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WR-1065 as a chemoprotector in Adriamycin chemotherapy

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Abstract

The protective effect of WR-1065 against the side effects of Adriamycin was studied in tumor bearing mice. It has been noticed that WR-1065 pretreatment improved the Adriamycin toxicities such as increased enzyme levels, pathological lesions in lung, liver and heart and increased rate of lipid peroxidation, without reducing its anticancer property.

Keywords: Chemotherapy; Adriamycin; WR-1065

1. Introduction

Harmful side effects of anticancer drugs are the major limiting factor in cancer chemotherapy. Chemotherapy can result in significant depression of the lymphoid and hematopoitic systems of treated patients and the toxicity produced by anticancer drugs itself may become a major cause for death of the patients at a later stage. Consequently it would be extremely beneficial to find agents that would protect normal cells from the toxicity of chemotherapeutic agents.

Adriamycin (ADR), an anthracycline antitumor antibiotic is an important anticancer drug used widely in the treatment of a variety of human malignancies [4]. It acts as an electron acceptor and in the presence of flavoenzymes generates either oxiradicals or drug radicals, depending on the availability of oxygen. The antitumor activity of ADR and its toxicity may be related to the formation of specific metabolites. The most serious side effect of ADR is myelosuppression. Cardiotoxicity is a major problem in ADR chemotherapy which restricts its clinical usefulness [9]. So, the search for an ideal chemoprotector against the side effects of ADR is of prime importance in clinical cancer chemotherapy.

Our earlier investigations clearly indicate that thiol compounds are capable of reducing the toxicity caused by cyclophosphamide and ADR [1,2].

The objective of this study is to investigate the chemoprotective effect of WR-1065(2-3-aminoethane thiol) against the toxicity of ADR.

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2. Materials and methods

2.1. Drugs

Adriamycin (Adriblastina) was the product of M/S. Farmitalia Carlo Erba. WR-1065 was a kind donation from NCI, NIH, USA. Both the drugs were dissolved in double distilled water before use and the route of administration was intraperitoneal.

Experiments were carried out using adult mice of BALB/c strain from an inbred colony, weighing 22 ± 4 gm. The animals were maintained on standard mice feed and water ad libitum.

2.2. Tumor system

The tumor system used was Ehrlich carcinoma, in the solid form. The cells grown in ascitic form were collected, washed and resuspended in physiological saline to get a count of 1×10^6 cells/ml. From this 0.1 ml was injected subcutaneously for the tumor induction. Treatment was started on day 7 post-inoculation. Tumor measurements were taken using a vernier caliper and the volume of the tumor was calculated as $V = \pi/6\ a \times b \times c$, where a,b,c are the length, width and depth of the tumor.

The tumor bearing animals were divided into 4 groups of 15 each and treated as:

Group A: 4 mg/kg wt. of ADR for 6 consecutive days.
Group B: 4 mg/kg wt. of ADR + 50 mg/kg wt. of WR-1065, 10 min before each ADR injection.
Group C: 50 mg/kg wt. of WR-1065 on the same days as in group B.
Group D: tumor bearing animals receiving isotonic saline, served as control.

2.3. Biochemical studies

Blood for the biochemical estimations was collected from the orbital sinus and alkaline phosphatase (AP) and creatine phosphokinase (CPK) were analyzed in the serum [7,8].

2.4. Assay of lipid peroxidation rate

Animals were autopsied and the heart, liver and kidney were collected after perfusion, homogenised and microsomes were separated by differential centrifugation to assess the lipid peroxidation rate as described by Bushaji and Balasubramaniam [5].

2.5. Histopathological analysis

Heart, liver, lung, kidney, stomach and intestine were collected, fixed and processed for histopathological examinations.

2.6. Tumor response studies

Tumor response was assessed by taking tumor regression, volume doubling time and growth delay as end points. Tumor volume doubling time (VPT) was calculated according to the formula, $Td = \lg 2 \times (T_1 - T_0)/\lg V_1 - \lg V_0$, where V_0

Table 1
Effect of WR-1065 pretreatment on serum alkaline phosphatase levels after ADR chemotherapy

Drug dose (mg/kg, i.p.)		Alkaline phosphatase levels (KA units/l)		
ADR	WR-1065	Day 4	Day 11	Day 18
NIL	NIL	2.4 ± 0.64	2.8 ± 0.49	2.3 ± 0.95
NIL	50*6	2.9 ± 0.51	3.1 ± 0.27	2.6 ± 0.26
4*6	NIL	4.6 ± 0.12*	7.2 ± 0.83*	8.9 ± 0.66*
4*6	50*6	3.1 ± 0.12[a]	2.8 ± 0.76[a]	3.2 ± 0.26[a]

Fifteen animals in each group. Values are mean ± S.E.
*Significantly higher than the control $P < 0.001$.
[a]Significantly lower than the ADR-treated group.

Table 2
Effect of WR-1065 pretreatment on serum creatine phosphokinase (CPK) levels after ADR chemotherapy

Drug dose (mg/kg, i.p.)		CPK levels (mg%)		
ADR	WR-1065	Day 4	Day 11	Day 18
NIL	NIL	21.8 ± 1.87	22.2 ± 1.27	21.9 ± 1.08
NIL	50*6	26.4 ± 1.12	24.2 ± 1.14	23.8 ± 1.16
4*6	NIL	55.6 ± 1.23*	51.9 ± 1.64*	56.0 ± 1.28*
4*6	50*6	28.9 ± 1.34[a]	27.2 ± 1.45[a]	23.8 ± 1.16[a]

Fifteen animals in each group. Values are mean ± S.E.
*Significantly higher than the control $P < 0.001$.
[a]Significantly lower than the ADR-treated group.

was the first measured volume of the tumor and V_1 was the final volume of the tumor, at the termination of the experiment [14]. Tumor growth delay (GD) is the difference in number of days for the tumor to reach 200 mm^3 after treatment (T) compared to the untreated control (C), GD = T − C.

Statistical analysis of the results were carried out using Student's t-test.

3. Results

Serum AP and CPK levels were found to be increased significantly after adriamycine treatment. WR-1065 by itself did not produce this effect. WR-1065 pretreatment could bring down the elevated enzyme levels significantly in adriamycine-treated animals (Tables 1 and 2).

The lipid peroxidation rate after WR-1065 treatment showed no significant increase from normal, while it was significantly high in heart, liver and kidney of ADR treated animals. The peak level was observed between 8 and 12 h post-treatment. This high rate of lipid peroxidation was found to be reduced after WR-1065 pretreatment significantly (Table 3).

None of the organs under study revealed any pathological lesions after WR-1065 treatment. The stomach, intestine or kidney showed no notable pathological changes after ADR treatment. Liver of ADR-treated animals showed Kupffer cell hyperplasia. Sections from lungs after ADR treatment showed features of pneumonia, with congestion of the capillaries and a severe acute inflammation in the alveolar walls. Heart tissues revealed pathological lesions after ADR treatment

Table 3
Effect of WR-1065 pretreatment on lipid peroxidation rate after ADR chemotherapy

Drug dose (mg/kg, i.p.)		Lipid peroxidation rate (nm/mg protein)[b]		
ADR	WR-1065	Heart	Liver	Kidney
NIL	NIL	2.2 ± 1.12	3.1 ± 0.98	3.6 ± 0.88
NIL	50*6	2.8 ± 1.34	3.9 ± 1.23	4.1 ± 1.4
4*6	NIL	10.8 ± 1.36*	9.3 ± 1.25*	11.3 ± 1.55*
4*6	50*3	3.1 ± 1.33[a]	4.1 ± 1.27[a]	4.2 ± 1.28[a]

[b]Values given are the maximum rate observed. Other details are as in Table 1.

Table 4
Changes in volume doubling time (VDT) and growth delay (GD) after ADR administration with or without WR-1065 pretreatment

Drug dose (mg/kg, i.p.)		VDT	GD
ADR	WR-10065	(Days ± S.E.)	(Days ± S.E.)
NIL	NIL	3.5 ± 0.18	—
NIL	50*6	3.8 ± 0.13	—
4*6	NIL	6.8 ± 0.35*	12.63 ± 0.43*
4*6	50*3	7.1 ± 0.28*	12.11 ± 0.39*

Fifteen animals in each group. *Significantly different from the control $P < 0.001$.

characterized by vacuolisation of myocytes, damaged myocardial and endothelial cells, and swelling and partial occlusion of the capillary lumina. Tissues from animals receiving WR-1065 pretreatment appeared to be normal without any notable pathological lesions.

WR-1065 did not alter the tumor growth pattern (Table 4). The VDT and GP increased significantly after ADR treatment and was not altered by the WR-1065 pretreatment.

4. Discussion

ADR binds tightly to DNA by its ability to intercalate between base pairs. Intercalation results in inhibition of DNA synthesis and DNA-dependent RNA synthesis. In addition to this the anthracycline ring of ADR can undergo one electron reduction to form free radicals. These highly reactive free radicals may then react with biologically important molecules as cell membranes and proteins [10).

The drug is extensively metabolized in liver to its hydroxylated and conjugated metabolites. The free radicals formed during this activation process may react with unsaturated lipids leading to their peroxidation and with cellular DNA to induce DNA damage. These highly reactive species may also oxidise certain functional proteins. Damage to these multiple sites may ultimately lead to cell death [3]. In this study the hepatic, cardiac and lung toxicity must have resulted from the free radical attack to the vital molecules, which is evidenced by the increased enzyme levels, high rate of lipid peroxidation and pathology of lung, liver and heart. ADR induced free radicals through a redox cycling interaction must have an important role in cardiac and hepatic damage. The membrane integrity must have been lost due to peroxidation of membrane lipids and the damage caused by this might have lead to the pathological lesions in liver, lung and heart.

Protein leakage from the blood vessels, as a result of vascular damage, may result in pneumonitic lung injury [13]. In the present study the free radicals formed during ADR metabolism might have induced damage to the vascular membranes and must have resulted in pneumonitic lung injury.

In this study the phosphorothioate WR-1065 protected the normal tissues from the toxicity of ADR. Several mechanisms have been proposed by various authors for the protective effect of phosphorothioates to normal tissues which include free radical scavenging, hydrogen ion donation to facilitate repair and release of protein bound thiols [6,12,17]. Sulphydryl derivatives can also offer protection indirectly by releasing glutathione from protein bound mixed disulphides [11]. Reduced glutathione in turn protects against electrophylic attack by hydrogen donation or by converting the toxic radicals to less or non-toxic metabolites. Moreover phosphorothioates are having differential uptake [18,16,15]. The differential uptake of these synthetic compounds enables differential protection to the normal tissues which is evidenced by the tumor studies (Table 4) in the present study. Here WR-1065 might have reduced the damage to vital molecules by scavenging the free radicals or by repairing the damages, without altering the antitumor activity of ADR.

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