Cisplatin and Bleomycin Alter Organ Functions in an Animal Model of Halothane and Isoflurane Anaesthesia

Füsun Eroğlu, MD[1]; Erol Eroğlu, MD[2]; Tuna Özbağı, MD[3]; Aliye Sarı, MD[4]; Lütfi Yavuz, MD[1]

[1] *Süleyman Demirel University, Department of Anesthesiology and Reanimation, Isparta, Turkey*
[2] *Süleyman Demirel University, Department of General Surgery, Isparta, Turkey*
[3] *State Railways Hospital, Ankara, Turkey*
[4] *Süleyman Demirel University, Department of Pathology, Isparta, Turkey*

Abstract

Objective: The interactions between cytotoxic agents and inhalation anaesthetics were investigated.

Design: An experimental animal study which was conducted on 75 female "Wistar Albino" rats.

Setting: The animals were divided into five groups: only saline infusion (I), halothane administered group (II), isoflurane administered group (III), and groups which received chemotherapeutic agents cisplatin and bleomycin 21 days before administration of anaesthesics (Groups IV and V). Blood samples for biochemical and haematological examinations were taken before anaesthesia and 48 h after the anaesthesia. Bronchoalveolar lavage (BAL) with 40 ml of phosphate buffered saline was also performed 48 h after the anaesthesia and tissue samples were taken for pathological examination.

Results: They were evaluated with "Independent samples Student's *t*-test", and "One-Way ANOVA". $p<0.05$ values were accepted as significant. In the pathological examinations, alveolitis and terminal bronchiolitis were noted especially in groups which received halothane. An additional finding was pre-oedema in the groups which received chemotherapy and halothane. Additionally, polymorphonuclear leukocyte ratio was found to be higher in BAL fluid in chemotherapy applied groups. There were also some changes in the biochemical parameters in the chemotherapy applied groups.

Conclutions: The study showed that inhalation anaesthesia had some important effects on tissues in chemoterapy administered rats.

Turkish Respiratory Journal, 2003;4:(1):8-13

Key words: *chemotherapy, anaesthesia, adverse effect, pulmonary*

Introduction

The number of cancer patients and the incidence of the disease are increasing every year, but treatment possibilities are also increasing (1). A multi-disciplinary approach including a choice of chemotherapy, surgery, radiotherapy, immunotherapy and other treatment modalities used singly or in combination, can improve survival and quality of life in cancer patients. As a result, anaesthesiologists are now seeing more cancer patients than in previous years. Some of these patients have received chemotherapy or radiotherapy before surgery.

Since chemotherapy may have cytotoxic effects, it is important for the anaesthesiologists to be aware of the effects of chemotherapy on certain organs or organ systems and of the interactions of anaesthetic and chemotherapeutic agents. This study was designed to investigate in an animal model the relationship between two

Correspondence: *Dr. Füsun Eroğlu*
Turan Mah. Balıkçı Sokak No:1/12
32040, Isparta, Türkiye
Tel: +90 (0) 536 554 39 89
Fax: +90 (0) 246 237 02 40
e-mail: eroglufusun@hotmail.com

Table 1. Hematogical, biochemical, and cytological parameters in the study group.

	Group I		Group II		Group III		Group IV		Group V	
	A	B	A	B	A	B	A	B	A	B
Hb	12.84±1.4	12.69±1.3	10.97±1.8	11.67±2.1	12.30±1.7	11.21±1.1	12.95±1.3	11.59±1.7	12.57±1.2	11.44±1.8
Gl	86.09±5.3	86.34±5.6	82.17±4.0	85.93±5.1	89.12±4.7	90.7±3.4	87.54±5.4	96.57±5.8	81.4±4.8	91.81±5.5
Creat	0.54±0.06	0.53±0.07	0.45±0.01	0.48±0.11	0.52±0.06	0.56±0.06	0.58±0.06	0.74±0.07	0.51±0.1	0.70±0.07
SGPT	35.6±6.0	36.93±6.8	30.26±2.3	32.06±1.2	38.26±7.8	42.53±6.3	33.40±4.5	40.40±4.7	38.93±6.6	43.6±5.2
SGOT	39.3±6.5	39.5±6.0	40.20±4.6	43.73±5.8	36.13±7.7	39.73±5.4	39.53±3.8	46.13±6.1	34.53±6.4	39.66±2.6
LDH	116.6	120.7	120.26	125.13	127.0	128.33	126.06	162.0	129.06	141.73
BAL AM	91.8±5.7		85.6±7.3		89.53±4.9		81.66±5.3		85.46±6.4	
BAL PMNL	8.2±0.9		14.4±1.3		10.46±2.0		18.33±2.4		14.53±2.1	

Hb: Hemoglobin, Gl: Glucose, Creat: Creatinin, BAL AM: Broncho Alveolar macrophage in bronkoalveolar lavage, BAL PMNL: Polymorphonuclear leukocytes in bronchoalveolar lavage.
A: Before Anesthesia, B: After Anesthesia

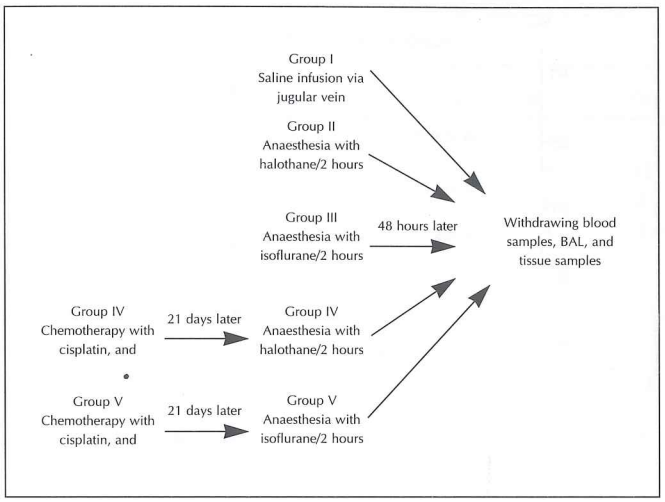


Figure 1. *Study groups,*

widely used cytotoxic agents, cisplatin and bleomycin, administered preoperatively, and halothane and isoflurane.

Material and Method

Approval was obtained from the local ethical committee before the study and animal care policies were complied with. Seventy five female "Wistar Albino" rats aged at least three months were used in the study. The mean weight of the animals was 284.24±28.1 g. For evaluating the interactions of preoperatively used cytotoxic agents with inhaled anaesthetic agents, the animals were divided into five groups (Figure 1). Only saline infusion was applied in Group I. Inhalation agents halothane and isoflurane were used in Groups II and III. Cisplatin and bleomycin were applied to animals 21 days before anaesthesia in Groups IV and V, a procedure similar to that used in a neoadjuvant setting in cancer patients.

Saline infusion (0.9% NaCl 2 ml given in 20 min) was applied with an infusion pump (Abbott Life Care® 5000 Infusion System, Ireland FINISKLIN, SLIGO) via the right jugular vein by using a 24G branule under general anaesthesia with ketamine (6 mg/kg) and xylazine (4 mg/kg) applied intraperitoneally. Peripheral blood samples were taken initially and 48 hours after the infusion.

Anesthesia with halothane and isoflurane was administered to the animals in Groups II and III. A plastic box was used for this inhalation anaesthesia. Nitrous oxide:oxygen ratio was adjusted as 70 to 30% and halothane was applied for a period of two hours in a concentration of 1.5% in Group II and isoflurane in a concentration of 2% in Group III. Blood samples were taken before anaesthesia and 48 h after inhalation anaesthesia via the right jugular vein. Cisplatin, in a dose of 1.4 mg/kg administered as a 20 min infusion with a perfusion pump was given to Group IV, and a 0.21 mg/kg bolus injection of bleomycin was applied to Group V. In both groups, these agents were administered via the right jugular vein under general anaesthesia with ketamin and xylazine intraperitoneally. Twenty one days after the chemotherapy, the animals were anaesthetised with halothane in Group IV and with isoflurane in Group V using the same dosage administered in the same duration as in Groups II and III. Blood samples were taken before chemotherapy and 48 h after anaesthesia via the right jugular vein.

In order to evaluate the effects of the anaesthesia and chemotherapy on the lungs, bronchoalveolar lavage (BAL) was performed 48 h after inhalation anaesthesia. BAL was performed to recover cells from alveolar spaces. The lavage was performed via an intratracheal angiocatheter with cold phosphate buffered saline (PBS). PBS contains 8 mM sodium phosphate, 2 mM potassium phosphate, 0.14 M sodium chloride, and 0.01 M potassium chloride, pH 7.4, with 0.1 mM ethylenediaminetetraacetic acid (EDTA). A volume of 40 ml PBS was collected from each rat by lavage

Table 2. Renal histopathological findings					
Groups	I	II	III	IV	V
Glomerulus	N	N	N	N	N
Tubules	N	N	N	-	-
Basophilic material	-	-	-	2	1
Ground glass appearance	-	-	-	1	2
Interstitial Tissue					
Hyperemia in medulla	-	2	2	4	5
Hemosiderin granules	-	2	1	1	-
Edema+PMNL	-	-	-	-	-
Perivascular lymphocyte	-	2	3	4	3
Cortical perivascular edema	-	-	-	3	1
Lymphocyte in cortex and medulla	-	-	-	-	-
Erythrocyte in medulla+ basophilic material	-	-	-	-	-
Siderophage, lymphocyte and plasmocytes in cortex	-	-	-	-	-
Calyx, Papilla, Pelvic Region, Prepelvic Structures					
PMNL+lymphocyte	-	2	3	4	2
Renal Capsule					
Edema+PMNL	-	-	-	3	2
N: Normal					

and this fluid was centrifuged at 700 rpm continuously for 15 min. The supernatant fraction was discarded and the pelleted cells were resuspended in 1 ml volume of the same solution. Smears from this solution were stained with Wright-Giemsa and differential cell counts were made. A total number of 100 cells were counted in each BAL fluid sample and the percentage of neutrophils and alveolar macrophages were calculated (2).

Haemoglobin (Hb) levels and biochemical parameters [serum glucose, creatinine, glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase (SGPT), lactic dehydrogenise (LDH) levels] were analysed in the blood samples. Laparotomy and sternotomy were performed 48 h after the anaesthesia and samples were taken from the left kidney, left hepatic lobe and the right lung. A pathologist, blind to the study groups, examined the tissue samples. Glomeruli, tubules, interstitial tissue, calyces, papilla, pelvic region, prepelvic structures and renal capsule were examined in the renal tissue samples. In hepatic samples hepatic capsule, connective tissue under the capsule, portal triad, limiting plate, central vein and hepatocelluler structures were analysed. Examination of the samples prepared from the right lung included examinations of the alveolar septa, alveolar sac, bronchioles, extraparenchymal tissues (nerve, blood vessels, connective tissue) and pleura.

The "One-Way ANOVA test" and "Independent Samples Student's *t*-test" were used in the statistical

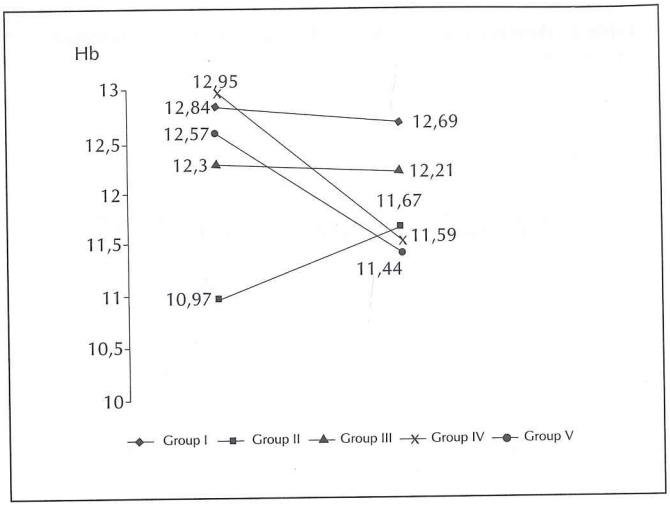


Figure 2. *Hb levels of the groups before and after anaesthesia.*

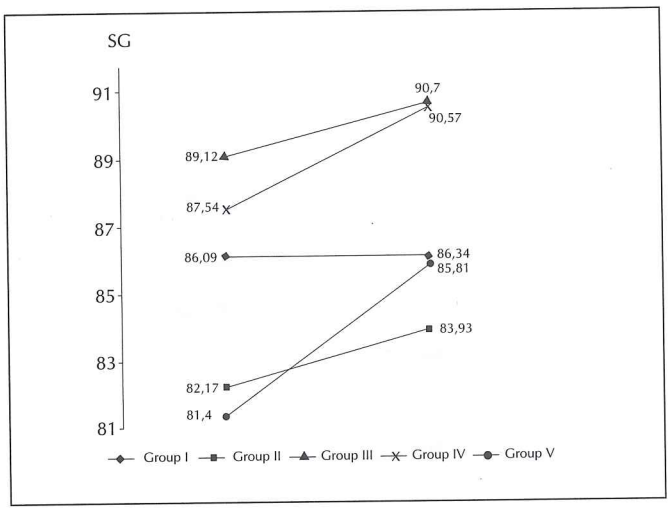


Figure 3. *Serum glucose levels of the groups before and after anaesthesia.*

analyses. A P value of <0.05 was accepted as statistically significant.

Results

No statistical differences were found between the groups in the analysis results of the blood samples taken before anaesthesia or chemotherapy (p>0.05). A One-Way ANOVA analyses of the results on samples obtained after 48 h from anaesthesia showed that Hb levels were significantly low in Groups III and IV (Table 2, p=0.015), while there were no important changes in glucose and creatinine levels. SGPT and SGOT levels showed an increase in all groups except in Group I, but the differences were of no statistical significance (p<0.05). An increase was also noted in LDH levels in all groups but this was significant only in Groups IV and V.

Analysis of the results by the Student's t-test in the halothane administered groups (Groups II and IV) revealed statistically significant differences (p<0.05) from initial values in all parameters. Similarly, significant differences

Table 3. Hepatic histopathological findings

Groups	I	II	III	IV	V
Liver Capsule					
Thickening in capsule	-	-	-	-	-
Subcapsullary Connective Tissue and Portal Triad					
Lymphocyte	-	2	1	4	1
Lymphocyte+PMNL	-	1	1	2	1
Plasma cell+lymphocyte+PMNL	-	1	-	1	-
Plasmocyte	-	-	-	-	-
Limiting Plate	N	N	N	N	N
Central Vein					
Minimal congestion	-	2	1	3	1
Hepatocellular Changes					
PMNL	-	1	1	2	1

N: Normal

Table 4. Pulmonary histopathological findings

Groups	I	II	III	IV	V
Alveolar Septa					
Alveolitis	-	8	9	15	12
Increase in cellularity	-	5	1	4	2
Increase in cellularity+PMNL	-	2	3	1	1
Lymphocyte+oamy macrophage +histiocytes	-	-	-	-	-
Edema+histiocytes	-	-	-	13	7
Alveolar Cavity					
Erythrocyte	3	4	5	14	11
Erythrocyte+alveolar macrophage	2	4	2	1	4
Alveolar macrophage+PMNL	3	3	3	5	3
Foamy macrophage	-	1	-	-	-
Bronchioles					
Terminal bronchiolitis	-	11	4	12	7
Lymphocyte	2	2	1	4	1
Lymphocyte+PMNL	2	8	4	7	5
Extraparanchymal Tissues					
Perivascular inflammatory infiltration	-	10	9	13	12
Pleura					
Hyperemia	-	-	1	-	-

($p<0.05$) were found in the isoflurane groups (Groups III and V), except in Hb, SGPT, and SGOT levels.

BAL fluid analyses also revealed statistical differences between Groups II vs. IV, and III vs. V ($p<0.05$). Polymorphonuclear leukocytes (PMNL) were found to be increased in the BAL fluid in chemotherapy applied groups (Figure 7).

In the histopathological evaluation of renal structures, hyperaemia in the medulla was noted in the groups which had received anaesthesia and chemotherapy. PMNL and lymphocyte infiltration were observed in the prepelvic structures of all anaesthetised groups. Another pathological finding was oedema in the renal capsule in the groups which had received chemotherapy. Results are indicated in Table 2.

In liver specimens the most significant finding was lymphocyte and/or PMNL infiltration in the portal triad, seen in all groups. Changes in these structures are listed in Table 3.

The most interesting findings of the study were obtained in the evaluation of pulmonary specimens. Alveolitis was seen in the alveolar septa in all groups which had received anaesthesia, but was more notable in the chemotherapy applied groups. Oedema and histiocyte infiltration were also seen in the groups which received chemotherapy and was most notable in Group IV (Figure 8). The appearance of erythrocytes in alveolar cavities was another finding which was noted in the chemotherapy groups. Terminal bronchiolitis and perivascular inflammatory infiltration were seen in all anesthesized animals with higher ratios in chemotherapy applied groups (Groups IV and V) (Figure 9). Results of examinations of pulmonary specimens are listed in Table 4.

Discussion

In recent years, neoadjuvant chemotherapy became a treatment modality widely used in cancer surgery. In this setting bleomycin and cisplatin are frequently applied agents. Nephrotoxicity, vomiting, high frequency hearing loss, anaphylactic reactions and hypomagnesaemia are among the potential side effects of cisplatin. Peripheral sensorial neuropathy and myelosupression can also develop when high doses are used (3,4).

Bleomycin is an agent which is widely used in various combinations because its suppressive effects on the immune system and bone marrow are minimal. The most important side effect of this drug is interstitial pneumonia (5). This effect can be potentiated by respiratory disease, by radiotherapy and by cumulative doses exceeding 400 mg (6,7). Although the pathogenesis of the pulmonary toxicity is not clear, animal studies appear to indicate that the toxicity may be due to terminal amines, produced as a metabolite of the drug (3,6).

We thought that the documentation of the histopathological changes in the lung due to interactions between anaesthetic and chemotherapeutic agents would contribute to clarifying the pathogenesis of the pulmonary toxicity.

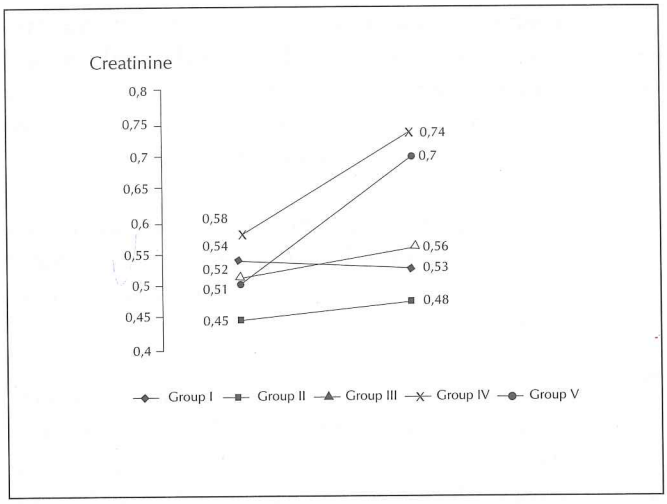
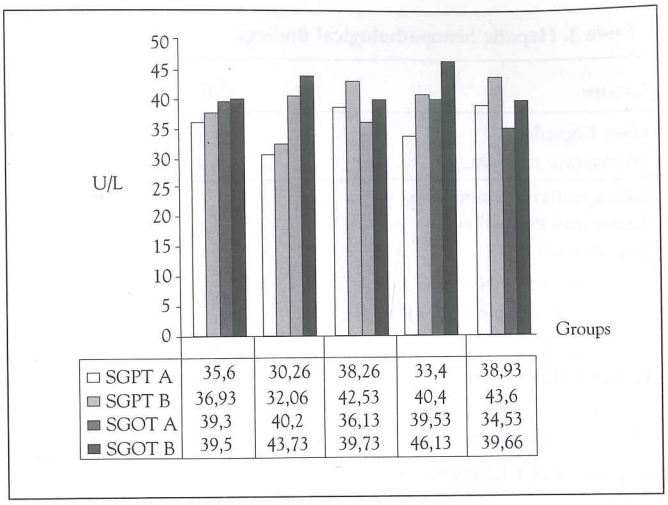


Figure 4. *Creatinine levels of the groups before and after anaesthesia.*



SGPT A	35,6	30,26	38,26	33,4	38,93
SGPT B	36,93	32,06	42,53	40,4	43,6
SGOT A	39,3	40,2	36,13	39,53	34,53
SGOT B	39,5	43,73	39,73	46,13	39,66

Figure 5. *SGPT and SGOT levels before and after anaesthesia.*

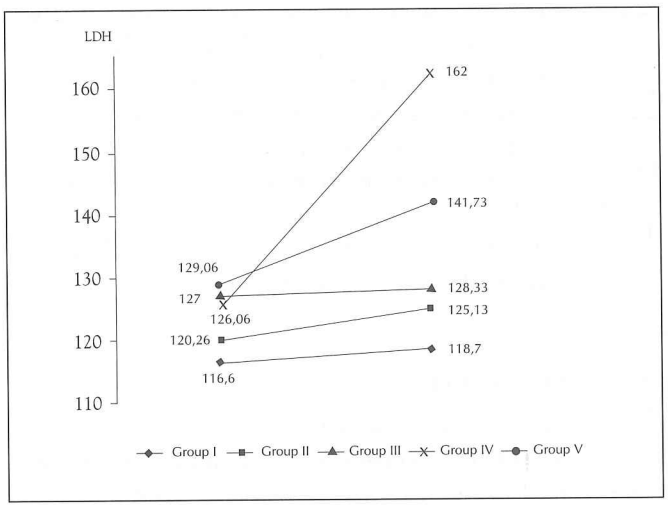
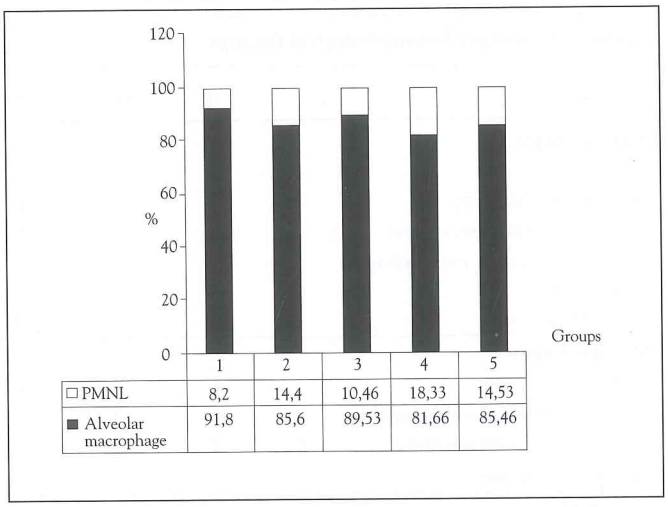


Figure 6. *LDH levels before and after anaesthesia.*



	1	2	3	4	5
PMNL	8,2	14,4	10,46	18,33	14,53
Alveolar macrophage	91,8	85,6	89,53	81,66	85,46

Figure 7. *PMNL and alveolar macrophage content of BAL.*

Alveolitis was more noticeable in the chemotherapy applied groups. While in Groups II and III the alveolitis findings were limited to a mild increase in cellularity in the alveolar septum and an increase in polymorphonuclear leukocytes and lymphocytes, oedema and histiocyte infiltration were added to these changes in Groups IV and V. PMNL count in BAL fluid was also found to be higher especially in Group IV. These findings were similar to previous reports on the pulmonary toxic effects of bleomycin (8-10). Postoperative pulmonary complications after treatment with bleomycin were first reported in 1978 in oesophagus cancer patients who were treated with bleomycin and radiotherapy (10). Goldiner et al (11) also reported severe pulmonary insufficiency which developed 3-5 days after bleomycin therapy and death in five of their patients due to interstitial pneumonia. In our study, terminal bronchiolitis developed in all groups, including 11 animals in Group I. While polymorphonuclear leukocytes and lymphocytes infiltrating the bronchial wall was a dominant finding in Groups I and II, a dense mononuclear cell infiltration was present in the bronchial walls in Groups III and IV. This finding indicated that bleomycin negatively affected the development of terminal bronchiolitis with anaesthetic agents. Terminal bronchiolitis was more significant in the halothane inhaled group than in isoflurane inhaled groups. Examination of extraparenchymal tissues showed that perivascular cell infiltration was also more noteable in chemotherapy applied groups.

In nephrotoxicity of cisplatin there is a heavy metal accumulation and the histopathology shows coagulation necrosis in the collecting tubules and also in the proximal tubules (12). In this study, hyperaemia, with erythrocyte accumulation in renal interstitial fields, was noted especially in chemotherapy applied groups. We believe we were not able to see the nephrotoxic effects of these agents clearly due to the short duration of the anaesthesia and to the limitation of chemotherapy to only one course.

In the examination of the hepatic specimens, lymphocytes and polymorphonuclear leukocytes were seen in some cases

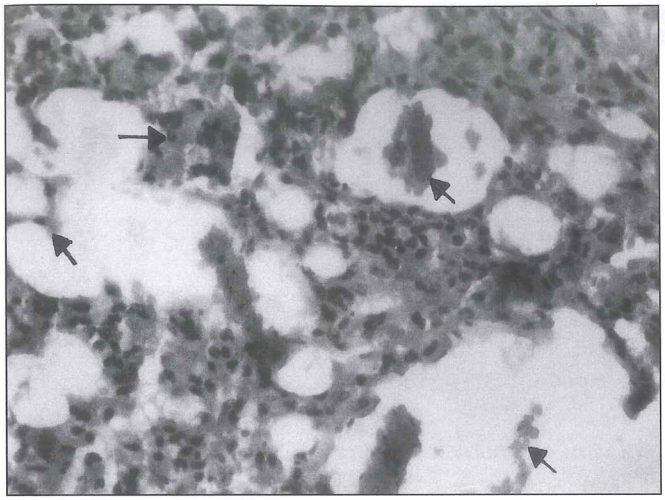


Figure 8. *Erythrocytes, alveolar macrophages and cellularity are increased in alveolar septa (H&E 100x).*

Figure 9. *Terminal bronchial walls infiltrated by lymphocytes and PMNLs (H&E 100x).*

in Groups II and IV. There were no specific changes in Groups III and V. These findings probably indicate that isoflurane has no or minimal hepatotoxic effects. Chemotherapeutic agents in this study also showed no major side effects on the liver. These histhopathological findings were supported by SGOT and SGPT levels which showed a mild elevation only in halothane applied groups.

Hb levels were significantly lower in chemotherapy applied groups compared to the other two groups. This may be due to toxic effects of the chemotherapeutic agents on bone marrow. In Groups IV and V, the decline of Hb levels was attributed to the effect of cisplatin The anaemia possibly developed as a result of the decrease in erytropoietin, caused by cisplatin (13).

In this study the effect of chemotherapy administered before anaesthesia was studied in an effort to contribute to the understanding of the interactions of anaesthetic and chemotherapeutic agents and the effects of these interactions on some tissues. It was observed that pulmonary, urologic, hepatic, and haemopoetic systems were affected in various degrees. In conclusion we may state that care in the selection of an anaesthetic agent for cancer patients who received chemotherapy before the anaesthesia is very important. We believe that further studies are needed to clarify the interactions between these agents.

References

1. Sikora K. Developing a global strategy for cancer. Eur J Cancer 1999; 35: 24-31.
2. Fan J, Kapus A, Li YH, et al. Priming for enhanced alveolar fibrin deposition after hemorrhagic shock role of tumor necrosis factor. Am J Resp Cell Mol Bio 2000; 22: 412-21.
3. Finley RS, Fortner CI, Grove WR. Cisplatin nephrotoxicity. A summary of preventive interventions. Drug Intell Clin Pharm 1985; 19: 362-367.
4. Nanji AA, Stewart DJ, Mikhael NZ. Hyperuricemia and hypoalbuminemia predipose to cisplatin-induced nephrotoxicty. Cancer Chemother Pharmacol 1986; 17: 274-276.
5. Goldiner PL, Carlon Gd, Cvitkovic E, et al. Factors influencing postoperative morbidity and mortality in patients treated with bleomycin. Br Med J 1978; 24: 1664-1667.
6. Conley NS, Yarbro JW, Ferrari HA, et al. Bleomycin increases superoxide anion generation by pig peripheral alveolar macrophages. Mol Pharmacol 1986; 30: 48-52.
7. Adamson IY, Bowden DH. The pathogenesis of bleomycin-induced pulmonary fibrosis in mice. Am J Pathol 1974; 77: 185-189.
8. Luna MA, Bedrossian CWM, Lichtiger B, et al. Interstitial pneumonitis associated with bleomycin therapy. Am J Clin Pathol 1972; 58: 501-510.
9. De Lena M, Guzzon A, Monfardini S. Clinical radiological and histopathological studies on pulmonary toxicity induced by treatment with bleomycin. Cancer Chemother Rep 1972; 56: 343-356.
10. Patchefsky AS. Nonneoplastic pulmonary disease. In: Sternberg SS ed. Diagnostic Surgical Pathology. New York: Raven Press, 1994; 981-1044.
11. Nygaard K, Smith-Erichsen N, Hatlevoll R, Refsum SB. Pulmonary complications after bleomycin, irradiation and surgery for esophageal cancer. Cancer 1978; 41:17-22.
12. Hout AE, Hacker MP. Role of reactive nitrogen intermediate production in alveolar macrophage-mediated cytostatic activity induced by bleomycin lung damage in rats. Cancer Res 1990; 50: 7863-7866.
13. Patricia A, Wood J, William J, et al. Cisplatin-associated anemia: An erythropoietin deficiency syndrome. J Clin Invest 1995; 95: 1650-1659.