide variety of tumors (Park et al., 2009). Despite its success, cisplatin has several disadvantages, which include severe side effects including nephrotoxicity, neurotoxicity as well as testicular damage (Salem et al., 2012). Cisplatin administration induces overproduction of reactive oxygen species (ROS) such as hydrogen peroxide and hydroxyl radicals, which abstract a hydrogen atom from polyunsaturated fatty acids (Kadikoylu et al., 2004) and depletes the cellular antioxidant capacity (Giaccone, 2000).
Oxidative evaluation:
Oxidative stress, a condition of an imbalance between free radicals and antioxidant defense system, is an important factor in the pathogenesis system that has high content of polyunsaturated membrane lipid (Acar et al., 2012). Recent study indicated that cisplatin led to neurotoxic effects in human and animals via induction of lipid peroxidation (Kamisli et al., 2013). Our present work showed that cisplatin produced a significant increase in serum CKBB and MDA levels that induced lipid peroxidation in the brain tissues accompanied with suppression in SOD activity. According to Aksenova et al. (1999) the results of CK BB expression may be an early indicator of oxidative stress in neurons. On the other hand, study of Qu et al. (2012) and Kamisli et al., (2013), explained the increase in MDA and the SOD activity decrement may be due to cisplatin and other complex producing oxidative changes in the nervous system.

On the other hand, therapeutic effects of cisplatin based on the interaction with DNA in the cell, preventing proliferation and inducing apoptosis in tumor cells. In fact, renal insufficiency is the major and most severe form of toxicity associated with use of cisplatin as a chemotherapeutic agent. Renal toxicity, which is manifested by an increase in serum urea and creatinine, can result both from doses that are higher than recommended and an accumulation of cisplatin in the body (Mukhopadhyay et al., 2011). According to Mukhopadhyay et al. (2011); cisplatin induced mitochondrial ROS generation triggered inflammatory response, cell death and kidney dysfunction/nephropathy. Cisplatin triggers oxidative stress in the mitochondria of kidney proximal tubule and endothelial cells which is followed by a secondary wave of ROS/RNS (reactive nitrogen species) generation, deterioration response (Ognjanovic et al., 2012). Moreover, cisplatin induces serious inflammatory changes and enhances renal expression TNF-α (Ramesh and Reeves, 2004). TNF-α induces apoptosis that produces reactive oxygen species and coordinate the activation of large network of chemokines and cytokines in kidney (Ramesh and Reeves, 2002). In addition several studies demonstrated that cisplatin induced acute nephrotoxicity is mediated by impaired activity of SOD as well as an increase in renal lipid peroxidation (Gonzalez et al., 2004). It has been suggested that cisplatin is able to generate reactive oxygen species by inducing glucose-6-phosphate dehydrogenase and hexokinase activity and inhibit the activity of antioxidant enzyme in renal tissue such as SOD, CAT, GSH-Px (Naziroglu et al., 2004). In addition, according to Atasayar et al. (2009) the increase in MDA level in the cisplatin treated group are also responsible for the renal oxidative stress.

In the present study, testicular damage induced by cisplatin treatment was characterized by decrease in serum testosterone level and significant reduction in testis SOD activity as well as elevated MDA production compared with the untreated control animals. The decreased serum testosterone level in cisplatin treated rats could be attributed to the impairment of Leydig cells (Ilbey et al., 2009). It has also been reported that the cisplatin -induced changes in testosterone are associated with decreased numbers of LH receptors on Leydig cells (Maines et al., 1990). Besides, Khaki et al. (2009) reported that spermatozoa are highly susceptible to damage by excessive concentration of ROS in the cisplatin treated rats due to the high content of
polyunsaturated fatty acids within their plasma membrane. Lipid peroxidation destroys the structure of spermatozoa with loss of its motility and impairment of spermatogenesis (Sharma and Agarwal, 1996).

**Antioxidant evaluation:**
In the biological system, one of the active sources of oxygen radicals is the ionizing radiation. Cells can be injured, and even killed under the most severe condition of radiation exposure, when the content of reactive oxygen species (ROS) gets uncontrolled by the cellular antioxidant (Pathak et al., 2007). On the other hand, low-dose radiation, may have different beneficial effects, it may decrease the cancer risk according to “linear hypothesis” and increase the immunity of the cells (Kojima et al., 2004). The present study has investigated the efficacy of low dose of radiation exposure which is probably induced adaptive protection. The delayed and temporary adaptive protection at low doses involves damage prevention, damage repair and immune responses. They appear to operate primarily against DNA damage from non-radiation sources. According to the results recorded by Pathak et al., (2007), the antioxidant enzyme activities and the reduced glutathione (GSH) content increased significantly almost in general to the generation of lipid peroxides after exposure to whole body low dose γ- radiation (0.5 Gy) (Yamaoka et al., 1991). This investigation was in agreement with the present work since exposure to 0.3 Gy caused a non significant increase in lipid peroxidation in brain tissue. In addition, the increase in GSH levels may be due to the activation of protective response in the organs to counteract the excessive formation of ROS. Moreover, it was interesting that this effect was observed over a range of 0.25- 0.5 Gy Kawakita et al. (2003). It is evident from the results of the present work that exposing to low dose of radiation after cisplatin administration protected animals from toxic effects of cisplatin in general and oxidative stress in particular. This study is in confirmation with the previous report suggested the preventive effects of low dose of radiation against exposure to chemical compound (Gharib et al., 2012). In addition, according to Yamaoka et al. (2004) low- dose irradiation induces various stimulating outcomes such as increase in resistance toxicity, enhancement of immune function; these effects may be related to the induction of antioxidant enzymes and the degeneration of $O_2^-$, $H_2O_2$ and *OH is inhibited by low dose irradiation.

**CONCLUSION**
These findings are of immense support in understanding the possible role of low radiation dose in rendering protection against cisplatin inflicted damages to Brain, Kidneys and testes tissues. Additional studies using different low dose of gamma radiation will be needed to address this issue.

**REFERENCES**


