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Modification of Adriamycine/Cyclophosphamide Induced Immune Suppression by an Aminothiol

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ABSTRACT

Cancer chemotherapeutic drugs are toxic to normal tissues and this toxicity is a limiting factor in the usage of higher doses. Another drawback of antineoplastic agents is their immunosuppressing nature. In the present study an aminothiol WR-1065 showed an antagonistic action against the immunosuppressent nature of two commonly used anticancer drugs, cyclophosphamide and adriamycine.

Introduction

The toxicity of cancer chemotherapy is an important limiting factor in the use of drugs that might be curative if higher doses could be used. Therapeutically effective doses of many anticancer drugs may produce irreversible changes in normal tissues, which may prove toxic to the patients. In addition to this immunosuppression is another limitation which prevents the use of higher doses of the antineoplastic drugs.

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Adriamycine (ADR), a commonly used anthracycline antineoplastic agent is reported to have severe and generalised hypersensitivity reactions like urticaria, angioedema, rashes and hypotension (1). Cyclophosphamide (CYD), an alkylating agent, another common antineoplastic drug also reported to cause injury to several cell types resulting in immune suppression (2).

In our previos study we observed that WR-1065, an aminothiol, pretreatment reduced the cardiotoxicity induced by ADR (3). The present investigations are aimed to analyse the immunopotentiating efficacy of WR-1065 (2-[(aminopropyl)amino]ethane thiol) in ADR and CYD treatment in mice.

Materials and Methods

Adult Swiss albino mice weighing 20 ± 5 gms were used. The animals were maintained under controlled temperature and humidity with sterile bedding material and food and water *ad libitum*.

ADR is a product of MS Farmitalia Carlo Erba. CYD is purchased from Khandelwal Laboratories, Bombay, India. WR-1065 is a kind donation from N C I, USA. All drugs were dissolved in double distilled water just before use and the route of administration was intraperitoneal. All other chemicals used were of analytical grade.

Animals were divided into 6 groups and treated as follows -

Group A : 0.84 mg/kg body wt of ADR

Group B : 50 mg/kg body wt of CYD

Group C : 50 mg/kg body wt of WR-1065

Group D : 0.84 mg/kg body wt of ADR + 50 mg/kg body wt of WR-1065

Group E : 50 mg/kg body wt of CYD + 50 mg/kg body wt of WR-1065

Group F : Isotonic saline, as control.

All the groups were immunised with 2.5 * 10^6/ml sheep red blood cells (SRBC) on the second day of drug treatment.

The spleen from each group was collected on day 2,3,4, and 5 after immunisation in chilled RPMI-1640 culture medium. A single cell suspention was made, centrifuged and the pellet was resuspended in cold RPMI medium and kept at 4°C for 15 min. Centrifuged and washed 3 times in cold RPMI medium and the final count was adjusted to $8*10^6$ cells/ml. This cell suspention was used for plaque assay as described by Jern and Nordin (4). The number of plaques formed were counted. Duplicates were kept for each group and the mean value was taken and multiplied by 2.5 and the values were expressed as plaque forming cells (PFC) per million spleen cells. The experiments were repeated 5 times. The statistical analysis of the data were done using students "t" test.

Blood was collected from the orbital sinus on day 2, 3 & 4 and the lymphocytes were seperated. Percentage T and B lymphocytes were assayed as described by Hunt (5).

Results

No PFC were observed in any of the experimental or control groups on day 2. The number of PFC were significantly less in both ADR and CYD treated groups (Fig 1). In both groups the least number of PFC were observed on day 3. WR-1065 could induce the formation of PFC, significantly higher than the normal and the

Fig 1

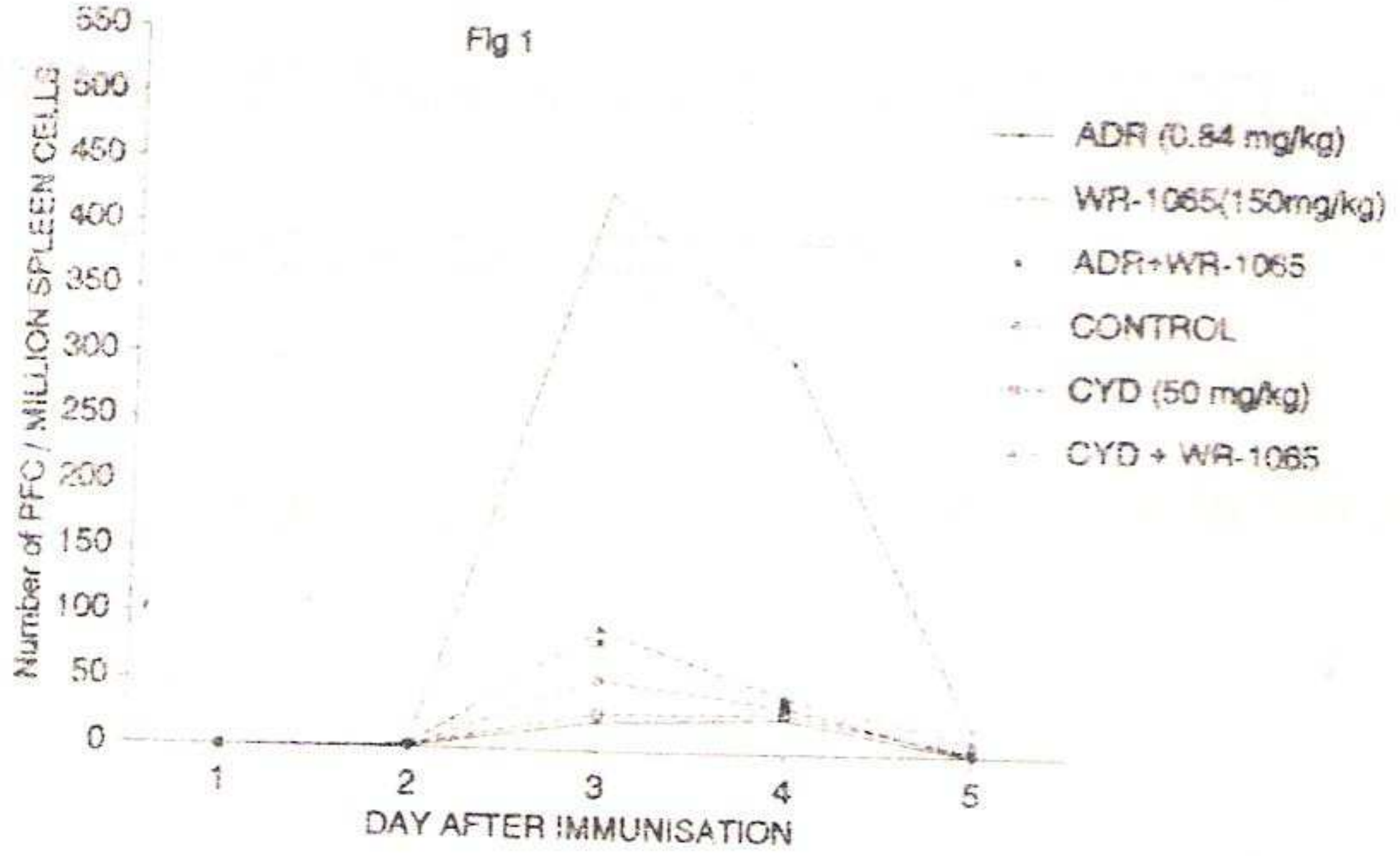

Fig 1

Modification of Adriamycine/Cyclophosphamide induced Immune Depression by WR-1065.

maximum number of PFC in this group were observed on day 3 and then declined. In all the experimental and control groups there were no PFC on day 5. WR-1065 pretreatment modified the decreased number of PFC in both ADR and CYD treated groups.

On day 4 there was no significant change in the % of T or B cells. The % T and B lymphocytes on day 2 and 3 were as given in Table 1. ADR induced a 74% decrease in the B lymphocytes and 63 % in T lymphocytes, on day 3. Whereas CYD produced a 60 % and 57% decrease in B and T lymphocytes respectively on the same day. WR-1065 stimulated the T and B lymphocyte production with a 20% increase in B and 5% increase in T lymphocytes on day 3. WR-1065

pretreatement resulted in 67% increase in B and 60% increase in T lymphocytes after ADR and 46% B and 56% T lymphocytes after cyclophosphamide treatment, than the respective drug controls.

Table 1

Influence of WR-1065 on T and B populations in ADR treatment on day 2 and day 3.

Experimental Groups	% B Lymphocytes		% T Lymphocytes	
	Day 2	Day 3	Day 2	Day 3
Normal	15	15	75	75
ADR	8	4	33	28
CYD	7	6	36	32
WR-1065	19	18	82	79
WR-1065 + ADR	16	14	73	73
WR-1065 + CYD	19	16	76	74

Discussion

Alkylating agents and ADR cause immune suppression (1, 2, 6 & 7) . This is due to the injury caused by the drugs to several cell types, including the T and B lymphocytes (2) which is evidenced in this study by the reduction in PFC and T and B lymphocytes. ADR and CYD might have suppressed the B lymphocytes from producing lytic antibodies to react against the antigen and hence the less number of PFC in those groups. The drugs also might have induced an inhibition on stem cell differentiation into T and B cells.

WR-1065 might have accelerated the antibody production by stimulating the antibody forming cells, which is indicated by the increased number of PFC and T and B lymphocytes in the WR-1065 pretreated group.

WR-1065 showed an antagonistic action against the immunosuppression induced by ADR and CYD. The point of action of WR-1065 may be mainly by the protection and preservation of antibody forming cells from the toxic metabolites of the drugs. Various other mechanisms may also be involved which needs furthe investigations.

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