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EFFECTS OF ANTICANCER DRUGS DOXORUBICIN AND ETOPOSIDE ON CYTOCHROME P450 AND B5 IN RATS

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ABSTRACT: Aim: To investigate the effects of anticancer drugs Doxorubicin and Etoposide on cytochrome P450 and b5 in rats. Methods: Doxorubicin was injected intraperitoneally to the rats of 1mg/kg /thrice a week (18 doses) and Etoposide was injected intraperitoneally to the rats of 1 mg/kg/day for 8 weeks (52 doses). Control group given saline solution intraperitoneally to rats at the doses of 0.5 ml/day/animal for 8 weeks (52 doses). Then contents of cytochrome P450 and b5 were determined. Results: Contents of cytochrome P450 and b5 in livers of rats (CY P450: 334.889±29.777, 656.99±31.7) in first group of CYP450 was significantly decreased and second group was significantly increased as compared with the control group (CYP450: 444±37.46, P<0.05). Contents of cytochrome b5 in livers of rats (b5: 837.177±61.197, 475.53±42.4) first group was higher while second group was lower than those in the control group (b5: 615±37.0). Contents of cytochrome P450 in kidneys of rats (CYP450: 288.762±25.806, 681.18±66.95) in Doxorubicin treatment was significantly decreased but in Etoposide treatment not significantly different with control group (CYP450: 683.4±40.5, P<0.05). Contents of cytochrome b5 in kidneys of rats of both treated groups (b5: 447.685± 35.215, 555.8±49.57) were higher than those in control (b5: 2605.5±259.2). Contents of cytochrome P450 in hearts of rats (CYP450: 312.311±24.261, 8±0.37) in Doxorubicin group significantly increased while Etoposide significantly decreased as compared with control counterpart (CYP450: 25±0.24, P<0.05). Contents of cytochrome b5 in hearts of rats (b5: 165.352±8.7, 71±0.3) in first group is higher significantly but second group lower significantly as compared with control group (b5: 88±0.4, P<0.05). Conclusion: Evaluating the level of toxicity at given doses by studying the changed of antioxidant enzymes cytochrome P450 and b5 and total protein profile of hepatic, renal and cardiac tissues.

Key words: *Doxorubicin; Etoposide; Cytochrome P450; Cytochrome of b5.*

INTRODUCTION :

Doxorubicin is one of the most effective and widely used chemotherapeutic drugs since late 1960's and commonly called as Adriamycin. It was originally isolated from fungus *streptomyces var. caesius* (Di Marco et al., 1969). It is a tetracyclic anthraycline compound**,** which linked to carbohydrate; removal of carbohydrate from it usually leads to loss of anti-tumor activity. Anti-Tumor activity of Doxorubicin is thought to derive from its interaction with DNA and inhibition of DNA topoisomerase II; leading to inhibition of nuclear DNA replication and transcription. It is effective as single or in combination with other antineoplastic drugs in treatment of a variety of malignancies afflicting both pediatric and adult patients. Doxorubicin play a very important role in treatment of cancer such as solid tumor, multiple myelomas, breast cancer, stomach cancer, ovarian cancer and bone cancer (Andringa, 2001).

Etoposide is a semi synthetic glucoside derivative of Podophyllotoxin. It was first anticancer drug to be demonstrated to work through inhibition of topoisomerase II. Etoposide is cell cycle specific and it works by arrest of cell in late S or early G2 phase of the cell cycle (Hande, 1998; Stalelin, 1973). It is used during chemotherapies for a wide range of malignancies e.g., small cell lung cancer, acute leukemia, lymphoma, and testicular cancer as a single or one of the constituents of standard therapeutic regimens (Clark and Slevin, 1987). Anti-Tumor properties of Etoposide involve, a one –electron oxidation leads to the Etoposide –phenoxyl radical that can be redox-cycled by proteins thiols (Kagen et al., 1999). Etoposide one of the constituents of standard therapeutic regimens has been reported in combination with Doxorubicin, cisplatin, 4-hydroxycyclophosphamide and vindesine (Henwood, 1990).

Cytochrome P450 and cytochrome b5 are important groups of enzymes among the eukaryotes. Both enzymes are in the same subcellular fraction of microsome [i.e.] in the microsomal lipids, the two hemoproteins behave independently in oxidative functions. Reduced form CYT P450 is very rapidly reoxidised in the presence of molecular oxygen to CYT P420 while CYT b5 autoxidies slowly.

Primary purpose of this investigation is to explore, activity of Doxorubicin and Etoposide on the contents of cytochrome P450 and b5 in liver, kidney and heart in rats to provide some useful information to understand toxic effects of commonly used anticancer drugs on architecture antioxidant enzymes and detoxification system of experimental rat models and applied it in clinical therapy.

MATERIALS AND METHODS:

Animals: Proven Fertile Adult male albino rats of Wistar strains of about (240–250 g) body weights were obtained from (Haffkine Research Institute. Mumbai, India). Rats were acclimatized to laboratory conditions for 2 weeks where they were maintained under a regulated photoperiod (12-hrs light and 12-hrs dark). Temperature was maintained constant at (26-28 ºC). Humidity was ideally maintained at 55±15 % R.H. They were allowed ad libitum access to tap water and food standard pellet feed from (Amrut Laboratory Animal Feed. Mumbai, India). Rats were divided into three groups as (I) Control, (II) Doxorubicin, (III) Etoposide.

Experimental Drugs : Doxorubicin is an anticancer drug (Trade name ADRIM) is manufactured by Dahur India Ltd. (Pharmaceutical Division, Solan-173205) India. It was intraperitoneally (i.p.) administrated into experimental rats group no. (I) at doses of 1mg /kg thrice a week for 18 doses (Ward et al., 1988). Etoposide is an anticancer drug (FYTOSID) is manufactured by Dabur India Ltd. Experimental rats group no.(II) were injected with 1 mg of Etoposide per kg intraperitoneally (i.p.) daily for period of 8 weeks (52 doses), (Mittal et al., 2001) with modification. Control group received physiological saline intraperitoneally (i.p.) 0.5 ml/day, for a total of 52 days, (52 doses). Changes of the body weight were measured every week until the end of the experiment.

Analytical Procedures

Extraction of Tissue : After treatment, animals were kept at the same laboratory condition for 2-3 days. Animals were sacrificed following anesthetization with ether and liver, kidney and heart were dissected and washed with chilled normal saline. 10 % homogenate were prepared by from 0.5 gm of tissue in 5 ml of (0.1M Sodium phosphate buffer pH 8.0). Homogenate was centrifuged at 9000 rpm for 20 minutes. The protein content of the homogenate was estimated by Lowry's method (Lowry et al., 1951). The homogenates were also estimated for antioxidant enzymes as per the methods of Omura and Sato (1964). The microsomal fractions obtained after centrifugation at 105,000×g were used for the estimation of cytochrome P450 and cytochrome b5 enzyme. The final microsomal fraction in both the cases was suspended in 0.1M-phosphate buffer [pH 7.4] to achieve a protein concentration of 2 mg/ml, as described by Omura and Sato (1964).

Microsomal enzyme assays : Cytochrome P450 (CYP450, EC 14.14.1) and cytochrome b5 (CYb5, EC 1.6.2.2).

The levels of Cytochrome b5 and p450 were estimated by the method of Omura and Sato (1964).

Approximately 3 ml of the suspended microsomal fraction was taken in 2 cuvettes and the baseline was adjusted to zero at 427 nm. A few mg (~ 10 mg) of sodium dithionate was added to the sample cuvette which resulted in rapid reduction of cytochrome b5. This was characterized by increase in absorbance at 427 nm. Then a few mg (~ 10mg) of sodium dithionite was added to the reference cuvette and the base line was adjusted to zero at 450 nm. The contents of the sample cuvette were gently gassed with co purged of oxygen for about 50 sec. Since the co bound reduced form of Cyt p450 has an absorbance band at 450 nm the change in absorbance at 450 nm relative to 490 nm was converted to a concentration of Cyt p450 using the extinction coefficient of 91/cm/mM.

The levels of cytochrome b5 were calculated using the extinction coefficient of 171/cm/mM at 427 nm. The specific activities of both the enzymes b5 & p450 are expressed as nmoles/mg of protein.

Calculation

1. $\frac{\text{O.D at 450nm-O.D at 490}}{\text{Mol.wt coff cyt p450 (91)}} \times \text{Dil factor}$

2. $\frac{\text{O.D at 427nm}}{\text{Mol.wt coff cyt b5 (171)}} \times \text{Dil factor}$

STATISTICAL ANALYSES: Data were calculated separately in male rats and expressed as mean ± SEM. Statistical analysis was carried out by student's *t*-test, difference was considered significance when $p < 0.05$.

RESULTS AND DISCUSSIONS :

General Observations : After injecting Doxorubicin and Etoposide (i.p) separately for eight weeks there were no injuries at the site of injections. In both drugs Doxorubicin treated rats as well as Etoposide treated rats; body weight was significantly lowered as compared to the control rats (Table 1). Control rats registered uniform increase in their body weight, after injecting the saline (i.p) (0.5 ml/ day /animal for 8 week) as compared to Doxorubicin and Etoposide treated groups (Table 1).

Body organs such as liver weight showed non-significant increase in both anticancer drugs treated rats as compared with control group (Table 1). Kidneys weights showed non-significant decrease in both Doxorubicin and Etoposide treated groups as compared to control (Table 1). Weight of heart treated groups in both drugs treated cases found decreasing compared with control group (Table 1).

Table 1. Doxorubicin and Etoposide induced changes on body weight and organ weight of male rats (mean ± SEM)

PARAMETER	GROUPS		
	CONTROL	DOXORUBICIN	ETOPOSIDE
Body weight (g)	443 ± 4.35	314 ± 4*	330 ±18.5*
Liver weight (g)	11.46 ± 0.654	11.781 ± 0.419#	12.286 ± 0.797#
Kidney weight (g)	1.261 ± 0.045	1.054 ± 0.398#	1.168 ± 0.027#
Heart weight (g)	0.928 ± 0.045	0.884 ± 0.028#	0.826 ± 0.017#

Doxorubicin and Etoposide rats separately versus matched control rats: *Represents significant change from the control values as ($p < 0.05$). #Represent non- significant different from the counter parts as ($p < 0.05$).

Antioxidant Enzyme Activity : *Antioxidant Enzymes Activity {Hepatic renal and cardiac}*

After eight weeks of Doxorubicin and Etoposide were injected intraperitoneally to rats, separately, contents of cytochrome P450 and b5 in liver, kidney and heart were determined. In Doxorubicin treated group, level of CYP450 in hepatic tissue showed significant decrease as compared to control (Table 2). In Etoposide treated group; level of CYP450 in hepatic tissue showed significant increase as compared to control (Table 2). In Doxorubicin treatment, level of CYb5 significant rise is seen in hepatic tissue as compared to control (Table 2). In Etoposide treatment, the level of CYb5 significant depletion monitored when compared with control (Table 2). In case of Doxorubicin, total protein in hepatic tissue showed significant increase as compared to control counterpart (Table 2). In case of Etoposide, total protein in hepatic tissue registered non-significant increase as compared to control counterpart (Table 2).

In Doxorubicin model; CYP450 level in renal tissue showed significant depletion as compared to control rats while the CYP450 in renal tissue of Etoposide treated group showed non-significant decrease as compared to control rats (Table 2). In Doxorubicin and Etoposide treated groups, level of CYb5 in renal tissue was significantly lowered as compared to control counterpart (Table 2). In Doxorubicin as well as Etoposide treated rats, total protein content in renal tissue showed significant increase as compared with control group (Table 2).

In Doxorubicin and Etoposide treatments, level of CYP450 level in cardiac tissue registered significant increase as compared to control counterpart (Table 2). In Doxorubicin treated group; level of CYb5 in cardiac tissue as compared to control counterpart (Table 2). In Etoposide treated group; level of CYb5 in cardiac tissue as compared to control rats part (Table 2). Total protein content in heart fraction, in Doxorubicin treated group showed non-significant decrease, but in case of Etoposide treated group showed significant decrease as compared to control group (Table 2).

Table 2. Effect of Doxorubicin and Etoposide separately on hepatic, renal and cardiac antioxidant enzymes and protein profile of male rats (mean ± SEM)

PARAMETER	GROUPS		
	CONTROL	DOXORUBICIN	ETOPOSIDE
Protein Liver (mg/g tissue)	371.068 ± 14.927	406.053 ± 18.615*	375.86 ± 10.23#
Protein Kidney (mg/g tissue)	262.736 ± 17.356	356.206 ± 21.959*	325.27 ± 19.11*
Protein Heart (mg/gm tissue)	334.26 ± 13.129	326.69 ± 16.266#	287.778 ± 14.25#
CYP450 Liver (nmoles of CO bound /mg protein)	444 ± 37.46	334.889 ± 29.777*	656.99 ± 31.7*
CYb5 Liver (nmoles of CO bound /mg protein)	615 ± 37.0	837.1765 ± 61.197*	475.53 ± 42.4*
CYP450 Kidney (nmoles of CO bound /mg protein)	683.4 ± 40.5	288.762 ± 25.806*	681.18 ± 66.95#
CYb5 Kidney (nmoles of CO bound /mg protein)	2605.5 ± 259.2	447.685 ± 35.215*	555.81 ± 49.57*
CYP450 Heart (nmoles of CO bound /mg protein)	25 ± 0.24	312.31 ± 24.261*	8 ± 0.37*
CYb5 Heart (nmoles of CO bound /mg protein)	88 ± 0.4	165.352 ± 8.7*	71 ± 0.3*
Doxorubicin and Etoposide rats separately versus matched control rats: *Represents significant change from the control values as ($p < 0.05$). #Represent non- significant different from the counter parts as ($p < 0.05$).			

In our recent studies, Body weight of anticancer drugs treated rats showed significant weight reduction at completion of doses while compared with body weight of control rats. Data obtained in our research also agree with those reported by these authors (Imahie et al., 1995 and Manabe et al., 1996) about significant decrease in body weight after injection of Doxorubicin to rats. Body weight was significantly decreased after Doxorubicin treatment might be due to loss of appetite and also due to accelerated utilization of the Doxorubicin in the body. Or a high dose of the Doxorubicin leads to drug toxicity (at least partly). Whereas in Etoposide may be because of associated with emesis and diarrhea, which occur occasionally, beside moderately higher dose.

In the present study, we have determined organs weights, in Doxorubicin and Etoposide models. Liver weight was slightly higher than controls after eight weeks of treatments but in case of kidney and heart weights for the both models; they was no significant decrease in comparison with control rats. Data obtained in our studies agree with those reported by Oguro et al., (1973a and 1973b) showing that Doxorubicin has adverse effect on hematopoietic organs, liver and kidney. Branden et al., (2000) observed that body weight was significantly decreased after Doxorubicin treatment as compared with control rats and they reported that kidney weight was higher after Doxorubicin treatment as compared with matched control rats. Bolaman et al., (2005) reported that non-significantly decrease of heart weight after Doxorubicin induced and pretreatment with amifostine when compared with control.

In Doxorubicin group, level of CYP450 in liver and kidney tissues showed significant decrease, whereas the level of CYP450 level in heart registered significant increase as compared to counterpart. In Etopoisde treated group, level of CYP450 in liver and heart tissues showed significant increase, however in kidney tissue level of the enzyme showed non-significant depletion as compared to control counterpart.

Omura and Sato first characterized the Cyt p450 system in 1964. Cytochrome p450 play a very important role in drug metabolism especially in phase one for comprise the oxidase. Bachur et al., (1979) reported that the enzyme CYTp450 is responsible for conversion of Doxorubicin into a semi-quinone free radical via a one –electron reaction. Our result is matching with (Schellen's et al., 2000); reported Bioavailability of anticancer drugs is very poor because of the high activity of CYTp450 in gut wall and liver in case of Etoposide model. Reid et al., (1999) approved that the most activity of Dacarbazine a widely used anticancer agent, in the treatment of cancer patients has been attributed in part to lower activity of CYTp450 in human liver microsomes.

The relevance of CYT p450 has long implicated in the metabolism of anticancer drugs such as cyclophosphamide, procarbazine and etoposide. Increased level of CYP450 perhaps has resulted into increased drugs metabolism whereas lowered level of CYP450 might be resulting into lowered drugs metabolism.

In Doxorubicin treatment, level of CYb5 in liver and heart tissues were significantly increased, whereas level of CYb5 in kidney tissue was significantly lowered as compared to control. In Etoposide treatment, level of CYb5 in liver, kidney and heart tissues were significantly lowered as compared to control. Perhaps significant depletion in cytochrome b5 indicates the involvement of ROS in enzyme inactivation in case of etoposide whereas the cytochrome b5 in case of doxorubicin might be there are high interactions of this enzyme with various CYT p450 species. The flavin reductase, including cytochrome p450, cytochromeb5 reductase, NADH dehydrogenase, and xanthine oxidase, have the capacity to reduce doxorubicin to doxorubicin semiquinone free radial.

Total protein content in hepatic, renal and cardiac tissues of Doxorubicin treated rats were significantly increased to that of control set of rats, but hepatic protein content in Etoposide treated rat was not significantly increased compared to control set of rats; whereas total protein of renal and cardiac tissues of Etoposide model were significantly increased to that of normal saline treated group. Changes in protein levels could a rise by inducible synthesis of existing or new proteins and or denaturation and degradation of the existing proteins in response to the Doxorubicin or Etoposide.

CONCLUSION:

It can be concluded from the results obtained in the present study, that two anticancer drugs have differential effects on the organs studied. For instance; extent of toxicity of these drugs varies from organ to organ. There are reports about combination of doxorubicin and etoposide is succeful *in vitro* in killing of synergistic anti-tumor activity along with cisplatin, 4hydroxyperoxycyclophamide and vindesine (Henwood and Brogden, 1990). According to our result, it can be suggested that the combination of these drugs Doxorubicin and Etoposide, can improve activity and minimize the side effects.

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