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RESEARCH ARTICLE

IMPACT OF DOXORUBICIN AND VITAMIN E, A SEPARATELY AND IN COMBINATION ON SELECTED ENZYMATIC ACTIVITY LEVELS INVOLVED IN THE ENERGY METABOLISM IN THE MAJOR TISSUES OF ALBINO RAT

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Abstract

Doxorubicin (DOX) is an anthracyclin antibiotic with broad spectrum of antitumour activity. Use of this agent has been shown to cytotoxicity. The effect of 20 mg per Kg weight of Dox and 100 mg per Kg weight of vitamin E and 50 IU per Kg weight of Vitamin A separately and in combination treated rat tissues for 10 weeks period (weekly doses). The state of rat tissues based on energy metabolic enzymes like ATPase and cytochrome-c-oxidase activity levels are reported. The data indicated decreased levels of ATPase and cytochrome-c-oxidase under Dox stress were observed. Above two Dox altered rat tissues energy metabolic enzymes to be neutralized by in administration of Dox plus vitamin E and Dox plus vitamin A. From the result it is reported that Dox impair the overall energy metabolism of rat tissues and that antioxidants like vitamin E and A by their antioxidant mechanism might be neutralize the Dox altered rat tissues like ATPase and cytochrome-c-oxidase activity levels *in vivo*.

INTRODUCTION

The anthracycline antibiotic Doxorubicin (Dox) is one of the most effective chemotherapeutic agents against a wide variety of cancers (Adam et al., 2012). However, its use is seriously limited by the development of acute and chronic toxic side effects such as cardiomyopathy and heart failure, dizziness, lack of concentration etc., (Buzdar et al., 1985). The mechanism by which Dox causes myocardial injury is not fully understood. Several explanations accounts for the Dox toxicity. e.g free radical production, calcium overloading, mitochondrial disfunction and peroxy nitrite formation have been proposed (Shuai et al., 2007; Arawala et al., 2010). Among followed strategies to attenuate Dox toxicity are doasage optimization, synthesis and use of analogues or combined therapy with antioxidants. The most promising results come from the combination of the drug delivery together with an antioxidant in order to reduce the toxic stress. Many antioxidants have been assayed with very different results. In view of this, attempt has been made to study the effects of doxorubucin, vitamin E and A separately and in combination on the levels of certain key enzymatic activities like ATPase and cytochrome-c-oxidase involved in energy metabolism of various tissues of albino rats.

MATERIALS AND METHODS

The study utilized adult swiss albino rats of the weight range of 125±5 gm. They were divided into 6 groups of seven each and were kept under standard laboratory conditions. They were fed with commercial pellet diet and water. The room temperature was maintained at 25±5°C. Dox and vitamins E & A at recommended doses were employed in the current study

ANIMALS

The albino rat colony was divided into 6 groups of Seven Each. Animal Ethics Resolution Number: 24/2012-2013(i)/a/CPCSEA/IAEC/SVU/KVK-BV dt. 01-07-2012.

TREATMENT OF ANIMALS

Group – 1 Rats acted as untreated control.

Group – 2 Rats were received 100 mg/kg body wt of vitamin E dissolved in 100 microlitres of olive oil over 10 weeks through gavages (weekly doses)

Group – 3 Rats received 50 IU/kg body wt of vitamin A dissolved in 100 microlitres of olive oil over 10 weeks through gavages (weekly doses).

Group – 4 Rats were administered with 20 mg/kg body wt of Doxorubicin dissolved in 100 microlitres of sterile water over 10 weeks. Through tail vein (weekly doses)

Group – 5 Rats were administered with 100 mg/kg wt vitamin – E followed by 20 mg/kg body wt of Doxorubicin over 10 weeks (Weekly doses).

Group – 6 Rats received 50 IU/kg body wt of vitamin A followed by 20 mg/kg body wt of doxorubicin over 10 weeks (weekly doses).

After treatment of animals the control and experimental animals were individually anaesthetized with pentabarbitone (10 mg/kg) ether and were sacrificed and the major tissues like brain, heart, liver and kidney were isolated blotted on a filter paper and were placed in a liquid nitrogen and finally were stored at -80^0C till used. Whenever necessary the blood samples were collected through cardiac puncture.

CHEMICALS:

Doxorubicin hydrochloride (Adrim) is a product from Dabur indica Ltd (Pharmaceutical division) 19, industrial area, Baddi, Dsit Solar Himachal Pradesh India.

METHODS

In the control and experimental samples ATPase activity was measured by Fritz and Hamrich (1966) modified by Desaih and Ho (1979) and the cytochrome-c-oxidase activity levels were measured by the procedure of Oda et al., (1958).

STATISTICAL ANALYSIS

For each parameter, the mean of individual observations (for both control and experimental groups) were taken into consideration. Statistical significance of the data was anlaysed through two way ANOVA (Analysis of variance); student Newman Keuls test and regression analysis. P value < 0.01 was considered significant.

RESULTS

The results shown in tables 1 and 2, the ATPase and cytochrome-c- oxidase activity levels in the control, vitamin E and A Dox separately and in combinations treated rat tissues, all the rat tissues showed decreased levels of their ATPase and cytochrome-c-oxidase activity levels upon Dox administration per 10 weeks period (weekly doses) and changes were found to be statistically significant over the control. The two dose combinations first one 100 mg/kg wt Vitamin E + 20 mg/kg wt Dox per 10 weeks (weekly doses) and second one 50 IU/kg wt Vitamin A + 20 mg/kg wt Dox per 10 weeks (weekly doses) treated the reversed Dox inhibited rat tissues ATP ase and cytochrome-c-oxidase activity levels and The reversal was more in rat tissues receiving to 100 mg/kg wt Vitamin E + 20 mg/kg wt Dox than compared to rat tissues receiving 50 IU/kg wt Vitamin A + 20 mg/kg wt Dox per 10 weeks (weekly doses).

Table 1: Effect of Doxorubicin, Vitamin E & A separately and in Combination on Rat Tissues Total ATPase Activity Levels (Values Expressed as µ moles Pi Liberated / mg protein / hr).

Name of the Tissue		Untreated Control	Vitamin E Treated	Vitamin A Treated	Dox Treated	Vitamin E + Dox Treated	Vitamin A + Dox Treated
Brain	AV	22.16	21.24	22.24	14.24	17.02	15.94
	SD	±0.65	±0.32	±0.16	±0.14	±0.16	±1.74
	PC		4.28 NS	0.40 NS	-35.74 *	19.52 *	11.93 *
Heart	AV	15.71	15.73	15.60	8.46	12.08	11.59
	SD	±1.05	±0.84	±0.75	±0.02	±0.74	±0.92
	PC		0.12 NS	-0.70 NS	-46.14 *	42.78 *	36.99 *
Liver	AV	26.38	27.05	28.22	9.32	22.46	20.79
	SD	±0.22	±0.19	±0.87	±0.96	±0.49	±0.77
	PC		2.53 NS	6.97 NS	-64.67 *	140.98 *	123.06 *
Kidney	AV	18.43	18.42	18.10	8.36	15.36	12.42
	SD	±0.92	±0.29	±0.36	±0.16	±0.99	±0.73
	PC		-0.05 NS	-1.79 NS	-54.63 *	83.73 *	48.79 *

Each value is the mean ±SD of seven samples
AV: Average Value
SD: Standard Deviation
PC: Percent Change over the control/Dox and Vitamin E & A treated ones
NS: Not Significant
*: $p<0.01$

Table 2: Effect of Doxorubicin, Vitamin E & A Separately and in Combination on Rat Tissues Cytochrome-c-oxidase activity levels (Values Expressed as µg Formazan formed /mg protein / hr).

Name of the Tissue		Untreated Control	Vitamin E Treated	Vitamin A Treated	Dox Treated	Vitamin E + Dox Treated	Vitamin A + Dox Treated
Brain	AV	56.04	56.06	56.68	20.24	42.72	39.08
	SD	±2.44	±0.66	±0.59	±1.61	±1.08	±0.96
	PC		0.03 NS	0.07 NS	-63.88 *	201.06 *	93.08 *
Heart	AV	43.92	44.14	44.08	18.69	35.20	32.60
	SD	±1.62	±1.49	±0.41	±0.36	±0.22	±0.74
	PC		0.20 NS	0.36 NS	-57.44 *	88.33 *	74.42 *
Liver	AV	82.40	81.93	82.93	30.50	75.36	70.89
	SD	±1.29	±0.08	±0.74	±0.52	±0.82	±0.36
	PC		-0.57 NS	0.64 NS	-62.98 *	147.08 *	132.42 *
Kidney	AV	54.79	53.07	52.56	20.26	40.49	38.22
	SD	±0.95	±0.45	±0.62	±0.35	±0.38	±0.24
	PC		-3.13 NS	-4.07 NS	-63.02 *	99.85 *	88.64 *

Each value is the mean ± SD of seven samples
AV: Average Value
SD: Standard Deviation
PC: Percent Change over the control/Dox and Vitamin E & A treated ones
NS: Not Significant
*: $p<0.01$

DISCUSSION

All living organisms transform energy taken from their surroundings. This energy is required for the synthesis of macromolecules to be used and in growth and differentiation of the organism. These transformations are achieved via the action of a large number of enzymes catalyzing a complex network of chemical reactions (Kuchel and Ralston, 1983). The proportion of energy utilized depends largely on the tissue and the physiological state of that tissue.

The essential linkage between the enzymes producing and utilizing pathways is maintained by the nucleotide triphosphate. The energy produced is stored as ATP molecules, synthesized in mitochondria through oxidative phosphorylation is hydrolyzed by ATPase which also belong to the category of enzymes known as phosphotases (Lehninger, 2004). The Na^+, K^+ATPase is accompanied by Mg^{2+}ATPase activity and differential assay is not possible. The enzyme activity is usually determined by the release of inorganic phosphate in the presence of Mg^{2+}, Na^+, K^+ and total ATPase. Cytochrome-c-oxidase is a heme containing protein with the properties of interacting directly with oxygen and hence is called a respiratory enzyme (Oser, 1965). The cytochrome-c oxidase is a mitochondrial enzyme, present in the terminal segment of the respiratory chain; it plays a significant role in oxidative phosphorylation to yield ATP. Its activity indicates prevalence of oxidative phosphorylation and also reflects oxygen dependent energy synthesis.

In view of the key role played by the enzymes, ATPases and cytochrome-c-oxidase in energy metabolism, an attempt was made to study the activity levels in rat tissues under Dox, vitamin E and A separately and in combination. From the current study it is evident that Dox at doses of 20 mg/kg wt over 10 weeks period impairs the rat tissue energy metabolism as evidenced by depleted levels of rat tissue ATPase and cytochrome-c-oxidase activities *in vivo*, since these enzymes are well known key enzymes necessary for maintenance of tissue energy metabolism.

Huang et al., (2003) have demonstrated Dox as to decrease SR Ca^{2+} ATPase from rabbit cardiomyocytes. Karunakaran et al., (2009) reported that Dox inactivates myocardial cytochrome oxidase activity in rats. As reported by Marcillat et al., (1989) mitochondrial damage is a major component of adriamycin cardiotoxicity, and rival oxidative and non-oxidative mechanisms for inactivation of the electron transport chain. The findings of Olson et al., (1988) speaks that Dox interact with rabbit ventricular SR membrane ATPase system. The studies of Elif et al., (2009) also reported the decreased Na^+ K^+ ATPase activity in rats. Guzy et al., (2003) have cited that treatment with anthracyclines can be associated with different types of cardiotoxicity including acute toxicity, chronic toxicity and delayed toxicity and these proceeds through impaired mitochondrial enzyme activities including ATPase and cytochrome-c-oxidase and dehydrogenase systems. All the above cited literature shows that many of these reports address only Dox induced cardiotoxicity.

As expected, Dox at the dose and time intervals tested markedly altered the two enzymatic activities where decreased activity of total ATPase and cytochrome-c-oxidase was observed (Table:1,2) compared to the control values. In control animals, ATPase activity was higher for liver tissue followed by brain > heart > kidney. Dox decreased kidney and liver ATPase activities more when compared to other tissues especially in 20 mg/kg wt for 10 weeks Dox treated tissues, so is the trend for cytochrome-c-oxidase activity. Vitamin E and A treatments reversed the Dox altered these enzyme levels. The energy produced is stored in ATP molecules, synthesized mitochondria through oxidative phosphorylation, inhibition of cytochrome-c-oxidase is responsible for disruption in the mitochondrial functions causing a decrease in oxidative phosphorylation, thus supporting a decrease in ATPase activities. Increased ammonia level may also be responsible for the inhibition of ATPase activity (Vijayakumari, 1979). Increased ca^{2+} and loss of potassium further inhibition of ATPase activity and cytochrome-c-activity sometimes may also be due to impaired glycolytic and Krebs cycle enzymes and like situations which has already cited by the author (Vijayudu, 2013). Dox as mentioned above causes cellular / tissue damage impairs oxygen consumption, ca^{2+} homeostasis and other metabolic functions in experimental models and any of these might be responsible for the present observed trend of inhibition of rat tissue ATPase and cytochrome-c-oxidase activities in Dox treated rat tissues. Vitamin E and A by way of maintaining the Dox treated rat tissues integrity may be normalizing the Dox altered ATPase and cytochrome-c-oxidase activities to some extent.

Vitamin E plus Dox and vitamin A plus Dox treatments reversed the Dox altered ATPases and Cytochrome-c-oxidase activities. The reversal of Dox altered enzyme levels could be due to the antioxidant properties of the vitamin E and A (Ciaccio et al., 1993; Tesoriere et al., 1994). Dox altered ATPase, Cytochrome-c-oxidase indicates disruption in the mitochondrial functions causing a decrease in oxidative phosphorylation. The essential groups of

these enzymes located in membranes. It is reasonable to assume that Vitamin E and A protects critical cellular structures and membranes against damage caused by free radicals and reactive product of lipid peroxidation. Upaganlawar et al., (2010) reported that Vitamin E application as protecting the –SH groups, essential for ATPase activity. Vitamin E has role in stabilizing the swollen morphology of mitochondria (Sebrell and Harris, 1972) might be possible reason for reversal of Dox altered enzyme levels.

CONCLUSION

Keeping in mind the toxic side effects induced by the cause of Dox and the protective role as afford by vitamin E and A in humans and experimental animals, the current study carried out with the objective of investigating the *in vivo* effect of Dox and vitamin E and A separately and in combination on selected enzymatic profiles in albino rat major tissues. Rats were administered in with selected doses of Dox 20 mg/kg wt and 100 mg/kg wt of vitamin E and 50 IU/kg wt of vitamin A separately and in combinations and the treatments period selected was 10 weeks (weekly doses). The control and experimental rat tissues like brain, heart, liver and kidney tissues were subjected for biochemical analysis.

In this paper the key enzymatic activities like ATPase and cytochrome-c-oxidase activities in control, vitamin E and A, Dox separately and in combination treated rat tissues were presented. Both these enzymes were found to be severely affected by Dox were their activities were found to be decreased in all the above tissues of rat lowered energy metabolism. Vitamin E and A reversed Dox altered rat tissues ATPase and cytochrome-c-oxidase activity levels to a considerable extent.

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