Reduction of hematotoxicity and augmentation of antitumor efficacy of cyclophosphamide by dopamine*

C. LAKSHMI, C. DEB, C. RAY, M. R. RAY**

Experimental Hematology Unit, e-mail: manasrray@rediffmail.com, Chittaranjan National Cancer Institute, Kolkata 700 026, India

Received May 30, 2004

Modulatory effects of dopamine (DA) on hematotoxicity and antitumor efficacy of cyclophosphamide (CY) were studied in Swiss mice bearing transplantable Ehrlich ascites carcinoma (EAC). DA was administered i.p. at a dose of 50 mg/kg/day for 5 consecutive days beginning day 3 after tumor transplantation. CY (200 mg/kg i.p.) was injected 24 hour after completion of DA treatment. DA pretreatment reduced the suppressive effects of CY on hemoglobin, RBC, total WBC, neutrophil, platelet, and bone marrow nucleated cell counts. Likewise, DA partially prevented the CY-induced fall in pluripotent (CFU-S_{12}) and lineage-specific stem cells for granulocytes (CFU-C) in bone marrow. Moreover, mice receiving a combination of DA and CY illustrated greater reduction in tumor volume, viable tumor cell count and mitotic index along with upregulation of tumor cell apoptosis than CY-only group. As a result, the former group demonstrated prolonged hosts' survival. Thus, DA protected to a great extent the hematopoietic cells of tumor bearing hosts from the suppressive action of CY and concomitantly augmented its antitumor efficacy resulting in improved hosts' survival.

Key words: cyclophosphamide, dopamine, myeloprotection, tumor inhibition

Myelosuppression and peripheral blood cytopenias are common side effects of cancer chemotherapy [7, 10]. To circumvent this problem, hematopoietic growth factors (HGFs) such as granulocyte-, and granulocyte-macrophage colony stimulating factors (G-CSF and GM-CSF, respectively) are used [29]. However, several studies have illustrated certain disadvantages of HGFs. For instance, HGFs may promote hematopoiesis of a particular lineage at the cost of other, such as increase in granulocytopoiesis with concomitant thrombocytopenia and associated hemorrhage by G-CSF treatment [29]. Several human tumors express the receptors for HGFs, and many human cancer cells produce G-CSF and GM-CSF that act as tumor growth enhancer via autocrine growth loop [1, 17, 25]. GM-CSF, on the other hand, can facilitate tumor metastasis [17]. Moreover, the HGFs are too expensive for routine clinical use, particularly in the developing countries and their beneficial effect on overall survival of the patients is questionable [24]. These observations highlight the need for suitable alternative to HGFs that will protect the hematopoietic cells from the toxic effects of anticancer drugs and would improve hosts' survival. In the present study we have examined the potential of dopamine (DA) in this regard.

DA, the precursor of norepinephrine, is synthesized from amino acid tyrosine. It acts as a neurotransmitter in the central nervous system, while peripheral DA is believed to be involved in cardiovascular, immune and renal functions [14]. The rationale for considering DA for this study is its stimulatory action on multilineage hematopoiesis [20, 21], while it inhibits tumor growth in laboratory animals [20]. Therefore, it seems possible that DA could protect the hematopoietic organs from the toxic effects of anticancer drugs and concomitantly augments their tumoricidal efficacy when used in combination. We have tested this hypothesis in mice bearing Ehrlich ascites carcinoma by using DA in combination with cyclophosphamide, a commonly used anticancer drug with profound hematotoxicity [30].

*The authors are thankful to Indian Council of Medical Research, New Delhi, for financial assistance.

**Corresponding author

Material and methods

Animal and tumor. Male Swiss mice, 8–10 week old and weighing 20–22 g were obtained from the Institute's own breeding facility. Food (pellet) and water were given *ad libitum*. Ehrlich ascites carcinoma (EAC) was maintained in Swiss mice by weekly intraperitoneal (i.p.) transplantation of 10^7 viable tumor cells suspended in 0.5 ml of phosphate-buffered saline (PBS). The tumor-bearing animals survived for an average period of 3 weeks after transplantation. The Institutional Animal Ethics Committee approved the study protocol.

Treatment. Dopamine hydrochloride (TTK Pharma, Chennai, India) was dissolved in PBS and injected i.p. at a dose of 50 mg/kg/day for 5 consecutive days beginning day 3 after tumor transplantation. Cyclophosphamide (CY, Endoxan-Asta, German Remedies, Mumbai, India) was dissolved in sterile water and injected in a volume of 0.5 ml i.p. at a dose of 200 mg/kg on day 8 of tumor bearing, i.e. 24 hr after completion of DA treatment. Age- and sex-matched control mice received saline instead of DA and CY.

Hematology, bone marrow and spleen count. Hematological parameters were evaluated from free-flowing tail vein blood by standard procedures [2]. The animals were sacrificed thereafter by cervical dislocation. Both femurs and spleen were surgically removed, and bone marrow (BM) cells were flushed from the femoral shaft into PBS. Spleen cells were harvested in PBS by teasing the organ in steel wire mesh. Nucleated cell counts in BM and spleen cell suspensions were done under microscope using hemocytometer. Granulocytes, lymphocytes and their precursors were identified and counted from Geimsa-stained smears. Erythroblasts and megakaryocytes were identified and counted following benzidine [15] and acetylcholinesterase [11] staining, respectively.

Erythropoiesis. The rate of erythrocyte production was assessed by measuring 72-hr RBC ^{59}Fe incorporation following intravenous injection of 37 kBq of $^{59}FeCl_3$ (BARC, Mumbai, India) assuming total blood volume as 7% of body weight [4].

CFU-S_{12} and CFU-C. Pluripotent hematopoietic stem cells were assessed as day 12 colony-forming unit in spleen (CFU-S_{12}) of irradiated mice (9.1 Gy from ^{60}Co source) following the procedure of TILL and MCCULLOCH [27]. Myeloid stem cells (CFU-C) were assayed *in vitro* [19]. In brief, $2x10^5$ BM cells were cultured for 10 days at 37 °C and 5% CO_2 in RPMI-1640 (GIBCO, USA) supplemented with 10% fetal bovine serum (GIBCO, USA) in 0.3% bacto-agar (DIFCO, USA) and 500 U of GM-CSF (GIBCO, USA) as growth stimulus. CFU-C colonies (>50 cells) were scored under inverted microscope.

Tumor growth measurement and survival. The animals were sacrificed by cervical dislocation at day 10 after transplantation and tumor ascites was collected from the peritoneal cavity using Pasteur pipette and the volume was recorded. Viable tumor cell count was done by 0.4% trypan blue dye exclusion under microscope using hemocytometer. For measurement of mitotic index, the animals were injected i.p. with 0.04% colchicine (Bubeck, Switzerland,) two hours before sacrifice to arrest the proliferating cells in metaphase. Tumor cell smears were made on clean glass slides, fixed in methanol for 10 min and stained with Giemsa. Mitotic cells in metaphase were identified and counted after screening at least 2000 tumor cells. Survival of control and treated EAC-bearing mice was recorded daily for 12 weeks. Prolongation of hosts' survival was recorded as percentage increase in life-span (% ILS) calculated by the formula % ILS = (T/C – 1) x 100, where T and C are median survival in days of treated and control groups respectively [28].

Enzyme activity. Succinate dehydrogenase (SDH) and β-glucuronidase enzyme activities in EAC cells were evaluated cytochemically [9, 16]. Enzyme activities were evaluated by subjective grading [5] of the intensity of color reaction (0 to 4 +) after examining at least 200 tumor cells in each slide.

Apoptosis and cell cycle analysis of tumor cells. Apoptosis of tumor cells were determined by the *in situ* nick-end labeling (TUNEL) method [8] using *in situ* death detection kit, POD (Boehringer Mannheim, Germany) following the manufacturer's protocol. For cell cycle analysis, EAC cells were washed twice in PBS and fixed in methanol at 4 °C for 90 min. Thereafter the cells were treated with RNase (10 μg/ml, ICN, USA), labeled with propidium iodide (5 μg/ml, Sigma Chem. USA) and analyzed on a flow cytometer (FACS Calibur, Becton Dickinson, USA) using Cell Quest software (Becton Dickinson, USA).

Toxicity study. Serum aspartate transaminase (AST), alanine transaminase (ALT), urea, and urea nitrogen were measured spectrophotometrically [13] using commercially available kits (Span Diagnostics, Surat, India).

Statistical analysis. The results were statistically analyzed by Student's t test and $p<0.05$ was considered significant.

Results

Hematological improvements. Tumor growth was associated with decline in hemoglobin and RBC values with concomitant rise in neutrophil and platelet counts. CY injection reduced the WBC and platelet counts along with reductions in hemoglobin and RBC values. In contrast, DA plus CY-injected mice elicited improvements in all these parameters as the values showed a change towards normalcy (Tab. 1). DA elicited significant protection to circulating neutrophils against the suppressive action of CY, and the protection was not at the cost of lymphocytes as the

Table 1. Hematological changes in EAC-bearing mice following treatment with CY and DA

	EAC-bearing mice treated with			
	Saline	CY	DA	DA+CY
Hemoglobin (g/dl)	10.5±1.3	10.2±0.5	11.0±0.4	12.7±0.5*
RBC (x10^6/μl)	4.7±0.3	4.3±0.4	5.0±0.6	5.6±0.3*
WBC (x10^3/μl)	34.2±2.2	11.5±1.0	22.5±1.8	14.7±1.1
Neutrophil (x10^3/μl)	22.5±1.7	3.9±0.8	15.9±1.1	6.8±0.8*
Lymphocyte (x10^3/μl)	10.3±0.8	6.9±1.4	5.9± 0.7	7.4±0.7
Platelet (x10^5/μl)	10.5±0.8	3.2±0.6	10.5±1.0	8.3±1.2*

Results are mean ±S.E.; 10 animals were used in each group; DA was injected on day 3–7 post-transplantation, CY on day 8; *p<0.05 compared with mice that received only CY.

Table 2. Total and differential counts of bone marrow and spleen

	EAC, day 10	EAC+ CY	EAC+ DA	EAC+ CY+DA
FEMORAL MARROW				
Erythroblast (%)	4.3±0.3	3.0±0.2	8.4±0.6	8.8±0.7*
Granulocytic cell (%)	80.9±1.2	43.7±2.5	62.9±2.9	60.9±2.3*
Lymphoid cell (%)	12.2±1.5	49.7±2.2	26.4±1.9	26.3±1.6*
Megakaryocyte (%)	0.5±0.1	0.2±0.1	0.6±0.1	0.5±0.1*
TNC (x10^6/femur)	14.8±1.2	7.5±0.8	13.8±1.0	13.5±1.2*
SPLEEN				
TNC/spleen, x10^7	22.3±1.6	12.1±1.3	17.5±1.7	16.0±1.0*
Lymphoid cell (%)	80.3±2.5	87.2±3.0	81.7±2.6	80.5±3.1
Granulocytic cell (%)	15.5±0.5	8.2±0.5	13.3±0.9	14.3±0.7*

Results are mean ±S.E; *p<0.05 compared with EAC+CY group; TNC – total nucleated cell.

Table 3. Pluripotent hematopoietic stem cells in EAC-bearing mice after DA and CY treatment

Group	CFU- S_{12}/femur	CFU-S_{12}/spleen
EAC, day 10	1422±98	2732±88
EAC+CY	447±17	315±13
EAC+DA	1160±145	2434±106
EAC+DA+CY	875±87*	1016±118*

Results are mean ±SE; *p<0.05 compared with EAC+CY group.

Table 4. Granulocytic stem cells in femoral marrow of tumor bearing mice treated with DA and CY

Group	CFU-C/femur (x10^3) %	Change over control
EAC control, day 10	67.3±2.6	–
EAC+CY	11.8±1.8	–82.5
EAC+DA	54.3±3.5	–19.3
EAC+DA+CY	21.8±2.2*	–67.6

Results are mean ±S.E; *p<0.05 compared with EAC+CY group.

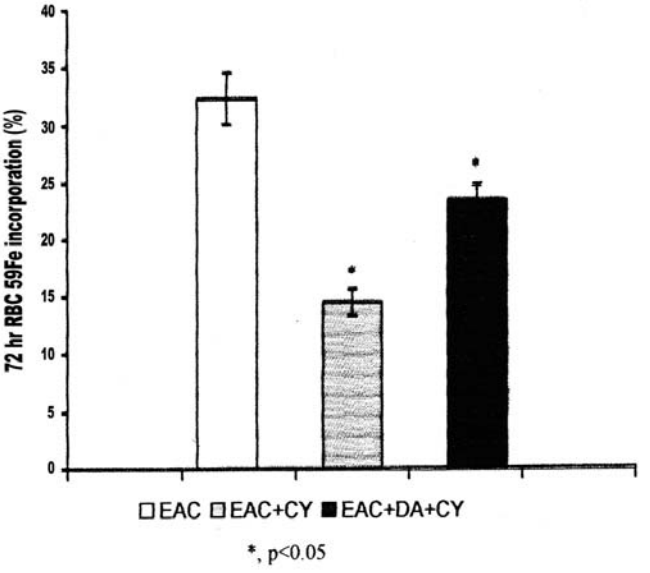

Figure 1. Assessment of erythropoiesis in terms of 72 h RBC ^{59}Fe incorporation (expressed as percentage of injected radioactivity) shows depression of red cell production in CY-injected EAC-bearing mice. Pretreatment with DA (50 mg/kg/day x 7) appreciably prevented the suppression of erythropoiesis.

absolute number of circulating lymphocytes also showed a mild increment (Tab. 1).

BM and spleen cellularity. EAC-bearing mice showed profound decline in nucleated cell count in the femoral marrow but marked increase in cell number in the spleen. CY administration further reduced the number of nucleated cells in femur. It also elicited decline in spleen cell count. DA treatment before CY substantially lessened the suppressive action of CY on BM and spleen cell counts (Tab. 2). Differential counts of BM following CY treatment revealed marked depression of cells of the granulocyte lineage, erythroblasts and megakaryocytes. DA pretreatment elicited appreciable protection to these cells. DA treatment before CY also prevented the fall in granulocytic cells in the spleen (Tab. 2).

Erythropoiesis. Erythropoiesis, assessed in terms of 72-hr RBC ^{59}Fe incorporation, was drastically reduced in CY-injected mice. DA pretreatment substantially improved the rate of RBC production (Fig. 1).

Stem cells. CY injection caused 69% and 89% fall in the number of pluripotent hematopoietic stem cells (CFU- S12) in femur and spleen, respectively. Prior administration of DA lessened the suppressive action of CY on these stem cells (Tab. 3). Besides pluripotent stem cells, CY elicited suppressive effects on CFU-C in the BM, and partial protection was given to these cells by DA (Tab. 4).

Table 5. Tumor inhibition by cyclophosphamide and dopamine

Treatment	Tumor volume (ml)	Total viable tumor cell count (x10^7)
CY only	0.6±0.2	10.5±1.5
DA only	1.2±0.2	20.1±2.1
DA+CY	0.2±0.1*	3.3±0.4*
Saline	2.8±0.4	86.8±1.4

Results are mean ±S.E of day 10 after tumor transplantation; *p<0.05 compared with CY-only group.

Table 6. Succinate dehydrogenase (SDH) and β-glucuronidase enzyme activities in tumor cells

Treatment	High SDH activity (2–4+)	High β-glucuronidase activity (2–4+)
CY alone	66.8±3.2	25.7±2.7
DA alone	76.4±2.6	14.8±1.5
DA+CY	42.8±2.4*	38.8±2.3*
Saline	94.9±1.2	10.5±0.7

Results are expressed as percentage of positive cells; enzyme activity was evaluated by subjective grading (0–4+); tumor cells were harvested on day 10 post-transplantation; *p<0.05 compared with CY-alone group.

Table 7. Effects of dopamine and cyclophosphamide on cell cycle of EAC cells

Treatment	Sub-G_1%	G_0/G_1%	S%	G_2M%
Saline	0.2±0.1	60.4±1.1	19.1±1.4	20.3±0.6
DA	3.9±0.2	57.9±2.8	11.4±0.5	26.8±1.4
CY	1.6±0.2	5.0±0.8	4.9±0.3	88.5±2.2
DA+CY	9.6±0.3*	12.0±1.3*	6.5±0.2*	71.9±3.2*

Results are mean ±S.E; *p<0.05 compared with CY-only group.

Table 8. Cell cycle analysis of bone marrow cells of EAC-bearing mice

Treatment	Sub-G_1%	G_0/G_1%	S%	G_2M%
Saline	0.1±0.02	65.3±2.8	14.5±1.3	19.1±1.7
CY	1.0±0.1	90.5±1.1	3.6±0.4	4.9±0.5
DA	0.2±0.04	57.9±2.7	11.4±0.8	30.5±1.6
DA+CY	0.5±0.05*	72.5±1.9*	4.5±0.7	22.5±1.8*

Results are mean ±S.E; *p<0.05 compared with CY-only group.

Table 9. Effect of cyclophosphamide and dopamine treatment on tumor cell mitosis, apoptosis, and host survival

Treatment	Mitotic index (%)	Apoptotic index (%) %	ILS
CY	0.5±0.1	1.2±0.2	74
DA	1.2±0.2	0.7±0.1	48
DA+CY	0.1±0.03*	2.6±0.3*	115
Saline	3.1±0.2	0.2±0.04	–

%ILS – percentage increase in life span; 8 animals were used for mitosis and apoptosis studies and 25 animals for survival in each group; *mitosis and apoptosis values were obtained from mice at day 10 post-transplantation; p<0.05 compared to saline-injected control.

Table 10. Liver and kidney function

Mice	Serum AST (U/ml)	Serum ALT (U/ml)	Serum urea (mg/dl)	Serum urea nitrogen (mg/dl)
Normal mice	33.0±1.6	22.6±3.4	17.8±0.2	8.3±0.2
EAC, control	54.6±2.2	36.7±1.6	24.7±1.4	11.6±1.1
EAC+DA	51.6±18	39.4±1.9	27.5±1.7	10.7±0.8
EAC+CY	68.7±2.6	45.9±2.7	33.6±2.3	16.6±1.5
EAC+DA+CY	55.6±2.1*	40.7±2.8	28.8±2.0	13.6±1.5

Values are mean ±SE; *p<0.05 compared with EAC+CY, 8 animals were used in each group.

Tumor response. The mean (±S.E.) tumor volume of control EAC-bearing mice at day 10 after tumor transplantation was 2.8±0.4 ml. Administration of CY, DA and a combination of both reduced the tumor volume by 67.8%, 57.1% and 78.6%, respectively. Thus, reduction of tumor volume was greatest in DA plus CY-injected group. Similarly, highest reduction (96.2%) in tumor cell number was recorded in mice receiving DA and CY while CY and DA alone were able to reduce tumor cell count by 87.9% and 76.8%, respectively (Tab. 5).

Tumor regression was accompanied by decrease in SDH and increase in β-glucuronidase enzyme activity in tumor cells, suggesting inhibition of cell growth and acceleration of cell death, respectively. Suppression of SDH and rise in β-glucuronidase activity were most remarkable in mice that received DA before CY (Tab. 6). This group also demonstrated lowest mitotic index of tumor cells compared with other treatment groups (Tab. 9).

CY injection in tumor bearing mice caused S-phase reduction and blockade of EAC cells in G2/M phase. Table 7 shows that DA and CY in combination mediated remarkable increase in sub-G1 peak suggesting induction of apoptosis. Apoptosis-enhancing effect of DA on EAC cells was further established by TUNEL assay. Compared to saline-treated control tumor bearing mice, DA and CY recipients displayed 3.5- and 6-fold rise in the percentage of TUNEL-positive tumor cells, respectively. In comparison, administration of DA and CY in combination resulted in 13-fold increase in programmed cell death of tumor cells (Tab. 9).

Cell cycle analysis of BM. CY blocked the BM cells in G1, as a result S- and G2/M populations were markedly reduced. In addition, it increased the percentage of sub-diploid cells as evident from rise in the proportion of cells in sub-G1 (Tab. 8). DA treatment before CY partially removed the G1 blockade resulting in increase in S and G2/

M fractions, and reduced the percentage of sub-G1 cells from 1% to 0.5% (Tab. 8).

Liver and kidney toxicity. Tumor growth was followed by alterations in liver and kidney functions as evidenced by rise in serum AST, ALT, urea and urea nitrogen. CY raised these values further. DA pretreatment reduced the severity of CY-induced changes in biochemical parameters of liver and kidney functions, and the improvement in serum AST level was statistically significant (Tab. 10).

Host survival. Tumor inhibition by DA and CY was followed by prolongation of hosts' life span. The percentage increase in life span (%ILS) was 68 and 47 in CY-injected and DA-treated groups, respectively. Further increment in life span (98% ILS) was recorded when DA and CY were used in combination (Tab. 9).

Discussion

Although chemotherapy constitutes the principal mode of treatment for leukemia, lymphoma and many solid tumors, their toxic effects on normal cells have been of monumental concern. Bone marrow cells are among the first to be adversely affected by chemotherapeutic drugs, leading to decline in circulating blood cells. The HGFs, which were earlier thought to control these problems, have several disadvantages. It is therefore highly desirable to search for agent (s) that can effectively destroy cancer cells but has minimal or no side effects on normal proliferating cells. We have earlier reported the potential of DA in this regard [20]. In the present preclinical study we have investigated the activity of DA in combination with commonly used anticancer drug CY. We found that DA substantially reduced the hematotoxicity of CY, augmented the antitumor effect of this alkylating agent, and more importantly, prolonged the lifespan of the tumor-bearing animals.

Inhibition of tumor by DA has been widely reported [6, 20, 28]. How DA inhibits the growth of tumor cells is a topic of current research. Our previous studies have shown stimulation of natural killer (NK) cells [3] and induction of apoptosis in tumor cells by DA [22]. It is possible that DA has upregulated NK cell activity and concomitantly augmented tumor cell apoptosis in CY recipients, leading to acceleration of tumor cell destruction. Indeed, apoptosis of EAC cells was maximal in mice that received a combination of DA and CY. Alternatively, the action of DA could be mediated by its effect on cell growth-related enzymes. SDH is an important respiratory enzyme and its activity is considered an index of cell growth and metabolism, whether normal or neoplastic [5]. DA selectively inhibits the actions of SH-group-containing enzymes to which SDH belongs [12]. Hence inhibition of SDH and resultant suppression of tumor cell growth may play a role in the mechanism of tumor regression by DA. The activity of lysosomal enzyme β-glucuronidase, on the other hand, is associated with cell lysis, and is used in assessing the efficacy of antitumor agents [5]. Therefore, marked increase in the activity of this enzyme in DA-treated mice suggests enhancement of cell lysis. This is confirmed by increased percentage of sub-G1 fraction and TUNEL-positive EAC cells, indicating rise in tumor cell apoptosis following DA treatment. In conformity with the present finding, induction of apoptosis by DA has been reported in human oral carcinoma cells [26].

How bone marrow cells are unaffected when tumor cells are killed or checked from further proliferation by DA is not known. Compared to BM cells, more tumor cells were in sub-G1 stage of cell cycle, suggesting that EAC cells were predominantly killed via upregulation of apoptosis. We have earlier shown that the present dose schedule of DA (50mg/kg/d x 5) significantly induces apoptosis in tumor cells, while the bone marrow and spleen cells were relatively unaffected by its action [22]. Irrespective of as-yet-unknown protective mechanism(s) operating in bone marrow cells, it is clear from our present and earlier [22] *in vivo* studies that DA has the novel property of preferentially killing tumor cells leaving hematopoitic cells of the host relatively unharmed.

DA has a LD_{50} value of 1100 mg/kg body weight in normal mice [6]. Therefore, the total dose of DA used in this study (250 mg/kg) represents about one fourth of LD_{50} dose. Admittedly it is high enough to elicit some toxicity in normal mice. But in tumor-bearing animals, we did not observe any adverse reaction in biochemical parameters of liver and kidney function. In fact, DA provided modest protection to liver and kidney from the adverse effects of CY on the functioning of these vital organs. In agreement with the present observation, DA induces recovery from IL-2 impaired renal function [18] in humans. Since toxicological studies in mice accurately predict the effects in man [23], hematoprotection by DA against the toxic effects of CY in tumor bearing mice is expected to be also effective in human cancer patients. Therefore, the present report describing hematoprotective action of DA appears highly encouraging with obvious clinical relevance.

References

[1] BABA M, HASEGAWA H, NAKAYABU M, SHIMIZU M. Establishment and characteristics of a gastric cancer cell line (Hu GC-OOHIRA) producing high levels of G-CSF and GM-CSF and IL-6: presence of autocrine growth control by G-CSF. Am J Hematol 1995; 49: 207-215.

[2]] BAIN BJ. Basic Hematological Techniques. In: JV Dacie and SM Lewis, editors. Practical Hematology. 8th Ed, Edinburgh, Churchill-Livingstone, 1996: 49–82.

[3] BASU S, DASGUPTA PS, RAY MR, LAHIRI T. Stimulation of NK

activity in Earlich ascites carcinoma bearing mice following dopamine treatment. Biogenic Amines 1992; 8: 191–197.

[4] BRODSKY I, DANNIS LH, KAHN SB, BRADY LW. Normal mouse erythropoiesis. I. The role of spleen in mouse erythropoiesis. Cancer Res 1966; 26: 198–201.

[5] CHOWDHURY TJ, BHATTACHARYA S, LAHIRI T. Studies on growth and enzymatic characteristics of human mammary tumors grown in heterogeneous host. Exp Clin Endocrinol 1983; 82: 356-360.

[6] DASGUPTA PS, LAHIRI T. Antitumor effect of i.p. dopamine in mice bearing Ehrlich ascites carcinoma. J Cancer Res Clin Oncol 1987; 113: 363–368.

[7] DOLL DC, WEISS RB. Chemotherapeutic agents and the erythron. Cancer treat Rev 1983; 10: 185–200.

[8] GAVRIELI Y, SHERMAN Y, BEN-SASSON SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 1992; 119: 493–501.

[9] HAYASHI M, NAKAJIMA Y, FISHMAN WH. The cytologic demonstration of β-glucuronidase and hexazolium pararosanilin: a preliminary report. J Histochem Cytochem 1963; 12: 293–295.

[10] HOAGLAND HC. Hematological complications of cancer chemotherapy. Semin Oncol 1982; 9: 95–102.

[11] KARNOVSKY MJ, ROOTS L. A 'direct coloring' thiocholine method for cholinesterase. J Histochem Cytochem 1964; 12: 219–221.

[12] KNOCK RW, GALT RM, OESTER YT. Inhibition of DNA polymerase and neoplastic cells by selected SH inhibitors. Oncology 1972; 113: 363–367.

[13] KOLMER JA, SPAULDING EH, ROBINSON HW. Approved Laboratory Techniques. Ist Indian Edition, New Delhi, Appleton Century Croft, 1969.

[14] LANDSBERG L, YOUNG JB. Physiology and pharmacology of the autonomic nervous system. In: Isselacher KJ, Braunwald E, Wilson JD, Martin JB, Fauci AS, Kasper DL, editors. Harrison's Priciples of Internal Medicine, New York, McGraw-Hill, 1994: 412–425.

[15] LOBUE J, DORNFEST BS, GORDON AS, HURST J, QUASTLER H. Marrow distribution in rat femur determined by cell enumeration and 59Fe labeling. Proc Soc Exp Biol Med 1963; 112: 1058–1062.

[16] NACHLAS MM, TOSU KC, DESOUZA F, CHENG CS, SELIGMAN AM. Cytochemical demonstration of succinate dehydrogenase by the use of new P-nitrophenyl substituted ditetrazole. J Histochem Cytochem 1957; 5: 420–436.

[17] NODA I, FUJIEDA S, OHTSUBO T, TSUZUKI H, TANAKA N et al. Granulocyte-colony stimulating factor enhances invasive potential of human head-and-neck carcinoma cell lines. Int J Cancer 1999; 80: 78–84.

[18] PALMIERI G, MORABITO A, LAURIA R, MONTESARCHIO V, MATANO E et al. Low-dose dopamine induces early recovery of recombinant interleukin-2-impaired renal function. Eur J Cancer 1993; 29A: 1119–1122.

[19] PETURSSON, CHERVENICK PA. Megakaryocytopoiesis and ganulocytopoiesis of W/Wv mice studied in long-term bone marrow culture. Blood 1985; 65: 1460–1468.

[20] RAY MR, DASGUPTA PS, BASU S, LAHIRI T. Dopamine inhibits tumor growth and concomitantly stimulates erythrocyte and platelet production in experimental tumor bearing mice. Biogenic Amines 1988; 5: 475–481.

[21] RAY MR, NANDI SK, LAHIRI T. Effect of dopamine on pluripotent hematopoietic stem cells (CFU-S) of mice. Biogenic Amines 1994; 10: 555–559.

[22] RAY MR, LAHIRI T. Dopamine induces apoptosis in murine tumor cells in vivo. Biogenic Amines 2000; 16: 21–28.

[23] SCHURIG JE, FLOREZYK AP, BRADNEI WT. The mouse as a model for predicting the myelosuppressive effect of anticancer drugs. Cancer Chemother Pharmacol 1986; 16: 243–246.

[24] SIDDIQUI T, BURNEY IA, KAKEPOTO GN, KHURSHID M, SALAM A, SMEGO RA JR. Lack of benefit of granulocyte macrophage or granulocyte colony stimulating factor in patients with febrile neutropenia. J Pak Med Assoc 2002; 52: 206–210.

[25] TACHIBANA M, MIYAKAWA A, TAZAKI H, NAKAMURA K, KUBO A, HATA J. Autocrine growth of transitional cell carcinoma of the bladder induced by granulocyte colony stimulating factor. Cancer Res 1995; 55: 338–3443.

[26] TERASAKA H, TAMURA A, TAKAYAMA F, KASHIMTA M, OHTOMO K et al. Induction of apoptosis by dopamine in human oral tumor cell lines. Anticancer Res 2000; 20: 243–250.

[27] TILL JE, MCCULLOCH EA. A direct measurement of radiation sensitivity of normal mouse bone marrow cells. Radiat Res 1961; 14: 213–221.

[28] WICK MM. Dopamine: novel antitumor agent against B-16 melanoma in vivo. J Invest Dermatol 1978; 71: 163–164.

[29] WOLF M. Colony-stimulating factors in clinical trials. Lung Cancer 1989; 5: 296–304.

[30] YEAGER AM, LEVIN FC, LEVIN J. Effects of cyclophosphamide on murine bone marrow and splenic megakaryocyte-CFC, granulocyte-macrophage CFC, and peripheral blood cell levels. J Cell Physiol 1982; 112: 222–228.