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Research Article

Paracetamol Supplementation Does Not Alter The Antitumor Activity and Lung Toxicity of Bleomycin

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ABSTRACT

Bleomycin (BLM) is well known by its antitumor activity both in vitro and in vivo. However, pulmonary fibrosis has been considered the dose limiting toxicity of the drug. Hyperpyrexia following injection of BLM was reported thus, paracetamol is sometimes administered with BLM as antipyretic drug. Actually, paracetamol was found to interfere with cytotoxicity of some drugs. This study was conducted to investigate the effect of paracetamol administration on the antitumor and lung toxicity of BLM. The antitumor activity was evaluated both in vitro and in vivo using Ehrlich ascites carcinoma (EAC) cells. Paracetamol did not alter the antitumor effect of BLM in vitro or in vivo. The lung toxicity of BLM was evidenced by decrease in the body weight, increase in the lung/body weight ratio, decrease in the response of pulmonary arterial rings to 5-hydroxytryptamine (5-HT) and increase in the contractility of tracheal smooth muscles induced by acetylcholine (ACh). The toxicity was also confirmed biochemically by marked increases in hydroxyproline and lipid peroxidation in rat lung and the decrease in reduced glutathione (GSH) level. Pretreatment with paracetamol did not significantly change lipid peroxidation, GSH level, percent survival of rats or the response of pulmonary arterial rings and tracheal smooth muscles to 5-HT and ACh respectively. The results of the present study indicated that paracetamol neither modified the antitumor effect of BLM nor changed drug-induced lung toxicity.

Keywords: Bleomycin (BLM), Paracetamol, Ehrlich Ascites Carcinoma (EAC), pulmonary fibrosis

1. Introduction

Bleomycin is a glycopeptide antibiotic with antineoplastic activity in a variety of human neoplasms (Lazo and Chabner, 1996). It is usually administered as a part of combination chemotherapy regimens either as a single dose or as a fractionated dose. However, the effective use of BLM in chemotherapy is greatly limited, since it causes a dose-dependent interstitial pneumonitis that often progresses to interstitial pulmonary fibrosis (Venkatesan et al., 1997). This is due to the lack of the BLM-inactivating enzyme, bleomycin hydrolase, in the lungs. The primary mechanism by which BLM induces pulmonary toxicity is thought to be related to redox cycling of an iron-BLM complex that catalyzes the formation of superoxide and hydroxyl radicals and causes DNA strand scission and lipid peroxidation.

Hyperpyrexia, shock and death following initial injection of BLM (7.5 units) for a poorly differentiated metastatic carcinoma was reported in some cases (Levy

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and Chiarillo, 1980). Leung *et al*. (1989) described a fatal hyperpyrexial reaction after bleomycin in a patient with T-cell lymphoma who had had no febrile response when receiving initial injection 3 weeks earlier. Paracetamol inhibits the synthesis of prostaglandins centrally, having an analgesic antipyretic but not anti-inflammatory effect (Doherty *et al*., 1990; Woollard *et al*., 1990).

The present study was undertaken to investigate whether paracetamol could modify the antitumor activity of BLM *in vitro* and *in vivo* against Ehrlich Ascites Carcinoma (EAC) cells. Moreover, its effect on lung toxicity produced by BLM was also studied. Firstly, by measuring the effect of chronic treatment with this combination on the response of the isolated pulmonary arterial rings and tracheal strips to 5-HT and ACh, respectively, as pharmacological markers of lung injury. Secondly, by measuring some biochemical parameters which reflect the severity of lung injury such as hydroxyproline level, lipid peroxidation, nitric oxide (NO), superoxide dismutase (SOD) activity and reduced glutathione level (GSH).

2. Materials and Methods

2.1. Experimental animals

Female Swiss albino mice weighing 20-30 g were used to study the cytotoxic effect of BLM alone and in combination with paracetamol. Sprague-Dawley rats of either sex weighing 110-160 g were used for evaluating lung toxicity. The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the Ethical Committee of Faculty of Pharmacy, Mansoura University, Egypt.

2.2. Drugs and chemicals

Bleomycin (BLM): as a white powder was obtained as a generous gift from Dr. Hiroshi Suda (Nippon Kayaku Co., LTD., Tokyo, Japan); Paracetamol: as a white powder was obtained from Sigma Aldrich chemical Co. (St. Louis, MO, USA); Roswell Park Memorial Institute (RPMI 1640) Medium (supplied by Sigma Aldrich chemical Co.); All other chemicals used in this study are of fine analytical grade.

2.3. Ehrlich Ascites Carcinoma Cells

Ehrlich ascites carcinoma cells were established in the Netherlands Cancer Institute. The Ehrlich tumor line was maintained in the laboratory of Faculty of Pharmacy, Mansoura University in female Swiss albino mice by serial intraperitoneal passage at 7-10 day intervals as described by Geran *et al*. (1972).

2.4. Cytotoxicity study

2.4.1. *In vitro* experiment

Cell culture. EAC cells suspended in RPMI 1640 medium supplemented with 10% fetal calf serum, containing various concentrations of drugs were cultured in cell culture sterile tubes at a density of 2×10^5 cells/ml/tube. The tubes were incubated in a humidified atmosphere containing 5% CO_2 at 37°C for 24 h. The trypan blue dye exclusion test was used to determine the rate of cellular growth inhibition. The dye stains the dead cells only (Weisenthal et al., 1983).

***In vitro* cytotoxic assay.** Ascitic fluid from the intraperitoneal cavity of the donor animal was aseptically aspirated, 7-8 days after EAC cells inoculation, and washed three times with N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid buffered Hanks' balanced salt solution. EAC cells were counted under the microscope using a haemocytometer and resuspended in normal saline so that each 0.1 ml contained 2×10^5 cells (Badary et al., 2000). EAC cells were incubated in RPMI 1640 medium for 24 h in cell culture tubes, each tube contained 0.1 ml cells and 0.9 ml medium (final concentration of the cells = 2×10^5 cells/ml). After 24-h incubation, the tubes were centrifuged at 1000 r.p.m. and cells were separated. EAC cells were resuspended in RPMI 1640 medium and drugs were added so that the content of each tube was 0.8 ml medium, 0.1 ml cells and 0.1 ml drug. The final drug concentrations of BLM were 25, 50, 100 and 200 $\mu g.ml^{-1}$.

The cytotoxicity dose response curve for BLM was constructed in order to determine the concentration that inhibits 50% of cell survival (IC_{50}). EAC cells were incubated with 50 μg/ml BLM (IC_{50}), paracetamol (400 μg/ml, Hayes et al., 1984), or their combination for 24 h. After incubation of cells with drugs, cells were separated, washed by PBS and resuspended in a drug-free medium. EAC cells were stained with trypan blue dye and the percent survival of cells was determined by trypan blue dye exclusion method. Cytotoxicity was determined 3 times and the mean was recorded.

Control experiments in which EAC cells were incubated in a drug-free medium were also conducted. Percent survival of cells = (T/C) X 100 was calculated where T and C represent the number of viable cells in a unit volume of the test drug tube and the control tube, respectively.

2.4.2. *In vivo* experiment

Ascitic fluid was withdrawn under aseptic conditions from tumor-bearing mice by needle aspiration from the peritoneal cavity, 7-8 days after EAC cells inoculation, and washed three times with normal saline by centrifugation at 1000 r.p.m. EAC cells obtained after washing were tested for viability using trypan blue. The cells were examined microscopically using a haemocytometer, suspended in normal saline so that each 0.1 ml contained 5×10^5 viable EAC cells. The cells were counted under the microscope.

Solid tumors were induced in mice by S.C inoculation of 0.1 ml containing 5×10^5 viable tumor cells on the left flank anterior to the hind leg (Raja Naresh et al., 1996). Tumor growth was determined by caliper measurement of the largest diameter and its perpendicular (Schirner et al., 1998).

Tumor size (mm^3) = $0.5 \times a \times b^2$.

Where a is the largest diameter and b is its perpendicular.

When the primary tumor reached a size of 50-100 mm^3, 40 mice were grouped into 4 groups (10 mice each). Group (1) received normal saline (EAC-bearing control, 5 mg/kg). Group (2) received BLM (5 mg/kg from 0.1% solution) I.P every other day for 10 days. Group (3) received paracetamol (200 mg/kg, orally, Hunskaar et al., 1985) for 10 consecutive days. Group (4) received BLM and paracetamol. Tumor size before (day 0, five days after tumor inoculation) and after treatment (day 10, fifteen days after tumor inoculation) was measured. Antitumor activity was calculated by the determination of ΔT (change of tumor size in the treatment group) and ΔC (change of tumor size in the control). The degree of tumor growth inhibition can be obtained from $\Delta T/\Delta C$ X 100 (Schirner et al., 1998).

2.4.3. Lung toxicity

Rats were grouped into 4 groups of 8 rats each. Group (1) received normal saline (2.5 mg/kg). Group (2) received BLM (mg/kg from 0.5% solution) I.P. three times/week for 4 weeks (Daba et al., 2002). Group (3) received paracetamol (150 mg/kg, orally, Chattopadhyay *et al*., 2002) daily for 4 weeks [13]. Group (4) received BLM and paracetamol. Twenty-four hours after the last dose of the specific treatment, all rats were weighed and then sacrificed by cervical dislocation. The pulmonary arteries, trachea and lungs were rapidly excised.

The vasoconstrictor effect of 5-HT on the pulmonary artery and the contractile response to ACh on trachea were tested. The percent survival of rats was also recorded at the end of treatment (after 4 weeks). Total lungs were weighed for determination of lung/body weight ratio. Sections of the isolated lungs, each weighing 100 mg, were then used for estimating collagen contents, while the other biochemical parameters (Lipid peroxide, nitrate/nitrite level, superoxide dismutase activity and GSH) were assessed in the lung homogenates. All died animals in the survival test were replaced to complete 8 rats/each group for other tests.

2.4.4. Preparation of pulmonary arterial rings

Segments of the main pulmonary artery were rapidly placed in warm physiological salt solution (PSS) (Kreb's Henseleit) and dissected free of surrounding tissue. The composition of PSS in mmol/L was NaCl 118; KCl 4.7; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4.7H_2O$ 1.2; $NaHCO_3$ 25 and glucose 10; pH 7.4 (Blom-Muilwijk et al., 1988). The pulmonary artery was trimmed and cut into rings about 2 mm in length. The rings were mounted horizontally between a clamp and a force transducer in an organ bath filled with 50 ml of the PSS. The PSS was kept at 37°C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The suspended pulmonary arterial rings were allowed to equilibrate for 30 min under a resting load of 3 g (El-Kashef, 1996).

The isometric tension was monitored by means of a displacement transducer (model 50-7905, Harvard Apparatus LTD., South Natick, MA, USA) connected to a two-channel physiograph recorder (model 50-8622, Harvard Apparatus LTD., USA). The contractile responses of the pulmonary arterial rings of each group of animals to different concentrations of 5-HT ranging from 10^{-7} to 10^{-5} M were recorded. The vascular responsiveness of the tissues to 5-HT was calculated after weighing the rings as g tension/g tissue.

2.4.5. Preparation of trachea

The trachea was rapidly removed, placed in Kreb's Henseleit solution gassed with 95% O_2 / 5% CO_2 and kept at 32°C. The Zig-Zag tracheal strips (Emmerson and Mackay, 1979) were then prepared. The tracheal tension was set at 1 g and a stabilization period of 1 h, was allowed before treatment with drugs. The contractile responses of the trachea of each group of animals to different concentrations of ACh ranging from 10^{-6} to 10^{-4} M were recorded. The trachea was weighed and the responsiveness to ACh was calculated as g tension/g tissue.

2.4.6. Biochemical measurements

2.4.6.1 Determination of collagen

The collagen content in lung was assayed indirectly as its hydrolytic product, hydroxyproline, HP (Woessner, 1961). The absorbance was read at 557 nm using colorimeter WPA colourwave (Model CO 7500, Cambridge, England) and the concentrations of collagen were calculated as mg HP/g wet tissue.

2.4.6.2 Preparation of lung homogenate

The isolated lungs were rinsed in chilled 1.15 % KCl (pH 7.4) and weighed quickly. Subsequently, the lung/body weight ratio was determined. Homogenization was carried out in ice-cold KCl (1.15%, pH 7.4) to yield 10% w/v tissue homogenates (Daba et al., 2002) and the following biochemical parameters were assessed.

2.4.6.3 Determination of Lipid peroxidation (LP)

The level of LP in the lung was estimated as thiobarbituric acid reactive substances (Ohkawa et al., 1979). The absorbance was determined at 532 nm spectrophotometerically and the concentrations were expressed as nmol/g wet tissue.

2.4.6.4 Determination of reduced glutathione (GSH)

The level of acid-soluble thiols, mainly GSH, in the lung was assayed colourmetrically, based on its reaction with Ellman's reagent (1959). The absorbance was measured at 412 nm and the concentrations were expressed μmol/g wet tissue.

2.4.6.5 Determination of superoxide dismutase (SOD)

The enzymatic activity of SOD was assessed according to Marklund (1985). SOD activity was expressed as U/g wet tissue. One unit of SOD activity is defined as the amount of the enzyme causing 50% inhibition of auto-oxidation of pyrogallol.

2.4.6.6 Determination of nitric oxide (NO)

The total amount of NO produced in the lung was indirectly estimated as the main metabolites, nitrate and

nitrite. After the conversion of nitrate to nitrite by enzyme nitrate reductase in the presence of the cofactor NADPH, NO was collectively determined as nitrite by the Griess reaction. In acid solution, nitrite is converted to nitrous acid (HNO_2) which diazotizes sulfanilamide. This sulfanilamide-diazonium salt then reacts with N- (1-Naphthyl)-ethylenediamine dihydrochloride (NEDA) to produce a chromophore which is measured at 540 nm and the concentrations of NO were calculated as μmol of nitrite and nitrate/g wet tissue (Tracy et al., 1995).

2.5 Statistical analysis

Data are expressed as mean ± S.E.M. (Significance was calculated at $p<0.05$). Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test , in addition to linear regression analysis for the best fitting line of all standard points (Daniel, 1991). Also paired Student's t-test was used as a test of significance for comparison between two arithmetic means of the same subject before and after treatment (Daniel, 1991). Chi-square test was used for comparison of two proportions (Daniel, 1991). Statistical calculations were carried out using Instat-2 computer program (GraphPad Software Inc. V2.04, San Diego, CA, USA).

3. Results

3.1. Effect of paracetamol on the cytotoxic effect of BLM on cultured EAC cells

The IC_{50} of BLM was found to be about 50 μg/ml. The cytotoxic effect of paracetamol was studied and the results showed no significant difference from the control. Combinations of BLM with paracetamol showed a significant difference when compared to the control group but non-significant when compared to BLM alone (table 1).

Table 1. Effect of paracetamol (400 μg/ml) on the cytotoxic effect of BLM (50 mg/ml) on cultured EAC cells

Treatment	% survival
Control	98.0 ± 0.3
BLM	49.0 ± 0.5*
Paracetamol	96.0 ± 0.6
BLM/ Paracetamol	48.0 ± 1.3*

* significantly different from control group using One-Way ANOVA followed by Tukey-Kramer multiple comparisons test ($p<0.05$).

3.2. Effect of bleomycin and/or paracetamol on tumor size after 10 days treatment

Implantation of EAC cells resulted in a solid palpable tumor mass that appeared after 5 days from inoculation (day 0). The size of tumor progressively increased with time and reached about 3.5-folds its initial mass after additional 10 days (day 10) that considered 100% tumor growth.

Treatment with BLM significantly decreased the relative tumor size compared to the control group, showing 19% tumor growth (i.e. 81% tumor growth inhibition). Mice treated with paracetamol showed a similar pattern like the control group. Administration of BLM with paracetamol showed an increase in tumor growth by only 7% (i.e. 93% tumor growth inhibition) compared to the control group with no significant difference from BLM group (table 2).

Table 2. Effect of bleomycin (BLM, 5 mg/kg) and/or paracetamol (200 mg/kg) on tumor size after 10 days treatment.

Treatment	Tumor size (mm^3)			
	Day 0	Day 10	ΔT/ΔC (%)	% inhibition
Control	91.7 ± 5.3	313.0 ± 28.4	100	0
BLM	87.5 ± 16.6	129.7 ± 24.6*	19	81
Paracetamol	97.11 ± 4.7	302.81 ± 17.6	93	7
BLM/ Paracetamol	74.80 ± 8.2	89.80 ± 13.5*	7	93

* significantly different from control group at day 10 using One-Way ANOVA followed by Tukey-Kramer multiple comparisons test ($p<0.05$).

3.3. Effect of BLM and/or paracetamol on the % survival of rats after 4 weeks treatment

At the end of the experiment, rats treated with saline and paracetamol, showed 100% survival. Rats treated with BLM showed a significant decrease in the percent survival (75%) compared to control non-treated rats. Administration of BLM with paracetamol showed a non-significant change in the percent survival of animals (75%) compared to BLM alone (Figure 1).

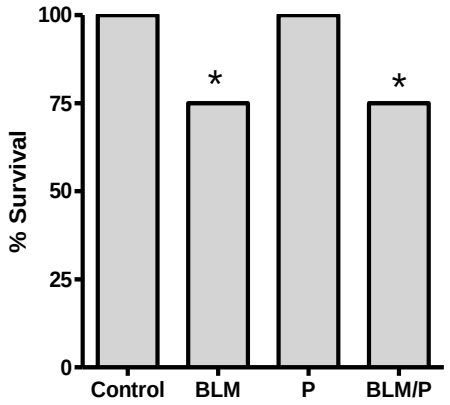

Fig. 1 Effect of bleomycin (BLM, 15 mg/kg) and/or paracetamol (150 mg/kg) on the percent survival of rats after 4 weeks treatment.
*Significantly different from control group using chi-square test ($p<0.05$).

3.4. Effect of BLM and/or paracetamol on body weight and lung/body weight ratio

The average body weight of normal control animals increased significantly after 4 weeks (37% increase). Administration of BLM alone induced a significant decrease in the average body weight (29% reduction). Paracetamol-treated group showed a similar increase as in the control group. Animals treated with BLM and paracetamol showed a significant decrease in their body weight by 37% which is non-significantly different from BLM-treated animals.

LM administration either alone or in combination with paracetamol showed a significant increase in lung/body weight ratio of the rats compared to control animals (table 3).

Table 3. Effect of bleomycin (BLM, 15 mg/kg) and/or paracetamol (150 mg/kg) on body weight and lung/body weight ratio of rats after 4 weeks treatment.

Treatment	Body weight (g)		Lung/body weight ratio ($\times 10^{-3}$)
	Before treatment	After treatment	
Control	100.0 ± 2.1	137.4 ± 4.4°	6.9 ± 0.15
BLM	136.3 ± 5.4	96.7 ± 5.5°	9.0 ± 0.36*
Paracetamol	160.3 ± 6.4	196.4 ± 7.6°	6.7 ± 0.27
BLM/ Paracetamol	123.1 ± 3.0	77.2 ± 4.5°	9.2 ± 0.32*

° significantly different from its corresponding initial value using paired Student's t-test ($p<0.05$).
* significantly different from control group using One-Way ANOVA followed by Tukey-Kramer multiple comparisons test ($p<0.05$).

3.5. Effect of BLM and/or paracetamol on the contractile response of pulmonary arterial rings of rats to 5-HT

Administration of BLM alone induced a significant decrease in 5-HT-induced contraction at all concentrations used (10^{-7} - 10^{-5} M) when compared to saline-treated rats. Rats treated with paracetamol showed a non-significant change in 5-HT-induced contraction at all concentrations used when compared to control group. A significant decrease in 5-HT-induced contraction at all concentrations used was produced when BLM was administered with paracetamol. The decrease was not significant from BLM alone (figure 2A).

3.6. Effect of BLM and/or paracetamol on the contractile response of tracheal strips of rats to ACh

Administration of BLM alone produced a significant increase in ACh-induced contraction at concentrations 10^{-6} and 10^{-5} M when compared to control group. Rats treated with paracetamol showed a non-significant change in ACh-induced contraction at all concentrations used when compared to control group. Administration of BLM with paracetamol showed a significant increase in ACh-induced contraction at 10^{-6} and 10^{-5} M with respect to control group (figure 2B).

3.7. Effect of BLM and/or paracetamol on lung content of hydroxyproline, TBARS, GSH, SOD and nitrate/nitrite after 4 weeks treatment

BLM treatment resulted in a significant increase in lung hydroxyproline content, TBARS and a significant decrease in lung GSH level compared to control non-treated group. Administration of paracetamol with BLM did not significantly change the measured parameters when compared to BLM alone (Table 4).

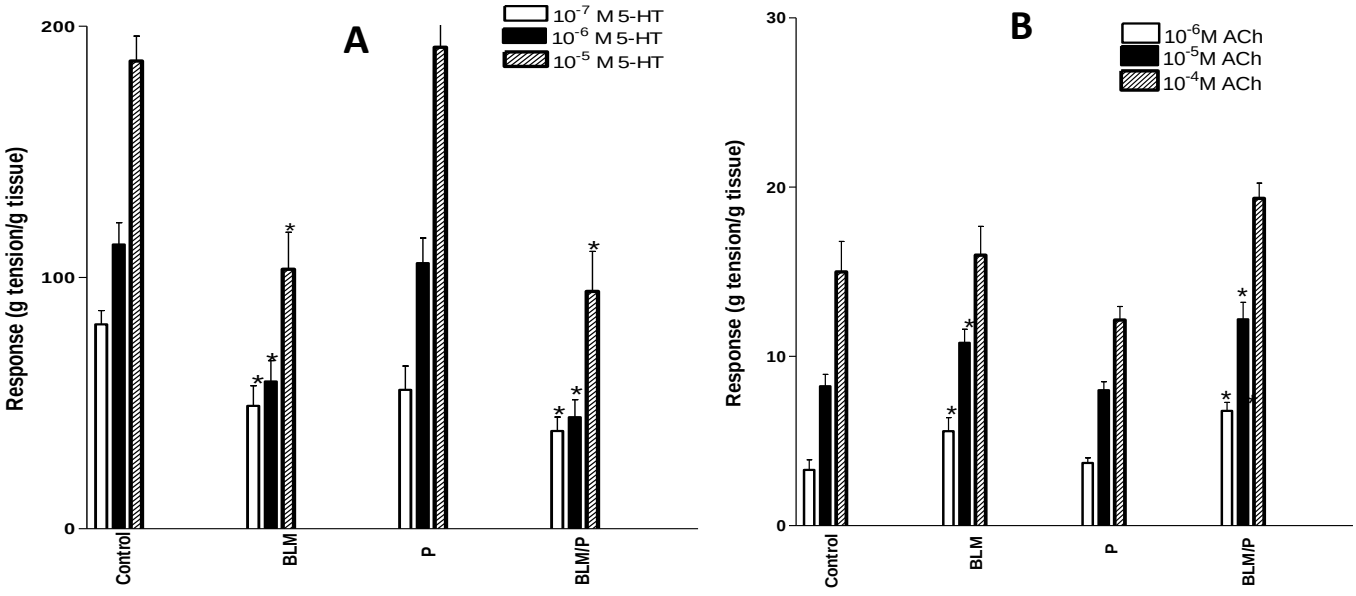

Figure 2. Effect of bleomycin (BLM, 15 mg/kg) and/or paracetamol (150 mg/kg) on the contractile response of pulmonary arterial rings of rats to 5-HT [A] and tracheal strips to ACh [B] after 4 weeks treatment.
* significantly different from control group using One-Way ANOVA followed by Tukey-Kramer multiple comparisons test ($p<0.05$).

4. Discussion

BLM is one of the most effective chemotherapeutic agents used in treatment of cancers of skin, penis, head, neck, lung, oesophageal carcinoma, malignant lymphomas, testicular carcinoma and malignant pleural effusions (Lazo and Chabner, 1996).

In this study, it was found that EAC cells are sensitive to BLM in a concentration-dependent manner. This observation is in agreement with the previously reported cytotoxicity of BLM on many other cultured experimental tumor cell lines such as V 79 fibroblasts and P 31 human lung cancer cell lines (Benham Motlagh *et al*., 1995) and human lung cancer cell lines DLKP, A 549, COR L23P and COR L23 R (Duffy *et al*., 1998). The cytotoxicity of BLM is believed to be through oxygen radical-mediated mechanisms (Bachur *et al*., 1978). Paracetamol was found to be non-cytotoxic *in vitro* at the concentration used and had no effect on BLM-induced inhibition of survival of EAC cells. This finding is consistent with the results of Heldestad *et al*. (1998). They found that acetaminophen (10 or 100 mg/L) was without effect on BLM cytotoxicity on Chinese hamster cultured fibroblasts (V 79). Thus, a combination of paracetamol with BLM was found to have no additional cytotoxic effect, indicating that BLM cytotoxicity is not modified by pretreatment with paracetamol.

This study has been extended further to explore if paracetamol could alter the antitumor effects of BLM *in vivo* and thus, modify its therapeutic activity. In this sense, a mouse model of solid tumor was used. The antitumor activity of BLM was evidenced by the reduction in the relative tumor size-compared to untreated tumor-bearing control animals. This is coping with previous studies in mice-bearing solid tumors such as Ehrlich carcinoma, B16 melanoma and Meth A fibrosarcoma (Yokoyama *et al*., 1991), Sarcoma-180 and Ehrlich carcinoma (Raja Naresh *et al*., 1996). BLM significantly decreased body weight of mice, a result in agreement with previous studies (Tanino *et al*., 2002; Genovese *et al*., 2005a).

Paracetamol did not interfere with BLM-induced antitumor activity in EAC-bearing mice meaning that BLM retains its full antitumor effectiveness in the presence of paracetamol.

The clinical usefulness of BLM has been, however, limited by detrimental side effects. Pulmonary fibrosis has been considered the dose limiting toxicity of the drug (Coker and Laurent, 1998). Therefore, it was of great interest to address whether or not paracetamol would have effects on BLM-induced lung toxicity. In the present study, BLM administration significantly decreased the body weight of rats, an observation agreed with others (Gong *et al*., 2004; Genovese *et al*., 2005b) Concomitant administration of BLM and paracetamol did not alter this effect. In addition, the results of this study revealed a significant increase in lung/body weight ratio of rats treated with BLM. Consistent with this observation, rats treated with intratracheal BLM developed a significant increase in mean lung wet/dry weight ratio confirming the development of pulmonary edema (Crawley *et al*., 1992; Habib *et al*., 1993). Concomitant administration of paracetamol with BLM did not alter this effect. It has been reported that after chronic exposure to BLM, body weight loss and an increase in the lung/body weight ratio are signs of lung toxicity (EL-Khatib, 2002). In the present study, administration of BLM resulted in a significant reduction in the percent survival of rats. This result is consistent with the results of others (Tanino *et al*., 2002; Genovese *et al*., 2005a).

The combined use of paracetamol with BLM exhibited no significant change in the percent survival of rats when compared to BLM alone.

BLM reduced significantly the response of isolated pulmonary arterial rings to 5-HT at all concentrations tested (10^{-7} to 10^{-5} M). These results are consistent with another study (EL-Khatib et al., 2001). Paracetamol did not alter the suppressor effect of BLM on 5-HT-induced pulmonary artery contraction.

Moreover, BLM increased significantly the response of isolated tracheal strips to ACh at concentrations 10^{-6} and 10^{-5} M. This result may be due to overexpression of acetylcholine receptors in fibrotic lung after BLM treatment. In the present study, paracetamol did not alter the effect of BLM on ACh-induced tracheal smooth muscle contraction.

Administration of BLM at a fibrogenic dose resulted in lung fibrosis clearly identified by biochemical parameters. BLM produced a significant increase in lung content of hydroxyproline. This observation is consistent with other reports (Pardo et al., 2003; Liu et al., 2005). Administration of paracetamol with BLM showed a similar increase in lung hydroxyproline content when compared to control value, while there was no significant change when compared to BLM alone.

In the present study, the production of malondialdehyde, which is an index of lipid peroxidation was increased after BLM treatment, a result which is agreed with many reported data that demonstrated apparent elevation in lung TBARS following administration of BLM (Daba et al., 2002; Gong et al., 2004).

Administration of BLM also reduced the lung content of GSH and this is in line with other reports (Arslan et al., 2002; Pardo et al., 2003). Reduced GSH level observed after BLM administration could be indirectly attributed to the increased level of TBARS.

BLM increased the lung activity of SOD, but it didn't reach a significant level. The level of nitrate and nitrite measured in the lung of BLM-treated group showed no significant change from the control, a finding in agreement with that of El-Khatib *et al*. (2001). The use of paracetamol with BLM did not significantly change the measured parameters when compared to BLM group.

Taken together, the results of the present study indicate the safe use of paracetamol with BLM.

References

Che Man, Y. B.; Mirghani, M. E. S. Detection of lard mixed with body fats of chicken, lamb, and cow by fourier transform infrared spectroscopy. *J. Am. Oil Chem. Soc.* **2001**, 78, 753 – 761.

Arslan, N.; Miller, T. R.; Dehdashti, F.; Battafarano, R. J. and Siegel, B. A. Evaluation of response to neoadjuvant therapy by quantitative 2-deoxy-2 (18F), fluoro-D-glucose with positron emission tomography in patients with esophageal cancer. *Mol. Imaging Biol*. **2002**, 4, 301-310.

Bachur, N. R.; Gordon, S. L. and Gee, M. V**.** A general mechanism for microsomal activation of quinone anticancer agents to free radicals. *Cancer Res*. **1978**, 38, 1745-1750.

Badary, O. A.; Sharaby, S. A.; Kenawy, S. A.; El-Denshary, E. E. and Hamada, F. M. Evaluation of cisplatin combined with ondansetron in Ehrlich ascites carcinoma *in vitro* and *in vivo*. *Tumori*. **2000**, 86, 153-156.

Benham Motlagh, P.; Henriksson, R. and Grankvist, K. Interaction of the antiemetics ondansetron and granisetron with the cytotoxicity induced by irradiation, epirubicin, bleomycin, estramustine and cisplatin *in vitro*. *Acta. Oncol*. **1995**, 34, 871-875.

Blom-Muilwijk, M. C.; Vriesendorp, R.; Veninga, T. S. Pulmonary toxicity after treatment with bleomycin alone or in combination with hyperoxia. Studies in the rat. *Br. J. Anaesth*. **1988**, 60, 91-97.

Chattopadhyay, D.; Arunachalam, G.; Mandal, A. B. and Mandal, S. C. Evaluation of antipyretic activity of leaf extracts of Mallotus peltatus (Geist) Muell. arg. var acuminatus: a folk medicine. Phytomedicine. 2002, 9, 727-730.

Coker, R. K. and Laurent, G. J. Pulmonary fibrosis: Cytokines in the balance. *Eur. Respir. J*. **1998**, 11, 1218-1221.

Crawley, D. E.; McCormack, D. G. and Evans, T. W. Bleomycin-induced acute lung injury in the rat does not influence pulmonary vascular responsiveness *in vitro*. *Crit. Care Med.* **1992**, 20, 641-644.

Daba, M. H.; Abdel-Aziz, A. H.; Moustafa, A. M.; Al-Majed, A. A.; Al-Shabanah, O. A. and El-Kashef, H. A. Effects of L-carnitine and Ginkgo Biloba extract (EGb 761) in experimental bleomycin-induced lung fibrosis. *Pharmacol. Res*. **2002**, 45, 461-467.

Daniel, W. W. Hypothesis testing. In: *Biostatistics: A Foundation for Analysis in the Health Sciences*: 5th ed. Jhon Wley & Sons. New York, Chichester, Brisbane, Toronto and Singapore, **1991**, P. 191.

Doherty, N. S.; Beaver, T. H.; Chan, K. Y.; Dinerstein, R. J. and Diekema, K. A. The antinociceptive activity of paracetamol in Zymosan-induced peritonitis in mice: the role of prostacyclin and reactive oxygen species. *Br. J. Pharmacol.* **1990**, 101, 869-874.

Duffy, C. P.; Elliott, C. J.; O'Connor, R. A. Enhancement of chemotherapeutic drug toxicity to human tumor cells *in vitro* by a subset of non-steroidal anti-inflammatory drugs (NSAIDs). *Eur. J. Cancer*. **1998**, 34, 1250-1259.

El-Kashef, H. A. Hyperglycemia increased the responsiveness of isolated rabbit's pulmonary arterial rings to serotonin. *Pharmacology*. **1996**, 53, 151-159.

El-Khatib, A. S. Possible modulatory role of nitric oxide in lung toxicity induced in rats by chronic administration of bleomycin. *Chemotherapy*. **2002**, 48, 244-251.

El-Khatib, A. S.; Moustafa, A. M.; Abdel-Aziz, A. H.; Al-Shabanah, O. A. and El-Kashef, H. A. Ginkgo biloba extract (EGb 761) modulates bleomycin-induced acute lung injury in rats. *Tumori* **2001**, 87, 417-422.

Ellman, G. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys*. **1959**, 82, 70-77.

Emmerson, J. and Mackay, D. The zig-zag tracheal strip. *J. Pharm. Pharmacol*. **1979**, 31, 798.

Genovese, T.; Cuzzocrea, S.; Di Paola, R. Inhibition or knock out of inducible nitric oxide synthase results in resistance to bleomycin-induced lung injury. *Respir. Res*. **2005a**, 6, 58.

Genovese, T.; Di Paola, R.; Mazzon, E.; Muia, C.; Caputi, A. P. and Cuzzocrea, S. Melatonin limits lung injury in bleomycin-treated mice. *J. Pineal Res*. **2005b**, 39, 105-112.

Geran, R. T.; Greenberg, N. H.; McDonald, M. M.; Schumacher, A. M. and Abbott, B. J. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. *Cancer Chemother. Rep*. **1972**, 3, 1-7.

Gong, L. K.; Li, X. H.; Wang, H. Feitai attenuates belomycin-induced pulmonary fibrosis in rats. *Biol. Pharm. Bull*. **2004**, 27, 634-640.

Habib, M. P.; Lackey, D. L.; Lantz, R. C. Vitamin A pretreatment and bleomycin induced rat lung injury. *Res. Commun. Chem. Pathol. Pharmacol.* **1993**, 81, 199-208.

Hayes, M. A.; Roberts, E.; Roomi, M. W.; Safe, S. H.; Farber, E. and Cameron, R. G. Comparative influences of different PB-type and 3-MC-type polychlorinated biphenyl-induced phenotypes on cytocidal hepatotoxicity of bromobenzene and acetaminophen. *Toxicol. Appl. Pharmacol.* **1984**, 76, 118-127.

Heldestad, A.; Jonsborg, S.; Henriksson, R. and Grankvist, K. Acetaminophen protection against estramustine-induced cytotoxicity on cultured fibroblasts. Pharmacol. Toxicol.1998, 82, 128-131.

Hunskaar, S.; Fasmer, O. B. and Hole, K. Formalin test in mice, a useful technique for evaluating mild analgesics. *J. Neurosci. Methods* **1985**, 14, 69-76.

Lazo, T. S. and Chabner, B. A. Bleomycin. In: *Cancer Chemotherapy and Biotherapy*. B. A. Chabner and D. L. Longo (Eds.), Lippincott – Raven Publishers, Piladelphia **1996**, PP. 379-393.

Leung, W. H.; Lau, J. Y.; Chan, T. K. and Kumana, C. R. Fulminant hyperpyrexia induced by bleomycin. *Postgrad. Med. J.* **1989**, 65, 417-419.

Levy, R. L. and Chiarillo, S. Hyperpyrexia, allergic-type response and death occurring with low-dose bleomycin administration. *Oncology* **1980**, 37, 316-317.

Liu, F.; Xu, Q. Y. and Ye, Y. Q. Effects of valsartan on bleomycin-induced pulmonary fibrosis in rats and the expression of hepatocyte growth factor in lung tissue. *Zhonghua Jie He He Hu Xi Za Zhi* **2005**, 28, 479-483.

Marklund, S. L. Superoxide dismutase isoenzymes in tissues and plasma from New Zealand black mice, nude mice and normal BALB/c mice. *Mutat. Res.* 1985, 148, 129-134.

Ohkawa, H.; Ohishi, N. and Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **1979**, 95, 351-358.

Pardo, A.; Ruiz, V.; Arreola, J. L. Bleomycin-induced pulmonary fibrosis is attenuated in gamma-glutamyl transpeptidase-deficient mice. *Am. J. Respir. Crit. Care Med*. **2003**, 167, 925-932.

Raja Naresh, R. A.; Ndupa, N. and Uma Devi, P. Effect of macrophage activation on niosome encapsulated bleomycin in tumor- bearing mice. *Ind. J. Pharamcol.* **1996**, 28, 175-180.

Schirner, M.; Hoffmann, J.; Menrad, A. and Schneider, M. R. Antiangiogenic chemotherapeutic agents: Characterization in comparison to their tumor growth inhibition in human renal cell carcinoma models. *Clin. Cancer Res*. **1998**, 4, 1331-1336.

Tanino, Y.; Makita, H.; Miyamoto K. Role of macrophage migration inhibitory factor in bleomycin-induced lung injury and fibrosis in mice. *Am. J. Physiol.* **2002**, 283, L156-L162.

Tracy, W. R.; Tse, J. and Carter, G. Lipopolysaccharide-induced changes in plasma nitrite and nitrate concentrations in rats and mice: Pharmacological evaluation of nitric oxide synthase inhibitors. *J. Pharmacol. Exp. Ther*. **1995**, 272, 1011-1015.

Venkatesan, N.; Punithavathi, V. and Chandrakasan, G. Curcumin protects bleomycin-induced lung injury in rats. *Life Sci.* **1997**, 61, 51-58.

Weisenthal, L. M.; Dill P. L.; Kunick, N. B. and Lippman, M. E. Comparison of dye exclusion assays with a cologenic assay in the determination of drug-induced cytotoxicity. *Cancer Res*. **1983**, 43, 258-264.

Woessner, J. F. Jr. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch. Biochem. Biophys*. **1961**, 93, 440-447.

Yokoyama, Y.; Ohmori, I.; Kohda, K. and Kawazoe, Y. Potentiation of antitumor activity of bleomycin towards solid tumors in mice by Bacillus thuringiensis subsp. *Israelensis toxin. Anticancer Res*. **1991**, 11, 1625-1628.