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Procollagen Gene Expression is Down-Regulated by Taurine and Niacin at the Transcriptional Level in the Bleomycin Hamster Model of Lung Fibrosis[1]

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ABSTRACT

Taurine (T) and niacin (N) have previously been found to block the accumulation of collagen in the bleomycin (BL) model of interstitial pulmonary fibrosis. The present study was designed to evaluate whether the mechanism for the antifibrotic effect of combined treatment with taurine and niacin involves the down-regulation of BL-induced overexpression of procollagen I and III messenger ribonucleic acid (mRNA) levels in lungs. Hamsters were intratracheally instilled with three consecutive doses of saline or BL at weekly intervals (2.5, 2.0, 1.5 units/5 ml/kg). Four groups of animals were fed a diet throughout the experiment containing either 2.5% taurine and 2.5% niacin or the same diet without the drugs. The four groups were saline-instilled with the control diet (SA + CD), saline-instilled with TN in the diet (SA + TN), BL-instilled with the control diet (BL + CD), and BL-instilled with the TN diet (BL + TN). Steady state transcript levels in total RNA prepared from lungs of all four groups were determined at 0, 3, 7, 14 and 21 days after the last BL instillation by slot blot and Northern blot analyses. Results indicate that procollagen I mRNA levels are elevated compared with saline control by 2.5-, 2.4- and 2.0-fold at 7, 14, and 21 days after the last dose of BL instillation, respectively. Dietary treatment with taurine and niacin decreased the steady state level of BL-induced increases of procollagen I mRNA from day 0 through 21. We observed a similar pattern of procollagen III inhibition by taurine and niacin from day 3 through day 21. Transcription of procollagen I and III genes was readily detected in nuclei prepared from BL-treated lung samples at 14 days after treatment. In contrast, transcription of procollagen I and III genes was barely detectable in nuclei prepared at the same time point from BL + TN treated lungs. Our results suggest that procollagen I and III gene expression in BL-induced lung fibrosis in hamsters is transcriptionally down-regulated by combined treatment with taurine and niacin.

Pulmonary fibrosis is a common final pathway for many forms of lung injury and it is characterized by alteration in the amount and organization of the extracellular matrix of the lung (Crouch, 1990). Bleomycin-induced lung fibrosis is a complex process and it involves the participation of many cell types. Increased synthesis and deposition of collagen result in an increased lung collagen content and distortion of the pulmonary structure and architecture (Goldstein and Fine, 1986; Laurent *et al.*, 1981). Disturbances in the timely expression of a variety of cytokines and growth factors are likely to contribute to the ineffective repair process that result in fibrosis (Kelley, 1990; Phan and Kunkel, 1992; Scheule *et al.*, 1992; Santana *et al.*, 1995).

Increased collagen deposition in lung results from alterations of both synthesis and degradation of newly synthesized collagen (Laurent and McAnulty, 1983). In BL-induced fibrotic lung, collagen synthesis is selectively increased while collagen degradation is decreased. Procollagen type I and procollagen type III steady state mRNAs are elevated in BL-induced ILF (Kelley *et al.*, 1985; Raghow *et al.*, 1985). These changes in type I mRNA accumulation are associated with an increases in the rates of procollagen gene transcription (Raghow *et al.*, 1985). Growth factors may increase the accumulation of type I procollagen mRNAs and collagen synthesis by transcriptional and/or post-transcriptional processes (Breen *et al.*, 1992).

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ABBREVIATIONS: ILF, interstitial lung fibrosis; i.t., intratracheal; BL, bleomycin; PC I, procollagen I; PC III, procollagen III; TN, taurine and niacin; SA, saline; CD, control diet; cpm, counts per minute; RNA, ribonucleic acid; mRNA, messenger RNA; DNA, deoxyribonucleic acid; cDNA, complementary DNA; GADPH, glyceraldehyde-3-phosphate dehydrogenase; SSPE, sodium chloride sodium phosphate ethylenediamine tetraacetic acid; SDS, sodium dodecyl sulfate; EDTA, ethylenediamine tetraacetic acid; DTT, dithiothreitol; Tris, tris (hydroxymethyl) aminomethane; tRNA, transfer RNA; UTP, uridine triphosphate; S.E.M., standard error of the mean; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

Therapeutic advances in the management of ILF have been less than satisfactory despite the evaluation of a diversified group of compounds to prevent collagen accumulation in rodent models of lung fibrosis (Giri, 1990). Taurine and niacin have previously been found to reduce the accumulation of lung collagen in a multidose BL hamster model of ILF (Wang *et al.*, 1991). However, the molecular mechanism for the antifibrotic effect of the combined treatment with taurine and niacin has not been examined. The present study was designed to evaluate whether taurine and niacin treatment blocks the BL-induced increased accumulation of procollagen I and III mRNAs at their gene transcriptional levels.

Materials and Methods

Treatment of Animals

Male Golden Syrian hamsters weighing 90 to 110g were purchased from Simonsens, Inc. (Gilroy, CA). 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 the National Institutes of Health for animal welfare. The hamsters were allowed to acclimate in facilities for 1 week before all treatments. A 12 hr/12 hr light/dark cycle was maintained, and hamsters had access to water and either pulverized Rodent Laboratory Chow 5001 (Purina Mills, Inc., St. Louis, MO) or the same pulverized chow containing 2.5% taurine and 2.5% niacin (w/w). The animals were fed these diets starting 3 days before the first i.t. instillation and continuing throughout the course of the experiment. Under pentobarbital anesthesia, hamsters were i.t. instilled with three consecutive doses of saline or BL at weekly intervals (2.5, 2.0, 1.5 units/5 ml/kg) as described in our earlier paper (Wang *et al.*, 1991). Animals were randomly divided into four experimental groups: the four groups were saline-instilled with a control diet (SA + CD); saline-instilled with the TN diet (SA + TN), BL-instilled with the control diet (BL + CD); and BL-instilled with the TN diet (BL + TN).

Animals were sacrificed at 0, 3, 7, 14, and 21 days after the last BL or saline instillation by decapitation, and their lungs were removed and quickly frozen in liquid N_2, then stored at −80°C until used for mRNA analysis. A major portion of each lung sample was used for direct RNA isolation, and the remainder was used for hydroxyproline measurement. Hydroxyproline was measured according to the procedure of Woessner (1961).

Molecular Probes

HF677, a 1.8-kb EcoRI fragment of procollagen I (α_1) cDNA, clone HF934, a 1.3kb EcoRI + HindIII fragment of procollagen III (α_1) cDNA, clone HHCPF19 containing the cDNA insert of GAPDH (1kb EcoRI fragment) and 18S ribosomal RNA clone PN29III with a 0.752kb BamHI and SphI cDNA insert were obtained from American Type Culture Collection (Rockville, MD). The plasmid pBR322 was purchased from Pharmacia (Piscataway, NJ). Using standard procedures, plasmids were isolated and inserts were purified with a Qiagen gel extraction kit (Qiagen, Chatsworth, CA) after complete restriction endonuclease digestion. We normalized the steady state mRNA levels of procollagen I and III relative to 18S rRNA levels because, in preliminary experiments, we found that mRNA levels of the so-called "housekeeping" genes β-actin and GAPDH increased 2-fold at 7 days after BL administration. The induction of these genes after lung injury has been previously reported (Raghow *et al.*, 1985). 18S rRNA has been used as an alternative standard to normalize the amount of RNA applied to slot and Northern blots.

Total RNA Isolation and Hybridization Analyses

The single-step method of RNA isolation using acid guanidinium thiocyanate-phenol-chloroform extraction was used to isolate cellular RNA from hamster whole lung samples (Chomczynski and Sacchi, 1987). Slot blot analysis was used to determine the quantitative differences in procollagen mRNA levels. Five μg of total RNA was directly spotted onto a Nytran (Schleicher and Schuell, Keene, NH) membrane using a Minifold II slot blot system (Schleicher and Schuell, Keene, NH). The Nytran membrane was UV cross-linked and hybridized. The specific activities of the probes were $1–2 \times 10^8$ cpm/μg of cDNA. RNA hybridization and washings were conducted as described previously (Gurujeyalakshmi and Giri, 1995). Band intensities were quantified by densitometric scanning using a dual-wavelength flying spot scanning densitometer (Model CS-9301PC, Shimadzu, Columbia, MD) and expressed as arbitrary units per μg of total RNA.

Northern blot experiments were performed to determine the effect of dietary intake of taurine and niacin on the levels of procollagen I and III mRNA in BL-treated hamster lungs. Briefly, total RNA (10 μg/lane) was electrophoresed through 1% agarose/2.2 M formaldehyde gels and transferred to a nylon membrane. The samples were prehybridized at 42°C for 2 hr in a solution containing 50% formamide, 5 × SSPE, 0.3% SDS and 200 μg/ml sheared salmon sperm DNA. The membranes were hybridized either with procollagen I or III cDNA probe (2×10^6 cpm of probe per ml of hybridization solution) at 42°C for 20 hr. Radiolabeled probes were prepared by the random primer method (Bio-Rad, Richmond, CA). After hybridization, the membranes were washed as described before (Gurujeyalakshmi and Giri, 1995) and exposed to Fuji X-ray film for 24 to 48 hr at −80°C with an intensifying screen.

Nuclear Runoff Transcription Analysis

Relative rates of nuclear transcription were assessed by following the method of Celano *et al.*, (1989). Nuclei were isolated from BL and BL + TN treated lungs (14 days after the last BL instillation) by the method of Marzluff and Huang (1984). The nuclear pellet was resuspended in nuclear storage buffer (40% glycerol; 5 mM magnesium acetate; 0.1 mM EDTA; 5 mM DTT; 50 mM Tris-HCl, pH 8) at a density of approximately 1×10^8 nuclei/ml. The nuclei were then counted with a hemocytometer and diluted into aliquots of approximately 1×10^7 nuclei. The nuclei were quick-frozen in liquid nitrogen and stored at −80°C or processed directly for the next step.

Transcription assays were performed in parallel using an equal number of nuclei isolated from the lung of BL or BL + TN treated hamsters. Transcription reactions were initiated by mixing 100 μl of the nuclei ($1–2 \times 10^7$) with 100 μl of the reaction buffer containing 10 mM Tris-HCl, pH 8; 5 mM $MgCl_2$; 0.3 M KCl; 5 mM DTT; and 1 mM each of adenosine triphosphate, cytidine triphosphate and guanosine triphosphate and 10 μl of (α-^{32}P) UTP (3,000 Ci/mmol, DuPont, DuPont NEN, Boston, MA) and then incubated at room temperature for 30 min. The incubation was terminated by incubation with ribonuclease free deoxyribonuclease I (10 μl of 20,000U/ml; Boehringer Manheim, Indianapolis, IN) and 20 μl of 20 mM $CaCl_2$ at room temperature for 5 min. The mixture was subsequently incubated with 25 μl of 10 × SET buffer (5% SDS; 50 mM EDTA; 10 mM Tris-HCl, pH 7.4), 5 μl of 10 mg/ml yeast tRNA and 2 μl of 10 mg/ml proteinase K at 37°C for 30 min. After the incubation, the RNAs were purified by the single step acid guanidinium thiocyanate-phenol-chloroform method.

Slot blot membrane containing procollagen I, III, GAPDH and 18S rRNA were prepared before RNA extraction. Ten μg of plasmid with cDNA inserts were used for each slot and an insert free pBR322 vector was included as a control for nonspecific binding. Plasmids were linearized using appropriate restriction enzymes, denatured by treatment with NaOH at 37°C for 10 min, then neutralized and blotted onto Nytran membrane by using a slot blot Minifold II. Membranes were air dried at room temperature for 30 min and UV cross-linked. Membrane strips were prehybridized in 6xSSPE, 0.5% SDS and 200 μg/ml sheared salmon sperm DNA for 2 hr at 65°C and hybridized with equal numbers of counts of ^{32}P-labeled RNA. After hybridizing for 36 hr at 65°C, the strips were washed twice in 2xSSC, 0.1% SDS at room temperature for 15 min, followed by two 30-min

washes at 65°C in 2xSSC, 0.1% SDS and one 30-min wash at 65°C in 0.1xSSC and 0.1% SDS. The strips were air dried and exposed to Fuji (Fisher Scientific, Pittsburgh, PA) X-ray film. Specific DNA-RNA binding was evaluated by autoradiography and densitometry.

Statistical Analysis

All values are expressed as the mean ± one S.E.M.. The data were compared within the four groups at the corresponding times using a one-way analysis of variance and the Fisher's Least Significant Difference test (SAS Institute Inc., 1985). A value of $P \leq .05$ was considered to be the minimum level of statistical significance.

Results

Regulation of Collagen Production by Taurine and Niacin

Hydroxyproline is measured as an index of collagen content. We investigated the relative rates of collagen accumulation in whole lung extracts at various time points from saline or BL instilled hamsters with and without dietary intake of taurine and niacin. Hydroxyproline content in the lungs of all control hamsters averaged 1,046 ± 152 μg/lung (fig. 1). At 3, 7, 14, and 21 days after the last BL instillation, the hydroxyproline content of the injured lungs increased to 133%, 145%, 166% ($P < .05$), and 210% ($P < .05$) of their corresponding control groups (SA + CD), respectively. In contrast, the combined treatment with taurine and niacin reduced the BL-induced increases of hydroxyproline content to the level of control values; significant reductions ($P < .05$) were observed from 1,734 ± 307 μg/lung (BL + CD group) to 1,052 ± 20 μg/lung (BL + TN) at 14 days and from 2,204 ± 390 μg/lung to 1,285 ± 213 μg/lung at 21 days in the corresponding groups.

Regulation of Procollagen I mRNA Accumulation by Taurine and Niacin

Studies were undertaken to determine whether taurine- and niacin-mediated reduction in collagen accumulation in the BL + TN group was associated with comparable alterations in procollagen I mRNA accumulation. Total RNA preparations from three hamsters in each of the four groups: SA + CD; SA + TN; BL + CD; and BL + TN were analyzed by slot blot hybridization. Duplicate samples for each hamster were assayed and serial dilutions for a subset of the samples were also analyzed to ensure that all measurements were made under linear conditions. As shown in figure 2, i.t. instillation of saline in SA + CD and SA + TN groups did not cause any change in procollagen I mRNA. The level of procollagen I mRNA expression in the lungs of BL-instilled hamsters in BL + CD and BL + TN groups during the early time points (0–3 days) was not significantly different from that in the saline-treated hamster lungs in SA + CD or SA + TN group. However, the message for procollagen I was increased in lung tissue at day 7 after instillation of BL, peaking at day 14 and remaining high until day 21 in BL + CD group. Procollagen I mRNA was significantly ($P < .05$) up-regulated in the lung of this group of hamsters at 7,14 and 21 days, as compared with hamsters in all other groups. Taurine and niacin treatment was found to down-regulate the BL-induced overexpression of the steady state levels of procollagen I mRNA in lung samples. This was statistically significant ($P < .05$) compared with the corresponding BL + CD groups at 7, 14 and 21 days after the last BL-instillation. The down-regulation at these time points ranged from 35% to 50% compared with the BL + CD group. Hybridization with the 18S rRNA control probe confirmed that equivalent amounts of RNA were loaded in each slot. According to these results, it took at least 7 days for the combined treatment with taurine and niacin to cause a significant down-regulation of procollagen I mRNA after the last dose of BL instillation. Northern blot analysis also confirmed the effect of taurine and niacin in down-regulating the BL-induced increases of procollagen I mRNA expression (fig. 3).

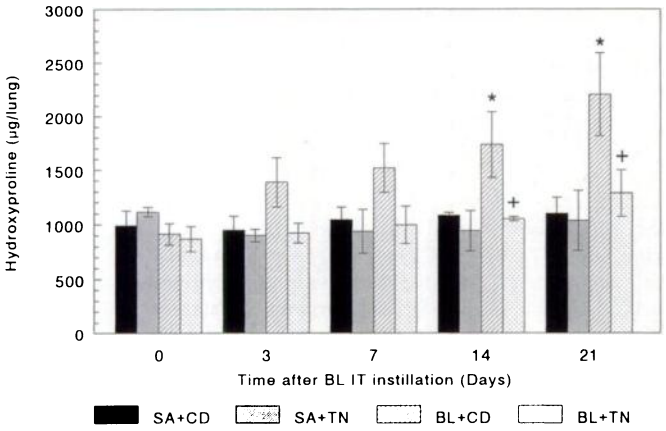

Fig. 1. Effect of i.t. instillation of saline (control) or bleomycin with and without dietary intake of taurine and niacin on hydroxyproline content of hamster lungs at different times after instillation. See the Materials and Methods for treatment details. Briefly, SA + CD: saline control; SA + TN: saline+ taurine + niacin; BL + CD: bleomycin alone; and BL + TN: bleomycin + taurine + niacin. Each value represents the means ± S.E.M. of three animals in duplicate experiments. (*) Significantly higher ($P < .05$) than all other groups at the corresponding times, and (+) significantly lower ($P < .05$) than the BL + CD group at the corresponding time.

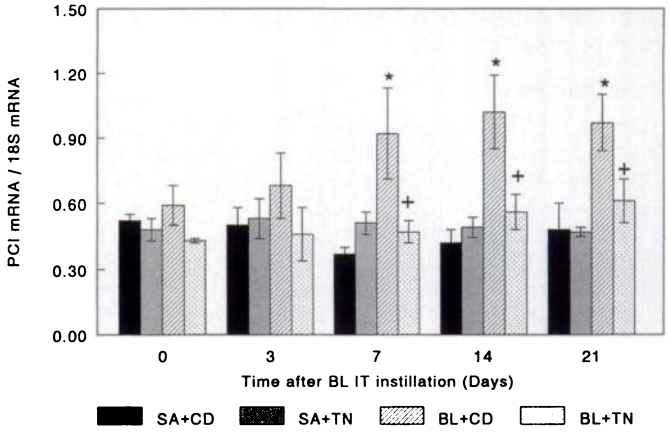

Fig. 2. Effect of dietary intake of taurine and niacin on BL-induced increases on the steady state level of procollagen I mRNA in hamster lungs. Procollagen I mRNA levels were examined at 0, 3, 7, 14 and 21 days after the last dose of saline or BL instillation. Zero (0) time represents expression after the last dose of saline or BL instillation. Procollagen I mRNA was quantitated as described in Materials and Methods. See the legend to figure 1 for the explanation of abbreviations and experimental details. Each value represents the mean ± S.E.M. of three animals in duplicate experiments. (*) Significantly higher ($P < .05$) than all other groups at the corresponding times, and (+) significantly lower ($P < .05$) than the BL + CD group at the corresponding time.

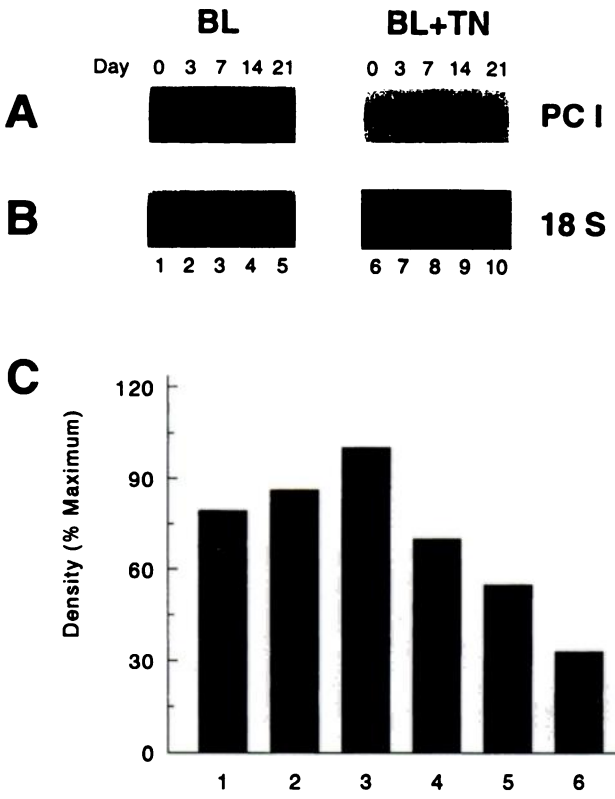

Fig. 3. Demonstration of the effect of taurine and niacin on procollagen I mRNA accumulation in BL-instilled hamster lungs. Total RNA was extracted and (A) Procollagen I mRNA was quantitated as described in Materials and Methods. The membrane shown in panel A was hybridized with procollagen I cDNA and then rehybridized with a cDNA probe for 18S rRNA as shown in panel B. Panel C shows a densitometric analysis of the autoradiogram shown in panel A.

Regulation of Procollagen III mRNA Accumulation by Taurine and Niacin

Previous data from our laboratory have suggested that the extent of procollagen I mRNA accumulation is greater than the extent of procollagen III mRNA levels in the lungs of BL-treated mice (Gurujeyalakshmi and Giri, 1995). To ascertain the specificity of the increased procollagen III mRNA quantities in BL-treated hamster lungs, we also quantified the levels of procollagen III mRNA by slot blot analysis. Procollagen III mRNA levels in BL + CD group of hamsters were significantly ($P < .05$) elevated at 3, 7, 14 and 21 days, after the last dose of BL instillation as compared with SA + CD, SA + TN and BL + TN groups at the corresponding times. (fig. 4). As compared with the BL + CD group at the corresponding time, the dietary intake of taurine and niacin in BL + TN group also caused reductions in the steady state level of lung procollagen III mRNA by 46% ($P < .05$), 51% ($P < .05$), 32% and 35% ($P < .05$) at 3, 7, 14 and 21 days after the last dose of BL instillation, respectively. Northern blot analysis also supported these findings (fig. 5).

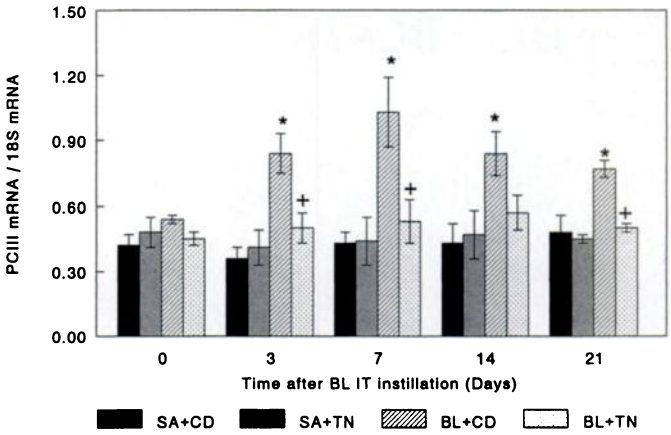

Fig. 4. Effect of dietary intake of taurine and niacin on BL-induced increases in the steady state level of procollagen III mRNA in hamster lungs. Procollagen III mRNA expression was examined at 0, 3, 7, 14, and 21 days after last dose of saline or BL instillation. Zero (0) time represents expression after the last dose of saline or BL instillation. Procollagen III mRNA was quantitated as described in Materials and Methods. See the legend to figure 1 for the explanation of abbreviations and experimental details. Each value represents the mean ± S.E.M. of three animals in duplicate experiments. (*) Significantly higher ($P < .05$) than all other groups at the corresponding time points, and (+) significantly lower ($P < .05$) than BL + CD group at the corresponding time.

Regulation of Procollagen I and Procollagen III Genes by Taurine and Niacin at the Transcriptional Level

Nuclear run-off analysis was performed to determine whether the inhibitory effect of taurine and niacin on BL-induced increases in the procollagen I and III mRNAs, which encode increased production of corresponding collagen I and III types, is mediated by an alteration in the gene transcription. The specificity of the assay was demonstrated by the inhibition of the incorporation of [α-^{32}P] UTP by α-amanitin, an inhibitor of RNA polymerase II (data not shown) and by a lack of hybridization of these transcripts to the plasmid pBR322 DNA. Newly synthesized RNA transcripts from lung nuclei of hamsters in SA + CD, SA + TN, BL + CD and BL + TN groups were isolated at 14 days after i.t. instillation of saline or BL as described in the Methods section. As shown in figure 6 and table 1, the labeled transcripts that hybridized to the cDNAs of procollagen I and III were decreased more than 10- and 4.5-fold, respectively, in BL + TN group of hamsters when compared with the BL + CD group. Transcription of procollagen I and III was readily detected in nuclei prepared from the lungs of hamsters in the BL + CD group. In contrast, transcription of pro-

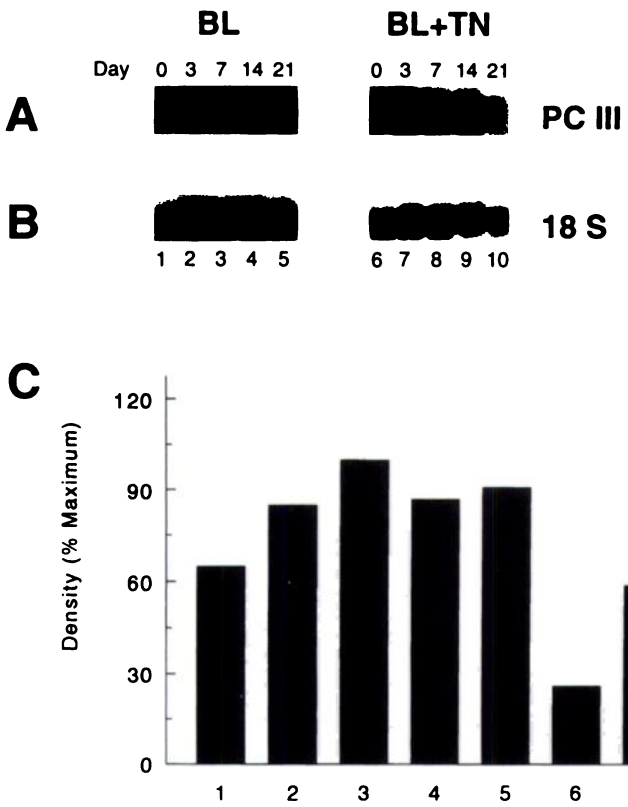

Fig. 5. Demonstration of the effect of taurine and niacin on procollagen III mRNA accumulation in BL-instilled hamster lungs. Total RNA was extracted and (A) Procollagen III mRNA was quantitated as described in Materials and Methods. The membrane shown in panel A was hybridized with procollagen III cDNA and then rehybridized with a cDNA probe for 18S rRNA as shown in panel B. Panel C shows a densitometric analysis of the autoradiogram shown in panel A.

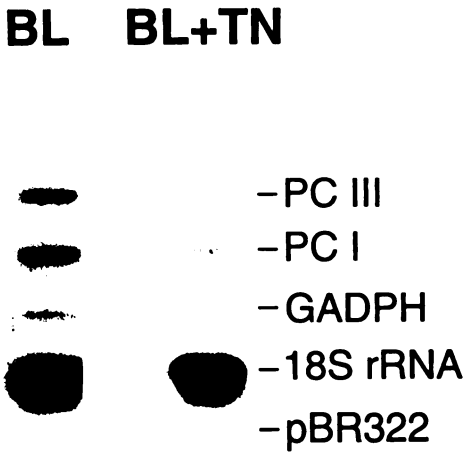

Fig. 6. Effect of taurine and niacin on procollagen I and III in BL-instilled hamster lungs. Nuclei were isolated from the lungs of hamsters in BL + CD and BL + TN groups from four animals at 14 days after last i.t. instillation of BL. *In vitro* transcription assays were performed with [α-^{32}P] UTP as described in Materials and Methods. The nascent ^{32}P-labeled RNAs (3–5 × 10^6cpm) from each assay were hybridized to 10 μg of plasmid procollagen I, III, GADPH and 18S rRNA containing the cDNA inserts, which had been denatured and immobilized on Nytran membranes. Plasmid pBR322 with no insert served as a control for nonspecific binding. The autoradiography of the strips were performed at −80°C with intensifying screen for 1–2 days.

TABLE 1

Rates of transcription of procollagen I (α_1), III (α_1), GAPDH and 18S rRNA genes in nuclei isolated from BL + CD- and BL + TN-treated hamster lungs

Nuclei were prepared from lungs of BL + CD or BL + TN group at 14 days after the last i.t. instillation of BL. They were incubated in a reaction mixture containing all ingredients for run-off transcription. The nascent ^{32}P-labeled RNAs (3–5 × 10^6 cpm) from each assay were hybridized to 10 μg of plasmid procollagen I, III, GAPDH and 18S rRNA containing the cDNA inserts, which had been denatured and immobilized on nytran membranes. Mean average values of ^{32}P-labeled nascent transcripts specifically hybridized to a given plasmid DNA were derived from four individual animals, and the peak areas of each signal were scanned densitometrically. The peak area values are presented as mean ± S.E.M. of four animals.

Probe	Densitometry Reading	
	BL + CD	BL + TN
Procollagen I (α_1)	2,016 ± 98	205 ± 25
Procollagen III (α_1)	1,335 ± 75	302 ± 18
GAPDH	295 ± 18	143 ± 14
18S rRNA	17,831 ± 1,240	14,766 ± 1,332

collagen I and III genes was barely detectable in nuclei prepared from the lungs of hamsters in BL + TN group. The hamsters in SA + CD and SA + TN groups also exhibited levels of gene transcription similar to the BL + TN group (data not shown). There was no alteration in the rate of transcription of the gene encoding for 18S rRNA in the lung nuclei of hamster either in BL + CD or BL + TN groups. These studies demonstrate that taurine and niacin treatment down-regulates the BL-induced overexpression of procollagen I and III mRNAs, at least partially, at the transcriptional level.

Discussion

Our laboratory has previously reported that dietary intake of taurine and niacin before and after BL-instillation is effective in preventing lung collagen accumulation in the multidose BL-hamster model of lung fibrosis (Wang *et al.*, 1991) and amiodarone-induced lung fibrosis (Wang *et al.*, 1992). In the present study, attempts were made to investigate the mechanism(s) by which the combined treatment with taurine and niacin ameliorates BL-induced lung fibrosis. To determine whether tran-

scriptional and/or post-transcriptional processes were involved, the effects of dietary intake of taurine and niacin on procollagen mRNA accumulation and gene transcription were evaluated. The results of the present study demonstrate that the ameliorating effect of taurine and niacin against BL-induced lung collagen accumulation possibly resides in their ability to cause a proportionate reduction in the BL-induced overexpression of procollagens I and III mRNA accumulation. Because accumulation of mRNA is a function of gene transcription, it is likely that taurine and niacin down-regulate the BL-induced overexpression of mRNA at the transcriptional level. However, a decreased rate of mRNA degradation cannot be ruled out at this time.

It has been reported that production of TGF-β (Khalil *et al.*, 1989, Raghow *et al.*, 1987) TNF-α (Piguet *et al.*, 1989) and interleukin-1 (Suwabe *et al.*, 1988) are up-regulated in BL-induced pulmonary fibrosis. These cytokines may play a crucial role in the pathogenesis of lung fibrosis through stimulation of collagen and fibronectin production by fibroblasts and inhibition of the biosynthesis of proteases that degrade the extracellular matrix (Sporn *et al.*, 1987). The role of these cytokines in the development of BL-induced lung fibrosis is further strengthened by the finding that treatment with anti-TNF-α antibody (Piguet *et al.*, 1989) or anti-TGF-β antibody (Giri *et al.*, 1993) caused a marked reduction in BL-induced lung fibrosis. In addition, a long-term dietary supplementation with vitamin E in animals undergoing CCL_4-induced liver fibrosis resulted in a net inhibition of both hepatic TGF-β_1 and procollagen α_2(I) mRNA levels (Parola *et al.*, 1992). In fact, taurine, which shares the antioxidant effect of vitamin E, in combination with niacin, was found to down-regulate the BL-induced overexpression of TGF-β mRNA in our preliminary study (Gurujeyalakshmi, Iyer, Hollinger, and Giri, unpublished). Therefore, it is possible that the combined treatment with taurine and niacin produces antifibrotic effect by way of down-regulating the synthesis of cytokines that are generally up-regulated in the BL-rodent model of lung fibrosis (Khalil *et al.*, 1989; Raghow *et al.*, 1989; Piguet *et al.*, 1989; Suwabe *et al.*, 1988). Whether taurine and niacin administered alone will produce a similar effect is not known at this time. However, it should be noted that these two compounds were demonstrated to produce antifibrotic effect independently in the single BL-hamster model of lung fibrosis (Wang *et al.*, 1989; Wang *et al.*, 1990).

Our study is the first to demonstrate the relative importance of the transcriptional mechanism by which the combined treatment with taurine and niacin inhibits collagen production. Additional investigations are underway to determine whether other mechanisms mediate the transcriptional effects of taurine and niacin on fibrogenic cytokines, including interleukin-1, TNF-α and TGF-β. The demonstration in the present study that dietary intake of taurine and niacin inhibits the production of collagen *via* a transcriptional mechanism stresses the importance of their interaction and supports the contention that taurine and niacin might modulate the fibrotic event by altering collagen mRNA levels acting directly at the gene transcriptional level and/or *via* fibrogenic cytokines.

References

Breen, E., Shull, S., Burne, S., Absher, M., Kelly, J., Phan, S. and Cutroneo, K. R.: Bleomycin regulation of transforming growth factor-β mRNA in rat lung fibroblasts. Am. J. Respir. Cell Mol. Biol. **6:** 146–152, 1992.

Celano, P., Berchtold, C. and Casero, Jr., R. A.: A simplification of the nuclear runoff assay. Biotechniques. **7:** 942–944, 1989.

Chomczynski, P. and Sacchi, S. N.: Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:** 156–159, 1987.

Crouch, E.: Pathobiology of pulmonary fibrosis. Am. J. Physiol. **259:** 159–184, 1990.

Giri, S. N., Hyde, D. M. and Hollinger, M. A.: Effect of antibody to transforming growth factor-β on bleomycin induced accumulation of lung collagen in mice. Thorax **48:** 959–966, 1993.

Giri, S. N.: Pharmacologic perspective in pulmonary fibrosis research. *In*: Focus on Pulmonary Pharmacology and Toxicology. ed. by M. A. Hollinger, pp. 19–55, CRC, Boca Raton, 1990.

Goldstein, R. H. and Fine, A.: Fibrotic reactions in the lung: the activation of the lung fibroblast. Exp. Lung Res. **11:** 245–261, 1986.

Gurujeyalakshmi, G. and Giri, S. N.: Molecular mechanisms of antifibrotic effect of interferon gamma in bleomycin-mouse model of lung fibrosis: Down-regulation of TGF-β and procollagen I and III gene expression. Exp. Lung. Res. **21:** 791–808, 1995.

Kelley, J., Chrin, L., Shull, S., Rowe, D. W. and Cutroneo, K. R.: Bleomycin selectively elevates mRNA levels for procollagen and fibronectin following acute lung injury. Biochem. Biophys. Res. Commun. **131:** 836–843, 1985.

Kelley, J.: Cytokines of the lung. Am. Rev. Respir. Dis. **141:** 765–788, 1990.

Khalil, N., Berezary, O., Sporn, M. B. and Greenberg, A. H.: Macrophage production of transforming growth factor-β and fibroblast collagen synthesis in chronic pulmonary inflammation. J. Exp. Med. **170:** 727–737, 1989.

Laurent, G. J., Mcanulty, R. J., Corrin, B. and Cockerill, P.: Biochemical and histological changes in pulmonary fibrosis induced in rabbits with intratracheal bleomycin. Eur. J. Clin. Invest. **11:** 441–448, 1981.

Laurent, G. J. and Mcanulty, R. J.: Protein metabolism during bleomycin-induced pulmonary fibrosis in rabbits. Am. Rev. Respir. Dis. **128:** 82–88, 1983.

Marzluff, W. F. and Huang, R. C. C.: Transcription of RNA in isolated nuclei. *In*: Transcription and Translation: A Practical Approach, ed. by B. D. Hames and S. J. Higgins, p. 89, IRL press, Oxford, England, 1984.

Parola, M., Muraca, R., Dianzani, I. and Barrera, G.: Vitamin E dietary supplementation inhibits transforming growth factor-β_1, gene expression in the rat liver. FEBS Lett. **308:** 267–270, 1992.

Phan, S. H. and Kunkel, S. L.: Lung cytokine production in bleomycin-induced pulmonary fibrosis. Exp. Lung Res. **18:** 29–43, 1992.

Piguet, P. F., Collart, M. A., Grau, G. E., Kapanci, Y. and Vassali, P.: Tumor necrosis factor/Cachectin plays a key role in bleomycin-induced pneumopathy and fibrosis. J. Exp. Med. **170:** 655–663, 1989.

Raghow, R., Lurie, S., Seyer, J. M. and Kang, A. H.: Profiles of steady state levels of mRNAs coding for type I procollagen, elastin and fibronectin in hamster lungs undergoing bleomycin-induced interstitial pulmonary fibrosis. J. Clin. Invest. **76:** 1733–1739, 1985.

Raghow, R., Postlethwaite, A. E., Keski-Oja, J., Moses, H. L. and Kang, A. H.: Transforming growth factor-β increases steady state levels of type I procollagen and fibronectin mRNAs posttranscriptionally in cultured human dermal fibroblasts. J. Clin. Invest. **79:** 1258–1288, 1987.

Santana, A., Saxena, B., Noble, N. A., Gold, L. I. and Marshall, B. C.: Increased expression of transforming growth factor β isoforms (β_1, β_2, β_3) in bleomycin-induced pulmonary fibrosis. Am. J. Respir. Cell Mol. Biol. **13:** 33–34, 1995.

Sas Institute Inc. SAS/STAT™ Guide for Personal Computers. Version 6. ed. by SAS Institute Inc., Cary, NC, 1985.

Scheule, R. K., Perkins, R. C., Hamilton, R. and Holian A.: Bleomycin stimulation of cytokine secretion by the human alveolar macrophage. Am. J. Physiol. **262:** L386–L391, 1992.

Sporn, M. B., Roberts, A. B., Wakefield, L. M. and deCrombruggle, B.: Some recent advances in the chemistry and biology of transforming growth factor-β. J. Cell. Biol. **105:** 1039–1045, 1987.

Suwabe, A., Takahashi, K., Yasui, S., Arai, S. and Sendo, F.: Bleomycin-stimulated hamster alveolar macrophages release interleukin-1. Am. J. Pathol. **132:** 512–520, 1988.

Wang, Q., Giri, S. N., Hyde, D. M. and L. I., C.: Amelioration of bleomycin-induced pulmonary fibrosis in hamsters by combined treatment with taurine and niacin. Biochem. Pharmacol. **42:** 1115–1122, 1991.

Wang, Q., Giri, S. N., Hyde, D. M. and Nakashima, J. M.: Effect of taurine on bleomycin-induced lung fibrosis in hamsters. Proc. Soc. Exp. Biol. Med. **190:** 330–338, 1989.

Wang, Q., Giri, S. N., Hyde, D. M., Nakashima, J. M. and Javadi, I.: Niacin attentuates bleomycin-induced lung fibrosis in the hamster. J. Biochem. Toxicol. **5:** 13–22, 1990.

Wang, Q., Hollinger, M. A. and Giri, S. N.: Attenuation of amiodarone-induced lung fibrosis and phospholipidosis in hamsters by taurine and/or niacin treatment. J. Pharmacol. Exp. Ther. **262:** 127–132, 1992.

Woessner, J. F., Jr.: The determination of hydroxyproline in tissue and protein samples containing small proportion of this amino acid. Arch. Biochem. Biophys. **93:** 440–444, 1961.

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