

COMPARITIVE STUDIES OF GLUTATHIONE-S-TRANSFERASE KINETICS IN CONTROL, CISPLATIN AND ETOPOSIDE TREATED HEPATIC AND RENAL TISSUE OF MALE RAT

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ABSTRACT

Cisplatin and etoposide are anticancer drug's used for treatment of various human malignancies. Cisplatin is used for treating solid tumors. The beneficial use of drug is often been mitigated due to its long term toxicities, hepatotoxicity and nephrotoxicity. The drug acts on proximal convoluted tubules of kidney reported as a major side-effect. Etoposide is plant derivative of podophyllotoxin also used for treatment of testicular cancer. In our previous studies we have documented cisplatin drug regimen showed changes in histopathological and antioxidant status of rat. We reported decreased reduced glutathione (GSH) level and increase in lipid peroxidation compared to controls. In present studies we have attempted to study the kinetics of Glutathione-S-Transferase (GST) after cisplatin and etoposide treatment in male wistar rat.

GST is a enzyme that metabolizes various reactive intermediates also includes anticancer drugs cisplatin, bleomycin by utilizing significant amount of glutathione.

Thus it can be said that cisplatin and etoposide at given dose level showed alteration in GST activity where cisplatin is responsible for inducing the toxic action to liver and kidney and etoposide did not cause any damage to both organs.

Keywords: Cisplatin, GST, Etoposide, Hepatotoxicity, Nephrotoxicity

INTRODUCTION

During past decade enormous progress has been made in defining the toxicological and potential physiological role of GST. Although the ability of GST to detoxify foreign compounds has been known, quinone metabolites of endogeneous molecules may also act as a substrate for GST in protecting against oxidative tissue damage. The ability of certain GST's and its enzymes to activate some of xenobiotics including anticancer drugs to its reactive forms and detoxifying others also presents additional challenge in toxicology¹.

Cisplatin and etoposide are two such anticancer drugs well-known for its application in the treatment of various human cancers^{2,3}. The drugs are also used for salvage therapy for solid tumors. However, the mechanism by which these two drugs undergoes bioactivation makes them different in action and its use in various cancers⁴.

Cisplatin (CDDP) is a planer-molecule that contains a central platinum atom surrounded by two chloride atoms in Cis-configuration and two ammonium moieties. Cisplatin is found to be inactive in its parent form and requires to undergo aqution/hydroxylation reaction to form potentially activated species. The dissociation of chloride ions from platinum at low salt concentration equivalent to the intracellular concentration of chloride creates a electrophilic form of platinum which binds to cellular nucleophiles such as DNA, RNA and negatively charged proteins⁵.³ Cisplatin also interacts with reduced glutathione and forms Cis-glutathione conjugates a reaction that takes place in presence of GST beneficial for biotransformation reactions of drugs⁶⁻⁷.

Similarly, etoposide is semisynthetic glucoside derivative of podophyllotoxin, is used in testicular cancer, refractory malignant lymphoma and currently in small cell lung cancer [SCLC] therapies⁸ and regarding etoposide bioactivation through GST pathway in catalyzing reactions is not yet been reported in literature. Previous studies reported that drug is activated by Cytochrome p450 (CYP3A4) forming catechol, semiquinone and quinones that can cause oxidative damage leading to lipid peroxidation. The quinone compounds and MDA, 4HNE formed after lipid peroxidation reaction can also participate in causing DNA adducts and also inhibit DNA

topoisomerase II activity in the cells⁹.

Based on our previous studies our current attempts are made to study the kinetics of GST in cisplatin and etoposide treated rat. These studies would also explain the mechanistic role of both the drugs cisplatin and etoposide at given dose level and its beneficial or detoxification process.

MATERIALS AND METHODS

Animals: Adult male albino rats of Wistar strain were obtained from Rajudyog Biotechnology division India were used for the study. Institutional animal ethical committee approved for the study.

Drugs: Cisplatin and Etoposide was obtained from Dabur India Ltd. Bovine serum albumin (BSA), reduced glutathione (GSH), thiobarbituric acid (TBA), Butylated hydroxytoluene (BHT), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB) were obtained from SRL India. All other chemicals used were of analytical grade.

Procedure : Rats were maintained in optimal condition and received standard pellets and water ad libitum. All animals were acclimatized for a period of 2 weeks and were then treated. They were coded in the group of three per cage and then were subsequently examined for further study. Experimental rats were divided into three groups. Group 1 were injected with 0.4 mg of cisplatin per kg i.p. daily, and group 2 were injected with 1mg of etoposide per kg i.p daily, for a period of 8 weeks. Control group received 0.5 ml of saline i.p.daily along with the treated set of the rats. The change in the body weight was monitored per week. At the end of the treatment, rats were given light ether anesthesia and sacrificed. Liver was perfused with saline to avoid any blood contamination and was then diced with scissors, homogenized in 0.1M potassium phosphate buffer (pH 6.5) containing 1mM EDTA and centrifuged. Kidney was excised and homogenates were used for studies.

GST activity was measured using various concentrations of cDNB as substrate and double reciprocal plots of 1/enzyme activity versus 1/[S] were generated to determine km values for GSH and cDNB. All the three groups control, cisplatin and etoposide were compared with each other for their action.

RESULTS

Comparative studies of Cisplatin and Etoposide treated bodyweight, organ weight followed with antioxidant levels and Lipid Peroxidation of male rat.

Results show that long-term treatment of animals with cisplatin (0.4mg/kg) i.p for 8 weeks caused evident decrease in body weight and organ weight of rats (Table 1). Etoposide treatment showed significant decrease in weight as compared to control rats (Table 1).

Table 2 indicates change in liver and kidney protein content in cisplatin and etoposide treated group of rat. Cisplatin showed significant decrease in both the tissues compared to controls whereas, etoposide did not cause any change.

Table 3 indicates significant decrease in cisplatin treated liver and kidney GSH levels of rat whereas, lipid peroxidation showed significant increase compared to control.

However, etoposide treatment showed non-significant change in liver and kidney GSH level and lipid peroxidation values (Table 3).

Table 1: Cisplatin and Etoposide induced changes in body weight and organ weight of male rat

Groups	Change in body weight (gms)	Change in organ weight (gms)	
		Liver	Kidney
Control (n=5)	367 ± 1 2.31	11.46 ± 0.65	1.261±0.0447
Cisplatin (n=5)	208 ± 8.46 *	6.526 ± 0.4 *	1.432±0.123 *
Etoposide (n=5)	304 ± 14.352 *	12.82 ± 0.7 #	1.168 ± 0.0269 #

Cisplatin and Etoposide rats versus control rats: *p<0.05, # non-significantly

Values are mean ± SE

Table 2: Cisplatin and Etoposide induced changes in Protein Content of male rat

Groups	Parameters	
	Protein Content (mg/gm tissue)	
	Liver	Kidney
Control (n=5)	371.06±14.92	262.73±17.35
Cisplatin (n=5)	346.79±17.54 *	138.85 ± 12.46*
Etoposide (n=5)	375.86±10.23 #	325.27 ± 19.11*

Cisplatin and Etoposide rats versus control rats: *p<0.05, # non-significantly

Values are mean ± SE

Table 3: Cisplatin and Etoposide induced changes in antioxidant and Lipid Peroxidation (MDA content) of male rat

Groups	Parameters			
	GSH	GSH	Lipid Peroxidation	Lipid Peroxidation
	(µmol/mg Protein)	(µmol/mg Protein)	(nmoles of MDA formed/mg protein)	(nmoles of MDA formed/mg protein)
	Liver	Kidney	Liver	Kidney
Control (n=5)	13.818 ± 1.3	8.06±0.74	0.2 ± 0.02	0.31±0.03
Cisplatin (n=5)	8.2 ± 0.1 *	5.45 ± 0.49 *	1.1 ± 0.07 *	0.62 ± 0.04*
Etoposide (n=5)	13.818 ± 0.91 #	7.724±0.732 #	0.239 ± 0.021 #	0.258 ± 0.015 #

Cisplatin and Etoposide rats versus control rats: *p<0.05, # non-significantly

Values are mean ± SE

Comparative Kinetics of GST activities between Control, Cisplatin and Etoposide treated rat.

Cisplatin treatment showed subsequent decrease in liver km and Vmax values of GST as compared to control set of rat shown in Table 4. Etoposide treatment did not cause any significant change compared to control liver.

However, renal tissue showed difference in its activity at given dose the km and Vmax values for GST in cisplatin treated rat showed lowered values as compared to controls however, etoposide treatment showed increase in km and Vmax compared to control group shown in Table 4.

Table 4: Comparative Kinetics characteristics of GST between Control, Cisplatin and Etoposide treated male rat

Groups (n=5)	Km (mM) for cDNB	Vmax for cDNB (mol/mol/min)	Km (mM) for cDNB	Vmax for cDNB (mol/mol/min)
	Liver		Kidney	
Control	77	0.03	102.78	0.01
Cisplatin	68.4	0.01	61.56	0.01
Etoposide	77.8	0.03	106.2	0.01

DISCUSSION

GST catalyze the general reaction $GSH + R-X \rightarrow GSR + HX$. The function of the enzyme is to (1) bring the substrate in close proximity with GSH by binding both GSH and the electrophilic substrate to the active site of the protein, and (2) activate the sulfhydryl group on GSH, thereby allowing for nucleophilic attack of GSH on the electrophilic substrate (R-X)¹⁰. Various types of catalytic reactions have been identified, including epoxide ring openings, nucleophilic aromatic substitution reactions, reversible Michael additions to α,β -unsaturated aldehydes and ketones, isomerization, and for a few GST's, peroxidase reactions. The electrophilic functional centre of the substrates can be a carbon, nitrogen or sulfur. The formation of thioester bond between the cysteine residue of GSH and the electrophile usually results in a less reactive and more water-soluble product, and thus GST's are usually involved in detoxification reactions. However, for certain compounds GST-mediated conjugation with GSH may result in formation of highly reactive intermediates, and thus catalyze activation reactions¹.

Previous reports of ours showed elevated levels of cytochrome p450 that emphasizes the metabolism of cisplatin by the cytochrome p450 converts into intermediated hydroxylated form which is capable of reacting with cellular macromolecules¹¹.

GST is an accessory enzyme that has been found to be involved in the metabolism of various reactive intermediates of various xenobiotics including certain anticancer drugs such as bleomycin, cisplatin etc by utilizing significant amount of glutathione. Cellular levels of GSH are important in the metabolism of alkylating xenobiotics and of reactive intermediates formed via; cytochrome p450 system thereby depleting levels of GSH in the tissue¹².

Moron et al (1979) have said as a consequence, the conjugation of xenobiotics and their metabolites with GSH is probably considerably lower in lungs. They stated for rabbit the apparent K_m of lung 6.21 mM, whereas for liver enzymes the apparent K_m is 1.5 mM which suggests the spontaneous (non-enzymatic) reaction of GSH with xenobiotics in liver and their metabolites is slower in lungs and that lung pool of GSH might be depleting more rapidly¹³.

An comparative analysis of controls, cisplatin and etoposide probably suggests that cisplatin is biotransformed into its hydroxylated form and is conjugated to reduced GSH. Thus it probably illustrates high enzyme substrate affinity i.e strong binding. Cisplatin treated liver shows K_m of 68.4 mM compared to control shown as 77.0, whereas, etoposide treatment showed 77.8 mM suggestive of weak binding.

However, these results prompt us to address the question, do these depletion in GSH status results in increased oxidative stress on tissue or biotransformed product Cis-GSH conjugates, is suggestive of the susceptibility of the organ to cytotoxic action of cisplatin and its metabolites as L_{px} values in cisplatin treated liver is increased compared to controls. Etoposide treatment did not cause much effect to liver suggestive of lower L_{px} reactions and non-significant change in GSH levels.

As it is very well known that cisplatin is nephrotoxic compound and Cis-GSH conjugates are responsible nephrotoxicants. Our present studies we have shown K_m of 61.56 mM in cisplatin treated kidney compared to control 102.78 mM indicating strong binding reactions and increased formation of conjugates. This is supported by increased L_{px} levels and decreased GSH levels in kidney treated with cisplatin. Studies suggest that cisplatin might also be undergoing biotransformation reactions spontaneously to form cis-GSH conjugates. In addition to increased lipid peroxidation, studies by Khanam et al. (2011) reported that cisplatin metabolism increases serum creatinine, blood urea nitrogen and alkaline phosphatase levels in rats and alcoholic *Tinospora cordifolia* extract can serve curative action¹⁴. Other anticancer drug such as

doxorubicin is also known to alter GST and GSH content in cardiac tissue of rat indicates that GST serves as oxidative marker in the tissue of rabbits¹⁵. Furthermore, studies also reveals that treatment with antioxidants such as morin, vitamin-E, rutin and quercetin would replenish the drug-induced oxidative action and prevent heart from any oxidative stress¹⁵.

Nevertheless, the toxicity of these anticancer drugs cisplatin can be controlled by combining it with other cancer chemotherapeutic etoposide. Our studies shows the K_m value for etoposide treated kidney as 106.2 mM compared to controls 102.78 mM. This suggests etoposide has lower affinity with GST. This could be supported with lower levels of GSH and L_{px} in renal tissue suggest that etoposide is least toxic.

To sum up it can be said that cisplatin serves both hepatotoxicant as well as nephrotoxicant whereas, etoposide showed non-significant actions on both the organs. Both the drugs act through different mechanism of action which could serve beneficial role if given for salvage cancer chemotherapeutic.

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