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CREATININE PHOSPHOKINASE AND CARDIOTOXICITY IN ADRIAMYCIN CHEMOTHERAPY AND ITS MODIFICATION BY WR-1065

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Abstract

The effect of adriamycin (ADR) chemotherapy on creatinine phosphokinase (CPK) and pathological lesions in heart was studied with Balb/c mice. WR-1065 was used as a chemoprotector against the cardiotoxicity of ADR. CPK levels were measured at different intervals and the hearts of the treated animals were analyzed for pathological changes. The results indicate that there is a significant relationship between the CPK levels and pathological lesions. WR-1065 could reduce the cardiotoxicity induced by ADR, without altering its antineoplastic activity.

Adriamycin (ADR), an anthracycline antibiotic is clinically used against a variety of human tumors and is one of the most effective drugs against breast tumors (1). The antitumor activity of ADR has been explained by different mechanisms, namely, (a) intercalation into DNA (2), (b) interaction with biomembranes to cause membrane lipid damage (3), and (c) bioreductive activation leading to the formation of both drug and oxygen free radicals (4).

A major limitation in ADR chemotherapy is its cardiotoxicity which reduces the clinical efficacy of the drug. Therefore any agent which can protect normal tissue, such as heart, against the toxicity of ADR would be expected to increase its efficacy. Agents which give protection to normal cells but at the same time do not reduce the clinical efficacy of anticancer drugs are called chemoprotectors. Chemoprotectors, in theory, enable the use of anticancer drugs in higher doses, which may be pharmacologically more effective, without notable side effects.

In our previous study we found that the sulfhydryl derivative, 2-mercaptopropionylglycine, is an effective chemoprotector against the alkylating agent cyclophosphamide (5). Phosphorothioates, another group of sulfhydryl derivatives, are also reported to have the ability to protect normal cells against the cytotoxicity of alkylating agents (6).

The present study was undertaken to investigate creatinine phosphokinase (CPK) levels and histopathological lesions as indicators of cardiotoxicity in ADR chemotherapy and the chemoprotective effect of WR-1065 (2-3 amino propylaminoethanethiol) against the cardiotoxicity of ADR.

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Materials and Methods

ADR (injection) was the product of M/s Farmitalia Carlo Erba. The drug was dissolved in isotonic saline to obtain a 1.0 mg/mL solution. WR-1065 (National Cancer Institute, USA) was dissolved in double distilled water and the pH was adjusted to 7.2 with N NaOH.

Balb/c mice of either sex, 22 $\pm$ 4 g, inbred, were maintained on commercial mice chow and offered water ad lib.

Sarcoma-180 tumor cells were grown in ascitic form. Cells were collected, washed three times, and suspended in isotonic saline. One million cells were injected subcutaneously for tumor induction. Tumor measurements were taken with a vernier caliper and the volume of the tumor was calculated from the equation $V = (\pi/6)*a*b*c$, where a,b,c are length, width and depth of the tumor, respectively.

Tumor bearing mice were divided into 4 groups with 15 mice in each group. Group A received 0.84 mg ADR/kg for 3 consecutive days, repeated weekly for 3 weeks. Group B received 0.84 mg ADR/kg for 3 consecutive days, repeated weekly for 3 weeks and 50 mg WR-1065/kg daily, for 21 days. Group C received 50 mg WR-1065/kg daily for 21 days. Group D received isotonic saline and served as the control. Drugs were administered intraperitoneally. In combination therapy WR-1065 was injected 20 minutes prior to ADR.

Creatinine phosphokinase was measured by the method of Huges (7) in serum of blood collected from the orbital sinus.

Following the course of treatment the heart was fixed in 10% formalin and slides were prepared for histological examination.

The mean and standard deviation of the data were calculated. The significance of the difference between means was determined by Students t-test.

Results

CPK levels in the ADR treated mice was significantly higher than the CPK level in controls. WR-1065 treatment lowered the elevated levels of CPK (Table 1).

Histopathological examination of hearts from rats treated with ADR without WR-1065 pretreatment revealed pathological lesions characterized by vacuolization of myocytes, damaged myocardial and endothelial cells, and swelling and partial occlusion of the capillary lumina. Mice which received WR-1065 had no pathological lesions of the heart.

WR-1065 had did not reduce the antineoplastic efficacy of ADR (Table 2).

Discussion

Adr is extensively metabolized to its hydroxylated and conjugated metabolites. The free radicals formed during this activation process may react with unsaturated lipids causing lipid peroxidation. These free radicals may react with cellular DNA to produce DNA damage and may also oxidize certain functional proteins. Damage to these multiple sites may ultimately lead to cell death (8). In the present study, the cardiotoxicity evidenced by high CPK serum levels and pathological lesions of the heart, may

be the result of multiple site injury caused by free radicals.

TABLE 1

Creatinine Phosphokinase Levels

Group	Creatinine Phosphokinase (mg%)		
	Day 7	Day 14	Day 21
A	55.59 ± 1.23	51.85 ± 1.64	56.02 ± 1.28
B	28.92 ± 1.34	27.22 ± 1.45	28.32 ± 1.62*
C	26.44 ± 1.12	24.28 ± 1.14	28.83 ± 1.16
D	24.80 ± 1.14	20.01 ± 1.22	22.20 ± 1.27

Normal CPK serum level: 21.80 ± 1.87 mg%.
* $P < 0.001$.

TABLE 2

Tumor Volume

Group	Tumor Volume on Day 60 (cm^3)
A	1.063 ± 0.547*
B	1.115 ± 0.532*
C	8.947 ± 0.922
D	9.081 ± 0.903

* $P < 0.001$

WR-1065 reduced the cellular damage caused by ADR and thereby lowered the elevated CPK level and eliminated the pathological lesions.

Several mechanisms have been proposed to explain the protective effects of phosphorothioate to normal tissue. The possible mechaisms include free radical scavenging (9) and hydrogen ion donation to repair free radical damage in target molecules (10). Normal cells take up WR-1065 more rapidly than tumor cells (11,12) and thus get protected against free radical damage (13). Sulfhydryl compounds may also offer protection indirectly by releasing protein bound glutathione from protein bound mixed disulfides. Reduced glutathione in turn protects against electrophilic attack by hydrogen ion donation.

In the present study, WR-1065 may have reduced DNA damage and lipid peroxidation induced by free radicals and may also have increased the intercellular glutathione level. Studies to determine the mechanism whereby WR-1065 reduces the cardiotoxicity of ADR are in progress.

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