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INFLUENCE OF DESFERRIOXAMINE ON CYTOTOXIC AND BIOCHEMICAL CHANGES INDUCED BY DOXORUBICIN IN NORMAL AND EHRLICH ASCITES CARCINOMA CELL BEARING SWISS ALBINO MICE

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ABSTRACT

Normal and Ehrlich ascites carcinoma cell (EAC cell) bearing Swiss albino mice, 6-8 weeks old were treated with desferrioxamine (DFO) at doses of 125 and 250 mg/kg/day body weight. Some of the mice in each group were injected with doxorubicin (DOXO) (15 mg/kg) and killed 30 h after the last DFO treatment. The total proteins and nucleic acids were analyzed in the liver and testes of normal mice and EAC cells in the ascetic mice. DFO treatment was found to be devoid of any significant effect on protein and nucleic acids in hepatic and testicular cells of normal mice and the EAC cells of ascetic mice. Pre treatment with DFO at a single dose failed to protect the biochemical changes induced by DOXO, whereas DFO treatment for 7 days was found to provide significant protection against the DOXO induced changes in nucleic acids in normal mice, but it does not interfere with the antineoplastic effect of doxorubicin. The protective effect of DFO may be advantategous in cancer therapy involving drugs which binds to DNA and cause neoplastic changes.

INTRODUCTION

The anthracycline antibiotic doxorubicin (DOXO), isolated from Streptomyces peucetius var Cesius is used as a potent anticancer agent against a variety of neoplastic lesions (Di Marco *et al.*, 1969; Wang *et al.*, 1971; and Alberts and Salmon, 1975). However, DOXO, like most anticancer drugs, is known to induce genetic effects in mammalian cell systems (Marquardt *et al.*, 1976 and Rosselli *et al.*, 1990) and Xenopus laevis (Risely and Phorenec, 1991) and produce renal tumors, mammary fibroadenoma, breast carcinomas and adenocarcinoma in female rats (Sternberg *et al.*, 1972 and Bertazzoli *et al.*, 1971) and hepatocellular carcinoma in Bufo regularis (El-Mofty *et al.*, 1991).

Recurrence of cancer due to clastogenecity of cytotoxic drugs is major draw back in long term survivors of both radiotherapy and chemotherapy (Harris, 1976). Several chemical agents including vitamins glutathione, propylgallate, retinylacetate, germanium oxide, β-carotene, cinnamaldehyde, phenols, coumarins etc., have been experimented for their antimutagenic potentials (Ames, 1983). Since iron is implicated in the production of free radicals and stimulation of lipid peroxidation (Weitberg and Corverse, 1989), considerable efforts are focused on using chelators to alleviate the genotoxic effects of mutagens, carcinogens and cytotoxic drugs. Desferrioxamine (DFO, deferoxamine methan-sulfonic, desferal$^{(R)}$) is an iron chelator isolated from Streptomyces pilosus and treated chemically to obtain the metal free ligand. DFO is used immensely in the treatment of chronic iron over load diseases like thalassemia and haemochromatoris (Hussain *et al.*, 1976; Propper *et al.*, 1977 and Mordente *et al.*, 1990). Although a large number of papers have been published on the effect of DFO o DNA single strand breaks (ssb) induced by different known mutagens in cultured cells or isolated tissues (Wahba *et al.*, 1989, 1990 and Carstens *et al.*, 1990) and the protective effect of DFO against DOXO induced cardiac and haematological toxicity (Al-Harbi *et al.*, 1992), the literature on biochemical effects of DFO against anticancer drugs *in vivo* is scanty. Since iron- anthracycline complex is known to generate free radicals, which induces genetic damage through lipid peroxidation (Weiberg and Corverse, 1989). The present study was designed to investigate the effect of desferrioxamine on the biochemical changes induced by doxorubicin in liver and testes of normal and Ehrlich ascetic carcinoma cell bearing mice.

MATERIAL SAND METHODS

Drugs and Chemicals:

Desferrioxamine (DFO) was obtained from Ciba Geigy Ltd., Basle, Switzerland and Doxorubicin (DOXO) was provided by Farmitalia Carolo Erba, Italy. All other chemicals were obtained either from Sigma Chemicals (St. Louis, MO, USA) or BDH Chemicals Ltd. (Poole, England).

Animals:

Female and male Swiss albino mice (SWR) aged 5-6 weeks and weighing 20-25 g were obtained from the Experimental Animal Care Centre, King Saud University, College of Pharmacy, Saudi Arabia. The animals were fed on a purina Chow diet and water *ad libitum* and were maintained under controlled temperature (24°C ± 1), humidity range 40 to 45 percent and 12 h light/dark cycle (light from 0600 to 1800 h).

Dose and Route of Administration:

The doses of DFO are 125 and 250 mg/kg/day which are approximately 1/8 and 1/4 of LD_{50} of DFO (Pitt, 1981). Doxorubicin dose (15 mg/kg) was selected as

reported in previous study (Al-Harbi, 1993). The route of administration of DFO was intraperitonal in normal mice and subcutaneously in Ehrlich ascetic mice. Doxorubicin was administered intraperitonally in both normal and Ehrlich ascetic mice.

Studies on hepatic and testicular cells of normal mice:

The study constituted acute (single dose) and subacute (seven days) treatment of aqueous solution of DFO. A total of 60 male mice were randomly assigned into 12 groups (5 mice in each group). Either acute or subacute treatments constituted 6 groups of mice. The different groups under each treatment were: (1) untreated control (distilled water); (2) DFO (125 mg/kg/day); (3) DFO (250 mg/kg/day); (4) DOXO (15 mg/kg); (5) DFO (125 mg/kg/day) pretreatment plus DOXO (15 mg/kg); (6) DFO 9250 mg/kg/day pretreatment plus DOXO (15 mg/kg). Groups 2, 3, 5 and 6 were treated with DFO either with a single dose or 7 repeated doses. Groups 4, 5 and 6 were injected i.p. with DOXO 30 h before sacrifice. After sacrificing the animals under ether anesthesia, the liver and testes were quickly excised, frozen in liquid nitrogen and stored at -20°C till analyzed for total proteins and nucleic acids.

Estimation of protein and nucleic acids:

Total protein was determined by method of Lowry *et al.*, 1951. The method described by Bregman (1983) was used to determine the levels of nucleic acids. Tissues were homogenized and the homogenate was suspended in ice-cold trichloroacetic acid (TCA). After centrifugation, the pellet was extracted with ethanol before a hot TCA extraction. The levels of DNA were determined by treating the nucleic acid extraction with diphenylamine reagent and reading the intensity of blue colour at 600 nm. For quantification of RNA levels, the nucleic acid extract was treated with orcinol and the green colour was read at 660 nm. Standard curves were used to determine the amounts of nucleic acids present.

Studies on Ehrlich ascites carcinoma cell bearing mice:

Ehrlich ascites carcinoma cells (EAC cells) supplied through the courtesy of Dr. C. Benckuijsen, Amsterdam, Holland. EAC cells were maintained by serial implantations in female Swiss albino mice(SWR, bred at the Experimental Animal Care Centre, King Saud University, Saudi Arabia) every 8 days. A total of 60 female mice were randomly allotted to different control and treatment groups (10 mice in each group) for evaluation of the parameters on cytotoxicity and biochemistry. EAC cells (2.5×10^6 cells/mouse) were implanted (i.p.) into all experimental mice which were used in the present study. The treatment was started after an incubation period of 6 days. The experimental groups of Ehrlich ascetic mice consisted of the following: (1) positive control (distilled water); (2) DFO (125 mg/kg/day); (3) DFO (250 mg/kg/day); (4) DOXO (15 mg/kg); (5) pretreatment with DFO (125 mg/kg/day) plus DOXO (15

mg/kg); (6) pretreatment with DFO (250 mg/kg/day) plus DOXO (15 mg/kg). Group 2, 3, 5 and 6 were treated with 7 repeated doses of DFO. DOXO was injected simultaneously with the last dose of DFO. Then the animals were killed, under ether anesthesia, 30 h after the last treatment. Samples of peritoneal fluid were collected for analysis of cytotoxicity, viability and for biochemical parameters.

Cytotoxicity and viability:

The samples of peritoneal fluid were collected and studied for the viability and cytotoxicity of EAC cells with hemocytometer using a dye exclusion technique (Kaltenbach *et al.*, 1958).

Biochemical parameters:

The frozen peritoneal fluid samples were used for estimation of nucleic acids and total proteins.

RESULTS AND DISCUSSION

The results showing the effect on nucleic acids and proteins in hepatic and testicular cells after treatment with DFO and DOXO in normal mice are presented in Table 1 and 2. DFO treatment did not show any significant effect on proteins, RNA and DNA concentrations of hepatic and testicular cells in both the acute and subacute studies. The known cytotoxic and prooxidant property of DOXO are confirmed (Voest *et al.*, 1993; Tewey *et al.*, 1994). Thus the inhibitory effect of DOXO on proteins and nucleic acids is attributed to the iron-DOXO complex which the later generates free radical (Mayers *et al.*, 1982). The pretreatment with a single dose of DFO failed to inhibit the DOXO induced reduction of proteins, RNA and DNA levels. However, pretreatment with DFO for 7 days was found to protect the inhibition of nucleic acids in both hepatic and testicular cells induced by DOXO treatment. The protection in DNA levels of liver ($p < 0.01$) and testis ($p < 0.05$) was statistically significant only at the higher dose of DFO 9250 mg/kg). Although related studies on DFO are scanty, this protective effect of DFO is supported by earlier reports where DFO was found to inhibit DNA single strand breaks induced by hydroxy- and hydroperoxy- eicostatraenoic acids in human lymphocytes (Weitbergand Corvese, 1989), tetrachlorodibenzo-p- dioxin in hepatic nuclei of rats (Wahba *et al.*, 1989), tetrachlorohydroquinone in human fibroblasts (Carstens *et al.*, 1990). The exact mechanism of inhibition of DOXO induced biochemical changes observed in the present study is not known. However, it appears that the chelating action of DFO might have prevented the iron dependent formation of hydroxyl radicals and lipid peroxidation (Halliwell, 1985). Further, the pretreatment of DFO may be a result of the conversion of divalent form of iron to trivalent which the later is known to inhibit the prooxidant activity by forming a stable coordination complex with DFO (Graf *et*

al., 1984). The result obtained in this studies on EAC cells bearing mice after treatment with DFO and DOXO in different experimental group are presented in Table 3 and 4. The treatment with DFO failed to reduce the total number of EAC cells and alter their viability. The concentration of nucleic acids and proteins were also not significantly affected. However, the treatment with DOXO reduced significantly the number of viable cells and inhibit their nucleic acids and proteins levels. These results confirmed the known antineoplastic activity of DOXO (Di Marco *et al.*, 1969; Wang *et al.*, 1971 and Alberts Salmon, 1975). Pretreatment with DFO for 7 days failed to alter the inhibition of proteins and nucleic acids in the EAC cells of DOXO treated Ehrlich ascetic mice. The lack of inhibition of nucleic acids and proteins in EAC cells in not in agreement with our earlier observation in hepatic and testicular cells of normal mice in the same experiment, where DFO was found to protect the DOXO induced depletion of nucleic acids and proteins. The discrepancy between the results obtained in the studies on hepatic and testicular cells of normal mice and those on malignant EAC cells might have been due to increased production of free radicals in the later (Borek, 1988). Previous reports have suggested that drug sensitivity may reflect differences in the intracellular concentration of enzymes and mediators of various target biochemical processes or repair mechanism (Biuck, 1984).

The results obtained in the present study suggested that the pretreatment with DFO protects the DOXO induced inhibition of nucleic acids in normal hepatic and testicular cells. However, DFO does not interfere with the antineoplastic effect of doxorubicin. In view of the fact that DOSO binds strongly to DNA (Halliwell and Guttridge, 1987) and causes neoplastic changes (Strenberg *et al.*, 1972; Bertazzoli *et al.*, 1971; El-Mofty *et al.*, 1991), the protective effect of DFO observed in the present study may be advantageous to minimize the toxicity of doxorubicin in these organs and in particular the testes. Further studies are warranted to elucidate the protective effects of DFO on the functions of these organs and elucidate exact made of action before possible clinical use as an adjunct in cancer therapy.

Table 1
Effect of acute treatment of desferrioxamine (DFO) on doxorubicin (DOXO) induced biochemical changes in hepatic and testicular cells of normal mice

	Treatment & dose (mg/kg)	Organ	Total Protein	RNA	DNA
1.	Control (distilled water)	a- liver	15.80 ± 0.23	617.77 ± 3.47	214.29 ± 3.88
		b - testes	10.98 ± 0.24	261.23 ± 11.73	341.24 ± 11.29
2.	DFO (125)	a- liver	15.55 ± 0.21	612.56 ± 20.16	219.80 ± 9.50
		b- testes	11.06 ± 0.21	265.67 ± 9.76	337.58 ± 12.80
3.	DFO (250)	a- liver	15.65 ± 0.19	618.48 ±18.38	224.26 ± 7.15
		b- testes	11.10 ± 0.14	269.45 ± 8.36	330.62 ± 13.26
4.	DOXO (15)	a- liver	14.11 ± 0.17[b]	519.80 ± 16.30[c]	170.92 ± 4.72[c]
		b- testes	9.76 ± 0.19	220.24 ± 7.12[a]	287.64 ± 11.68[a]
5.	DFO (125) plus DOXO (15)	a- liver	14.08 ± 0.12	501.17 ± 29.30	169.74 ± 4.03
		b- testes	10.05 ± 0.24	218.55 ± 8.42	285.23 ± 10.24
6.	DFO (250) + DOXO (15)	a- liver	14.17 ± 0.18	493.82 ± 30.47	170.04 ± 4.51
		b- testes	9.88 ± 0.46	228.12 ± 8.79	286.45 ± 10.24

A total of 5 mice were used in each group.
Groups 2, 3 and 4 were statistically compared with group 1.
Groups 5 and 6 were statistically compared with group 4.
Results are expressed as mean ± S.E. of proteins (mg/100 mg) and nucleic acids (μg/100 mg).
a - $p < 0.05$; b - $p < 0.01$; c - $p < 0.001$ (Student's t-test).

Table 2
Effect of subacute treatment of desferrioxamine (DFO) on doxorubicin (DOXO) induced biochemical changes in hepatic and testicular cells of normal mice

Treatment & dose (mg/kg)	Organ	Total Protein	RNA	DNA
1. Control (distilled water)	a- liver	16.68 ± 0.42	623.33 ± 17.67	239.33 ± 5.06
	b- testes	11.04 ± 0.57	264.67 ± 6.23	326.67 ± 7.69
2. DFO (125)	a- liver	16.27 ± 0.37	617.18 ± 16.50	224.60 ± 6.48
	b- testes	9.79 ± 0.32	231.36 ± 11.76	304.78 ± 9.86
3. DFO (250)	a- liver	16.07 ± 0.20	608.17 ± 27.71	228.67 ± 7.07
	b- testes	9.69 ± 0.26	244.00 ± 7.43	314.67 ± 11.57
4. DOXO (15)	a- liver	15.02 ± 0.29[a]	466.50 ± 13.86[c]	178.67 ± 8.71[c]
	b- testes	9.05 ± 0.15[a]	226.67 ± 4.96[b]	286.83 ± 10.99[a]
5. DFO (125) plus DOXO (15)	a- liver	15.29 ± 0.19	476.00 ± 11.72	201.67 ± 7.14
	b- testes	9.31 ± 0.35	239.67 ± 7.96	306.67 ± 11.76
6. DFO (250) plus DOXO (15)	a- liver	15.39 ± 0.08	511.67 ± 17.8	221.83 ± 6.31[b]
	b- testes	9.29 ± 0.18	251.33 ± 9.17	329.50 ± 8.03[a]

A total of 5 mice were used in each group.
Groups 2, 3 and 4 were statistically compared with group 1.
Groups 5 and 6 were statistically compared with group 4.
Results are expressedas mean ± S.E. of proteins (mg/100 mg) and nucleic acids (μg/100 mg).
a - $p < 0.05$; b - $p < 0.01$; c - $p < 0.001$ (Student's t-test).

Table 3
Effect of subacute treatment of desferrioxamine (DFO)[a] on the cytotoxicity and viability induced by doxorubicin (DOXO)[b] in Ehrlich ascites carcinoma cells in mice

	Treatment & Dose (mg/kg)	Total number of cells (mean ± S.E.) x 10^6	Percentage of viable cells (mean ± S.E.) X 10^6
1.	Positive control (distilled water)	452.4 ± 38.43	91.27 ± 1.49
2.	DFO (125)	471.25 ± 37.11	90.26 ± 2.15
3.	DFO (250)	401.2 ± 28.32	88.75 ± 2.12
4.	DOXO	311.4 ± 36.71*	82.55 ± 2.74*
5.	DFO (125) plus DOXO (15)	288.8 ± 22.81	85.05 ± 1.67
6.	DFO (250) plus DOXO (15)	275.6 ± 16.48	82.65 ± 2.77

A total of 5 mice were used in each group.
a = Treatment with DFO (for 7 days) was started after an incubation period of 6 days of tumor implantation.
b = DOXO was injected simultaneously with last dose of DFO.
Groups 2, 3 and 4 were statistically compared with group 1.
Groups 5 and 6 were statistically compared with group 4.
*$p < 0.05$.

Table 4

Effect of subacute treatment with desferrioxamine (DFO)[a] on doxorubicin (DOXO)[b] induced depletion of nucleic acids and total protein levels of Ehrlich ascites carcinoma cells in mice

	Treatment & Dose (mg/kg)	DNA	RNA	Total protein
1.	Positive control (distilled water)	176.27 ± 9.92	427.76 ± 26.24	14.75 ± 0.41
2.	DOXO (15)	112.19 ± 13.89**	190.67 ± 11.21***	12.74 ± 0.17**
3.	DFO (125)	182.32 ± 10.11	405.44 ± 29.03	14.45 ± 0.37
4.	DFO (250)	190.46 ± 11.67	431.25 ± 29.21	14.51 ± 0.42
5.	DFO (125) plus DOXO (15)	132.68 ± 13.59	221.98 ± 17.67	13.00 ± 0.39
6.	DFO (250) plus DOXO (15)	141.33 ± 10.42	232.01 ± 17.07	13.12 ± 0.21

A total of 5 mice were used in each group.

a = Treatment with DFO (for 7 days) was started after an incubation period of 6 days of tumor implantation.

b = DOXO was injected simultaneously with last dose of DFO

Groups 2, 3 and 4 were statistically compared with group 1.

Groups 5 and 6 were statistically compared with group 2.

**$p < 0.01$ and *** $p < 0.001$ (Student's t-test).

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