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Modulatory Effect of Dopamine on Doxorubicin-induced Myelosuppression

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Abstract. Bone marrow suppression is a dose-limiting toxicity of anticancer drugs. We have investigated in this study whether dopamine (DA), a catecholamine neurotransmitter, could alleviate the haematotoxicity of doxorubicin (DXN), an established anticancer drug having profound myelotoxicity. DA was injected intraperitoneally at a dose of 50 mg/kg/day for five consecutive days in Swiss mice. The animals received a single intravenous injection of DXN (25 mg/kg) 24h after last injection of DA. DXN caused an immediate and sharp fall in total leucocyte, neutrophil, lymphocyte and platelet counts in peripheral blood, and nucleated cells in femur and spleen. DA treatment before DXN substantially reduced the degree and duration of these abnormalities, and also mediated a significant rise in the number of cells of granulocyte and erythroid lineage in the spleen. DA-pretreated mice showed early recovery of megakaryocytes (MK) and their precursors (SAChE+ cells), and stimulation of pulmonary MK. Thrombopoiesis, assessed in terms of incorporation of ^{35}S by platelets, was stimulated in DA-treated mice. Moreover, DA pretreatment partially protected the day 12 colony-forming unit spleen ($CFU\text{-}S_{12}$) from the lethal effects of DXN, and substantially reduced the mortality of the treated animals. The results demonstrate protection by DA against the myelosuppressive effect of DXN.

Keywords: Dopamine; Doxorubicin; Haematotoxicity; Mice; Protection

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Introduction

Cancer treatment is often limited by the potentially lethal effects of radiation and chemotherapy on the bone marrow (Hoagland 1982). Resultant neutropenia and thrombocytopenia are of particular concern because of the chances of opportunistic infection and haemorrhage (Pizzo 1984). Cycling haematopoietic cells as well as stromal cells in the haematopoietic microenvironment are highly susceptible to the effects of high-dose chemotherapy, whereas primitive haematopoietic cells may be less sensitive because many of these cells reside in G_0 (Sprangude and Johnson 1990). Taking advantage of this differential sensitivity of haematopoietic cells, haematopoietic growth factors (HGFs) namely colony stimulating factors for granulocytes (G-CSF) and granulocyte–macrophage (GM-CSF) are used in clinical medicine with considerable success for alleviation of the toxic effects of anticancer radiochemotherapy (Wolf 1989; Henry 1997). Indeed, the use of HGFs has allowed chemotherapy in high-dose schedules, with better therapeutic success (Bronchud et al. 1989).

In recent years, however, some disturbing aspects of HGF use have surfaced. G-CSF has been shown to damage the self-renewal capacity after multiple courses of cytotoxic drugs (Van Os et al. 1998), and HGFs also stimulate growth of a variety of non-haematopoietic cells, either normal (Dedhar et al. 1988) or neoplastic (Sagawa et al. 1991; Lopez-Marure et al. 1998). In addition, GM-CSF stimulates proliferation of leukaemic bone marrow cells (Schmetzer et al. 1999). Hence, simultaneous proliferation of haematopoietic progenitors and tumour cells could not be ruled out in tumour-bearing subjects receiving HGFs. Recent investigations have convincingly proved that this hypothesis is a fact, as G-CSF and GM-CSF have been shown to stimulate

the proliferation of bladder carcinoma (Tachibana et al. 1995), melanoma (Ciotti et al. 1995), human skin cell carcinoma (Mueller and Fusenig 1999), gastric carcinoma (Baba et al. 1995) and non-small cell lung carcinoma (Oshika et al. 1998). Interleukin (IL)-3 also stimulates clonal growth of non-haematopoietic tumour cells (Berdel et al. 1989). Equally important are the findings that G-CSF and IL-6 augment the metastatic potential of human head-and-neck carcinoma (Nishino et al. 1998; Noda et al. 1999) and oesophageal squamous cell carcinoma (Nishino et al. 1998). Moreover, a correlation between GM-CSF gene expression and metastasis has been demonstrated in murine tumours (Takeda et al. 1990). These reports underscore the need to apply discretion in using HGFs to prevent the chemotherapy-related leucopenia/neutropenia, and also emphasised the need to explore suitable alternative(s) for haematopoietic protection and recovery. The fact that HGFs are too expensive to be used routinely, especially in the developing countries, further underlines the importance of such a study.

With this perspective in mind, we have investigated whether dopamine (DA) could be used as a protective agent against cytotoxic drug-induced haematotoxicity. DA, the precursor of noradrenline, is a catecholamine neurotransmitter of the central nervous system. It is also found in the peripheral tissues, where it may be involved in the regulation of cardiovascular and immune functions (Bergquist et al. 1994). The rationale for using DA as a haematoprotective agent is based on our earlier observations that DA stimulates multilineage haematopoiesis in mice (Ray et al. 1988, 1994). Moreover, DA and its analogue, 3,4-dihydroxybenzylamine inhibit tumour growth and concomitantly improve the haematological profile of the host (Ray et al. 1988; Lahiri et al. 1990). We therefore hypothesised that DA could be effective in alleviating the adverse haematological effects, and augmenting the antitumour efficacy of commonly used anticancer drugs. To test the first part of this hypothesis, the present work has been conducted in Swiss mice using doxorubicin, a potent anticancer agent having profound haematotoxicity (Pannacciulli et al. 1989), as the myelosuppressive agent.

Materials and Methods

Animals

Male Swiss mice, 6–8 weeks of age and weighing 20–22 g were used. The animals, obtained from the Institute's own animal breeding facility, were kept in plastic cages (five mice per cage) in a temperature- and humidity-controlled environment with alternate cycles of light and darkness (12 h each). They had free access to food (standard mouse pellet) and water. Experiments were carried out strictly adhering to the guidelines of the Institutional Animal Ethics Committee.

Treatment

Dopamine hydrochloride (DA, TTK Pharma, Chennai, India) was diluted in sterile 0.9% saline and injected i.p. at a dose of 50 mg/kg body wt/day for five consecutive days. Control animals received equivalent volumes (0.25 ml) of saline. At 24 h after the last injection of DA, the animals received a single intravenous injection, via the tail vein, of 25 mg/kg of doxorubicin (DXN, Dabur India Ltd., Solan, India) in 0.25 ml of sterile saline.

Haematology

Blood samples were collected from control and DA-treated mice at different time intervals beginning 24 h after doxorubicin injection. Haematological parameters (haemoglobin, erythrocyte, leucocyte and platelet counts) were determined from free-flowing tail vein blood without anticoagulant using standard procedures (Bain 1996). Cell counts were performed with a microscope using a haemocytometer. Differential white blood cell (WBC) counts were performed from Leishman-stained blood smears, counting at least 200 cells. Red cell morphology was examined under an oil immersion objective.

Bone Marrow and Spleen Cell Count

The animals were killed by cervical dislocation, and both femurs and spleen were removed. Bone marrow was flushed from the femoral shaft into graduated plastic tubes using 5 ml disposable syringes containing phosphate buffered saline (PBS). Nucleated cell counts were carried out using haemocytometers, after destroying the non-nucleated mature erythrocytes by using WBC diluting fluid containing 3% glacial acetic acid. Nucleated cells per femur was estimated from cell count/ml and total volume of marrow suspension.

The spleen cells were harvested into PBS by teasing the organ in steel wire mesh. Cell counts were performed as for the marrows.

Differential Distribution of Haematopoietic Cells

BM and spleen cell suspensions were smeared on to clean glass slides for identification of haematopoietic cells of different lineage by a panel of lineage-specific cytochemical stains. Granulocytes and their precursors were detected by positive reaction with Sudan black B staining (Catovsky 1996). Erythroid cells were identified by benzidine reaction after staining with 1% benzidine (Sigma Chem, USA) for 1 min and incubation with a mixture of 70% ethanol and 30% H_2O_2 (3:1, v/v) for 1 min (Lobue et al. 1963). Megakaryocytes (MK) and their precursors, the small acetylcholinesterase-positive (SAChE +) cells were detected by the presence of marker enzyme acetylcholinesterase (AChE, Jackson

1973). For AChE staining, the procedure of Karnovsky and Roots (1964) using acetylthiocholine iodide (Sigma Chem, USA) as the substrate was followed. Diameters of the AChE-positive cells was determined by eye-piece reticule. Cells having a diameter of less than 18 μm were designated as SAChE + cells, (MK precursors), whereas the larger cells were considered as mature MK (Young and Weiss 1987). The slides were examined under light microscopy and the percentage of cells of a particular lineage was calculated after counting at least 500 cells. The total number of cells of each type was estimated from relative distribution (%) and number of nucleated cells per femur or spleen.

^{35}S Incorporation into Platelets

Quantitative assessment of platelet production was done by estimating the incorporation of ^{35}S into circulating platelets (McDonald 1973). The animals were given a single intravenous injection of 30 μCi $Na_2\ ^{35}SO_4$ (BARC, India), and blood samples were collected into acid citrate–dextrose solution 24 h later. The samples were centrifuged at 1000 ***g*** for 10 min and the platelet-rich plasma (PRP) was collected. Radioactivity in the PRP was measured in a scintillation counter. The percentage of ^{35}S incorporation into platelets was calculated by the method described by McDonald (1973).

MK and SAChE Cells in Lung

After killing the animals by cervical dislocation, the trachea was exposed and the lungs rinsed in situ with 2.0 ml of sterile saline at a hydrostatic pressure of 30 cm of water. The lavaged lungs were dissected out into cold Dulbecco's modified Eagle's medium (DMEM) and gently washed thrice in this medium to remove, as far as possible, the blood cells. Thereafter, the lung tissues were minced with fine scissors in DMEM and the cell clumps were dispersed by gently teasing through fine steel-wire mesh. An aliquot of cell suspension was used for the total cell count using a haemocytometer, after destroying the mature red cells with 3% glacial acetic acid. The remaining cell suspension was used to prepare cell smears for AChE staining. Three slides were prepared for each lung, and the number of MK and SAChE+ cells was calculated from their relative distribution (%) and the nucleated cell count per lung.

Day 12 CFU-spleen Assay In Vivo

Pluripotent haematopoietic stem cells were assayed as day 12 colony-forming unit, spleen (CFU-S_{12}) in lethally irradiated mice, according to the procedure of Till and McCulloch (1961). In essence, assay mice were irradiated with 9.0 Gy from a ^{60}Co source. Within 2 h they were transfused via the tail vein with 1×10^5 marrow or 2.5×10^5 spleen cells harvested from control and from tumour-bearing mice. The recipients were killed 12 days later and their spleens were removed and fixed in Bouin's solution. Colonies on the surface of the spleen were counted as CFU-S_{12}. The total number of CFU-S_{12} per femur or spleen was calculated as: number of CFU-S_{12} counted $\times$ cells per femur or spleen/number of cells injected into assay mice.

Survival

The survival of DXN only and DA+DXN groups were compared over a period of 30 days after completion of treatment. Thirty mice were used in each group for the purpose.

Statistical Analysis

The results were statistically analysed by Student's 't' test and $p < 0.05$ was considered as significant.

Results

Changes in Peripheral Blood

Table 1 presents peripheral blood leucocyte counts of control (DXN only) and DA pretreated (DA+DXN) mice. DXN caused a sharp fall in WBC count from a mean normal value (derived from a simultaneous study of age and sex matched normal untreated animals from the same colony as the treated mice) of $14.3 \times 10^3/\mu l$ to 6.5×10^3 and $4.1 \times 10^3/\mu l$ at day 2 and day 4 post-treatment, respectively. The corresponding leucocyte counts in DA pretreated mice were 29% and 112% higher than the DXN only group. Likewise, DXN-induced neutropenia and lymphocytopenia were less severe in the DA-treated group ($p < 0.05$, Table 1). DA also shortened the duration of leucopenia by initiating early recovery. As a result, total leucocyte and neutrophil counts returned to the normal level by day 6 post treatment, 3 days earlier than the DXN-only group. The recovery of lymphocyte count was also nearly complete by day 6. Lymphocytes with the morphology of large granular lymphocytes (LGL) were abundant in mice receiving DA before DXN (Fig. 1). Accelerated neutrophil recovery in DA-treated mice was associated with an elevated number of band cells (Table 2).

A progressive decline in erythrocyte and haemoglobin values was observed following DXN injection, and platelet count was suppressed for a week (Table 3).

Thrombocytopenia was soon followed by a rebound thrombocytosis, but the erythrocyte and haemoglobin levels remained subnormal in the second week after treatment. DA administration before DXN prevented thrombocytopenia completely. In fact, circulating plate-

Table 1. Circulating leucocyte counts following chemotherapy

	Normal mean	Days after DXN				
		2	4	6	9	12
WBC (10^3/μl)						
DXN	14.3±1.2	6.5±0.7	4.1±0.6	8.3±0.8	16.5±1.3	14.7±1.5
DA+DXN		8.4±0.6	8.7±0.8*	14.6±1.4*	17.5±1.3	19.8±1.4*
Neutrophil (10^3/μl)						
DXN	5.0±0.4	2.0±0.3	1.0±0.2	3.1±0.4	6.9±0.5	6.7±0.8
DA+DXN		2.7±0.2	2.6±0.3*	5.2±0.5*	7.3±0.6	8.9±0.8
Lymphocyte (10^3/μl)						
DXN	9.1±0.7	4.2±0.5	2.9±0.4	4.9±0.4	9.2±0.8	7.6±0.8
DA+DXN		5.3±0.4	5.8±0.6*	8.8±1.0*	9.5±0.9	10.4±0.7*

Results are mean ±SE; eight to ten animals were used in each group at each individual time point.
*$p<0.05$ compared to DXN-only group.

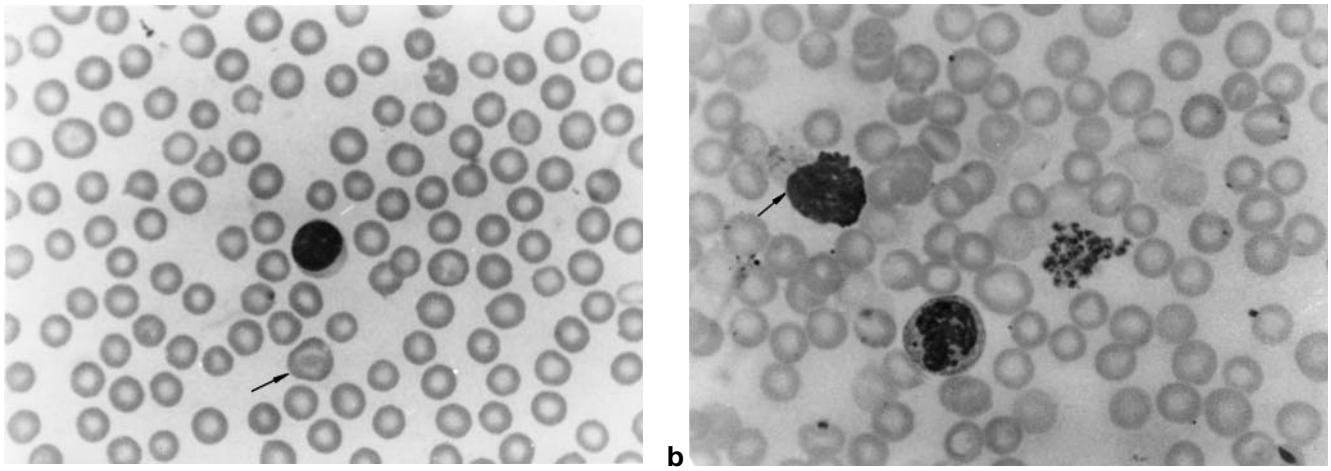

Fig 1. Peripheral blood smears of (**a**) DXN-only and (**b**) DA+DXN groups showing red cell abnormalities. DXN treatment was associated with any abundance of poikilocytes and 'target' cell (*arrow*). In contrast, DA pre-treated animals (**b**) had fewer poikilocytes. The smear also shows a large granular lymphocyte (*arrow*), neutrophil and aggregates of platelets. Leishman-stained ×1000.

let count of the DA+DXN group remained far above the normal level throughout the period of observation. DA also conferred moderate protection against DXN-induced depression in RBC and haemoglobin values (Table 3). Besides numerical changes, DXN caused abnormalities in RBC morphology, with both anisocytosis and poikilocytosis being observed. Anisocytosis was primarily due to the elevated percentage (20- to 30-fold over control) of 'target' cells having larger cell size. Red cell fragments, echinocytes and acanthocytes were abundant (15- to 27-fold over control) in peripheral blood smears from DXN-treated mice (Table 2, Fig. 1), but these abnormalities were less prevalent in DA pretreated mice (Table 2), suggesting a protective effect of DA on DXN-induced alterations in red cell morphology.

Changes in Bone Marrow and Spleen

The number of nucleated cells in the femur diminished markedly shortly after DXN injection, even in DA pretreated mice (Table 4). In the latter group, however, the recovery process started two days earlier. As a result, the nucleated cell count in the femur returned to normal level by day 12 after DXN injection whereas the DXN-only group took another three days to achieve this. The difference between DXN-only and DA+DXN groups becomes further apparent from the number of granulocytic cells in femoral marrow. DXN caused remarkable suppression of progenitor cells of every haematopoietic lineage in the marrow, but the depression was more acute in granulocytic and erythroid compartments. The depletion of granulocytic

Table 2. Distribution (%) of immature neutrophil and morphologically abnormal erythrocytes in peripheral blood

Cell type	Normal mice	DXN only		DA+DXN	
		Day 2	Day 6	Day 2	Day 6
Neutrophil band	0.5±0.02	3.1±0.4	6.7±0.8	6.5±0.5*	10.7±1.2*
Erythrocyte target	0.03±0.01	0.6±0.1	0.9±0.2	0.2±0.1*	0.3±0.1*
Echinocyte and acanthocyte	0.3±0.01	4.7±0.3	8.2±0.8	2.8±0.3*	4.6±0.4*
Fragmented RBC	0.1±0.02	1.3±0.2	1.7±0.3	0.5±0.1*	0.6±0.2*

The results are mean±SE; eight to ten blood smears were examined in each group at each individual time point.
* $p<0.05$ compared to corresponding DXN-only mice.

Table 3. Red cell and platelet counts following doxorubicin administration

	Normal mean	Days after DXN				
		2	4	6	9	12
RBC (10^6/μl)						
DXN	7.3±0.2	7.3±0.4	6.4±0.5	6.2±0.4	5.8±0.5	6.2±0.5
DA+DXN		7.2±0.5	6.6±0.5	6.8±0.5	6.9±0.4	7.5±0.5
Hb (g/dl)						
DXN	14.6±0.3	14.8±0.4	14.3±0.3	13.8±0.4	13.2±0.4	13.5±0.5
DA+DXN		14.5±0.4	13.9±0.3	14.0±0.4	14.1±0.4	14.5±0.4
Platelet (10^5/μl)						
DXN	3.9±0.3	3.3±0.5	1.8±0.2	3.7±0.3	6.8±0.4	8.8±0.5
DA+DXN		4.6±0.4	7.5±0.5*	8.9±0.4*	12.3±1.8*	8.2±0.7

Results are mean +SE; eight to ten animals were used in each group at each individual time point.
* $p<0.05$ compared to DXN-only group.

Table 4. Bone marrow cellularity following chemotherapy

	Normal mean	Days after DXN				
		2	4	6	9	12
Granulocyte/femur(10^6)						
DXN	10.5±0.5	1.8±0.2	0.7±0.1	3.7±0.2	8.8±0.7	11.9±0.8
DA+DXN		2.3±0.1	3.0±0.2*	8.4±0.4	11.7±0.9	14.5±1.8
Lymphocyte/femur(10^6)						
DXN	4.8±0.2	2.5±0.2	1.3±0.1	3.9±0.2	3.1±0.2	2.6±0.2
DA+DXN		1.8±0.1	3.1±0.2*	4.3±0.3	3.6±0.2	5.0±0.3*
Erythroblast/femur(10^6)						
DXN	4.6±0.2	0.8±0.1	0.2±0.03	1.1±0.06	1.4±0.1	2.5±0.2
DA+DXN		0.9±0.05	1.2±0.08*	2.1±0.1*	2.1±0.2*	3.8±0.2*
TNC/femur (10^6)						
DXN	20.2±1.2	5.3±0.5	2.3±0.3	8.8±0.5	13.5±1.1	17.6±1.2
DA+DXN		5.1±0.3	7.5±0.5*	15.3±0.7*	17.8±1.4*	23.8±1.3*

The results are mean±SE; eight to ten mice were used at each individual time point.
TNC, total nucleated cell count.
* $p<0.05$ compared to DXN-only group.

cells was of lesser magnitude in the DA pretreated group and the animals recovered completely by day 9 compared to day 12 in the DXN-only group (Table 4). Similarly, DA mediated faster recovery from DXN-induced lymphocytopenia in the femur. However, DA failed to mediate complete recovery of marrow erythroid cells within the 12-day observation period although DA recipients demonstrated a higher erythro-

blast number in the femur compared to animals that received only DXN.

Nucleated cell counts and organ weights of the spleen were also greatly reduced following DXN treatment, but the magnitude was less pronounced in DA pretreated mice (Table 5). Normal cell counts and spleen weights were restored by day 9 in DA recipients, whereas the DXN-only mice took another three days for recovery (Table 5). DA-treated mice showed a striking increase in the number of granulocytic cells and erythroblasts in the spleen. The magnitude of increment was so high that granulocyte and erythroblast numbers were greater even than the normal values, despite the suppression of total nucleated cell count per spleen (Table 5). DA treatment before DXN protected, to a great extent, the splenic lymphoid cells from the cytotoxic effects of this anticancer drug. As a consequence, DA-treated mice had a significantly greater number of lymphoid cells than the DXN-only group. The results suggest partial protection of lymphoid cells and concomitant stimulation of granulo- and erythropoiesis in the spleen of mice that received DA before DXN.

Table 5. Total and differential counts of spleen

	Normal mean	Days after DXN				
		2	4	6	9	12
Lymphocyte/spleen(10^6)						
DXN	155.0±8.0	40.5±5.6	30.7±4.6	87.9±9.2	120.3±11.6	155.0±9.4
DA+DXN		58.6±4.5*	77.1±4.4*	126.4±10.5*	150.9±9.9	166.1±11.7
Granulocyte/spleen(10^6)						
DXN	3.2±0.2	1.5±0.2	1.4±0.2	4.1±0.4	5.6±0.5	5.5±0.4
DA+DXN		4.1±0.3*	6.4±0.4*	9.7±0.8*	9.6±0.6*	9.8±0.7*
Erythroblast(10^6)						
DXN	2.9±0.2	0.9±0.1	0.8±0.1	2.4±0.2	2.2±0.2	3.3±0.2
DA+DXN		2.7±0.2*	4.0±0.2*	6.1±0.5*	6.5±0.7*	7.6±0.5*
TNC/spleen(10^7)						
DXN	16.2±0.7	4.3±0.6	3.3±0.5	9.5±1.0	12.4±1.2	16.4±1.0
DA+DXN		6.5±0.5	8.8±0.5*	14.3±1.2*	16.8±1.1*	18.5±1.3
Spleen wt (mg)						
DXN	122±8	36±4	33±5	82±6	97±8	132±9
DA+DXN		45±5	64±6*	103±8	144±12*	175±14*

The results are mean±S.E; eight to ten mice were used at each individual time point.
TNC, total nucleated cell count.
* $p<0.05$ compared to DXN-only group.

Table 6. Megakaryocytes and their precursors in bone marrow and spleen of anticancer drug-treated mice.

	Normal mean	Days after DXN				
		2	4	6	9	12
MK/femur(10^3)						
DXN	21.4±1.4	10.6±1.2	6.9±0.8	24.7±2.3	52.6±2.8	68.7±4.2
DA+DXN		12.3±1.3	22.5±2.1*	48.6±3.4*	72.7±4.5*	97.5±6.2*
MK/spleen(10^3)						
DXN	44.7±2.5	14.3±1.3	17.6±1.7	47.7±2.6	71.8±3.9	84.3±6.8
DA+DXN		36.8±2.5*	58.3±3.2*	78.6±4.7*	93.7±5.3	108.4±9.3
SAChE+ cell/femur(10^3)						
DXN	4.2±0.4	1.5±0.4	0.8±0.3	3.6±0.6	5.7±1.2	7.8±0.5
DA+DXN		2.2±0.6	3.1±0.4*	7.4±0.8*	8.3±0.7*	10.5±0.7
SAChE+ cell/spleen(10^3)						
DXN	13.6±1.5	4.1±0.6	3.6±0.5	10.4±1.2	22.8±2.3	28.9±2.7
DA+DXN		8.4±1.3*	14.1±1.5*	27.4±1.6*	34.5±2.5	41.7±2.8

The results are mean±SE; eight to ten animals were used in each group at individual time point.
* $p<0.05$ compared to DXN-only group.

Changes in Megakaryocyte Number and Platelet Production

Injection of DXN was followed by significant reduction ($p < 0.05$) in the number of MK and SAChE+ cells in bone marrow and spleen within 48 h. DA protected these cells to a large extent, especially in the spleen (Table 6). Compared to other cell lineage, MK recovery from the suppressive effect of DXN was rapid, and normal values were restored within a week. DA treatment further accelerated the recovery process as these animals exhibited normal MK and SAChE+ cell numbers in femur and spleen (except SAChE+ cell count in marrow) by day 4 after DXN injection.

Table 7. MK and SAChE+ cells in lung

	MK/lung ($\times 10^3$)	SAChE+ cell/lung ($\times 10^3$)
Control	0.23±0.03	0.42±0.05
DXN-only	0.48±0.04*	0.73±0.03*
DA+DXN	1.42±0.21*	1.68±0.24*

Results are mean ± SE; eight to ten animals were used in each group; samples collected at day 2 after DXN injection.
* $p<0.05$ compared to control.

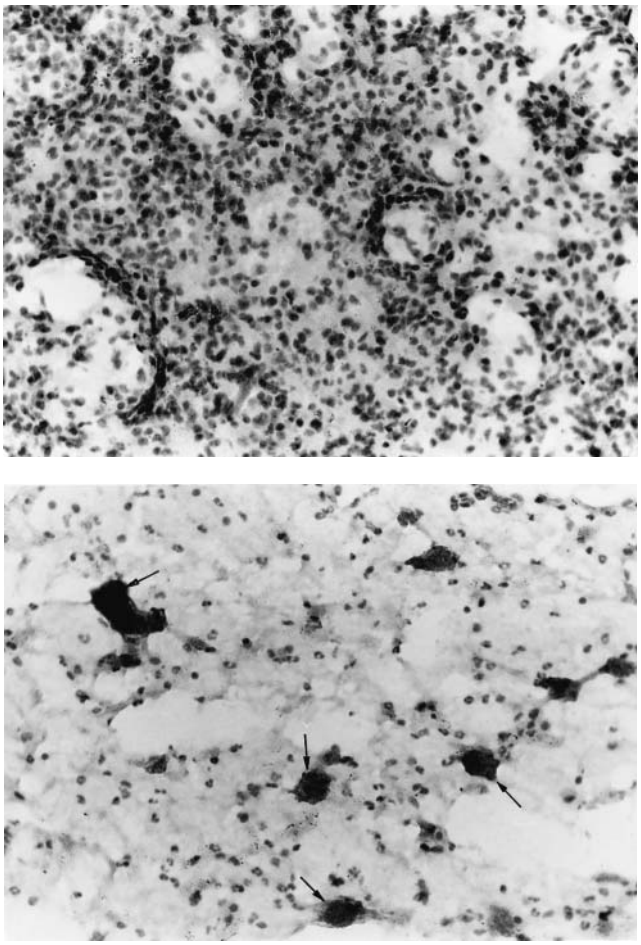

Fig. 2. Cryostat sections (5 μm) of lung of DXN-only (**a**) and DA+DXN (**b**) groups stained for the presence of acetylcholinesterase (AChE). Note the abundance of AChE-positive megakaryocytes (*arrow*) in dopamine pretreated mice (**b**). AChE and haematoxylin-stained × 250.

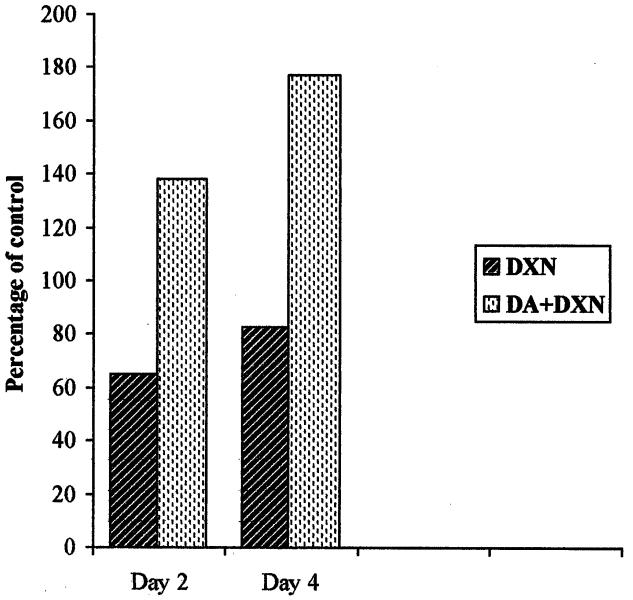

Fig. 3. ^{35}S incorporation (% control) in newly formed platelets at 24 h after isotope injection of mice receiving DXN. In DA-pretreated mice, ^{35}S incorporation was elevated above the normal level on both day 2 and day 4 after DXN suggesting stimulated thrombopoiesis.

DXN injection was associated with increased numbers of MK and SAChE+ cells in the lung. The rise was much more in the DA pretreated group (Table 7, Fig. 2).

Platelet production was remarkably stimulated in DA pretreated mice. The animals showed 38% and 77% increase in ^{35}S incorporation over the normal values at days 2 and 4, respectively. In contrast, the DXN-only group showed 35% and 17% decline in ^{35}S incorporation on days 2 and 4 post-treatment, respectively (Fig. 3).

CFU-S_{12}

Profound depression in the number of day-12 CFU-S was observed at day 2 after DXN injection. DA pretreated mice also showed depleted number of CFU-S_{12} in femoral marrow and spleen, but the magnitude of suppression was much less compared to that of seen in DXN-only mice (Table 8).

Table 8. Day 12 CFU-S in bone marrow and spleen

	Total CFU-S_{12} femur	Total CFU-S_{12} spleen
Control	2683±98	1832±88
DXN-only	447±17*	315±13*
DA+DXN	675±23*	616±18*

Results are mean±SE; eight to ten animals were used in each group; samples collected at day 2 after DXN injection.
* $p<0.05$ compared to control.

Survival

A 30-day survival analysis showed 23% mortality (seven out of 30 mice) in the DXN-only group. In contrast, the mortality was 10% (3/30) in animals receiving DA before DXN.

Discussion

It is generally recognised that the degree of tumour cell killing is related to the dose of antitumor agent used – the higher the dose, the greater the effect. But high-dose chemotherapy for optimal antitumour effect often cannot be contemplated because of the toxicity on proliferating normal cells. DXN, like several other anticancer drugs, has toxic effects on cells of the haematopoietic system resulting in peripheral blood leucopenia, neutropenia and thrombocytopenia (Pannacciulli et al. 1989). For reasons described earlier, the use of G-CSF and GM-CSF can also be disadvantageous. Against this background, we have examined whether DA can be used as a protective agent against the haematotoxic effects of DXN. The results strongly suggest haematoprotection by DA against the adverse effects of DXN.

DA caused overall improvement of the haematological profile of DXN-injected mice, which is in contrast to the effects of lineage-specific HGFs. G-CSF and GM-CSF, for example, may stimulate granulopoiesis at the cost of platelet production (Wolf 1989), and the resultant thrombocytopenia may lead to haemorrhage. The multilineage action of DA could be traced to its stimulatory action on pluripotent haematopoietic stem cells (Ray et al. 1994). In the present study, we have also observed more CFU-S_{12} in marrow and spleen in the DA+DXN group compared to that of DXN alone. Differentiation from stem and progenitor cells requires lineage-specific stimulatory molecules like erythropoietin (Epo), thrombopoietin (Tpo) and G- or GM-CSF. Hence, the activity of DA in the generation of terminally differentiated cells is probably indirect, via the action of HGFs. The rise in the number of erythroblasts, for instance, could be mediated by stimulation of Epo synthesis and/or activity by DA. Alternatively, DA can mediate its haematostimulatory activity directly; evidence is accumulating on the neural and neuroendocrine regulation of haematopoiesis (Maestroni 1998). Substantial amounts of DA, released from sympathetic nerve endings, are known to be present in the bone marrow (Marino et al. 1997), and specific uptake of DA by D2 receptors have been demonstrated in bone marrow and spleen cells of normal and tumour-bearing mice (Basu et al. 1993). Human neutrophils also synthesise and store catecholamines like dopamine, noradrenaline and their metabolites suggesting an autoregulatory adrenergic mechanism in these cells (Cosentino 1999). In view of these reports, the observed hematostimulatory effect of dopamine could have been mediated by the direct action of this catecholamine on cells of the marrow and spleen. Whatever the mechanism, the rise in the number of erthroblasts and circulating erythrocytes, the elevation in haemoglobin level and the substantial reduction in the number of morphologically aberrant RBCs have obvious clinical relevance. Anaemia is a common complication of cancer (Spivak 1994), and a majority of anticancer drugs, including DXN, are toxic to the erythron (Doll and Weiss 1983). As a result, the pre-existing anaemia in cancer patients may be aggravated following chemotherapy, causing further complexity to treatment. In this context, the present results demonstrating the erythroprotective effect of DA are really encouraging.

Another common problem of anticancer chemotherapy is thrombocytopenia and consequent haemorrhage. It is worthwhile mentioning, therefore, that DA has completely prevented DXN-induced thrombocytopenia. The action of DA was perhaps mediated via elevation of Tpo levels, because a rise in SAChE+ cells, as observed in DA-treated mice, is generally associated with high Tpo levels (Kalmaz and McDonald 1982). An important point in this regard is that the platelet count of DA recipients was above normal throughout the post-DXN period, whereas the number of MK and SAChE+ cells was below the normal level until day 2. An increase in MK size and resultant platelet yield per cell may explain this discrepancy. However, we did not find any substantial rise in MK diameter in the DA group. Alternatively, the observation may point to the possibility of extramedullary thrombopoiesis outside the spleen, such as in the lungs. We have also examined this aspect and found striking increases in the number of pulmonary MK and SAChE+ cells in DA-treated mice. Thus megakaryocytopoiesis was expanded considerably in the lungs of the DA-treated group. It is not known, however, whether this can affect the function of the lung. Likewise, we have not analysed systemic toxicity, if any, associated with DA treatment. However, DA has shown protective effects against glycerol-induced acute renal failure in rats (Gomez-Garre et al. 1996) and amphotericin B-induced nephrotoxicity in humans (Camp et al. 1998). Indirect evidence in support of the hepatoprotective effect of DA comes from the results of this study, showing a reduction in 'target' cell numbers in the DA+DXN group. 'Target' cells with enhanced surface-to-volume ratio signify liver toxicity with respect to cholesterol metabolism. Histology of the liver, kidney and gastrointestinal tract of DA-treated mice did not show any adverse cellular reaction (unpublished data). Taken together, the results may suggest little or no toxic effects of DA in this dose schedule on functions of the important organs. On the contrary, DA partially prevented the adverse effect of DXN on the liver as evident from depleted 'target' cell number. The findings have important clinical relevance because toxicological studies in rodents accurately predict the effects in humans (Schurig et al. 1986). Moreover, being an antitumour agent itself, DA might augment the net antitumour effect when used in combination with established anticancer drugs. Investigations are now being carried out in our laboratory in this direction, using a wide spectrum of experimental tumours in mice.

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