

## Attenuation of bleomycin-induced lung fibrosis in rats by mesna

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### Abstract

Lung fibrosis is a common side effect of the chemotherapeutic agent, bleomycin. Current evidence suggests that reactive oxygen species may play a key role in the development of lung fibrosis. The present study examined the effect of mesna on bleomycin-induced lung fibrosis in rats. Animals were divided into three groups: (1) saline control group; (2) Bleomycin group in which rats were injected with bleomycin (15 mg/kg, i.p.) three times a week for four weeks; (3) Bleomycin and mesna group, in which mesna was given to rats (180 mg/kg/day, i.p.) a week prior to bleomycin and daily during bleomycin injections for 4 weeks until the end of the treatment. Bleomycin treatment resulted in a pronounced fall in the average body weight of animals. Bleomycin-induced pulmonary injury and lung fibrosis was indicated by increased lung hydroxyproline content, and elevated nitric oxide synthase, myeloperoxidase, platelet activating factor, and tumor necrosis factor- $\alpha$  in lung tissues. On the other hand, bleomycin induced a reduction in reduced glutathione concentration and angiotensin converting enzyme activity in lung tissues. Moreover, bleomycin-induced severe histological changes in lung tissues revealed as lymphocytes and neutrophils infiltration, increased collagen deposition and fibrosis. Co-administration of bleomycin and mesna reduced bleomycin-induced weight loss and attenuated lung injury as evaluated by the significant reduction in hydroxyproline content, nitric oxide synthase activity, and concentrations of myeloperoxidase, platelet activating factor, and tumor necrosis factor- $\alpha$  in lung tissues. Furthermore, mesna ameliorated bleomycin-induced reduction in reduced glutathione concentration and angiotensin activity in lung tissues. Finally, histological evidence supported the ability of mesna to attenuate bleomycin-induced lung fibrosis and consolidation. Thus, the findings of the present study provide evidence that mesna may serve as a novel target for potential therapeutic treatment of lung fibrosis.

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**Keywords:** Bleomycin; Lung fibrosis; Mesna

### 1. Introduction

Bleomycin is a commonly used chemotherapeutic agent that can cause dose-dependent pulmonary fibrosis (Jules-Elysee and White, 1990). Interstitial pulmonary fibrosis is characterized by an altered cellular composition of the alveolar region with excessive deposition of collagen. The etiology of this disease is unknown. However, lung inflammation is a major underlying component of a wide variety of pulmonary fibroproliferative disorders. Reactive oxygen species such as superoxide, hydrogen peroxide,

peroxynitrite, and hydroxyl radical are major mediators of lung inflammatory processes (Kinnula et al., 1995). Yet, the direct linkage of reactive oxygen species formation and pulmonary fibrosis has not been firmly established.

Bleomycin-induced pulmonary injury and lung fibrosis has been documented in several animal species (Wang et al., 1991; Tzurel et al., 2002). This model has been widely used for studying the mechanisms involved in the progression of human pulmonary fibrosis and the impact of various drugs on this progression (Yara et al., 2001; El-Khatib, 2002). The mechanisms of bleomycin-induced pulmonary injury and fibrosis are not completely understood. Bleomycin is known to generate reactive oxygen species upon binding to DNA and iron, which cause DNA

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damage. The interaction of bleomycin with DNA appears to initiate the inflammatory and fibroproliferative changes via a concerted action of various cytokines leading to collagen accumulation in the lung. BLM also promotes the depletion of endogenous antioxidant defenses thus exacerbating oxidant mediated tissue injury (Atzori et al., 2004).

The lung is selectively affected by bleomycin because this tissue lacks an enzyme that hydrolyzes the  $\beta$ -amino-alanine moiety of bleomycin, which prevents its metabolite from binding metals such as iron (Filderman et al., 1988). Strategies aimed at reducing oxidative stress have been successful in decreasing bleomycin-induced lung injury and fibrosis such as superoxide dismutase (Galvan et al., 1981), glutathione (Pdi et al., 1999), dimethylurea (Trush et al., 1982), and metalloporphyrin (Oury et al., 2001). The role of reactive oxygen species in many disease states has led to the development of new antioxidants.

Mesna (sodium 2-mercaptoethane sulfonate) is used clinically to prevent the nephrotoxicity and urinary tract toxicity caused by cyclophosphamides such as ifosfamide (Skinner et al., 1993; Mashiach et al., 2001). Mesna is a small molecule and by virtue of its sulfhydryl group, has the potential to scavenge reactive oxygen species (Gressier et al., 1994, 1995). When administered intravenously, mesna is auto-oxidized in blood to its unreactive disulfide form dimesna (Schoenike and Dana, 1990), which is taken up by renal tubular cells and reduced back to mesna by intracellular glutathione. Due to the small molecular size of mesna, its ability to concentrate in different tissues, and its antioxidant potential, it may be useful in minimizing tissue toxicity induced by reactive oxygen species generating insults.

The principal aim of our study was to evaluate the potential role of mesna in oxidative lung injury induced by bleomycin in rats. This was assessed using biochemical and histological methods.

## 2. Materials and methods

### 2.1. Animals

Adult male Wistar rats, weighing 220–250 g, were used in this study. They were obtained from the Animal Care Center, College of Pharmacy, King Saud University. All the animals were fed a standard rat chow and water ad libitum and kept in a temperature-controlled environment (20 to 22 °C) with an alternating cycle of 12-h light and dark. The animals used in this study were handled and treated in accordance with the strict guiding principles of the National Institution of Health for experimental care and use of animals. The experimental design and procedures were approved by the Institutional Ethical Committee for Animal Care and Use at the King Saud University, Riyadh, Kingdom of Saudi Arabia.

### 2.2. Experimental design

Rats were randomized into 3 groups, each consisting of 9 animals. Group 1 was the saline-treated control group. Animals in group 2 were treated with bleomycin (15 mg/kg, i.p.), three times a week for a total period of 4 weeks. The drug was freshly prepared in saline and the concentration was adjusted so that each animal received 0.1 ml/100 g body weight. Rats in group 3 received mesna (180 mg/kg/day, i.p.), one week before and during induction of lung fibrosis by bleomycin until the end of the experiment. The dose was chosen depending on the literature (Mashiach et al., 2001). Mesna is not toxic to rats at the dose tested in this study. The median lethal dose of intravenous mesna in rats lies between 1200 and 2000 mg/kg, which is much higher than the upper limit used in humans (60–70 mg/kg; Brock et al., 1982). The animals were weighed at the beginning, through, and at the end of experiments. The changes in body weight were determined.

### 2.3. Biochemical assays

#### 2.3.1. Preparation of lung tissue for biochemical studies

At the end of treatments, all rats were anaesthetized with ether, sacrificed by cervical dislocation, and the lung lobes were excised, washed in ice-cold saline, and then quickly frozen in liquid nitrogen before being stored at  $-20^{\circ}\text{C}$ . The frozen lung lobes of each animal were thawed and homogenized in isotonic saline. The homogenates were stored at  $-20^{\circ}\text{C}$  until assayed.

#### 2.3.2. Determination of lung hydroxyproline

Lung hydroxyproline content was determined as a biochemical index of parenchymal collagen content. Hydroxyproline was released from lung tissue homogenates by acid hydrolysis. The free hydroxyproline was then oxidized by chloramine to produce a pyrrole type compound. The addition of Ehrlich's reagent resulted in the formation of a chromophore with a wavelength maximum at 550 nm. The absorbances were read using water as reference and corrected for the reagent blank (Jamall et al., 1981).

#### 2.3.3. Determination of reduced glutathione

Lung reduced glutathione was determined as previously described by Owens and Belcher (1965) based on the reaction of 5,5-dithiobis-(2-nitrobenzoic acid) with reduced glutathione present. The absorbance was measured at 412 nm. The amount of reduced glutathione concentration was calculated using a standard solution of glutathione. The results were expressed as  $\mu\text{mol/g}$  wet tissue weight.

#### 2.3.4. Determination of nitric oxide synthase

The activity of total nitric oxide synthase was determined by conversion of [ $^3\text{H}$ ] L-arginine to [ $^3\text{H}$ ] L-citrulline as previously described by McNaughton et al. (1998). Frozen

lung specimens were homogenized in an ice-cold HEPES buffer (100 mg/ml). The homogenate was centrifuged at  $11,300 \times g$  for 10 min and supernatants were collected. Aliquots (50  $\mu$ l) of the supernatants were mixed with calcium-dependent assay mixture for total NOS activity in a final volume of 200  $\mu$ l and controls were prepared with distilled water instead of homogenate supernatants. The incubation was performed at 37 °C for 10 min. The final mixture was incubated for 30 min at 4 °C after addition of 0.5 ml distilled water. The tubes with the final mixture were centrifuged at 1500 rpm and 0.7 ml aliquots of supernatants were counted in a Beckman L-S- 7500 scintillation counter. The activity was calculated as picomoles of citrulline produced/min/mg protein.

#### 2.3.5. Measurement of myeloperoxidase activity

Myeloperoxidase activity had been used as an index of leukocyte adhesion and accumulation in several tissues. Myeloperoxidase was determined according to the method described by Mullane et al. (1985). In brief, lung tissues were homogenized in a solution containing 0.5% (w/v) hexadecyltrimethyl ammonium bromide dissolved in 10 mM sodium phosphate buffer (pH 7.4) in an ice bath using polytron homogenizer (50 mg tissue/ml). The homogenates were centrifuged for 30 min at  $20,000 \times g$  at 4 °C. An aliquot of the supernatant was allowed to react with a solution of tetramethyl benzidine (1.6 mM) and 0.1 mM hydrogen peroxide. The rate of change in absorbance was measured spectrophotometrically at 650 nm. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1  $\mu$ mol of peroxide/min at 37 °C and was expressed in milliunits/g wet tissue.

#### 2.3.6. Determination of platelet activity factor

Platelet activity factor activity in the different samples was measured in an aggregometer by the aggregation of aspirin-treated washed rabbit platelets in the presence of adenosine diphosphate scavengers after preparing the platelets by differential centrifugation. PAF activity is presented as pg/10 mg tissue (Wallace et al., 1989).

#### 2.3.7. Assessment of angiotensin converting enzyme activity

Lung angiotensin converting enzyme activity was determined by a kinetic assay at 37 °C using a test reagent kit for angiotensin converting enzyme (Sigma, MO, USA; Holmquist et al., 1979). The procedure utilizes the ACE calibrator i.e. lyophilized porcine ACE and the synthetic tripeptide substrate *N*-(3-(2-furyl) acryloyl)-L-phenyl alanil glycyl glycine. The latter is hydrolyzed by angiotensin converting enzyme to furyl acryloyl phenylalanine and glycyl glycine resulting in a decrease in absorbance read at 340 nm. The activity of angiotensin converting enzyme in the sample was assessed by comparing the sample reaction rate with that of the angiotensin converting enzyme calibrator and the results are expressed as mM/min/gm wet tissue.

#### 2.3.8. Measurement of tumor necrosis factor- $\alpha$

Tumor necrosis factor- $\alpha$  concentration was measured using an enzyme-linked immunosorbent assay kit (Amersham, RPN 2734). The minimum detectable level of the assay is <10 pg/mg (Espevik and Nissen-Meyer, 1986).

#### 2.4. Histological studies

Lung specimens were fixed in 4% formaldehyde for 24 h, dehydrated in ethyl alcohol, and embedded in paraffin. Sections of 5  $\mu$  were stained with hematoxylin and eosin (H and E) and Masson trichrome for histological evaluation of lung injury and fibrosis by light microscopy.

#### 2.5. Materials

Mesna was provided from Asta Pharma AG (Frankfurt, Germany). Bleomycin hydrochloride was supplied as bleomycin ampoules (15 mg) from Nippon Kayaku (Tokyo, Japan). *N*-nitro-L-arginine methyl ester and chloramine-T were provided by ICN Biomedicals (Aurora, Ohio, USA). L-Arginine, hydroxyproline, ethylene glycol monomethyl ether, thiobarbituric acid, 5,5-dithiobis-(2-nitrobenzoic acid), and P-dimethyl aminobenzaldehyde were purchased from Sigma, St. Louis (MO, USA). The last two chemicals were used to prepare Ellmann's and Ehrlich's reagents, respectively. All other chemicals were of the highest grade commercially available.

#### 2.6. Statistical analysis

Values were expressed as means  $\pm$ S.E.M. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test. The data were analyzed with SYSTAT statistical software (SYSTAT).  $P < 0.05$  was considered significant.

### 3. Results

#### 3.1. Changes in body weight

Treatment of rats with bleomycin (15 mg/kg, i.p.) 3 times a week for a total period of 4 weeks resulted in a marked decrease in their body weight with an average mean of  $234.3 \pm 11.97$  g,  $P < 0.05$  as compared to the saline treated control group ( $264.2 \pm 8.74$  g). Administration of mesna led to a significant increase in body weight with a mean value of  $256.83 \pm 8.39$  g as compared to the bleomycin group  $P < 0.05$  (Fig. 1).

#### 3.2. Hydroxyproline content

Evidence for toxicity in the lung tissue was determined by significant elevation of hydroxyproline content  $P < 0.05$  with mean values of  $17.5 \pm 0.56$ ,  $52.33 \pm 0.99$ , and

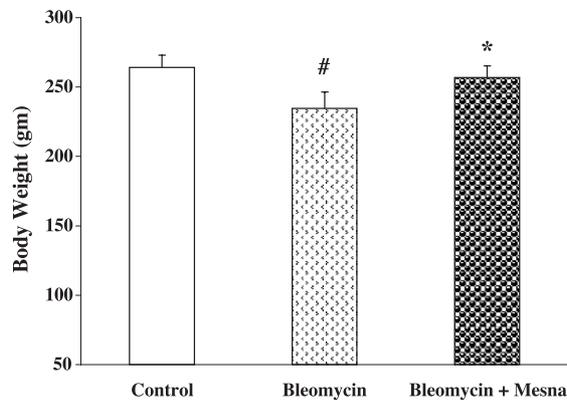


Fig. 1. Effect of bleomycin (15 mg/kg, i.p. 3 times a week) on the body weight of rats before and after treatment with mesna (180 mg/kg/day, i.p.) for 4 weeks. Values are expressed as mean  $\pm$  S.E.M.,  $n=6$ . <sup>#</sup> $P<0.05$  compared with the control group. <sup>\*</sup> $P<0.05$  compared with bleomycin group.

$40 \pm 0.58$  mg/g regarding control saline, bleomycin, and mesna-treated groups, respectively (Fig. 2).

### 3.3. Nitric oxide synthase activity

The nitric oxide synthase activity in lung tissue of the bleomycin group increased  $P<0.05$  with mean values of  $4.23 \pm 0.33$  nmol/g/min as compared to  $1.06 \pm 0.12$  nmol/g/min in the normal control group. Administration of mesna led to a significant decrease in nitric oxide synthase activity  $P<0.05$  with a mean value of  $2.67 \pm 0.15$  nmol/g tissue (Fig. 3).

### 3.4. Myeloperoxidase activity

Treatment of normal rats with bleomycin elicited a profound increase in myeloperoxidase activity in lung tissue  $P<0.05$  as compared to the control group with mean values

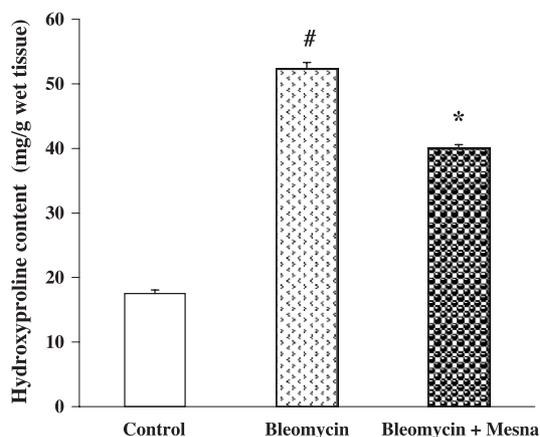


Fig. 2. Effect of mesna (180 mg/kg/day, i.p.) on lung hydroxyproline content in bleomycin-induced lung injury (15 mg/kg, i.p. 3 times a week) for 4 weeks in adult male rats. Values are expressed as mean  $\pm$  S.E.M.,  $n=6$ . <sup>#</sup> $P<0.05$  compared with the control group. <sup>\*</sup> $P<0.05$  compared with the bleomycin group.

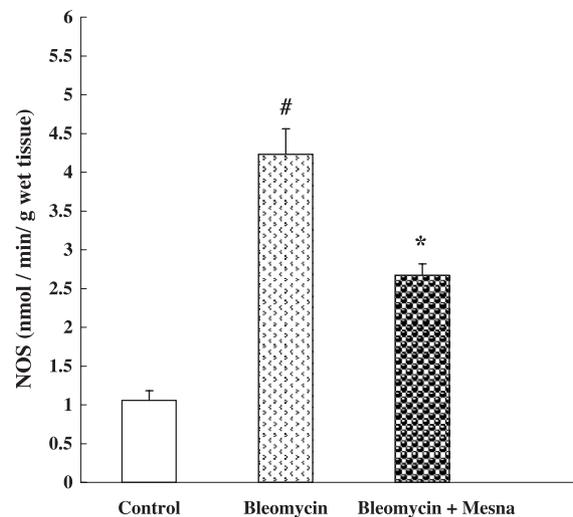


Fig. 3. Effect of mesna (180 mg/kg/day, i.p.) on lung nitric oxide synthase activity in bleomycin-induced lung injury (15 mg/kg, i.p. 3 times a week) for 4 weeks in adult male rats. Values are expressed as mean  $\pm$  S.E.M.,  $n=6$ . <sup>#</sup> $P<0.05$  compared with the control group. <sup>\*</sup> $P<0.05$  compared with the bleomycin group.

of  $0.59 \pm 0.02$  U/mg wet tissue and  $1.05 \pm 0.04$  U/mg wet tissue, respectively. Administration of mesna resulted in a significant decrease in myeloperoxidase in lung tissue  $P<0.05$  with a mean value of  $0.72 \pm 0.07$  U/mg wet tissue (Fig. 4).

### 3.5. Platelet activating factor concentration

The mean value of platelet activating factor content in the bleomycin-treated group was  $27.08 \pm 0.88$  pg/mg as compared to that of the control group  $12.6 \pm 0.48$  pg/mg,  $P<0.05$ , while co-administration of mesna with bleomycin resulted in mean value of  $16.92 \pm 0.51$  pg/mg,  $P<0.05$  as compared to bleomycin group (Fig. 5).

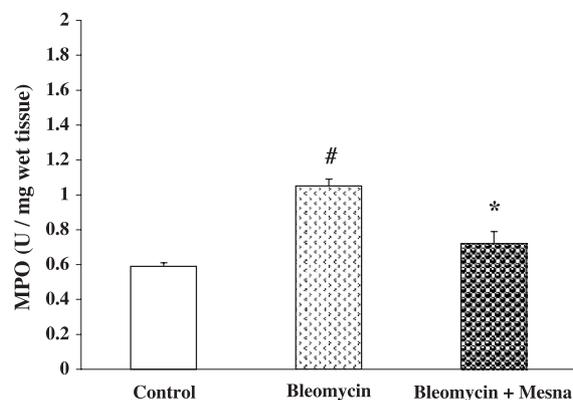


Fig. 4. Effect of mesna (180 mg/kg/day, i.p.) on lung myeloperoxidase activity in bleomycin-induced lung injury (15 mg/kg, i.p. 3 times a week) for 4 weeks in adult male rats. Values are expressed as mean  $\pm$  S.E.M.,  $n=6$ . <sup>#</sup> $P<0.05$  compared with the control group. <sup>\*</sup> $P<0.05$  compared with the bleomycin group.

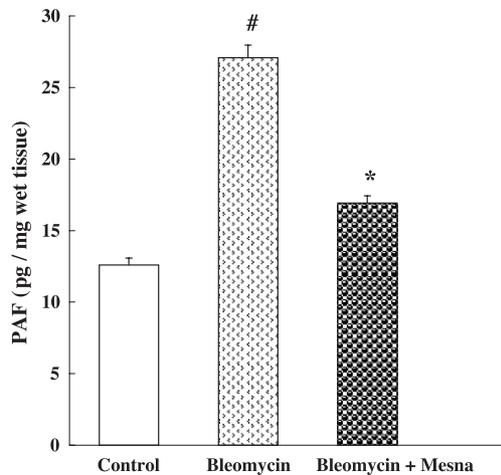


Fig. 5. Effect of mesna (180 mg/kg/day, i.p.) on lung platelet activating factor content in bleomycin-induced lung injury (15 mg/kg, i.p. 3 times a week) for 4 weeks in adult male rats. Values are expressed as mean  $\pm$  S.E.M.,  $n=6$ . <sup>#</sup> $P<0.05$  compared with the control group. <sup>\*</sup> $P<0.05$  compared with the bleomycin group.

### 3.6. Tumor necrosis factor- $\alpha$ concentration

A significant increase in tumor necrosis factor- $\alpha$  was observed in the BLM group,  $P<0.05$  with a mean value of  $104.17 \pm 1.17$  pg/mg wet tissue compared with the saline-treated control group ( $84.33 \pm 2.73$  pg/mg tissue). Addition of mesna to bleomycin resulted in a significant reduction in tumor necrosis factor- $\alpha$ ,  $P<0.05$  with a mean value of  $90.33 \pm 0.76$  pg/mg tissue in comparison with the bleomycin-treated group alone (Fig. 6).

### 3.7. Angiotensin converting enzyme activity

Fig. 7 shows a significant reduction in lung angiotensin converting enzyme activity in the bleomycin-treated group as compared to the normal control group  $P<0.05$  with mean values of  $12.33 \pm 1.05$  and  $19.5 \pm 1.26$  mmol/

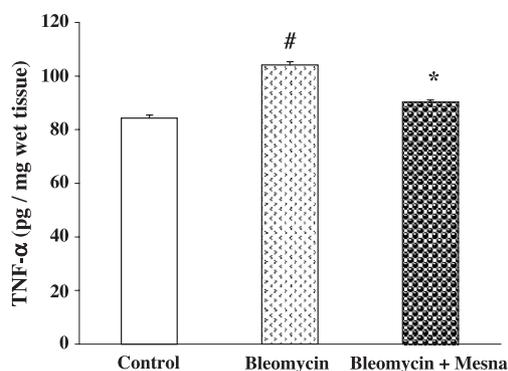


Fig. 6. Effect of mesna (180 mg/kg/day, i.p.) on lung tumor necrosis factor- $\alpha$  activity in bleomycin-induced lung injury (15 mg/kg, i.p. 3 times a week) for 4 weeks in adult male rats. Values are expressed as mean  $\pm$  S.E.M.,  $n=6$ . <sup>#</sup> $P<0.05$  compared with the control group. <sup>\*</sup> $P<0.05$  compared with the bleomycin group.

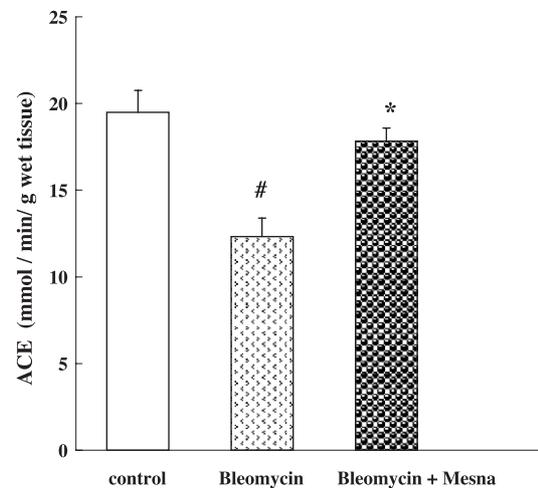


Fig. 7. Effect of mesna (180 mg/kg/day, i.p.) on lung angiotensin converting enzyme activity in bleomycin-induced lung injury (15 mg/kg, i.p. 3 times a week) for 4 weeks in adult male rats. Values are expressed as mean  $\pm$  S.E.M.,  $n=6$ . <sup>#</sup> $P<0.05$  compared with the control group. <sup>\*</sup> $P<0.05$  compared with the bleomycin group.

min/g tissue, respectively. Co-administration of mesna with bleomycin resulted in a significant increase of the angiotensin converting enzyme activity,  $P<0.05$  with a mean value of  $17.83 \pm 0.75$  mmol/min/g tissue.

### 3.8. Reduced glutathione content

Pulmonary reduced glutathione content was significantly decreased by bleomycin treatment as compared to the control group  $P<0.05$  with mean values of  $840 \pm 4.31$  and  $1122 \pm 6.76$   $\mu$ mol/g tissue, respectively. Addition of mesna resulted in a significant increase in reduced glutathione content, the mean value was  $1020.33 \pm 18.42$   $\mu$ mol/g as compared to the bleomycin-treated group  $P<0.05$  (Fig. 8).

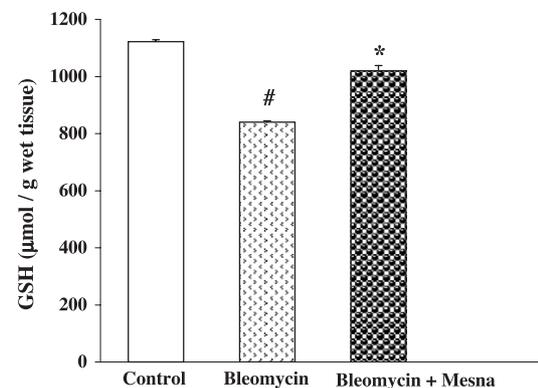


Fig. 8. Effect of mesna (180 mg/kg/day, i.p.) on lung reduced glutathione concentration in bleomycin-induced lung toxicity (15 mg/kg, i.p. 3 times a week) for 4 weeks in adult male rats. Values are expressed as mean  $\pm$  S.E.M.,  $n=6$ . <sup>#</sup> $P<0.05$  compared with control group. <sup>\*</sup> $P<0.05$  compared with bleomycin group.

### 3.9. Histological changes

Lungs of control rats showed normal alveolar spaces and normal thickening of alveolar septa (Fig. 9A). The amount and distribution of collagen in sections stained with trichrome showed normal reticulin distribution around the alveoli (Fig. 10A). The lungs of rats treated with bleomycin showed collapsed alveoli, marked thickening of the interalveolar septa, and dense interstitial infiltration by inflammatory cells mainly lymphocytes, neutrophils, and fibroblasts (Fig. 9B). In addition, there was an excessive amount of collagen deposited around the alveoli as manifested by Masson trichrome stain (Fig. 10B). On the other hand, rats treated with both BLM and mesna showed marked suppression of the bleomycin-induced inflammatory cellular infiltration as evidenced by reduced thickening of the interalveolar septa and more inflation of the alveoli (Fig. 9C). Furthermore, the amount of collagen deposited in the alveolar septa was markedly reduced compared to lungs of bleomycin-treated rats as indicated by Masson trichrome stain (Fig. 10C).

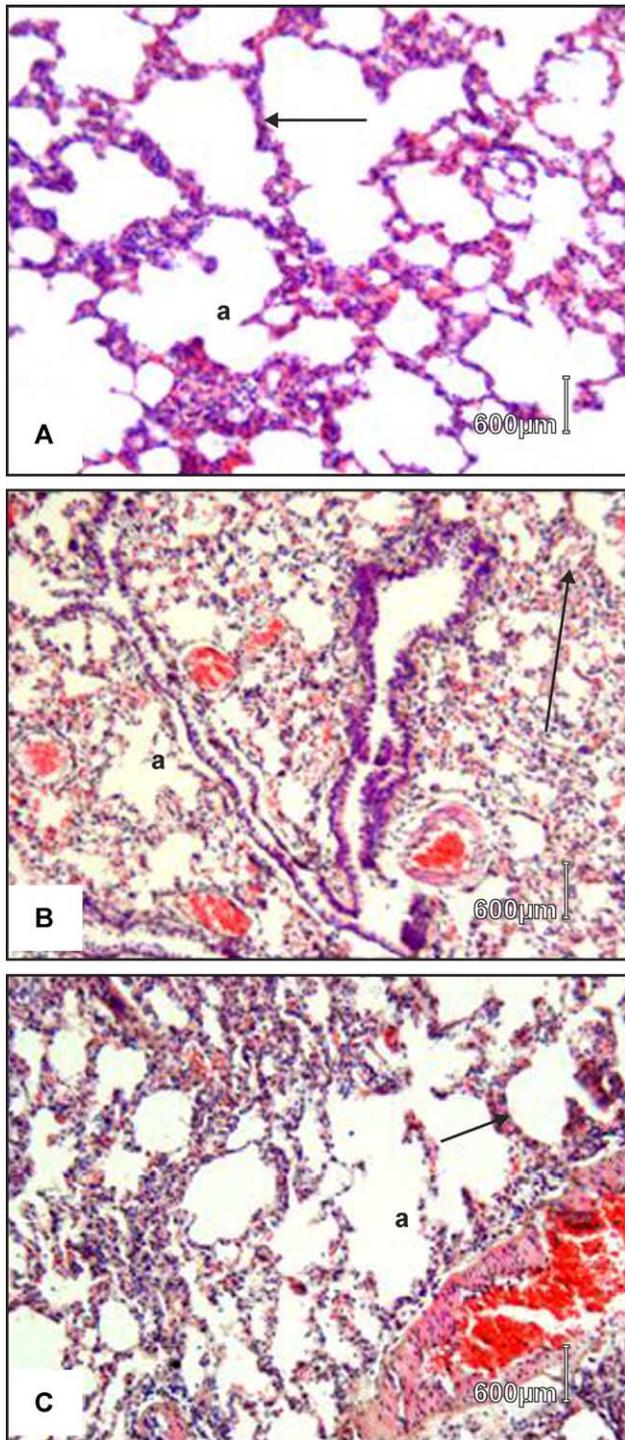


Fig. 9. Photomicrographs of rat lung sections. (A) Normal control rat showing alveolar spaces (a) and alveolar septum (arrow). (B) Bleomycin-treated rat showing collapsed and narrow alveolar spaces (a). The pulmonary interstitium is markedly infiltrated with inflammatory cells that leads to thickening of interalveolar septa (arrow). (C) Mesna plus bleomycin-treated rat lung showing more patent alveolar spaces (a) and reduced interstitial infiltrations (H and E stain  $\times 200$ ).

## 4. Discussion

The present study examined the effect of mesna on bleomycin-induced pulmonary injury and lung fibrosis in rats. Bleomycin-induced lung injury was evident biochemically by increasing in the hydroxyproline content, myeloperoxidase activity, tumor necrosis factor- $\alpha$ , platelet activating factor, and nitric oxide synthase activity in lung tissues. On the other hand, lung angiotensin converting enzyme activity and reduced glutathione content were reduced. Moreover, histological changes revealed that bleomycin-induced lung injury typically consisted of two overlapping stages; an early inflammatory phase characterized by leukocyte infiltration and injury to alveolar epithelial cells, and a subsequent fibroproliferative phase with matrix remodeling and fibrosis. These findings were consistent with previous reports (Oury et al., 2001).

It was clear from the data presented here that treatment of rats with bleomycin caused a significant weight loss. Administration of mesna resulted in a significant reduction in this loss. This result is in agreement with Brewer et al. (2003) who studied the protective effects of tetrathiomolybdate against bleomycin-induced lung fibrosis in mice.

Mesna minimized bleomycin-induced lung fibrosis as revealed by the significant reduction in lung collagen content measured as hydroxyproline, a biochemical index of parenchymal collagen accumulation. Other studies have shown that bleomycin-induced increased lung hydroxyproline content which was reduced by the use of different antioxidants including aminoguanidine (Xiao-Ling et al., 2001), curcumin (Punithavathi et al., 2000), melatonin (Arslan et al., 2002), and tetrathiomolybdate (Brewer et al., 2003).

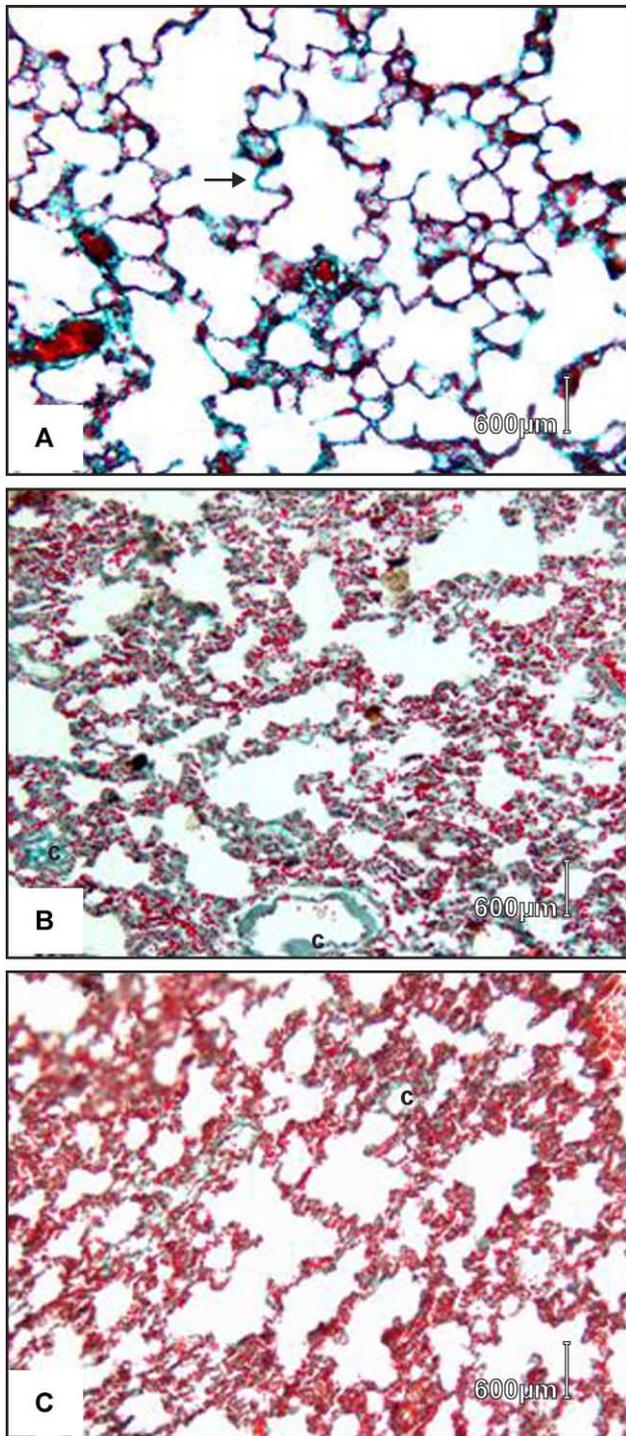


Fig. 10. Photomicrographs of rat lung sections. (A) Normal control rat showing the distribution of pulmonary interstitial reticulin indicated by thin green color (arrow). (B) Bleomycin-treated rat showing the excessive collagen deposition in the interstitial lung tissue. (C) Mesna plus bleomycin-treated rat lung showing marked reduction of interstitial deposition of collagen (Masson trichrome  $\times 200$ ).

The identification of tumor necrosis factor- $\alpha$  as a key player in the pathogenesis of inflammation is supported by the effects of various therapeutic strategies aimed at blocking tumor necrosis factor- $\alpha$  activity. A variety of drugs used to treat human inflammatory diseases had effects on tumor

necrosis factor- $\alpha$  (Tak and Firestein, 2001). It has been shown that during the process of bleomycin-induced pulmonary fibrosis sudden inflammatory responses are produced leading to an increase in the number of macrophages, which induce inflammatory cells in the injured tissue to produce cytokines (Phan and Kunkel, 1992; Ortiz et al., 1998; Kuroki et al., 2003). These cytokines have been linked to airway fibrosis because of their ability to regulate fibroblast and matrix production (Elias et al., 1999). In the present study, co-administration of mesna with bleomycin reduced bleomycin-induced elevation in lung tumor necrosis factor- $\alpha$ . These results are in accordance with that of Underwood et al. (2000), who showed that inhibition of inflammatory cytokines attenuated bleomycin-induced pulmonary fibrosis.

In the present study, bleomycin-induced lung fibrosis that was accompanied by a significant elevation in platelet activating factor level and myeloperoxidase activity in lung tissues. Giri et al. (1995) have shown that inhibition of platelet activating factor can ameliorate bleomycin-induced lung injury. The increase in myeloperoxidase activity obtained in our study may be explained by the increase in the oxidative stress elicited by bleomycin. The link between oxidative stress and inflammation has been shown in other studies (Venkatesan, 2000; Kruidenier and Verspaget, 2002).

In the current investigation, an increase in nitric oxide synthase activity in rats treated with bleomycin was observed. The increased nitric oxide synthase activity can lead to overproduction of nitric oxide, which is involved in diverse physiological and pathological processes (Moncada et al., 1991). Nitric oxide has been localized and identified as two different forms, i.e. a constitutive form present in endothelial cells and brain (Sessa et al., 1993) and an inducible form found in macrophages (Sherman et al., 1993).

Although low concentrations of nitric oxide are sufficient in most cases to affect the physiological processes including those of pulmonary functions (Kobsik et al., 1993), it is produced in high concentrations during infection and inflammation in vivo. Nitric oxide may produce deleterious effects either directly or indirectly (Wink et al., 1991). It may react with superoxide and form peroxynitrite, which is demonstrated to be present in the inflammatory lesions of acute lung injury (Kooy et al., 1995; Gurujeyalakshmi et al., 2000). In addition, nitric oxide production may enhance transforming growth factor- $\alpha$  activity, which is proven a strong stimulator of collagen, fibronectin, and proteoglycan synthesis (Vodovotz et al., 1999).

Several investigators have demonstrated that macrophages when activated in vitro by cytokines as tumor necrosis factor- $\alpha$ , secrete reactive nitrogen intermediate through an L-arginine-dependent pathway (Anderson et al., 1995; Stuehr and Nathan, 1989). Moreover, increased production of nitric oxide has been shown in acute lung injury and adult respiratory distress syndrome (Kooy et al., 1995).

The use of mesna in the present study caused a significant reduction in bleomycin-induced increase in nitric oxide synthase activity. Salch et al. (1997) and Giri et al. (2002)

showed similar observations using different antioxidants as lung protectives against bleomycin-induced pulmonary toxicity.

Angiotensin converting enzyme is a further evidence of bleomycin-induced pulmonary toxicity. It is considered as an index of endothelial cell metabolism (Suntres and Shek, 1997) and has been regarded as a sensitive monitor of tissue response to bleomycin (Newman et al., 1980). The reduction in angiotensin converting enzyme activity observed in the present study after treatment of rats with bleomycin corroborates the previously reported findings of Suntres and Shek (1997). Treatment with mesna in the present study showed an increase in angiotensin converting enzyme activity, which has been taken as a marker of endothelial cell integrity. Another study by El-Khatib (2002) who used arginine as a protective agent against bleomycin-induced lung toxicity in rats supports the previous observation.

Imbalance between oxidant and antioxidant defense mechanisms may contribute to incidence of pulmonary fibrosis (MacNee and Rahman, 1995). Glutathione is an intracellular thiol present in all tissues, including lungs. Besides maintaining cellular integrity by creating a reducing environment, reduced glutathione has multiple functions including detoxification of xenobiotics and synthesis of proteins, nucleic acids, and leukotrienes (Wang and Ballatori, 1998). In addition, it provides a protection to the lung from oxidative damage induced by endogenous or exogenous lung toxicants (Kinnula et al., 1995). However, its depletion in the lung by a fibrogenic agent, bleomycin as shown in the present study was associated with the risk of lung damage. This observation is in agreement with the results of Rahman et al. (1999) and Arslan et al. (2002). Co-administration of mesna with bleomycin reversed reduced glutathione depletion and subsequent lung damage. The ability of mesna to prevent depletion of lung glutathione stores suggests that its antifibrotic activity in the bleomycin model is mediated at least in part by its antioxidant properties. Mesna may save intracellular reduced glutathione by acting as either reactive oxygen species scavenger or by enhancing reduced glutathione synthesis (Multhoff et al., 1996; Lord-Fontaine and Averill, 1999).

Consistent with our findings, mesna has been shown to prevent injury ensued by oxidative situations as ischemic/reperfusion injury to kidney (Mashiach et al., 2001), ifosfamide urotoxicity in vivo (Siu and Moore, 1998) and in vitro (Zaki et al., 2003), and ferric nitrilotriacetate nephrotoxicity (Umemura et al., 1996). Moreover, mesna has been used as a useful adjuvant to anticancer agents (Dechant et al., 1991; Skinner et al., 1993; Meier et al., 1994).

The histological results of the present study clearly demonstrate that mesna exert a significant attenuation of the extent and severity of the histological signs of tissue damage. These results are in agreement with Daba et al. (2002) and Zhao et al. (2002) who used different antioxidant compounds as protective agents against bleomycin-induced lung fibrosis.

In conclusion, the data reported here reveal that mesna can attenuate the bleomycin-induced lung fibrosis and the mechanisms underlying this protective action may be attributed to either the increase in activities of enzymes that improve the total antioxidative defense capacity of the organism and/or the decline in neutrophil trafficking and activation in lung via reduction of pro-inflammatory cytokines. These findings suggest that mesna may be a promising candidate to prevent bleomycin-induced lung damage or other interstitial pulmonary fibrosis.

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