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Effects of Niacin on Bleomycin-Induced Increases in Myeloperoxidase, Prolyl Hydroxylase, and Superoxide Dismutase Activities and Collagen Accumulation in the Lungs of Hamsters

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ABSTRACT: It has been shown that lung nicotinamide adenine dinucleotide (NAD) depletion accompanies bleomycin (BL)-induced lung fibrosis in the hamster and that treatment with niacin (NA), a precursor of NAD, was found to attenuate lung fibrosis caused by this agent. Niacin was used in the present study to investigate changes in some biochemical parameters and enzymes involved in the development of BL-induced lung fibrosis in the hamster. Niacin (500 mg/kg, IP), or an equivalent volume of saline (SA, IP), was given daily 2 days prior to intratracheal instillation of BL (7.5 U/5 mL/kg) or SA and everyday thereafter throughout the study. Hamsters were killed at 1, 4, 7, 10, and 14 days after the BL or SA instillation and their lungs processed for various biochemical assays. Hydroxyproline content and superoxide dismutase (SOD) activity in SABL treated animals were significantly ($P \leq 0.05$) elevated at 7 and 10 days, peaking at 14 days to 161 ± 11% and 159 ± 11% of the SASA treated animals, respectively. Although the hydroxyproline level of NABL treated animals was significantly elevated at 7 and 10 days and peaked at 14 days to 123 ± 8% of the NASA control, these values were significantly lower than the SABL treated animals at the corresponding times. The lung SOD activity of NABL groups was significantly higher at 4 days but significantly lower at 10 and 14 days than the SABL groups at the corresponding times. Prolyl hydroxylase (PH) activity and total lung calcium in SABL treated groups were significantly elevated compared to SASA treated groups starting at 4 days, with PH peaking at 10 days to 163 ± 13% and calcium peaking at 7 days to 148 ± 8% of SASA treated groups. The NABL treated animals displayed a significant elevation in PH activity at 4 days only (132 ± 15%), while the calcium content in this group was significantly increased at 4 and 14 days compared to NASA treated animals. However, the activity of PH in the NABL treated animals was significantly lower than the SABL treated animals at 7, 10, and 14 days. The calcium content of the NABL group was significantly lower than the SABL group at 7 and 10 days. The thiobarbituric acid reactive substance equivalents (TBARS) content and myeloperoxidase (MPO) activity were significantly elevated at all time points in SABL groups as compared to SASA groups, with peak elevation of TBARS to 160 ± 9% at 4 days and MPO to 268 ± 40% at 1 day. The TBARS content of NABL groups was significantly elevated above NASA groups at 1, 4, 7, and 10 days. The MPO activity in NABL groups was significantly elevated above NASA groups at 1, 4, 7, and 14 days, but the activity was significantly lower than SABL groups at 4 and 14 days. The data suggest that NA inhibits the extent of oxidative damage or enhances processes involved in the repair of BL-induced alveolar epithelial damage.

KEYWORDS: **Lung Fibrosis, Bleomycin, Niacin, Hamster, Collagen, Prolyl Hydroxylase, Superoxide Dismutase, Myeloperoxidase, Calcium, Lipid Peroxidation.**

INTRODUCTION

Bleomycin (BL), a mixture of glycopeptides, is an effective antineoplastic agent, but unfortunately, it causes a dose-dependent pneumotoxicity that progresses to pulmonary fibrosis (1). Since BL-induced pulmonary fibrosis in hamsters resembles, histologically and biochemically, the interstitial pulmonary fibrosis (IPF) seen in humans (2), this agent has been used extensively in rodents as

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a model to define the underlying mechanisms for the development of IPF and to screen potential antifibrotic agents (3).

The exact mechanism of BL-induced lung damage is not completely understood, but it is believed that the initial injury caused by this agent involves binding to DNA and Fe^{2+} and this complex, under aerobic conditions, is thought to generate reactive oxygen species (ROS) (4,5). The DNA-BL-Fe^{2+} complex has been shown to cause DNA strand breaks *in vitro* (6) and lipid peroxidation *in vivo* (7). Additional evidence of DNA as the target of BL-induced lung injury has been shown by intranuclear ultrastructural changes and dysplasia of lung cells (8). Similarly, it has been shown that BL depletes lung NAD and induces the fragmentation of DNA, as reflected by elevated activity of poly (ADP-ribose) polymerase *in vivo* (9). Moreover, Schraufstatter *et al.* (10,11) have shown that H_2O_2 caused a depletion of NAD and ATP *in vitro* and suggested that the activation of poly (ADP-ribose) polymerase was probably responsible for the depletion. Bleomycin-induced depletion of NAD could compromise DNA repair due to a lack of the poly (ADP-ribose) polymerase coenzyme, NAD, or decrease ATP formation, since low NAD levels are associated with reduced activity of the glycolytic pathway (12).

It is therefore highly likely that maintenance of intracellular NAD content may minimize the damage caused by BL *in vivo*. Indeed, Wang *et al.* (13) have shown that hamsters treated with niacin (NA), [nicotinic acid], a precursor of NAD and NADP (14) attenuated collagen accumulation at 14 days and 21 days following BL instillation. Although it has been reported that long-term administration of NA has been associated with growth inhibition and fatty livers in rats (15), this compound is generally believed to be nontoxic since it is readily metabolized and excreted. In fact, this compound, at supraphysiological doses, has been used clinically as a vasodilator and hypolipidemic-hypocholesterolemic drug (16,17). The purpose of this study was to define the antifibrotic mechanisms of NA during the development of BL-induced lung fibrosis in hamsters with respect to its effects on lung hydroxyproline, total calcium, and thiobarbituric acid reactive substances along with the activities of superoxide dismutase, prolyl hydroxylase, and myeloperoxidase at varying times after BL treatment.

MATERIALS AND METHODS

Animals and Reagents

Male, Golden Syrian hamsters were obtained from Simonsens Inc. (Gilroy, CA) and weighed 90 to 110 g. The animals were housed four per cage in a special environmentally controlled room and had access to water and laboratory chow ad libitum. Bleomycin sulfate (Blenoxane®) was supplied by Bristol Laboratories (Division of Bristol-Myers, Syracuse, NY). Niacin, [nicotinic acid], was purchased from Sigma Chemical Co. (St. Louis, MO). The L-4-[^{3}H]Hydroxyproline (sp. act. 8 Ci/mmol) was obtained from NEN Research Products (Boston, MA). All other chemicals were purchased from standard commercial sources.

Treatment of Animals

Hamsters were acclimatized for 5 days before the start of the experiment. Two days prior to the intratracheal (IT) instillation of BL or saline (SA) and everyday thereafter until 1 day prior to sacrifice, animals received NA (500 mg/kg/day, IP) in sterile isotonic SA or an equivalent volume of SA alone. This dose of NA has been shown to attenuate collagen accumulation at 14 and 21 days after the intratracheal administration of BL in hamsters (13). Bleomycin (7.5 U/5 mL/kg), or an equivalent volume of sterile isotonic SA, was instilled under sodium pentobarbital anesthesia (60–90 mg/kg, IP). Animals treated with SA (IP) and SA (IT) were designated SASA. Animals treated with NA (IP) and SA (IT) were designated NASA. Animals treated with SA (IP) and BL (IT) were designated SABL. Animals treated with NA (IP) and BL (IT) were designated NABL. Additionally, six animals were allowed free access to laboratory chow and water (*ad lib*-fed) and six animals were partially fasted (control-fed) to the point when the percentage loss in their body weight matched the percentage loss in body weight of the SABL treated animals compared to the SASA treated animals at 14 days following BL treatment. The *ad lib*-fed and control-fed groups were included to evaluate the effect of decreased food intake on various biochemical parameters that were measured in this investigation.

Animal Sacrifice and Tissue Processing

Under sodium pentobarbital anesthesia (90–120 mg/kg, IP), animals were sacrificed at 1, 4, 7, 10, and 14 days after the instillation of BL or SA. The animals were exsanguinated by transecting the descending aorta and inferior vena cava. The thoracic cavity was quickly opened, and each lung lobe was rapidly removed and immediately frozen between two aluminum plates (4-cm diameter, 0.9-cm thick), prechilled in liquid nitrogen. The frozen lung lobes of each animal were quickly placed in

a pretared piece of aluminum foil, quickly weighed to the nearest 1 mg, and stored at −80°C until homogenized. Lungs were removed from −80°C and quickly transferred to a Bessman® stainless steel tissue pulverizer (Santa Clara, CA), prechilled in liquid nitrogen, and pulverized to a fine powder. The tissue powder was gently mixed with a stainless steel spatula, prechilled in liquid nitrogen, and transferred to a Potter-Elvehjem manual tissue grinder mortar, prechilled to 0–4°C. The powder was homogenized in approximately 8 ml of ice-cold isotonic saline using a teflon tipped pestle attached to a drill. Aliquots of the homogenate were stored at −80°C until used in various biochemical and enzyme assays.

Hydroxyproline Determination

One milliliter of the whole homogenate was precipitated in a final concentration of 10% trichloroacetic acid (TCA) at 0°C for 10 min and centrifuged at 2500 × *g* for 15 min. The precipitate was hydrolyzed in 6N HCl for 18–24 h at 110°C. [^{3}H]Hydroxyproline (1 × 10^5 dpm) was added to each sample to determine recovery, and hydroxyproline content was measured by a method described by Woessner (18). Recovery ranged from 89–100%. The hydroxyproline value for each sample was corrected for its recovery.

Prolyl Hydroxylase Activity Assay

Lung prolyl hydroxylase (PH) activity was determined by the method of Giri *et al.*, (19). Briefly, the PH substrate (procollagen) was prepared from the tibias of 10-day-old chick embryos. During the reaction, ^3H_2O is released in stoichiometric proportion to the hydroxylation of proline and is used as a measure of the enzyme activity (20).

Thiobarbituric Acid Reactive Substance Equivalents Determination

Thiobarbituric acid reactive substance equivalents (TBARS), an index of lipid peroxidation and possible DNA damage (21,22), was measured in whole lung homogenate by the method outlined by Ohkawa *et al.* (23).

Superoxide Dismutase Activity Assay

The lung superoxide dismutase (SOD) activity was determined from the inhibition of the rate of autoxidation of epinephrine to adrenochrome, as described by Misra and Fridovich (24). The reaction mixture contained $NaHCO_2$-CO_3 buffer (pH 10.2), EDTA, and epinephrine. The rate of formation of adrenochrome was 0.025 absorbance units (au)/min at 490 nm using a Varian Cary 219 spectrophotometer (Palo Alto, CA). Under these conditions, the volume of tissue homogenate required to inhibit the rate of formation of adrenochrome by 50% (i.e., 0.0125 au/min) was defined to contain 1 unit of SOD activity.

Myeloperoxidase Activity Assay

Myeloperoxidase (MPO) activity of the hamster lungs was determined by the method outlined by Bradley *et al.* (25). Briefly, 1 ml of whole lung homogenate was rehomogenized in 0.5% hexadecyltrimethylammonium bromide (HTAB) on ice. The homogenate was freeze-thawed 3 times and centrifuged at 30,000 × *g* for 20 min at 4°C. The resulting supernatant was decanted and the volume recorded. The reaction mixture contained 50 mM $NaH_2PO_4 \cdot H_2O$ buffer (pH 6.0), *o*-dianisidine dihydrochloride, and H_2O_2. Utilizing 0.1 ml of the whole lung supernatant, the rate of change in absorbance at 460 nm at 25°C (Varian Cary 219 spectrophotometer) was used as an index of MPO activity. The absorbance was converted to units according to the procedure outlined by Bradley *et al.*

Calcium Measurement

One milliliter of the lung homogenate was deproteinized with 0.25 ml of 50% TCA on ice for 10 min and centrifuged at 2500 × g for 15 min. The resulting supernatant was decanted and the volume recorded. The calcium content of the TCA supernatant was determined according to the method outlined by Thompson and Walsh (26), using inductively coupled plasma (ICP) atomic absorption with a Model 3510 ICP spectrometer (Applied Research Laboratories, Sunland, CA).

Data Presentation and Statistical Analysis

All data were expressed on the basis of whole lung. Expression of the data based upon the whole lung avoids artifactual lowering of the values in BL treated animals (27). All values were reported as the mean ± standard error of the mean (SEM) and were analyzed by one-way analysis of variance and Duncan's multiple range test (28). Comparisons were made among groups at each individual time point, with the $P \leq 0.05$ value being considered significant.

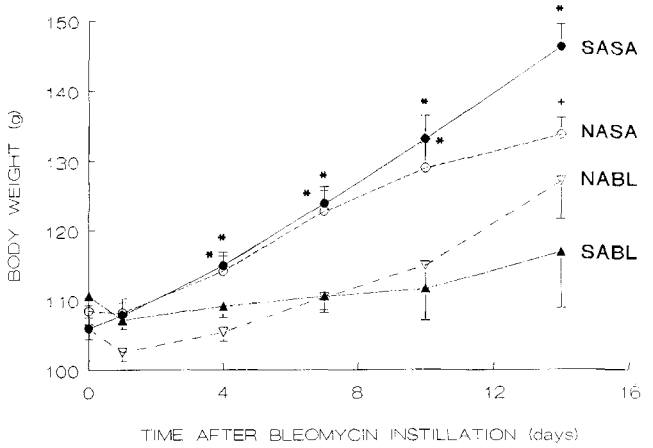

FIGURE 1. Effects of daily administration of NA (IP) on hamster body weight at 1, 4, 7, 10, and 14 days after intratracheal instillation bleomycin (7.5 U/kg). All data are expressed as mean ± SEM. Treatment groups are indicated along the right side of the *y*-axis and explained in the Materials and Methods section. SASA = saline + saline, 6 animals at each time in this group; NASA = niacin + saline, 5 to 6 animals at each time in this group; SABL = saline + bleomycin, 7 to 10 animals at each time in this group; NABL = niacin + bleomycin, 8 to 10 animals at each time in this group. *Significantly higher ($P \leq 0.05$) than the corresponding SABL or NABL group. ⁺Significantly lower ($P < 0.05$) than the corresponding SASA group.

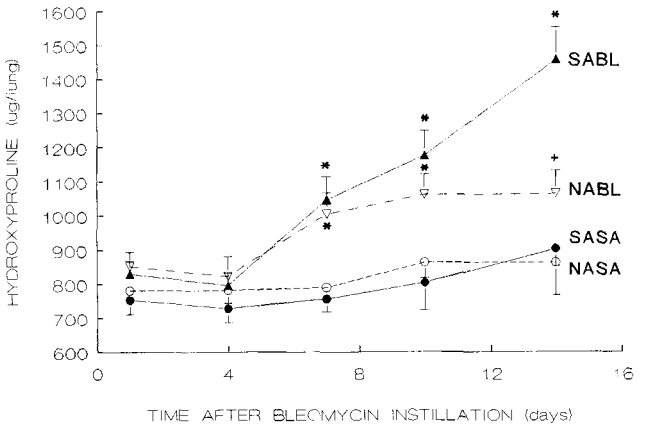

FIGURE 2. Effects of daily administration of NA (IP) on total lung hydroxyproline at 1, 4, 7, 10, and 14 days after intratracheal instillation bleomycin (7.5 U/kg). All data are expressed as mean ± SEM. The treatment groups are indicated along the right side of the *y*-axis and explained in the Materials and Methods section. See the explanation of abbreviations and the number of animals at each time for each group in Figure 1. *Significantly higher ($P \leq 0.05$) than the corresponding SASA or NASA group. ⁺Significantly lower ($P < 0.05$) than the corresponding SABL group but significantly higher ($P < 0.05$) than the corresponding NASA group.

RESULTS

Body Weight and Mortality

The mean body weights of the various groups are summarized in Figure 1. There were no significant differences in body weight at 0 and 1 days. The body weight was significantly lower in the SABL and NABL treated groups when compared to the SASA and NASA treated groups at 4, 7, and 10 days, respectively. The body weight of SABL treated hamsters was significantly lower than that of SASA treated hamsters at 14 days. Additionally, the NASA treated hamsters had a significantly lower body weight than the SASA treated hamsters at 14 days and their body weight did not significantly differ from the body weight of NABL treated animals.

The body weight of the control-fed animals was 82 ± 3% ($p \leq 0.05$) of that of the *ad lib*-fed animals at 7 days. The body weight of the SABL treated animals was 80 ± 5% of the SASA treated animals at 14 days. There was no significant difference in the body weight between the control-fed animals and the animals in the SABL group at 14 days.

The cumulative mortality of the SABL group was 2.3%, with one animal dying at 7 days. Mortality for NABL treated animals was 2.2%, with one animal dying at 13 days. No animals died in the SASA and NASA treated groups or in the *ad lib*-fed and control-fed groups.

Hydroxyproline Content

Lung hydroxyproline content, an index of collagen accumulation, in various groups is depicted in Figure 2. When compared to saline controls (SASA and NASA), there were no significant differences in mean hydroxyproline content in SABL and NABL treated animals at 1 and 4 days. However, significant increases in hydroxyproline levels were noted at 7, 10, and 14 days in SABL and NABL treated animals when compared to their respective saline control groups. In fact, the hydroxyproline levels in the SABL and NABL groups were maximally increased to 161 ± 10% and 123 ± 8% of their respective controls at 14 days after BL treatment. Additionally, the hydroxyproline level of NABL-treated hamsters was significantly lower than SABL treated animals at this time. There were no significant differences in the hydroxyproline levels between the two saline control groups at any point in the study and between the *ad lib*-fed and control-fed groups (Table 1).

Prolyl Hydroxylase Activity

Figure 3 summarizes the mean lung PH activities in various groups of hamsters. No significant

TABLE 1. The Effects of Ad Lib-Fed and Control-Fed Diets on Lung Hydroxyproline, TBARS, and Total Calcium Contents and the SOD, PH, and MPO Activities

Group	Hydroxy proline (μg/lung)	PH (dpm/30 min/lung)	TBARS (nmoles/ lung)	SOD (units/ lung)*	MPO (units/ lung)*	Calcium (μg/ lung)
Ad lib-fed (n = 6)	858 ± 46	120,000 ± 9,700	152 ± 7	201 ± 8	6.0 ± 0.3	18.8 ± 3.0
Control-fed (n = 6)	850 ± 95	116,000 ± 4,700	162 ± 8	193 ± 11	5.5 ± 0.4	16.9 ± 3.1

*Units as explained in the Materials and Methods section.

changes were noted among any of the four groups at 1 day and between the SASA and NASA groups at any day after IT instillation of SA. Additionally, no significant differences were noted between the *ad lib*-fed and control-fed animals in lung PH activity (Table 1). The PH activity at 4 days in lungs from both the SABL and NABL treated animals was significantly elevated when compared to the respective saline controls. The PH activity of the SABL treated animals was significantly elevated to 149 ± 6.9, 162 ± 11, and 152 ± 13% of the SASA treated animals at 7, 10, and 14 days, respectively. However, the enzyme activities at these time points in the NABL treated animals were nonsignificantly changed to 116 ± 10.4%, 121 ± 12%, and 97 ± 5% of the NASA treated animals, respectively, and these activity levels were significantly lower than the SABL treated animals at the corresponding time points.

Thiobarbituric Acid Reactive Substance Equivalents

There were no significant differences between SASA and NASA treated hamsters in TBARS content at any time during the study (Figure 4) and between the *ad lib*-fed and control-fed animals (Table 1). The TBARS contents in the SABL treated animals were significantly increased to 144 ± 10%, 160 ± 9%, 119 ± 4%, 130 ± 7%, and 146 ± 14% of the SASA treated controls at 1, 4, 7, 10, and 14 days, respectively. The TBARS contents in the NABL groups were significantly elevated to 151 ± 5%, 143 ± 14%, 126 ± 7%, and 128 ± 8% of the

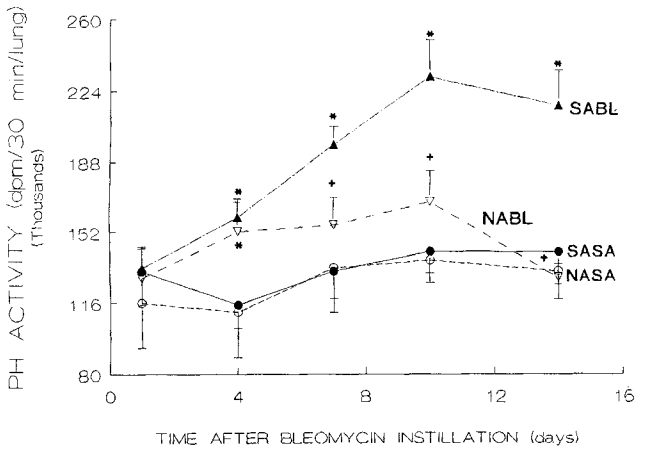

FIGURE 3. Effects of daily administration of NA (IP) on total lung PH activity at 1, 4, 7, 10, and 14 days after intratracheal instillation of bleomycin (7.5 U/kg). All data expressed as mean ± SEM. The treatment groups are indicated along the right side of the y-axis and explained in the Materials and Methods section. See the explanations of abbreviations and the number of animals at each time for each group in Figure 1. *Significantly higher ($P \leq 0.05$) than corresponding SASA or NASA group. †Significantly lower ($P \leq 0.05$) than corresponding SABL group.

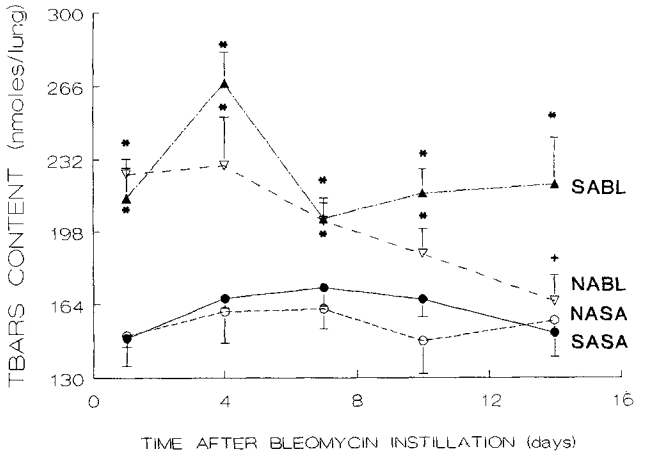

FIGURE 4. Effects of daily administration of NA (IP) on lung TBARS at 1, 4, 7, 10, and 14 days after intratracheal administration of bleomycin (7.5 U/kg). All data are expressed as mean ± SEM. The treatment groups are indicated along the right side of the y-axis and explained in the Materials and Methods section. See the explanations of abbreviations and the number of animals at each time for each group in Figure 1. *Significantly higher ($P \leq 0.05$) than the corresponding SASA or NASA group. †Significantly lower ($P < 0.05$) than corresponding SABL group.

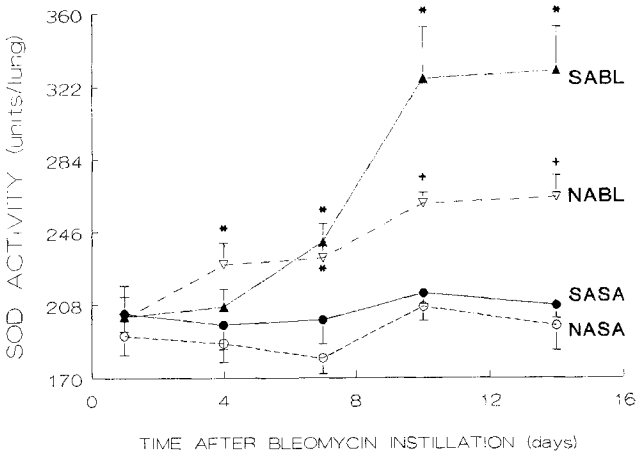

FIGURE 5. Effects of daily administration of NA (IP) on lung SOD activity at 1, 4, 7, 10, and 14 days after intratracheal instillation of bleomycin (7.5 U/kg). All data are expressed as mean ± SEM. The treatment groups are indicated along the right side of the *y*-axis and explained in the Materials and Methods section. See the explanations of abbreviations and the number of animals at each time for each group in Figure 1. *Significantly higher ($P \leq 0.05$) than the corresponding SASA or NASA group. ⁺Significantly lower ($P \leq 0.05$) than the corresponding SABL group but significantly higher ($P \leq 0.05$) than the corresponding NASA group.

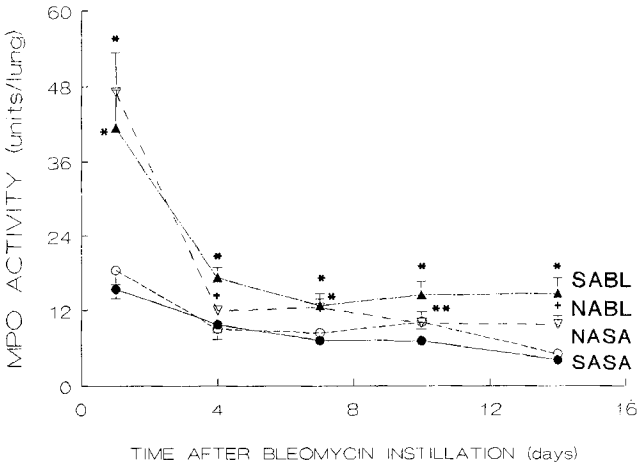

FIGURE 6. Effects of daily administration of NA (IP) on lung MPO activity at 1, 4, 7, 10, and 14 days after intratracheal administration of bleomycin (7.5 U/kg). All data are expressed as mean ± SEM. Treatment groups are indicated along the right side of the *y*-axis and explained in the Materials and Methods section. See the explanations of abbreviations and the number of animals at each time for each group in Figure 1. *Significantly higher ($P \leq 0.05$) than the corresponding SASA or NASA group. ⁺Significantly lower ($P \leq 0.05$) than the corresponding SABL group but significantly higher ($P \leq 0.05$) than the corresponding NASA group. **Significantly lower ($P < 0.05$) than the corresponding SABL group but not significantly different from the corresponding NASA group.

NASA groups at 1, 4, 7, and 10 days, respectively. However, the TBARS content in the NABL treated animals was significantly lower than that of the SABL treated animals at 14 days.

Superoxide Dismutase Activity

Lung SOD activities for the various groups are shown in Figure 5. There were no significant differences in the activity of this enzyme between the SASA and NASA group at any time point during the study and between the *ad lib*-fed and control-fed groups (Table 1). Similarly, at 1 day, there was no significant difference in SOD activity among the four treated groups. There was no significant difference in SOD activity between the SABL and SASA treated groups at 4 days, although the activity in the NABL treated group was significantly elevated (122 ± 6%) in comparison to the NASA treated group at this time. The SOD activities in the SABL treated groups were significantly elevated to 120 ± 5%, 152 ± 12%, and 159 ± 11% of the SASA treated groups at 7, 10, and 14 days, respectively. At the corresponding times, the SOD activity in the NABL treated animals was significantly elevated to 129 ± 4%, 126 ± 3%, and 134 ± 6% of the NASA treated animals, respectively. However, the activity in the NABL treated groups was significantly lower than that of the SABL groups at 10 and 14 days.

Myeloperoxidase Activity

Lung MPO activities in the various groups are summarized in Figure 6. There were no significant differences in MPO activities between the SASA and NASA treated animals at any time during the study and between the *ad lib*-fed and control-fed animals (Table 1). The MPO activities of the SABL and NABL treated groups were significantly elevated to 268 ± 40% and 255 ± 68% of their respective SA treated groups at 1 day. There was also a significant elevation of lung MPO activity in the SABL and NABL groups to 177 ± 16% and 131 ± 9%, respectively, of their SA controls at 4 days, but the increase in the NABL group was significantly less than the increase in the SABL group. The activity of this enzyme in SABL group was significantly elevated to 176 ± 28%, 203 ± 30%, and 358 ± 60% of the SASA control at 7, 10, and 14 days, respectively. Similarly, the MPO activity of the NABL treated group at 7 and 14 days was significantly increased to 147 ± 17% and 191 ± 20% of the NASA treated group, respectively. However, the increase in MPO activity in the NABL

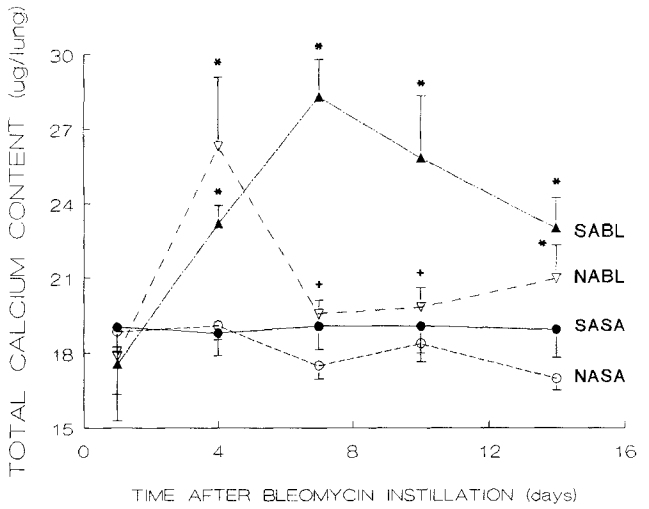

FIGURE 7. Effects of daily administration of NA (IP) on lung calcium content at 1, 4, 7, 10, and 14 days after intratracheal administration of bleomycin (7.5 U/kg). All data are expressed as mean ± SEM. Treatment groups are indicated along the right side of the *y*-axis and explained in the Materials and Methods section. See the explanations of abbreviations and the number of animals at each time for each group in Figure 1. *Significantly higher ($P \leq 0.05$) than the corresponding SASA or NASA group. †Significantly lower ($P \leq 0.05$) than the corresponding SABL group but significantly higher ($P \leq 0.05$) than the corresponding NASA group.

treated group was significantly less than the increase of MPO activity in the SABL group at 10 and 14 days.

Calcium Content

Total lung calcium data from the various groups are depicted in Figure 7. There were no significant differences between the SASA and NASA treated animals at any time during the study and between the *ad lib*-fed and control-fed animals (Table 1). There were also no significant differences in lung calcium content among all four treated groups at 1 day. At 4 days, however, there were significant increases in the lung calcium content in both the SABL (123 ± 4%) and NABL (138 ± 15%) treated animals when compared to their respective SA treated groups. The total lung calcium content in the SABL treated animals remained significantly elevated to 148 ± 8%, 136 ± 13%, and 122 ± 6% of the SASA treated animals at 7, 10, and 14 days, respectively, whereas the calcium content in the NABL treated animals gradually decreased to control levels at 7 and 10 days. The calcium level in the NABL group rebounded and was significantly increased over that of the NASA treated animals at 14 days (123 ± 8%).

DISCUSSION

The therapeutic uses of BL in humans have been limited by pulmonary toxicity that resembles IPF (29,30). Consequently, IT administration of BL in rodents has been used as a reproducible model of this disease (2). Under aerobic conditions, the BL-DNA-Fe^{2+} complex generates ROS which cause DNA strand breaks, lipid peroxidation, and necrosis of Type I epithelial cells (4,6). This event is followed by proliferation and differentiation of Type II epithelial cells for repair of the damaged alveolar epithelium (31). Normally, lung injury is self-limiting and ceases when adequate repair has been achieved. However, in the absence of the normal repair process, either from a sustained injury or subsequent injury from an overexuberant repair process that entails damage from immune cell reactions, fibrosis would result (32). Extended injury, or delayed repair of the epithelium, will activate fibroblasts to proliferate and produce excess collagen, a hallmark of lung fibrosis (33).

It has been shown that the depletion of NAD precedes BL-induced lung fibrosis in hamsters (9). It is possible that NAD depletion could compromise the repair of lung DNA damaged by BL, since NAD acts as a coenzyme for poly (ADP-ribose) polymerase. This enzyme, which is generally activated in response to damaged DNA (34,35), catalyzes the transfer of ADP-ribose residues from NAD onto acceptor proteins to form homopolymers of adenosine diphosphoribose (36). These polymers then activate DNA ligase (37) which maintains proper chromosomal conformation during DNA repair (38). In addition, NAD depletion would compromise many vital cellular functions secondary to a reduction in ATP formation due to a decrease in function of the glycolytic pathway (12).

Wang *et al.* (13) found that daily administration of niacin caused a marked attenuation of BL-induced increases in lung collagen accumulation at 14 and 21 days after BL treatment. In the present study, we confirmed our earlier findings and found a marked reduction in the deposition of lung collagen at 14 days after BL treatment. In fact, the rate of increase in the accumulation of lung collagen, measured as hydroxyproline, between 4 and 14 days after BL treatment was 64 μg/day (r^2 = 0.96) in the SABL group as compared to 23 μg/day (r^2 = 0.92) in the NABL group during the same period. In our earlier work, the effect of niacin treatment on the level of lung collagen in BL treated animals was examined only at two time points. In this study, measurement of the collagen in BL

treated animals at multiple time points with and without niacin treatment revealed that the latter compound slowed the rate of progression of the fibrotic process markedly with respect to the deposition of collagen in the lung. A similar trend was noted in the activity of prolyl hydroxylase which is responsible for post-translational hydroxylation of proline residues in newly synthesized collagen (39,40). Increases in the activity of this enzyme generally precede the accumulation of collagen in the lung, as demonstrated in several animal models of lung fibrosis including the BL rodent model (41,19). Therefore, it is not surprising that BL treatment in the SABL group caused a progressive increase in prolyl hydroxylase activity starting at 4–10 days and, thereafter, plateaued at 14 days after BL treatment. It is interesting that administration of NA in BL treated animals generally had a marked suppressing effect on BL-induced increases in the prolyl hydroxylase activity. A decrease in the activity of this enzyme coincided with a reduction in the accumulation of lung collagen in NABL groups relative to the SABL groups. Although the mechanism by which NA suppresses the BL-induced increases in the lung prolyl hydroxylase activity is not known, it is possible that a decrease in the activity of this enzyme will allow the accumulation of immature, uncross-linked collagen which is more susceptible to degradation by collagenase than the mature, cross-linked collagen, since hydroxylation of proline residues in the collagen chain is a prerequisite for its maturation.

The TBARS are generally used as an index of lipid peroxidation and DNA damage (21,22). Our findings that BL treated animals in both SABL and NABL groups had increased lung TBARS content, as compared to their respective controls, indicate that ROS resulting from BL-DNA-Fe^{2+} complex were probably involved in peroxidation of lipid membranes and DNA damage. However, the observed difference between SABL and NABL groups was that the extent of lipid peroxidation in the animals of the SABL group was of a sustained nature and lasted through the entire period of the study as compared to that of the animals in the NABL group. In fact, the extent of lipid peroxidation in the latter group steadily declined after 4 and reached control level at 14 days after BL treatment. The lung TBARS content in the NABL group was significantly less than that of the SABL group at 14 days. The protective effect of NA against lipid peroxidation at the later stage of BL-induced lung toxicity was also reflected by a decreased level of lung SOD activity in the NABL group, since the activity of this enzyme is thought to parallel the generation of superoxide radicals to protect tissue against their injurious effects (5).

Because of restricted metabolic capabilities, the Type I epithelial cells of the lung appear to be more vulnerable to deleterious effects of pneumotoxins, including BL, than the Type II cells (33,42). The latter cell type contains poly (ADP-ribose) polymerase (43), which helps to repair DNA damaged by BL treatment. It is conceivable that administration of NA in the BL treated animals would allow the repair of Type II cells by furnishing much needed NAD which acts as the substrate for poly (ADP-ribose) polymerase. The intact Type II cells then proliferate and differentiate to Type I cells which line the denuded alveoli brought about by BL insult (32). Thus, the inability of the Type II cells to repair damaged DNA in the absence of an adequate level of NAD might explain the sustained nature of the lung injury caused by the IT administration of BL in the animals of the SABL group as compared to the animals receiving NA in the NABL group. This hypothesis is strengthened by our previous findings that IT instillation of BL in hamsters in the single dose model of pulmonary fibrosis depleted lung NAD (9), whereas supplementation of NA in the same model prevented the lung NAD depletion and maintained its level (13).

The sequestration of neutrophils is a characteristic feature of the acute phase of lung inflammation caused by BL (32). Infiltration of an excess number of neutrophils could inflict further lung damage by generating ROS, releasing proteolytic enzymes and MPO. The latter enzyme converts Cl^- to hypochlorous acid (HOCl) which is known for its biocidal property in oxidizing a variety of vital cellular components (44). Thus, neutrophils appear to play a major role in the pathogenesis of the acute phase of BL-induced lung inflammation and the activity of MPO in the lung provides a good index of their involvement in this process (45). The lung MPO activity in the SABL group was significantly elevated as compared to that in the SASA control through the entire study, whereas the increases in activity of this enzyme in the NABL group were generally of a lesser magnitude than those of the SABL group except at 1 day. The increases in MPO activity among all four groups at day 1 could be due to the mechanical trauma of intratracheal instillation of both SA and BL. An attenuated increase in the lung MPO activity in the NABL group suggests a lower level of neutrophil sequestration. How NA treatment reduces the sequestration of neutrophils in the lung of BL treated

animals is not presently known. However, it is possible that NA treatment may facilitate removal of the sequestered neutrophils from the pulmonary vascular bed by virtue of its vasodilatory effect (16). Removal of the neutrophils from the pulmonary vasculature would help to minimize the subsequent tissue damage of animals in the NABL group relative to animals in the SABL group. Additionally, the removal of sequestered neutrophils may also partially explain the attenuation of increased lung SOD activity in the NABL treated animals compared to the SABL treated animals at 10 and 14 days since these cells are known to contain SOD (46).

We have measured total lung calcium, since this element has been shown to be involved in BL-induced lung fibrosis (47). Calcium is known to be an intracellular signal influencing many processes, including activation of phospholipase (PL) A_2 (48) and formation of ATP (49). Excess intracellular calcium has been associated with mitochondrial inhibition of ATP formation and ATP depletion due to maintenance of homeostatic ion gradiants. Calcium accumulates on the inner surface of the cell membrane and activates various membrane bound PLs and proteases which damage this barrier (49). The PLA_2 has been implicated in BL-induced lung fibrosis (48). This enzyme is responsible for the cleavage of arachidonic acid from various phospholipids (50) and stimulation of peroxidation of membrane lipids (51). Total lung calcium in the SABL was significantly elevated at 4 days and remained significantly elevated throughout the study. A sustained increase in the lung calcium content of the SABL group could be attributed to the alveolar epithelial cell death and continuing inflammatory reactions of a progressive nature in the lungs of this group of animals. Niacin treatment had a beneficial effect in reducing the accumulation of calcium in the lung of the NABL group except at 4 days. In fact, the lung calcium content in this group of animals was similar to that of the control group at 7 and 10 days after BL treatment. The protective effect of NA against BL-induced damage could possibly be related to its ability to prevent the influx of calcium and thereby minimize the calcium-dependent processes responsible for the inflammatory reaction of the lung caused by BL.

The biochemical changes reported in this study in BL-treated animals with and without NA treatment cannot be attributed to decreased food intake. This is based on the findings that there were no differences in any of the measured biochemical determinants between the *ad lib*-fed and control-fed groups. The animals in the latter group were partially fasted until they lost a percentage of their body weight comparable to the BL-treated animals at 14 days.

The findings reported in this study revealed that NA treatment attenuated the BL-induced lung fibrosis in hamsters. The possible mechanisms for the beneficial effects of niacin are not known. However, it is possible that this compound may be directly or indirectly involved in protecting the Type II epithelial cells against oxidative damage or enhancing the repair process of damaged Type II cells by providing NAD and ATP. The intact functional Type II cells will proliferate and differentiate into Type I epithelial cells which will reline the denuded alveolus and thereby minimize lung damage or enhance repair. However, further studies are warranted at the biochemical and morphological levels to define the exact mechanism of the antifibrotic effect of NA in the BL hamster model of lung fibrosis.

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