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AMELIORATION OF BLEOMYCIN-INDUCED PULMONARY FIBROSIS IN HAMSTERS BY COMBINED TREATMENT WITH TAURINE AND NIACIN*

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Abstract—Interstitial pulmonary fibrosis induced by bleomycin (BL) involves the production of reactive oxygen species and the impairment of repair of damaged epithelial cells. We have shown previously that taurine or niacin treatment partially attenuates BL-induced lung fibrosis in hamsters and that the two agents probably act through different mechanisms. In the present investigation, we have demonstrated that taurine and niacin in combination provide nearly complete protection against BL-induced pulmonary fibrosis. Based on the findings of this investigation, it is suggested that combined treatment with taurine and niacin offers the potential for a novel pharmacological approach in the prevention of lung fibrosis in humans.

Interstitial pulmonary fibrosis is a crippling disease that leads to a reduction of lung compliance and impairment of the vital gas exchange function due to an excessive accumulation of collagen resulting from inflammatory and fibroproliferative changes of the lung [1]. This disease may result from a wide range of processes including the use of bleomycin (BL) in chemotherapy. The BL-rodent model has been one of the most widely used animal models for the study of the pathogenesis of lung fibrosis [2]. Intratracheal (i.t.) instillation of BL in hamsters has been shown to cause lung fibrosis similar to that seen in humans and therefore this model has been widely used for studying mechanisms of fibrogenesis and for screening potentially desirable antifibrotic compounds [3–5].

BL has been used clinically against a variety of tumors such as squamous cell carcinomas, lymphomas and testicular cancers because of its effective antineoplastic activity and minimal hematopoietic toxicity or immunosuppressive activity [6]. Unfortunately, BL therapy exhibits a dose-dependent interstitial pneumonitis that often progresses into interstitial pulmonary fibrosis [6], and this greatly limits its use on a long-term basis. Thus, the search for effective antifibrotic agents is of great importance not only in elucidating the mechanisms of BL-induced lung fibrosis, but also in preventing pulmonary fibrosis induced by therapeutic agents.

The mechanisms of BL-induced pulmonary injury and the subsequent development of fibrosis are not understood completely. One hypothesis supported directly by *in vitro* and indirectly by *in vivo* studies is that upon binding to DNA and iron, BL generates reactive oxygen species (ROS) [5, 7] which cause DNA damage [8]. The interaction with DNA appears to initiate fibroproliferative changes leading to accumulation of collagen in the lung. Our laboratory has shown that BL-induced lung fibrosis is accompanied by an increase of total calcium and a decrease of NAD content in the lung in the first few days [9, 10]. It has also been hypothesized that pulmonary epithelial injury, in the presence of inefficient DNA repair, could result in the activation and proliferation of interstitial cells responsible for collagen production [11]. We have reported that administration of taurine partially reduces lung fibrosis in the BL-hamster model [12]. Taurine (2-aminoethanesulfonic acid) is a naturally occurring sulfur-containing amino acid present in high concentrations in many tissues including lung [12, 13]. This amino acid appears to have antioxidant and membrane-stabilizing properties [13, 14]. We have also demonstrated that niacin minimizes the pulmonary fibrosis in the same model [15]. Niacin (nicotinic acid), a B vitamin, is an established precursor of the nicotinamide nucleotide coenzymes, NAD and NADP [16] and is known to prevent cytotoxicity and DNA damage by maintaining NAD content of the cell [17]. Since taurine and niacin appear to produce their antifibrotic effect by different mechanisms, it was hypothesized that combined treatment with taurine and niacin might offer a complete amelioration of lung fibrosis in the BL-hamster model. The present studies were designed to test this hypothesis.

MATERIALS AND METHODS

Pathogen-free male Golden Syrian hamsters were purchased from Simonsen, Inc. (Gilroy, CA).

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Bleomycin sulfate (Blenoxane®) was a gift from Bristol Laboratories (Syracuse, NY). Taurine and niacin were obtained from the Sigma Chemical Co. (St. Louis, MO). L-4-[^{3}H]Hydroxyproline (sp. act. 8 Ci/mmol) and [^{14}C]NAD (labeled in the adenine moiety, purity 99.6%, sp. act. 518.0 mCi/mmol) were obtained from NEN Research Products (Boston, MA). All other reagents were of analytical grade and obtained from standard commercial sources.

Treatment of animals. This investigation consisted of two independent studies. Study One initially aimed at testing whether the combined use of taurine and niacin administered by the two routes employed in our previous studies, i.e. taurine in drinking water and niacin by intraperitoneal (i.p.) injection, could offer complete protection against BL-induced lung fibrosis in the hamster. After the completion of this study, Study Two was designed to test whether the protective effect of combined treatment with taurine and niacin against BL-induced lung fibrosis could be confirmed by administering these two compounds in the diet, since drug delivery for therapeutic purposes by the oral route is considered to be more practical and acceptable than any other route.

Hamsters were housed in groups of four in facilities with filtered air and constant temperature and humidity. All care was in accordance with the guidelines of NIH for animal welfare. The hamsters were allowed to acclimate in the facilities for 1 week prior to all treatments. A 12 hr/12 hr light/dark cycle was maintained and hamsters had access to water and Rodent Laboratory Chow 5001 (Purina Mills Inc., St. Louis, MO) *ad lib.* In each study, animals were divided randomly into four experimental groups: saline (SA); taurine + niacin + saline (TNSA); bleomycin (BL); and taurine + niacin + bleomycin (TNBL). In Study One, taurine (1%, w/v) was given in drinking water and niacin (250 mg/5 mL/kg) was administered i.p. for 2 days prior to the first i.t. instillation of saline or BL and thereafter daily throughout the study for TNSA and TNBL groups. Animals in SA and BL groups were treated in the same manner except that taurine was not added in the drinking water and saline instead of niacin was injected (i.p.). In Study Two, pulverized chow thoroughly mixed with taurine (2.5%, w/w) and niacin (2.5%, w/w) was given for 2 days before the first i.t. dose of saline or BL and thereafter throughout the study for TNSA and TNBL groups. Hamsters in SA and BL groups received only the pulverized chow. The regimen of BL administration in both studies was the same as described by Zia *et al.* [18]. Briefly, hamsters were placed under pentobarbital anesthesia (75–85 mg/kg) and BL (2.5, 2.0 and 1.5 units/5 mL/kg) or an equivalent volume of SA was instilled i.t. in three consecutive doses at weekly intervals. Twenty days after the last i.t. instillation, the hamsters were killed under pentobarbital anesthesia (90–120 mg/kg, i.p.).

Preparation of plasma and lung tissue for various assays. After anesthesia, the abdominal cavity was opened and blood was collected in a heparinized syringe from the caudal vena cava. The plasma was separated by centrifugation at 1500 *g* for 20 min at 4° and frozen at −80° until assayed for taurine concentration. After the thoracic cavity was opened, the lungs were perfused *in situ* via the right ventricle of the heart with ice-cold isotonic saline. The lung lobes were quickly dissected free of non-parenchymal tissue, immediately frozen in liquid nitrogen, and stored at −80°. Later, the frozen lungs were thawed and homogenized in 0.1 M KCl, 0.02 M Tris (pH 7.6) with a Polytron homogenizer (Brinkmann Instruments Inc., Westbury, NY). The homogenate was mixed thoroughly by repeated inversions and the final homogenate volumes (9–10 mL) were recorded. The samples were aliquoted and stored at −80° except for the samples for NAD, malondialdehyde equivalent and collagen assays, which were processed for their measurement on the same day the lungs were homogenated. Enzyme assays were performed on supernatant obtained by centrifugation of the lung homogenate at 12,000 *g* for 20 min at 4° unless specified otherwise.

Determination of malondialdehyde equivalent, NAD and superoxide dismutase (EC 1.15.1.1, *SOD) activity.* Lung malondialdehyde equivalent was estimated from the total amount of thiobarbituric acid-reacting products in unfractionated homogenate by the method of Ohkawa *et al.* [19]. The NAD content of the lung was estimated by the enzymatic assay of Bernofsky and Swan [20]. SOD activity of the supernatant of lung homogenate was determined from the rate at which it inhibits the auto-oxidation of epinephrine to adrenochrome, as described by Misra and Fridovich [21]. The rate of formation of adrenochrome was 0.025 absorbance units/min at 480 nm in a Varian Cary 219 spectrophotometer (Beckman Instruments, Palo Alto, CA). Under these defined conditions, the amount of tissue required to inhibit the rate of formation of adrenochrome by 50% (i.e. rate of 0.0125 absorbance units/min) was defined to contain 1 unit of SOD activity.

Determination of lung calcium and poly(ADP-ribose) polymerase (EC 2.4.2.30*) activity.* For calcium determination, 1 mL of lung homogenate was first deproteinized in a final concentration of 10% (w/v) trichloroacetic acid (TCA) on ice. After centrifugation, the TCA supernatant was decanted and its volume recorded. The calcium content in the TCA supernatant was determined by inductively coupled plasma (ICP) atomic emission spectrometry [22] using a model 3510 ICP spectrometer (Applied Research Laboratories, Sunland, CA). Lung poly-(ADP-ribose) polymerase activity was determined by measuring the incorporation of [^{14}C]ADP-ribose for 15 min at 37° into an acid-insoluble product as reported previously [10]. The activity of the enzyme is expressed as picomoles of [^{14}C]ADP-ribose incorporated per total lung per 15 minutes.

Determination of hydroxyproline and prolyl hydroxylase (EC 1.14.11.2*) activity.* For lung hydroxyproline assay, 1 mL of homogenate was precipitated with 0.25 mL of ice-cold 50% (w/v) TCA and centrifuged and the precipitate was hydrolyzed in 2 mL of 6 N HCl for 18 hr at 110°. [^{3}H]Hydroxyproline (1×10^5 dpm) was added to each sample to determine recovery and the hydroxyproline content was measured by the

technique described by Woessner [23]. The preparation of prolyl hydroxylase substrate (procollagen) and the method for the prolyl hydroxylase assay were essentially the same as described in our previous paper [5]. During the reaction, tritium is released in stoichiometric proportion to prolyl hydroxylation and ^3H_2O is used as a measure of the enzyme activity. The enzyme activity was determined as disintegrations per minute of ^3H_2O released per total lung per 30 minutes and is reported as percent of the control.

Measurement of taurine in plasma and lung tissue. The samples were processed and analyzed for taurine concentration by means of an amino acid analyzer (model 121 M amino acid analyzer, Beckman Instruments) as previously described [12].

Statistical analysis of data. All data are expressed on the basis of per total lung except for plasma taurine. Expression of the data on a per lung basis avoids the artifactual lowering of the values in treated animals due to presence of proteins of extrapulmonary origin [24]. All values are reported as the mean ± 1 SEM. Comparison was made only within either Study One or Study Two and the data were analyzed by a one-way analysis of variance and Duncan's Multiple Range test [25]. A value of $P \leq 0.05$ was considered significant.

RESULTS

Intratracheal instillation of a total dose of 6 units BL/kg administered in three divided doses over a period of 3 consecutive weeks caused 0 and 17% mortality in Studies One and Two, respectively. However, the same total dose of BL caused no mortality in hamsters receiving taurine and niacin in either study.

Malondialdehyde equivalent and SOD activity. Lung malondialdehyde equivalent was elevated significantly by three doses of BL instillation (Fig. 1). The SOD activity in the lungs of these animals was found to be stimulated by BL treatment (Fig. 2). Treatment with taurine + niacin significantly diminished the BL-induced increases in the lung malondialdehyde equivalent, although its level in the TNBL group was still significantly higher than those of the SA and TNSA groups in either study (Fig. 1). As shown in Fig. 2, taurine + niacin treatment prevented the BL-induced increases in lung SOD activity.

Lung calcium content and poly(ADP-ribose) polymerase activity. Intratracheal instillation of BL significantly increased lung calcium content (Fig. 3). BL-induced increases in lung calcium were decreased by the treatment with taurine and niacin. Lung calcium content in TNBL animals was not significantly different from that of the SA or TNSA groups of both studies. BL administration significantly elevated poly(ADP-ribose) polymerase activity in hamster lungs compared to the control groups (SA and TNSA) (Fig. 4). Taurine and niacin treatment (TNBL) significantly attenuated the increase in poly(ADP-ribose) polymerase activity in the BL-treated animals. There was no significant difference in the activity of this enzyme among the hamsters in the SA, TNSA and TNBL groups.

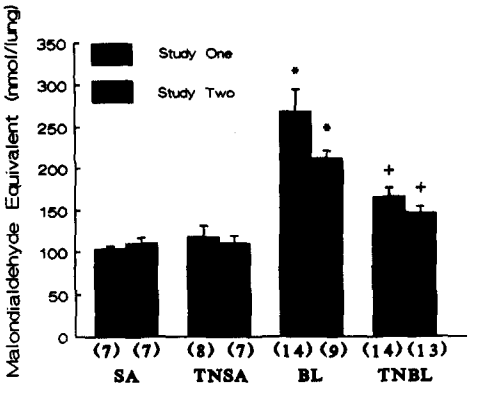

Fig. 1. Effects of combined treatment with taurine and niacin on bleomycin-induced increases in the malondialdehyde equivalent level of hamster lungs in Study One and Study Two. In Study One, hamsters were treated with taurine in drinking water (1%) and niacin 250 mg/kg, i.p. daily. In Study Two, hamsters were fed taurine (2.5%) and niacin (2.5%) in the diet. Two days after taurine and niacin treatment, hamsters were given bleomycin i.t. (2.5, 2.0 and 1.5 units/5 mL/kg) in three consecutive doses at weekly intervals. Twenty days after the last i.t. instillation, hamsters were killed and the lungs processed for the malondialdehyde equivalent assay as described in Materials and Methods. The number of animals in each group is shown in parentheses below each bar and treatment groups are indicated along the x-axis and explained in Materials and Methods. Briefly, SA: saline control; TNSA: taurine + niacin + saline; BL: bleomycin alone; and TNBL: taurine + niacin + bleomycin. Values are means ± SEM. Key: (*) significantly higher ($P < 0.05$) than all other corresponding groups, and (+) significantly lower ($P < 0.05$) than the corresponding BL group, but higher ($P < 0.05$) than the corresponding SA and TNSA groups.

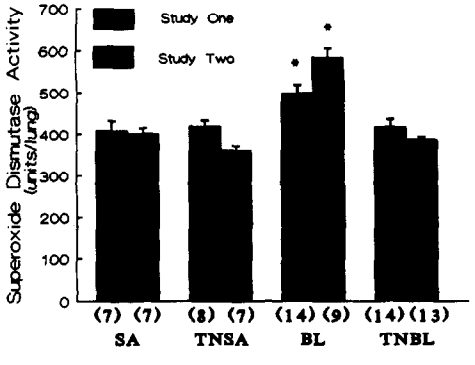

Fig. 2. Effects of combined treatment with taurine and niacin on bleomycin-induced increases in superoxide dismutase activity of hamster lungs in Study One and Study Two. See the legend to Fig. 1 for the explanation of abbreviations and experimental details. The lungs were processed for the superoxide dismutase assay as described in Materials and Methods. The number of animals in each group is shown in parentheses below each bar and treatment groups are indicated along the x-axis and explained in Materials and Methods. Values are means ± SEM. Key: (*) significantly higher ($P < 0.05$) than all other corresponding groups.

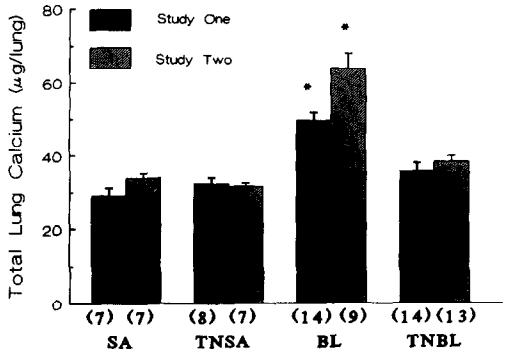

Fig. 3. Effects of combined treatment with taurine and niacin on bleomycin-induced increases in the calcium level of hamster lungs in Study One and Study Two. See the legend to Fig. 1 for the explanation of abbreviations and experimental details. The lungs were processed for the calcium assay as described in Materials and Methods. The number of animals in each group is shown in parentheses below each bar and treatment groups are indicated along the x-axis and explained in Materials and Methods. Values are means ± SEM. Key: (*) significantly higher ($P < 0.05$) than all other corresponding groups.

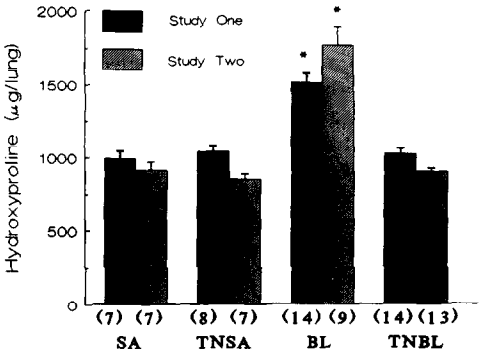

Fig. 5. Effects of combined treatment with taurine and niacin on bleomycin-induced increases in the hydroxyproline content of hamster lungs in Study One and Study Two. See the legend to Fig. 1 for the explanation of abbreviations and experimental details. The lungs were processed for the hydroxyproline assay as described in Materials and Methods. The number of animals in each group is shown in parentheses below each bar and treatment groups are indicated along the x-axis and explained in Materials and Methods. Values are means ± SEM. Key: (*) significantly higher ($P < 0.05$) than all other corresponding groups.

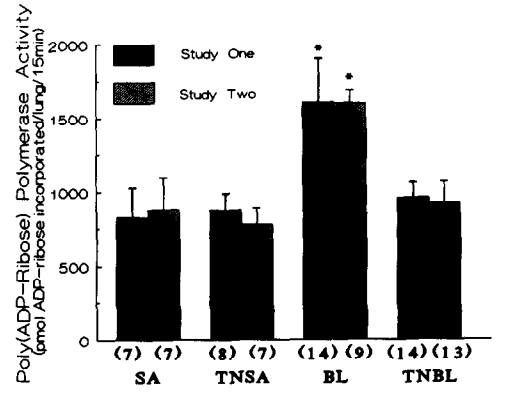

Fig. 4. Effects of combined treatment with taurine and niacin on bleomycin-induced increases in poly(ADP-ribose) polymerase activity of hamster lungs in Study One and Study Two. See the legend to Fig. 1 for the explanation of abbreviations and experimental details. The lungs were processed for the poly(ADP-ribose) polymerase assay as described in Materials and Methods. The number of animals in each group is shown in parentheses below each bar and treatment groups are indicated along the x-axis and explained in Materials and Methods. Values are means ± SEM. Key: (*) significantly higher ($P < 0.05$) than all other corresponding groups.

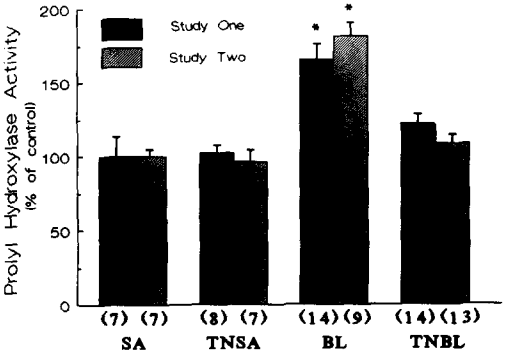

Fig. 6. Effects of combined treatment with taurine and niacin on bleomycin-induced increases in the prolyl hydroxylase activity of hamster lungs in Study One and Study Two. See the legend to Fig. 1 for the explanation of abbreviations and experimental details. The lungs were processed for the prolyl hydroxylase assay as described in Materials and Methods. The saline control activities of this enzyme in Study One and Study Two averaged 162,000 ± 21,600 and 112,000 ± 5250 dpm/30 min/lung, respectively. The number of animals in each group is shown in parentheses below each bar and treatment groups are indicated along the x-axis and explained in Materials and Methods. Values are means ± SEM. Key: (*) significantly higher ($P < 0.05$) than all other corresponding groups.

Lung hydroxyproline content and prolyl hydroxylase activity. Lung hydroxyproline content in various groups of hamsters is shown in Fig. 5. BL significantly elevated the lung hydroxyproline level to 152 and 194% of the saline controls in Study One and Study Two, respectively. Combined treatment with taurine and niacin blocked the BL-induced increases in lung hydroxyproline content in both studies. BL alone increased the lung prolyl hydroxylase activity to 166 and 181% of the saline controls in Study One and Study Two, respectively (Fig. 6). Likewise, combined treatment with taurine and niacin inhibited the BL-induced increases in lung prolyl hydroxylase activity to the levels that were near controls in both studies.

Plasma and lung taurine concentration, and lung NAD content. Plasma taurine concentrations in the TNSA and TNBL groups of hamsters receiving taurine and niacin were increased significantly as

Table 1. Effects of intratracheal instillation of bleomycin with or without combined treatment with taurine and niacin on taurine content in plasma and lung tissue of hamsters in Study One and Study Two

Treatment	Plasma taurine (nmol/mL)		Lung taurine (nmol/lung)	
	Study One	Study Two	Study One	Study Two
SA	115 ± 4 (7)	120 ± 8 (7)	7059 ± 412 (7)	5727 ± 542 (7)
TNSA	237 ± 20* (8)	345 ± 47* (7)	8569 ± 376 (8)	5939 ± 667 (7)
BL	110 ± 6 (14)	159 ± 16 (7)	7797 ± 328 (14)	7170 ± 578 (9)
TNBL	225 ± 17* (14)	374 ± 69* (7)	7296 ± 279 (14)	5851 ± 513 (13)

In Study One, hamsters were treated with taurine in drinking water (1%) and niacin 250 mg/kg, i.p., daily. In Study Two, hamsters were fed taurine (2.5%) and niacin (2.5%) in the diet. Two days after taurine and niacin treatment, hamsters were given bleomycin i.t. (2.5, 2.0 and 1.5 units/5 mL/kg) in three consecutive doses at weekly intervals. Twenty days after the last i.t. instillations, hamsters were killed and the plasma and lung tissue processed for taurine assay as described in Materials and Methods. The number of animals in each group is shown in parentheses. Abbreviations: SA = saline control; TNSA = taurine + niacin + saline; BL = bleomycin alone; TNBL = taurine + niacin + bleomycin. Values are means ± SEM.

* Significantly higher ($P < 0.05$) than corresponding SA and BL groups.

Table 2. Effects of intratracheal instillation of bleomycin with or without combined treatment with taurine and niacin on lung NAD levels in hamsters in Study Two

Treatment	NAD (pmol/lung)
SA	2183 ± 101 (7)
TNSA	2963 ± 114* (7)
BL	2100 ± 32 (9)
TNBL	2902 ± 119* (13)

Hamsters were fed taurine (2.5%) and niacin (2.5%) in the diet. Two days after taurine and niacin treatment, hamsters were given bleomycin i.t. (2.5, 2.0 and 1.5 units/5 mL/kg) in three consecutive doses at weekly intervals. Twenty days after the last i.t. instillation, hamsters were killed and the lungs processed for NAD assay as described in Materials and Methods. The number of animals in each group is shown in parentheses. See Table 1 for explanation of abbreviations. Values are means ± SEM.

* Significantly higher ($P < 0.05$) than SA and BL groups.

compared with their respective SA and BL groups in both studies (Table 1). However, there was no significant difference in the lung levels of taurine among all four groups in either study (Table 1). Lung NAD levels in all four groups of the hamsters were determined only for Study Two and the data are summarized in Table 2. The hamsters in the TNSA and TNBL groups ingesting taurine and niacin in their diet had significantly higher levels of NAD in the lungs than their counterpart controls.

DISCUSSION

In the present report, we have described the results of two independent studies which demonstrated that combined treatment with taurine and niacin abolished the BL-induced lung collagen accumulation. The mechanisms by which the combined treatment with taurine and niacin offered nearly complete protection against BL-induced lung fibrosis are not understood clearly. However, it is possible that the inhibitory effect of taurine and niacin on lung prolyl hydroxylase activity may be related to the reduction of BL-induced lung collagen accumulation, since a decreased activity of this enzyme paralleled a marked reduction in the accumulation of lung collagen content. Increases in the prolyl hydroxylase activity in various animal models of lung fibrosis, including BL-rodent, usually precedes the accumulation of collagen in the lung [26]. The enzyme-catalyzed hydroxylation of proline is an important posttranslational event in the processing of highly cross-linked mature collagen fibers [27]. The inhibition of BL-induced increases in prolyl hydroxylase activity would allow the deposition of a more pliable and soluble form of collagen which is more susceptible to degradation by intracellular collagenases than cross-linked, mature collagen fibers.

Although the underlying mechanisms for the protective effect of taurine are not understood clearly, its antioxidant and membrane-stabilizing properties seem to be somehow involved [13]. BL-induced pulmonary toxicity is usually attributed to the generation of ROS [28] and their involvement in the pulmonary toxicity is reflected by the increase in lung SOD activity, presumably as a defense mechanism to protect tissues against the deleterious effects of superoxide radicals [5]. Interestingly, taurine and niacin administered together significantly inhibited the BL-induced increase of SOD activity. This suggests that taurine and/or niacin may prevent the initial BL-induced production of ROS or block their injurious effects by virtue of the antioxidant property of taurine. However, it is unlikely that the antioxidant effect of taurine is the only mechanism since the use of the antioxidant compound dimethyl sulfoxide has yielded conflicting results in the BL-rodent model of lung fibrosis [29, 30]. In addition, there is no direct evidence that taurine scavenges free radicals. In fact, the results of an *in vitro* study indicated that taurine itself was a poor free radical scavenger [31].

It is possible that the beneficial effect of taurine against the BL-induced lung fibrosis may reside in its ability to stabilize the cell membrane and thereby prevent an excess influx of calcium known to be involved in this model of fibrosis as reported in our previous paper [9]. The ability of taurine to prevent an excess intracellular accumulation of calcium in the BL-hamster model of lung fibrosis is also supported by the biochemical [14, 32] and morphological [33] findings of other investigators. It has been suggested that taurine interacts uniquely with neutral phospholipids of biological membrane and thereby stabilizes the cell membrane by altering the membrane cation binding characteristics [14]. This hypothesis was advanced by ruling out the direct chemical interaction of calcium with taurine [34]. An excess level of intracellular calcium is known to impair the ability of mitochondria to synthesize ATP. In the absence of an adequate level of ATP, calcium accumulates at the inner surface of the cell membrane and activates the membrane bound phospholipases and proteases which in turn damage the cell membrane, leading to cell death [32]. It is also known that calcium is a stimulator of phospholipase A_2 (PLA_2), an enzyme proposed to play a role in the BL-induced lung fibrosis [35]. Activation of PLA_2 not only produces arachidonic acid metabolites, which are presumably involved in the fibrogenesis [28], but also stimulates the peroxidation of membrane lipids [36]. Malondialdehyde equivalent, as indicated by the amount of thiobarbituric acid-reacting products, has been used as an index of not only lipid peroxidation [4, 32, 37] but also DNA damage [38] since thiobarbituric acid-reacting products are also produced in BL-mediated DNA chain breakage in the presence of pure DNA [39]. The increase of malondialdehyde equivalent in the BL-treated hamster lungs could result from either BL-mediated lipid peroxidation or DNA damage. The significant suppression of BL-increased lung malondialdehyde equivalent by the taurine and niacin treatment in the present studies indicates that taurine and/or niacin may have inhibited BL-induced lipid peroxidation or DNA damage since taurine has been shown to inhibit lipid peroxidation [32, 37] and niacin to prevent DNA damage [17, 40].

It has been demonstrated that BL produces its toxicity by damaging DNA [8]. Niacin has been shown recently to protect against DNA damage caused by chemicals in cell culture [40]. Niacin has also been reported to offer protection against paraquat toxicity *in vivo* [41]. The beneficial effects of niacin in counteracting the tissue damage were attributed to its ability to increase tissue level of NAD and to inhibit overactivation of the chromosomal enzyme poly(ADP-ribose) polymerase, which is generally activated secondary to DNA scission [15, 41, 42]. The activation of poly(ADP-ribose) polymerase leads to intracellular depletion of NAD [42]. We have shown previously that i.t. instillation of BL increases lung poly(ADP-ribose) polymerase activity and causes a marked depletion of lung NAD during the first week of the study [10]. Comparable to our previous report, lung activities of poly(ADP-ribose) polymerase were increased in hamsters following i.t. instillation of three low doses of BL, whereas taurine and niacin in combination inhibited the BL-induced elevation of the activities in the present two studies. Lung NAD levels in the TNSA and TNBL groups of hamsters receiving taurine and niacin in diet were increased significantly over their respective controls as determined in Study Two. Although the lung NAD level in the BL-treated hamsters was decreased slightly, there was no evidence of any marked depletion of NAD at the time the animals were killed in the present study. It is possible that BL-induced lung NAD depletion is an early event followed by a gradual recovery phase responsible for restoring the normal NAD level as demonstrated in our earlier study [10]. On the contrary, BL-treated hamsters kept on a diet supplemented with taurine and niacin had significantly higher lung NAD levels and remarkably less collagen accumulation than hamsters kept on the same diet without taurine and niacin supplementation. Thus, the beneficial effect of niacin against BL-induced pulmonary fibrosis [15] may involve an adequate availability of NAD as found in the present study. The availability of NAD would maintain the critical vital cell functions including the DNA repair of injured pulmonary epithelial cells and this would tend to minimize the subsequent proliferation of collagen-producing interstitial cells [11].

It is not known whether treatment with taurine and niacin in combination compromises the antineoplastic activity of BL. However, this question should not undermine the significance of the antifibrotic effect of this combination since the combined treatment with taurine and niacin opens a new approach to understanding and interrupting the fibroproliferative changes induced by other chemicals. To the best of our knowledge, there is no report that taurine impairs the antineoplastic effect of cytostatic drugs. Niacin, on the other hand, provides significant protection against Adriamycin-induced cardiotoxicity without compromising its antineoplastic potency [43]. Similarly, it was reported that niacin protects normal cells from DNA strand breakage induced by adenosine deaminase inhibitors while maintaining the cytotoxic effect of these inhibitors in neoplastic cells [40]. It is, therefore, unlikely that a taurine and niacin combination would compromise the antineoplastic activity of BL.

It should be pointed out that the taurine and niacin treatment regimen in the present investigation should be considered as a prophylactic measure only. Further experiments are planned in our laboratories to evaluate the antifibrotic effect of the taurine and niacin combination at various stages of the development of lung fibrosis induced by BL. Taurine and niacin are considered relatively safe in humans at pharmacological doses and the combined treatment did not cause any overt systemic toxicity at any time during this investigation. Therefore, the combined treatment with taurine and niacin offers great potential in the pharmacological intervention of the development of chemically induced lung fibrosis in humans.

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REFERENCES

1. Watters LC, King TE, Schwarz MI, Waldrom JA, Stanford RE and Cherniack RM, A clinical, radiographic, and physiologic scoring system for the longitudinal assessment of patients with idiopathic pulmonary fibrosis. *Am Rev Respir Dis* **133**: 97–103, 1986.
2. Bowden DH, Unraveling pulmonary fibrosis: The bleomycin model. *Lab Invest* **50**: 487–488, 1984.
3. Snider GL, Celli BR, Goldstein RH, O'Brien JJ and Lucey EC, Chronic interstitial pulmonary fibrosis produced in hamsters by endotracheal bleomycin. *Am Rev Respir Dis* **117**: 289–297, 1978.
4. Giri SN, Chen Z, Younker WR and Schiedt MJ, Effects of intratracheal administration of bleomycin on GSH-shuttle enzymes, catalase, lipid peroxidation and collagen content in the lungs of hamsters. *Toxicol Appl Pharmacol* **71**: 132–141, 1983.
5. Giri SN, Misra HP, Chandler DB and Chen Z, Increases in lung prolyl hydroxylase and superoxide dismutase activities during bleomycin-induced lung fibrosis in hamsters. *Exp Mol Pathol* **39**: 317–326, 1983.
6. Crooke ST and Bradner WT, Bleomycin, a review. *J Med* **7**: 333–427, 1976.
7. Sugiura Y and Kikuchi T, Formation of superoxide and hydroxy radicals in iron (II)–bleomycin–oxygen system: Electron spin resonance detection by spin trapping. *J Antibiot (Tokyo)* **31**: 1310–1312, 1978.
8. Moseley PL, Augmentation of bleomycin-induced DNA damage in intact cells. *Am J Physiol* **257**: C882–C887, 1989.
9. Giri SN, Nakashima JM and Curry DL, Effects of intratracheal administration of bleomycin or saline in pair-fed and control-fed hamsters on daily food intake and on plasma levels of glucose, cortisol and insulin and lung levels of calmodulin, calcium, and collagen. *Exp Med Pathol* **42**: 206–219, 1985.
10. Hussain MZ, Giri SN and Bhatnager RS, Poly(ADP-ribose) synthetase activity during bleomycin-induced lung fibrosis in hamsters. *Exp Mol Pathol* **43**: 162–176, 1985.
11. Witschi H, Godfrey G, Frome E and Lindenschmidt RC, Pulmonary toxicity of cytostatic drugs: Cell kinetics. *Fundam Appl Toxicol* **8**: 253–262, 1987.
12. Wang Q, Giri SN, Hyde DM and Nakashima JM, Effects of taurine on bleomycin-induced lung fibrosis in hamsters. *Proc Soc Exp Biol Med* **190**: 330–338, 1989.
13. Wright CE, Tallan HH, Lin YY and Gaull GE, Taurine: Biological update. *Annu Rev Biochem* **55**: 427–453, 1986.
14. Huxtable RJ, From heart to hypothesis: A mechanism for the calcium modulatory actions of taurine. *Adv Exp Med Biol* **217**: 371–387, 1987.
15. Wang Q, Giri SN, Hyde DM, Nakashima JM and Javadi I, Niacin attenuates bleomycin-induced lung fibrosis in the hamster. *J Biochem Toxicol* **5**: 13–22, 1990.
16. Bender DA, Magboul BI and Wynick D, Probable mechanisms of regulation of utilization of dietary tryptophan, nicotinamide and nicotinic acid as precursors of nicotinamide nucleotides in the rat. *Br J Nutr* **48**: 119–127, 1982.
17. Weitberg AB, Effect of nicotinic acid supplementation *in vivo* on oxygen radical-induced genetic damage in human lymphocytes. *Mutat Res* **216**: 197–201, 1989.
18. Zia S, Hyde DM and Giri SN, Development of a subchronic bleomycin hamster model of lung fibrosis. *Toxicologist* **10**: 99, 1990.
19. Ohkawa H, Ohismi N and Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* **95**: 351–358, 1979.
20. Bernofsky C and Swan M, An improved cycling assay for nicotinamide adenine dinucleotide. *Anal Biochem* **53**: 452–458, 1973.
21. Misra HP and Fridovich I, The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* **247**: 3170–3175, 1972.
22. Thompson M and Walsh JN, *A Handbook of Inductively Coupled Plasma Spectrometry*. Blackie & Son Ltd., Glasgow, 1983.
23. Woessner JF Jr, The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch Biochem Biophys* **93**: 440–447, 1961.
24. Karlinski JB and Goldstein CH, Fibrotic lung diseases: A perspective. *J Lab Clin Med* **96**: 939–942, 1980.
25. SAS Institute Inc., *SAS/STAT™ Guide for Personal Computers*. SAS Institute Inc., Cary, NC, 1985.
26. Kelley J, Newman RA and Evans JN, Bleomycin-induced pulmonary fibrosis in the rat. Prevention with an inhibitor of collagen synthesis. *J Lab Clin Med* **96**: 954–964, 1980.
27. Miller RL and Udenfriend S, Hydroxylation of proline residues in collagen nascent chains. *Arch Biochem Biophys* **139**: 104–113, 1970.
28. Giri SN and Wang Q, Mechanisms of bleomycin-induced lung injury. *Comments Toxicol* **3**: 145–176, 1989.
29. Pepin JM and Langner RO, Effects of dimethyl sulfoxide (DMSO) on bleomycin-induced pulmonary fibrosis. *Biochem Pharmacol* **34**: 2386–2389, 1985.
30. Haschek WM, Baer KE and Rutherford JE, Effects of dimethyl sulfoxide (DMSO) on pulmonary fibrosis in rats and mice. *Toxicology* **54**: 197–205, 1989.
31. Aruoma OI, Halliwell B, Hoey BM and Butler J, The antioxidant action of taurine, hypotaurine and their metabolic precursors. *Biochem J* **256**: 251–255, 1988.
32. Azuma J, Hamaguchi T, Ohta H, Takihara K, Awata N, Sawamura A, Harada H, Tanaka Y and Kishimoto S, Calcium overload-induced myocardial damage caused by isoproterenol and by adriamycin: Possible role of taurine in its prevention. *Adv Exp Med Biol* **217**: 167–179, 1987.
33. Gordon RE, Heller RF and Del Valle JR, Membrane perturbations and mediation of gap junction formation in response to taurine treatment in normal and injured alveolar epithelia. *Exp Lung Res* **15**: 895–908, 1989.
34. Irving CS, Hammer BE, Danyluk SS and Klein PD, Coordination and binding of taurine as determined by nuclear magnetic resonance measurements on ^{13}C-labeled taurine. In: *Taurine in Nutrition and Neurology* (Eds. Huxtable RJ and Pasantes-Morales H), pp. 5–17. Plenum Press, New York, 1982.
35. Wang Q, Giri SN and Hyde DM, Characterization of a phospholipase A_2 in hamster lung and *in vitro* and *in vivo* effects of bleomycin on this enzyme. *Prostaglandins Leukotrienes Essent Fatty Acids* **36**: 85–92, 1989.
36. Chien KR, Abrams J, Serroni A, Martin JT and Farber JL, Accelerated phospholipid degradation and associated membrane dysfunction in irreversible, ischemic liver cell injury. *J Biol Chem* **253**: 4809–4819, 1978.
37. Nakashima T, Takino T and Kuriyama K, Therapeutic and prophylactic effects of taurine administration on

experimental liver injury. In: *Sulfur Amino Acids: Biochemical and Clinical Aspects* (Eds. Kuriyama K, Huxtable R and Iwata H), pp. 449–459. Alan R. Liss, New York, 1983.

38. Trush MA, Mimnaugh EG, Ginsburg E and Gram TE, Studies on the interaction of bleomycin A_2 with rat lung microsomes. II. Involvement of adventitious iron and reactive oxygen in bleomycin-mediated DNA chain breakage. *J Pharmacol Exp Ther* **221**: 159–165, 1982.
39. Giloni L, Takeshita M, Johnson F, Iden C and Grollman AP, Bleomycin-induced strand-scission of DNA: Mechanism of deoxyribose cleavage. *J Biol Chem* **256**: 8608–8615, 1981.
40. Weitberg AB and Corvese D, Niacin prevents DNA strand breakage by adenosine deaminase inhibitors. *Biochem Biophys Res Commun* **167**: 514–519, 1990.
41. Brown OR, Heitkamp M and Song CS, Niacin reduces paraquat toxicity in rats. *Science* **212**: 1510–1512, 1981.
42. Stubberfield CR and Cohen GM, NAD^+ depletion and cytotoxicity in isolated hepatocytes. *Biochem Pharmacol* **37**: 3967–3974, 1988.
43. Schmitt-Gräff A and Scheulen ME, Prevention of adriamycin cardiotoxicity by niacin, isocitrate or *N*-acetyl-cysteine in mice. A morphological study. *Pathol Res Pract* **181**: 168–174, 1986.