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Cisplatin or carboplatin caused suppression in anti–oxidant enzyme defense system in liver, kidney and testis of male albino rats

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Abstract

The present study aimed to investigate the possible interference of cisplatin or carboplatin on anti-oxidant enzyme defense system in liver, kidney and testis of male rats. Rats were divided into three groups consisting of eight animals in each group. The rats in the first group were served as control and received 0.9% of normal saline only. The rats in the second group were received cisplatin (3 mg/kg body wt) only. The rats in the third group were received carboplatin (10 mg/kg body wt) respectively. Injections were given intra-peritoneally to rats on 1st, 3rd and 5th day of experimentation. On 45th day of experiment, animals were sacrificed by cervical dislocation. GST activity in the cytosol fraction of the tissues were assayed by using 1-chloro-2, 4-dinitro benzene at 340 nm. Catalase activities in the tissues were assayed by Chance and Machly method. The levels of lipid peroxidation in the liver, kidney and testes were measured in terms of malondialdehyde content and determined by using the thiobarbituric acid reagent. From the study it may be concluded that male rats treated with platinum compounds causes reduction in activities of antioxidant enzyme system with an increase in products of lipid peroxidation in various tissues of rats which ultimately leads to cellular peroxidation and damage in the tissues

Keywords: Cisplatin, Carboplatin, Glutathione-S-transferase, Catalase, lipid peroxidation, liver, kidney, testes, Rats.

Introduction:

Despite its excellent anticancer activity, the clinical use of cisplatin or carboplatin is often limited by its undesirable side effects such as severe hepatotoxicity and nephrotoxicity. Oxidative stress may induces cellular damage by forming reactive oxygen species and activities of antioxidant enzymes such as catalase, glutathione-S-transferase could change in response to the amount of reactive oxygen species produced in the cell.[1]

Glutathione-S-transferases are a group of multifunctional enzymes involved in the detoxification of a wide spectrum of compounds. These enzymes are considered to be particularly important for detoxifying many xenobiotics including cytotoxic drugs, carcinogenic compounds and reactive intermediates. Glutathione-S-transferases play a prominent role in antioxidant defense mechanisms by their ubiquitous distribution in a wide range of species including mammals, birds, insects, plants and bacteria.[2]

Catalase is one of the major antioxidant enzymes, present in the peroxisomes of the cells. It plays a vital role in removing peroxy radicals and has been able to protect cells from toxic injury. However catalase activity is known to alter under certain physiological and pathological conditions. The increased activity level of glutathione-S-transferase and catalase will eliminate highly reactive free radicals and serve as a defense mechanism. Where as, the cell with deficient catalase is expected to have a reduced ability to detoxify oxidative stress products .[3]

Levels of malondialdehyde (MDA) in the tissue are used as an index of rate of lipid peroxidation. MDA is one of the products of lipid peroxidation, which seems to be synthesized in relatively constant proportion to lipid peroxidation and it is considered as a good indicator of the rate of lipid peroxidation. Since it is a major oxidation product of peroxidized polyunsaturated fatty acids (PUFA's) of the cell membrane, MDA has been demonstrated to cause cross linking and polymerization of membrane components and may contribute to genotoxic, mutagenic and carcinogenic effects.[4]

In the present study, malondialdehyde products were estimated in various tissues of rats to assess the extent of lipid peroxidation caused by platinum compounds. The MDA

content in tissues was estimated by TBA method, which is widely used for detection of the occurrence of lipid peroxidation.[5]

Materials and Methods:

Animals

Healthy adult male wistar rats of same age group (70±5 Days) were selected for the present study. Animals were housed in an air conditioned animal house facility at 26±1^{o} C, with a relative humidity of 75%, under a controlled 12 h light/dark cycle. The rats were reared on a standard pellet diet (HLL Animal Feed, Bangalore, India) and tap water adlibitum.

Test chemicals

Cisplatin was purchased from Sigma chemicals, St.Louis Co., MO, USA. This compound was dissolved in 0.9% normal saline to obtain the final concentration of the 3 mg/kg body wt. of the animal. Carboplatin was purchased from Sigma chemicals, St.Louis Co., MO, USA. This compound was dissolved in 0.9% normal saline to obtain the final concentration of the 10 mg/kg body wt. of the animal.

Experimental Design

The rats were divided into three groups consisting of eight animals in each group. The rats in the first group were served as control and received 0.9% of normal saline only. The rats in the second group were received cisplatin (3 mg/kg body wt) only. The rats in the third group were received carboplatin (10 mg/kg body wt). Injections were given intra-peritoneally to rats on 1^{st,} 3rd and 5th day of experimentation. On 45th day of experiment animals were sacrificed by cervical dislocation.

Glutathione S- tr ansferase (E.C: 2.5.11.8) activity

GST activity in the cytosol fraction of the tissues was assayed by using 1-chloro-2, 4-dinitro benzene (CDNB) (at 340 nm) as described by Habig *et al*. (1974).[6]

The tissues (Liver, Kidney and Testes) from control and experimental rats were isolated and homogenized (5% W/V) in 50 mM Tris HCl (pH 7.4), containing 1 mM EDTA and 1 mM DDC (diethy1 dithiocarbamate). The homogenized samples were centrifuged at 105,000 x g for 60 minutes at 4^{0}C.The supernatant fraction was used for the assay of an enzyme activity.

The reaction mixture in a final volume of 3.0 ml contained: 150 mM phosphate buffer (pH 7.5), 1 mM CDNB, 5 mM glutathione (GSH) and an appropriate amount of enzyme protein. The reaction was initiated by the addition of GSH and incubated at 37°C. The formation of a thioether by the conjugation of CDNB to GSH was monitored at 340 nm in a spectrophotometer (Hitachi model U, 2001). Thioether concentration was determined from the slopes of initial reaction rates. A molar extinction coefficient 9.6 x 10^{-3} cm^{-1} was used in the calculations.

The GST activity was expressed as µ moles of thioether formed/mg protein/h, where one unit of enzyme activity is defined as one µ moles of thioether formed/mg protein/min.

Catalase (E.C: 1.11.1.6) assay

Catalase activity in the tissues was assayed by the method of Chance and Machly (1955).[7]

Tissues (liver, kidney and testis) from control and experimental rats were isolated and homogenized (5% W/V) in 50 mM Phosphate buffer (pH 7.0). The homogenized sample was centrifuged at 105,000 x g for 60 minutes at 4^{0}C. The clear supernatant (cytosol) fraction was used for the assay of enzyme activity.

The reaction mixture in a final volume of 2.5 ml contained: 0.05 M phosphate buffer (pH 7.0), and appropriate amount of enzyme protein. The reaction was initiated by the addition of 19 mM H_2O_2. The decomposition of H_2O_2 was followed directly by measuring the decrease in absorbance at 240 nm in spectrophotometer (Hitachi model U, 2001).

The Catalase activity was expressed as μ moles of H_2O_2 metabolized/mg protein/min.

Determination of Lipid Peroxidation

The levels of lipid peroxidation in the liver, kidney and testes were measured in terms of malondialdehyde (MDA; a product of lipid peroxidation) content and determined by using the thiobarbituric acid (TBA) reagent. The reactivity of TBA is determined with minor modifications of the method adopted by Hiroshi *et al.* (1979).[8]

Tissues were homogenized (10% W/V) in 1.15% potassium chloride solution and centrifuged at 5000 rpm for 10 minutes. To 2.5 ml of supernatant, 0.5 ml of saline (0.9% sodium chloride), 1.0 ml of (20% W/V) trichloroacetic acid (TCA) was added. The contents were centrifuged for 20 minutes on a refrigerated centrifuge at 4000 x g at 4^0C.

To 1.0 ml of supernatant, 0.25 ml of TBA reagent was added and the contents were incubated at 95°C for 1 h. One ml of n-butanol was added to it. After thorough mixing the contents were centrifuged for 15 minutes at 4000 x g in a refrigerated centrifuge. The organic layer was transferred into a clear tube and its absorbance was measured at 532 nm. The rate of lipid peroxidation was expressed as μ moles of malondialdehyde formed/gm wet weight of tissue/h.

Results:

In the present study platinum based anticancer drugs induced remarkable changes in anti-oxidant enzymes such as glutathione-S-transferase (GST) and catalase in various tissues (liver, kidney, testes) of male albino rats.

The activity levels of GST and catalase were decreased significantly in liver tissue of rats exposed to platinum-based anticancer drugs when compared with the control rats (Table 1). Whereas the levels of lipid peroxidation products were significantly increased in the liver tissue of rats exposed to platinum-based anticancer drugs when compared with the control rats (Table 1).

The activity level of catalase was decreased significantly in kidney of rats exposed to platinum compounds, whereas the activity levels of glutathione-S-transferase in these tissues have recorded non-significant change when compared with the control rats**.** But the levels of lipid peroxidation products in these tissues were increased significantly when compared with the control rats (Table 2).

A significant decrease in the activity levels of glutathione-S-transferase and catalase were observed in testis of rats exposed to platinum compounds when compared with the control rats. Whereas the levels of lipid peroxidation products were increased significantly in the testes of rats exposed to platinum-based anticancer drugs when compared with the control rats (Table 3).

Discussion:

Oxidative stress has been defined as a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defenses, which may lead to tissue injury. It is a result of one of three factors- an increase in reactive oxygen species, an impairment of antioxidant defense systems or insufficient capacity to repair oxidative damage. Under physiological conditions a balance exists between the generation of reactive oxygen species and the level of endogenous antioxidants, which serve to protect tissue from oxidative damage. Disruption of this balance, either through increased production of ROS or decreased levels of antioxidants, results in a condition referred to as "oxidative stress".[9,10]

Many compounds are capable of causing an increase in the generation of various reactive oxygen species (or) free radicals such as superoxide ($O_2^{-\bullet}$), hydroxyl (OH), hydrogen peroxide (H_2O_2), semiquinone (Q), singlet oxygen (O_2^-), nitric oxide (NO), peroxy

Table 1: Effect of cisplatin or carboplatin on the changes in the activity levels of catalase and Glutathione S-transferase and levels of lipid peroxidation in the liver of rats.

Enzyme	Control	Cisplatin	Carboplatin	ANOVA
Catalase (n moles of H_2O_2 metabolized/mg protein/min)	78.28±3.32	59.4**±1.33 (-24.11)	72.11*±5.77 (-7.88)	$F_{2,21}$=48.265 P<0.0001
Glutathione S-transferase (μ moles of thioether formed/mg protein/min)	1.19±0.11	0.92**±0.11 (-22.68)	1.05*±0.06 (-11.76)	$F_{2,21}$=308.34 P<0.0001
Lipid peroxidation (μ moles of malondialdehyde formed/g wet wt. tissue)	14.76±1.18	18.11**±1.97 (+22.69)	20.14**±1.15 (+36.44)	$F_{2,21}$=26.858 P<0.001

Values are mean ± S.D of eight individuals. Values in parentheses are percent change from control. Values are significantly different at *p<0.05, **p<0.001.

Table 2: Effect of cisplatin or carboplatin on the changes in the activity levels of catalase and Glutathione S-transferase and levels of lipid peroxidation in the kidney of rats.

Enzyme	Control	Cisplatin	Carboplatin	ANOVA
Catalase (n moles of H_2O_2 metabolized/mg protein/min)	62.85± 4.10	35.83*±2.29 (+42.99)	45.43*±3.01 (-27.71)	$F_{2,21}$=144.72 P<0.0001
Glutathione S-transferase (μ moles of thioether formed/mg protein/min)	0.59 ± 0.16	0.47^{ns} ± 0.08 (-20.33)	0.50^{ns} ± 0.05 (-15.25)	$F_{2,21}$=8.959 P=0.0015
Lipid peroxidation (μ moles of malondialdehyde formed/g wet wt.tissue)	9.34± 1.19	12.62*±1.11 (+35.11)	14.34*± 1.52 (+53.53)	$F_{2,21}$=18.184 P<0.0001

Values are mean ± S.D of eight individuals. Values in the parentheses are percent change from control. Values are significantly different at * p<0.0001, ns = not significant.

Table 3: Effect of cisplatin or carboplatin on the changes in the activity levels of catalase and Glutathione S-transferase and levels of lipid peroxidation in the testis of rats.

Enzyme	Control	Cisplatin	Carboplatin	ANOVA
Catalase(n moles of H_2O_2 etabolized/mg protein/min)	47.28± 2.82	36.56**±2.74 (-22.67)	39.99**±2.26 (-15.41)	$F_{2,21}$=34.973 P<0.0001
Glutathione S-transferase (μ moles of thioether formed/mg protein/min)	0.95±0.11	0.71**±0.06 (-25.26)	0.68**±0.07 (-28.42)	$F_{2,21}$=25.575 P<0.0001
Lipid peroxidation (μ moles of malondialdehyde formed/g wet wt. of tissue)	19.72± 1.74	22.04*± 1.16 (+11.76)	23.78**± 2.02 (+20.58)	$F_{2,21}$=11.779 P=0.0004

Values are mean ± S.D of eight individuals. Values in the parentheses are percent change from control. Values are significantly different at *p<0.05,** p<0.001.

nitrite (ONOO-) etc. Free radicals can be defined as chemical species possessing one or more unpaired electrons in their outer orbital. Moreover, these free radicals formed by hemolytic cleavage of covalant bond of a molecule, by the loss of a single electron from a normal molecule or by the addition of a single electron to a normal molecule.[11]

In the present investigation, it was observed that treatment with either cisplatin (3 mg/kg body weight) or carboplatin (10 mg/kg body weight) induced profound alterations in the activity levels of selected anti-oxidant enzymes as well as lipid peroxidaton product levels in various tissues of rats. These results indicate platinum compounds caused oxidative stress in tissues due to increased production of reactive oxygen species with decrease in the activity of antioxidant enzymes such as glutathione-S-transferase and catalase in rats.

It is evident from the data obtained in the present study that the exposure to platinum-compounds has a remarkable effect on the antioxidant enzyme system in liver of experimental rats. Our results are in agreement with previous studies, where also cisplatin administration resulted in increased production of MDA levels with decreased levels of GSH, GSH-Px and catalase in liver of rats.[12]

In the present study, alternations in the anti-oxidant enzyme system in kidney of experimental rats indicating nephrotoxicity (signs of injury) caused by platinum compounds. Our results are in inconsonance with previous reports such as changes in urinary volume, body weight, glutathione status and increase of lipid peroxidation products. Moreover cisplatin is able to generate reactive oxygen species such as superoxide anion and hydroxyl radical and to inhibit the activity of antioxidant enzymes

in renal tissues as well as increase in lipid peroxidation products in kidney.[13, 14]

In the present study, decreased activities of GST, catalase with increased production of MDA was observed in testes of rats exposed to platinum-compounds. Our results are in consonance with previous investigations such as concurrent decrease in the activities of testicular GSH, catalase with elevated levels of MDA in rats. In addition, lipid peroxidation products in the testes were reported to be high for several days after a single dose of cisplatin.[15, 16]

From the above results it is easier to conclude that platinum compounds treatment caused significant reduction in activities of antioxidant enzyme system with an increase in MDA in various tissues of rats. On one hand, increased levels of lipid peroxidation products (MDA) and on the other, decreased activities of antioxidant enzymes namely glutathione-S-transferase and catalase, create severe oxidative stress, which leads to cellular peroxidation and damage in the tissues (liver, kidney and testes). However further studies are needed to evaluate the consequences of platinum-compounds induced generation of reactive oxygen species (ROS) in causation of tissue injury.

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