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CHOLESTEROL, BIOMARKERS AND CANCER THERAPY

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ABSTRACT

Background: Cytotoxic agents contributes an remarkable therapautic features and has a fundamental importance in current cancer chemotherapy. The therapy focuses mainly on minimizing cytotoxicity and therapautic potential of these drugs is dependent on cellular status. Results: Rats administered with 0.4 mg/kg bodywt ip cisplatin daily for 8 weeks showed significant increase in Liver cholesterol content compared to control group however, etoposide therapy at 1mg/kg bodywt ip daily for 8 weeks to rats showed significant decrease in liver cholesterol levels. The cisplatin and etoposide treated kidney showed significant decrease compared to control rats. Antineoplastic agent adriamycin at 1mg/kg bodywt to rats ip, three doses per week and total of 12 doses showed significant changes in body weight and organ weight. The biomarkers of kidney γ-glutamyl transpeptidase (GGT), drug-metabolizing enzyme Cytochrome p450 & b5 and Lipid peroxidation (MDA content) showed significant decrease compared to control counterpart. However, Glutathione-S-Transferase (GST) activity showed significant increase compared to control rats. Conclusion: We hypothesize that at given dose Cisplatin and Etoposide can inhibit the cholesterol synthesis and might control an increase in tumor cell growth. Additionly, an increase in GST and decrease in GGT significant biomarker's of kidney can represent the extent of oxidative stress on the organ.

KEYWORDS: Biomarkers, GGT, GST, Cholesterol, Adriamycin, Cisplatin, Etoposide.

1. INTRODUCTION

Cisplatin and Etoposide are antineoplastic agents long been used for treatment of human malignancies. Cisplatin is platinum-coordinated complex used for treatment of testicular and ovarian cancer. The application of the drug is limited due to its both short term side effects includes emesis, alopecia and long term side effects includes nephrotoxicity.[1] Cisplatin also interferes with mitochondrial function and causes injury. The mechanism of action of cisplatin is that the drug undergoes hydroxylation reaction by cytochrome p450 enzyme system to form hydroxylated complex. The drug also reacts with glutathione and forms GSH-Cisplatin complex which makes the drug more reactive.[2]

Etoposide is a plant derivative of podophyllum pelltatum and it is DNA topoisomerase II inhibitor. Etoposide is used for the treatment of small cell and non-small cell lung cancer, lymphoma and ovarian cancer. The mechanism of action of etoposide is it form's phenoxyl radicals. Reports suggests that these radicals in least form can also produce antioxidant action.[3, 4]

Our previous studies showed significant alterations in Reduced Glutathione (GSH) levels and GSH-related enzymes in hepatic and renal tissue. The morphological changes and SDS-PAGE protein studies also supported the biochemical findings for antioxidants in cisplatin and etoposide treated rats.[5-12]

Inaddition, adriamycin is hydrochloride of doxorubicin (14-hydroxydaunomycin). The drug is used for the treatment of cancer, such as leukaemia. The mechanism of action of adriamycin is that it acts as DNA topoisomerase II inhibitor. Adriamycin intercalates between base pairs of DNA and inhibits DNA-dependent RNA synthesis. It forms quinones and hydroquinones moieties.[13]

Here we report the effect of adriamycin treatment on kidney biomarker's such as GGT, GST and drug metabolizing enzymes like Cytochrome p450 & b5 levels and Lipid peroxidation (MDA)content in rats. Also further we studied cholesterol content in cisplatin, etoposide treated liver, and kidney of rat.

2. MATERIALS AND METHODS

2.1 Animals and Ethical Clearance

Adult male albino rats of Wistar strain were weighing about 220-250g were obtained from Rajudyog biotechnology division Maharashtra, India and was used for study. Animal studies

were carried out upon institutional animal ethical committee approval.

2.2 Drugs & Reagents

Cisplatin, Etoposide and Adriamycin (Doxorubicin) was purchased from Dabur India Ltd. Bovine serum albumin, reduced and oxidized GSH, Trichloroacetic acid (TCA), Thio barbituaric acid (TBA), Butylated hydroxytolune (BHT), L-gammaglutamyl-p-nitro-anilidehydrochloride, sodium azide, Dinitro thio benzoic acid (DNTB), Ferric chloride, Phosphoric acid, Sulphuric acid, NADPH, sodium dithionite, sodium formate were purchased from SRL India. All other chemicals used were of analytical grade.

2.3 Methodology

Cisplatin treated animals were administered with 0.4 mg per kg bodywt i.p daily for a period of 8 weeks and Etoposide treated rats received dose of 1.0 mg per kg bodywt i.p daily for a period of 8 weeks. Adriamycin treatment at 1mg per kg bodywt to rats ip, three doses per week and total of 12 doses. Control group received 0.5 ml of saline daily along with the treated set of rats. At the end of treatment rats were sacrificed using ether anaesthesia organs were perfused with 0.1M sodium phosphate buffer (pH 8.0) and tissues were dissected out, blotted and homogenate fractions were used for estimation. Cholesterol [Couchaine et al., (1959)] content was measured in all the three groups of Control, Cisplatin and Etoposide Liver, Kidney.

Estimation of Cholesterol [Couchaine et al., (1959)]

Reagents

Preparation of Color reagent: 2.5g of ferric chloride was dissolved in 100ml of conc (85%) phosphoric acid. 4ml of stock solution was diluted to 50ml with conc sulphuric acid before use.

Extraction: For extraction 0.3g of tissue was homogenized in a mixture of chloroform and methanol (2:1) to make a 10% homogenate. After 3h of extraction the homogenate was filtered and was used as lipid extract.

Estimation: Total cholesterol in the lipid extract was estimated by the method of Couchaine et al., (1959). 0.5 ml lipid extract was evaporated to dryness and 6 ml of glacial acetic acid was added and mixed. 4.0 ml of diluted colored reagent was added and mixed by inversion. After cooling the purple color developed was read at 550nm. Standard graph for cholesterol

was plotted using 0.1-0.5µg of cholesterol in glacial acetic acid.

Adriamycin treated rat kidney was used to study the biomarkers, GSH- related enzymes like GST[14], GGT.[15] Trichloroacetic acid (TCA) treated samples were utilized for the estimation of Lpx (MDA content).[16] The resulting supernatant fractions were recentrifuged for an additional 60 min at 105,000g. The microsomal pellets obtained were carefully collected and used for the estimation of Cytochrome p^{450} and Cytochrome b_5.[17] Total protein content was estimated by Lowry et al. (1951). All spectrophotometric readings were taken on Shimadzu UV-160 double beam spectrophotometer.

3. ANALYSIS

The significance of difference between the means was calculated by students t-test and results were expressed as mean ± SEM. Significance in all groups are shown as *$p<0.05$

4. RESULTS

Control rats registered 367±12.3 and adriamycin treated rats showed decrease as 314±4.0 in body weight when compared to controls. A noticeable effect of adriamycin treated rat is increase in fat deposition which was noticed at the time of dissection this could be one of the side effects caused by the drug. Kidney of adriamycin treated group of rats revealed non-significant decrease in weight shown as 1.054±0.1 compared to controls rats 1.26±0.04 (Table 1). In kidney lipid Peroxidation (MDA content) formed after adriamycin treatment showed significant decrease 0.015±0.01 compared to control rats shown as 0.31 ± 0.03 (Table 1). The mean values were expressed as nmol of MDA formed per mg protein. Total protein content registered significant increase of 355.27 ± 0.79 in adriamycin treated rats as compared to control rats reported as 262.73 ± 17.35 (Table 1).

Table 1 registered the biomarker's of kidney, GGT activity in adriamycin treated group revealed significant decrease shown as 6.67 ± 0.3 as compared to controls shown as 9.09 ± 0.02 which is expressed as µmol of p-nitroaniline/min/mgprotein.

GST showed significant increase of 6.46 ±0.1 in drug treated rats as compared to control rats shown as 1.46 ± 0.11 (Table 1). The mean values were expressed as nanomol of cDinitrobenzene bound/mg protein. The level of drug metabolizing enzyme namely Cytochrome p^{450} in kidney showed significant decrease in values reported as 288.76 ± 25 and controls showed 683.4 ±40.5 expressed as nmol/mg protein whereas, Cytochrome b_5 level

showed significant decrease shown as 447.6 ± 35.2 after drug treatment as compared to controls seen as 2605.5 ± 259.2 (Table 1). The mean values were expressed as nmol/mg protein.

Table 2 shows total cholesterol content in Liver of control, cisplatin and etoposide treated rats registered as 1.217±0.03, 1.851±0.04, 1.543±0.04. In kidney the mean values of control, cisplatin and etoposide treated groups are 1.792±0.08, 0.993±0.03, 1.422±0.07. All mean values are expressed as (µg/g tissue).

Table 1: Bodyweight, Kidney Weight and antioxidant status of Kidney in Control and Adriamycin treated male rats (mean ± SEM)

PARAMETER	GROUPS	
	Control (n=5)	Adriamycin (n=5)
Body weight (g)	367 ± 12.31	314±4.0*
Organ weight (g)	1.26±0.04	1.054±0.1#
Protein (mg/g tissue)	262.73 ± 17.35	355.27 ± 0.79*
Lipid peroxidation (nmol of Malondialdehyde formed per mg protein)	0.31 ± 0.03	0.015±0.01*
Glutathione-s-transferase (□mol of cDinitrobenzene bound/mg protein)	1.46 ± 0.11	6.46 ±0.1*
Gammaglutamyl transpeptidase (□mol of p-nitroaniline/min/mgprotein)	9.09 ± 0.02	6.67 ± 0.3*
Cytochrome p450 (nmol/mg protein)	683.4 ±40.5	288.76 ± 25*
Cytochrome b5 (nmol/mg protein)	2605.5 ± 259.2.	447.6 ± 35.2*

Adriamycin rats versus control rats: *p<0.05, # non- significant

Table 2: Total Cholesterol Content in Control, Cisplatin & Etoposide treated Liver and Kidney tissues of male rats (mean ± SEM)

PARAMETER	TISSUES	GROUPS		
		Control (n=5)	Cisplatin (n=5)	Etoposide (n=5)
Cholesterol (µg/g tissue)	Liver	1.217±0.03	1.851±0.04*	1.543±0.04*
	Kidney	1.792±0.08	0.993±0.03*	1.422±0.07*

5. DISCUSSION

Most of Cancer chemotherapautic drugs gain entry into the cellular system through the carrier-mediated mechanisms. Many drugs require activation before it can produce therapautic action. The activation process involves chemical and enzymatic reactions in normal or in tumor tissues. Cancer agents that are capable of intercalation includes

adriamycin, which inhibits topoisomerase II and produce DNA strand breaks. Cells also posses free radical scavenging system that protects cells from free radical induced damage. However, a constitutive cellular production of low levels of superoxide and H_2O_2 can stimulate cell proliferation and also can inhibit apoptosis.[19]

Our reports suggests adriamycin treatment produced sigificant decrease in bodyweight and kidney weight which can be said to be drug-induced action. However, protein levels were significantly increased which suggest that drug might not be causing protein oxidative damage by reactive free radicals or reactive oxygen species (ROS) which is also evident by decreased levels of Lpx (MDA content) levels.

Present studies we have also noticed that adriamycin at given dose produced significant increase in GST activity in kidney however, GGT activity was significantly decreased compared to controls. GST serves as a important biomarker[14] and its increase indicates that the enzymes can transfer -SH groups of reduced glutahione to adriamycin and increase the levels of oxidized glutathione (GSSG) therefore forming GSH-adriamycin conjugates. It can produce cytotoxic action to tumor cells. We also speculate that the mechanism of action can increase levels of H_2O_2 which can also counteract apoptotic stimuli. Further an significant decrease in Lpx (MDA content) signifies that there might be mild oxidative conditions that is not capable of inducing oxidative stress to tissue. Hence it can be said that at this dose adriamycin is found to be non-toxic/non-nephrotoxic. We also suggest that low H_2O_2 levels might be originating from GST activity that can act as a life-signal to the kidney as it can protect against apoptosis.

GGT, a plasma membrane-bound activity is in charge of metabolizing extracellular GSH. GGT is also efficient biomarker and acts as a indicator used to detect any free radical damage caused to kidney.[15, 20] An non-significant decrease in GGT activity in kidney shows that there might be least number of adriamycin formed reactive oxygen intermediates (ROI) that can induce oxidative stress. Bello et al (1999) reported that inhibition of GGT by acivin is a sufficient stimulus for the induction of cell growth arrest, and can induce cell death and DNA fragmentation. Further, it is known that GGT expression is important phenotypic change associated with neoplastic transformation, and it is known as a marker of neoplastic progression in several experimental models of chemical carcinogenesis, and occurs at significant levels in a number of human malignant neoplasms and their metastases.

Adriamycin is known to be involved in oxidation/reduction reactions : a number of NADPH-dependent cellular reductases are able to reduce the drug to semiquinone radicals.The cardiotoxic action of adriamycin in mouse can be prevented by N-acetylcysteine.[21]

Cytochrome p450 is a drug metabolizing enzymes that might bring about biotransformation of parent compound adriamycin into its activated form 13-OH-doxorubicinol. Aldo-keto reductase, also forms 13-OH-doxorubicinol, which posses a certain degree of antitumor activity. The terminal half-life of 13-OH-doxorubicinol is 20 to 48 hrs. Renal excretion is modest, accounts to 5% to 10% of administered dose in 5 days.[22] In our studies we reported that at given dose adriamycin increased Cytochrome p450 activity and decreased Cytochrome b5 indicating that Cytochrome p450 might be participating in drug metabolism to form 13-OH-doxorubicinol however, significant decraese in Cytochrome b5 indicates that amount of substrate formed might not be sufficient to induce the enzyme.

Thus therapautic dose of adriamycin in the treatment of various cancer's may potentially help to confirm drug dose and to lower drug-induced toxicity.

We have also determined the effects of drugs cisplatin and etoposide on cholesterol content of liver and kidney of rats. As it is reported sofar that cancerous cells requires more cholesterol and hypercholesterolaemia is also a risk factor for atherosclerosis. Therefore we hypothesize that whether at this dose both drugs can inhibit the levels of cholesterol. Cholesterol is synthesized from acetyl-CoA through mevalonate and from uptake of extracellular cholesterol via the Low density lipoprotein LDL-receptors. Moreover, by inhibiting the cholesterol synthesis or cholesterol availability the tumor growth can also be controlled which can be done by using proper diet and drugs shown previously in various studies in mice and rat.[23]

Buchwald (1992) reported that tumor weight was positively correlated with plasma cholesterol concentrations, and lower tumor weight is due to reduction in plasma cholesterol, LDL and VLDLc. The survival rate in rats was found to be more with lower plasma cholesterol. Lovastatin is efficient drug that has shown to suppress tumor growth and tumor-cells grown in drug-modified medium produced decrease ability to form metastatic lung nodules after injection to rats. Morphological alterations of cells showed rounded appearance with few cytoplasmic projections and also cells did not had ability to adhere. Lovastatin can be used as a adjuvant to mitomycin-C.

Present studies showed that cisplatin therapy significantly increased cholesterol levels in liver, but etoposide treatment produced significant decrease compared to control counterpart. Thus it can be predicted that both the agents, cisplatin and etoposide can be utilized as adjuvants/salvage therapy as it can be beneficial in inhiting the cholesterol synthesis and its availiability. Inaddition, in kidney both drugs at its part produced substantial depletion in cholesterol levels which is also a significant action of drugs.

CONFLICT OF INTEREST

No competing financial interests exist.

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