Protective effect of orally administered carnosine on bleomycin-induced lung injury

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Carnosine reduces proinflammatory and profibrotic cytokines such as TGF-β, IL-1, and TNF-α in different experimental settings. In the present study, we investigated the efficacy of carnosine on the animal model of bleomycin-induced lung injury. Mice were subjected to intratracheal administration of bleomycin and were assigned to receive carnosine daily by an oral bolus of 150 mg/kg. One week after fibrosis induction, bronchoalveolar lavage (BAL) cell counts and TGF-β1 levels, lung histology, and immunohistochemical analyses for myeloperoxidase, TGF-β1, inducible nitric oxide synthase, nitrotyrosine, and poly(ADP-ribose) polymerase were performed. Finally, apoptosis was quantified by terminal deoxynucleotidyltransferase-mediated UTP end-labeling assay. After bleomycin administration, carnosine-treated mice exhibited a reduced degree of lung damage and inflammation compared with wild-type mice, as shown by the reduction of body weight, mortality rate, lung infiltration by neutrophils (myeloperoxidase activity and BAL cell counts and TGF-β1 levels), lung histology, and apoptosis. Our results indicate that orally administered carnosine is able to prevent bleomycin-induced lung injury likely through its direct antioxidant properties. Carnosine is already available for human use. It might prove useful as an add-on therapy for the treatment of fibroblastic disorders of the lung where oxidative stress plays a role, such as for idiopathic pulmonary fibrosis, a disease that still represents a major challenge to medical treatment.

IDIOPATHIC PULMONARY FIBROSIS (IPF) is the most common among interstitial pneumonias of unknown origin and one of the most aggressive interstitial lung diseases. It is characterized by a chronic and progressive course leading to respiratory failure with a median survival under 3 yr (1, 43). Pathological findings in this disease include temporally and spatially non-homogeneous areas of inflammation, fibrosis, and honeycombing. Microscopically, the hyperplasia of type II pneumocytes and active fibroblasts proliferation leads to excessive matrix deposition resulting in the irreversible distortion of the lung architecture (56), which in turn is responsible for impaired gas exchanges and respiratory failure.

A number of exogenously administered agents, including commonly used drugs, are known to induce a iatrogenic form of pulmonary fibrosis (57). Bleomycin is an efficacious antitumor agent currently used in humans. Nevertheless, repeated administration of this drug may lead to lung inflammation and fibrosis as a side effect. Because this phenomenon is easily reproduced in different mammals, intratracheal administration of bleomycin has become the most widely used experimental model of lung fibrosis, although with certain limitations. This model is characterized by an early neutrophilic response, increased collagen deposition, and fibroblast proliferation (13). Bleomycin alters the balance between oxidants and antioxidant defense systems in the lung. In this particular organ, the selective absence of bleomycin hydrolase activity gives a high susceptibility to bleomycin-induced oxidative stress (20). Contemporarily, hydroxyl radicals, superoxide anion radical, hydrogen peroxide, and peroxynitrite are increased by bleomycin administration (51). Reactive oxygen species (ROS) overproduction ultimately results in tissue injury, with activation of several intracellular signaling pathways leading to the production of proinflammatory cytokines (33). DNA is a target for ROS activity as well. Radical oxygen species production, by determination of DNA damage, in turn activates poly(ADP-ribose) polymerase (PARP). This largely expressed nuclear protein contributes to the maintenance of genomic stability and to the repair of oxidative DNA damage (5). Although PARP activity promotes cell survival, PARP activation depletes NAD+ and decreases ATP levels, thus leading to cell death after extensive DNA strand breaks (14). Therefore, ROS produced in response to oxidative stress are able to contribute by multiple pathways to the pathogenesis of bleomycin-induced lung injury.

Carnosine is an endogenously synthesized dipeptide composed of β-alanine and l-histidine, which is present abundantly in muscle and nervous tissue in many species (52). It acts as a...
physiological buffer, a metal ion chelator, a free radical scavenger, and finally as an antioxidant (17, 35). Besides the known anti-aging properties of this dipeptide, it has been demonstrated that carnosine plays a role in inflammation. In fact, carnosine-inhibited hydrogen peroxide induced IL-8 release in vitro (58). IL-6 and TNF-α were reduced by the oral administration of carnosine in an animal model of diabetes (44), and finally carnosine proved to decrease the secretion of transforming growth factor-β (TGF-β) and of various extracellular matrix components induced by high doses of glucose in vitro (34).

Given the antioxidant and anti-inflammatory properties of carnosine, we sought to investigate carnosine efficacy on lung injury caused by bleomycin administration. To this end, we evaluated the following endpoints: 1) loss of body weight, 2) mortality rate, 3) infiltration of the lung with polymorphonuclear neutrophils (myeloperoxidase activity), 4) edema formation, 5) histological evidence of lung injury, 6) bronchoalveolar lavage (BAL) inflammatory cells counts, 7) TGF-β expression, inducible nitric oxide synthase (iNOS) activity, nitrotyrosine formation, and activation of the nuclear enzyme PARP, and 8) lung cells apoptosis [terminal deoxynucleotidyl-transferase-mediated UTP end-labeling (TUNEL) assay].

METHODS

Animals. Male CD mice (25–35 g; Harlan Nossan) were housed in a controlled environment and provided with standard rodent chow and water. The University of Messina Review Board (Italy) approved the study for the care of animals. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116192) as well as with the European Economic Community (EEC) regulations (Official Journal of EEC 358/1 12/18/1986).

Experimental groups. Mice were randomly allocated into the following groups. 1) Wild-type (WT) + bleomycin group: mice were subjected to bleomycin-induced lung injury (n = 15). 2) WT + saline group: this was a sham-operated group in which identical surgical procedures to the bleomycin group were performed, except that saline was administered instead of bleomycin (n = 15). 3) Carnosine group: mice in this group were treated the same as the WT + bleomycin group; however, mice were administered daily with an oral bolus of carnosine in PBS to a final dose of 150 mg/kg (Sigma, Milan, Italy) starting 30 min after bleomycin intratracheal administration. 4) Vehicle group: mice in this group were treated the same as carnosine group mice, except that only PBS was administered as control vehicle instead of carnosine (n = 15).

Induction of lung injury by bleomycin. Mice received a single intratracheal instillation of saline (0.9%), or saline containing bleomycin sulfate (1 mg/kg body wt) in a volume of 50 μl and were killed after 7 days by pentobarbital sodium overdose.

Measurement of fluid content in lung. The wet lung weight was measured after careful excision of extraneous tissues. The lung was exposed for 48 h at 180°C, and the dry weight was measured. Water content was calculated by subtracting dry weight from wet weight.

Histological examination. Lung biopsies were taken 7 days after injection of bleomycin. Lung biopsies were fixed for 1 wk in 10% (wt/vol) PBS-buffered formaldehyde solution at room temperature, dehydrated using graded ethanol, and embedded in Paraplast (Sherwood Medical, Mahwah, NJ). After specimens were embedded in paraffin, the sections were prepared and stained by trichrome stain. All sections were studied by light microscopy (Dialux 22 Leitz). The severity of fibrosis was semiquantitatively assessed according to the method proposed by Ashcroft et al. (2).

Briefly, the grade of lung fibrosis was scored on a scale from 0 to 8 by examining randomly chosen sections, with fields per sample at a magnification of ×100. Criteria for grading lung fibrosis were as follows: grade 0, normal lung; grade 1, minimal fibroblast thickening of alveolar or bronchiolar walls; grade 3, moderate thickening of walls without obvious damage to lung architecture; grade 5, increased fibrosis with definite damage to lung structure and formation of fibrous bands or small fibrous masses; grade 7, severe distortion of structure and large fibrous areas; and grade 8, total fibrous obliteration of the fields.

Immunohistochemical localization of iNOS, nitrotyrosine, PARP, and TGF-β. Tyrosine nitration, an index of the nitrosylation of proteins by peroxynitrite and/or ROS, was determined by immunohistochemistry as previously described (15). At the end of the experiment, the tissues were fixed in 10% (wt/vol) PBS-buffered formaldehyde, and 8-μm sections were prepared from paraffin-embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 0.3% hydrogen peroxide (vol/vol) in 60% (vol/vol) methanol for 30 min. The sections were permeabilized with 0.1% (wt/vol) Triton X-100 in PBS for 20 min. Nonselective adsorption was minimized by incubating the sections in 2% (vol/vol) normal goat serum in PBS for 20 min. Endogenous biotin or avidin binding sites were blocked by sequential incubation for 15 min with biotin and avidin (DBA, Milan, Italy), respectively. Sections were incubated overnight with anti-iNOS rat polyclonal antibody (1:500 in PBS, vol/vol), anti-nitrotyrosine rabbit polyclonal antibody (1:500 in PBS, vol/vol) or anti-poly(ADP-ribose) goat polyclonal antibody (1:500 in PBS, vol/vol), and finally anti-TGF-β rabbit polyclonal antibody (1:500 in PBS, vol/vol). Sections were washed with PBS and incubated with appropriate secondary antibodies. Specific labeling was detected with biotin-conjugated IgG and avidin-biotin peroxidase complex (DBA). To confirm that the immunoreaction for the nitrotyrosine was specific, some sections were also incubated with the primary antibody (anti-nitrotyrosine) in the presence of excess nitrotyrosine (10 mM) to verify the binding specificity. To verify the binding specificity for PARP, some sections were also incubated with only the primary antibody (no secondary) or with only the secondary antibody (no primary). In these situations, no positive staining was found in the sections, indicating that the immunoreaction was positive in all the experiments carried out.

TUNEL assay. TUNEL assay was performed using a TUNEL detection kit according to the manufacturer’s instructions (Apopatg, horse-radish peroxidase kit; DBA). Briefly, sections were incubated with 15 μg/ml protease K for 15 min at room temperature and then washed with PBS. Endogenous peroxidase was inactivated by 3% hydrogen peroxide for 5 min at room temperature and then washed with PBS. Sections were immersed in terminal deoxynucleotidyl transferase buffer containing deoxynucleotidyl transferase and biotinylated dUTP in terminal deoxynucleotidyl transferase buffer, incubated in a humid atmosphere at 37°C for 90 min, and then washed with PBS. The sections were incubated at room temperature for 30 min with anti-horse-radish peroxidase-conjugated antibody, and the signals were visualized with diaminobenzidine.

Myeloperoxidase activity assay. Myeloperoxidase activity, an indicator of polymorphonuclear leukocyte accumulation, was determined as previously described (49). At the specified time after injection of bleomycin, lung tissues were obtained and weighed, and each piece was homogenized in a solution containing 0.5% (wt/vol) hexadeccyltrimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 30 min at 20,000 g at 4°C. An aliquot of the supernatant was then allowed to react with a solution of tetramethylbenzidine (1.6 mM) and 0.1 mM hydrogen peroxide. The rate of change in absorbance was measured spectrophotometrically at 650 nm. Myeloperoxidase activity was defined as the quantity of enzyme degrading 1 μmol of peroxide per minute at 37°C and was expressed in milliunits per gram of wet tissue.

BAL. Seven days after bleomycin or saline solution instillation, mice were euthanized, and the trachea was immediately cannulated with an intravenous polyethylene catheter (Neo Delta Ven 2; delta Med, Viadana, Italy) equipped with a 24-gauge needle on a 1-mL
syringe. Lungs were lavaged once with 0.5 ml n-PBS (GIBCO, Paisley, UK). In >95% of the mice, the recovery volume was over 0.4 ml. The BAL fluid was spun at 800 rpm, the supernatant was removed, and the pelleted cells were collected. Total BAL cells were enumerated by counting on a hemocytometer in the presence of trypan blue. Cytospins were prepared from resuspended BAL cells.

Cytospins of BAL cells were made by centrifuging 50,000 cells onto microscope slides using Shandon Cytospin 3 (Shandon, Astmoore, UK). Slides were allowed to air dry and were then stained with Diff-Quick stain set (Baxter Scientific, Miami, FL). A total of 400 cells were counted from randomly chosen high-power microscope fields for each sample.

BAL supernatants were collected and analyzed in duplicate by ELISA for biologically active TGF-β1 quantification (TGF-β1 Emax immunoassay system, Promega Italia, Milan, Italy), in accordance with manufacturer’s instructions.

**Materials.** Unless otherwise stated, all compounds were obtained from Sigma-Aldrich (Poole, Dorset, UK). All other chemicals were of the highest commercial grade available. All stock solutions were prepared in nonpyrogenic saline (0.9% NaCl; Baxter Scientific).

**Statistical evaluation.** All values are expressed as means ± SE of n observations. For the in vivo studies, n represents the total number of animals studied; dead animals were replaced in further experiments to reach the specified number of observations. In the experiments involving histology or immunohistochemistry, results shown are representative of at least three experiments performed on different experimental days. The results were analyzed by one-way ANOVA followed by a Bonferroni post hoc test for multiple comparisons. A P value of <0.05 was considered significant. Statistical analysis for survival data was calculated by Fisher’s exact probability test. For such analyses, P < 0.05 was considered significant.

**RESULTS**

Histological examination of lung from bleomycin-treated animals revealed significant tissue damage (Fig. 1A). Compared with lung sections taken from saline-treated animals, these were characterized by extensive inflammatory infiltration of neutrophils, lymphocyte and plasma cells extending through
the lung epithelium, and fibrosis and granulomas visible in the perivascular region. The administration of the dipeptide carnosine in mice significantly prevented lung inflammation induced by bleomycin administration. This was confirmed by the histological grading of lung fibrosis according to criteria of Ashcroft et al. (2) executed on Masson’s trichrome-stained slides, showing a significant reduction of the fibrosis score in carnosine-treated animals (Fig. 1B).

Bleomycin in WT mice elicited an inflammatory response characterized by the accumulation of water in lung as an indicator of tissue edema and neutrophil infiltration in lung tissues as demonstrated by the myeloperoxidase assay (Fig. 2). Carnosine significantly reduced the fluid content and the neutrophil infiltration in bleomycin-treated animals.

Accordingly, with the extensive infiltration of inflammatory cells, bleomycin instillation in WT mice produced a significant increase of inflammatory cells compared with sham WT mice (10.2 ± 0.2 vs. 1.69 ± 0.3 cells × 10⁶/ml; P < 0.001) (Fig. 3A). Carnosine-treated WT mice that underwent bleomycin intratracheal instillation showed a reduced number of inflammatory cells in BAL compared with bleomycin WT mouse group (5.15 ± 0.6 vs. 10.2 ± 0.2 cells × 10⁶/ml; P < 0.05). Differential cell counts showed a similar profile compared with all of the sham groups. In bleomycin-treated WT mice vs. sham WT mice, respectively, it was evident that there were increased numbers of macrophages (5.77 ± 0.6 vs. 2.06 ± 0.3 cells × 10⁶/ml; P < 0.001), lymphocytes (1.49 ± 0.4 vs. 0.18 ± 0.1 cells × 10⁶/ml; P < 0.01), and neutrophils (3.66 ± 1.1 vs. 0.10 ± 0.04 cells × 10⁶/ml; P < 0.01).

Carnosine-treated mice that underwent bleomycin tracheal instillation showed a decreased content of BAL inflammatory cells when evaluated by differential cell count on cytospin preparations (Fig. 3B). In these mice, macrophages (4.09 ± 0.6 vs. 5.77 ± 0.6 cells × 10⁶/ml; P < 0.05), lymphocytes (0.52 ± 0.1 vs. 1.49 ± 0.3 cells × 10⁶/ml; P < 0.05), and neutrophils (0.77 ± 0.3 vs. 3.66 ± 1.1 cells × 10⁶/ml; P < 0.05) were significantly reduced compared with bleomycin-treated WT group. Eosinophils did not show any statistically significant difference among all groups.

Fig. 2. Effect of carnosine on tissue edema and myeloperoxidase activity in the lung. Bleomycin in WT mice elicited an inflammatory response characterized by the accumulation of water in lung (A) and increased myeloperoxidase activity (B). Carnosine significantly reduced the edema formation and myeloperoxidase activity. Black bar, vehicle group; gray bar, carnosine group. Data are means ± SE from 15 mice for each group. *P < 0.01 vs. sham. **P < 0.01 vs. bleomycin.

Fig. 3. Effect of carnosine on bleomycin-induced total and differential cellularity in bronchoalveolar lavage (BAL). A: total BAL cellularity for sham and bleomycin-treated mice with and without carnosine administration. B: differential cells counts for macrophages, lymphocytes, neutrophils, and eosinophils per ml of BAL fluid. Open bar, sham group; black bar, bleomycin group; gray bar, carnosine group. Data, expressed as means ± SE, are representative of 15 mice for each group. *P < 0.01 vs. sham; **P < 0.05 vs. bleomycin-treated WT; °P < 0.01 vs. sham; °°P < 0.05 vs. bleomycin-treated WT.
Immunohistochemical analysis of lung sections obtained from bleomycin-treated mice revealed a positive staining for iNOS in macrophages and neutrophils present in the alveolar space and in septal walls (Fig. 4A). Carnosine treatment abolished immunostaining for iNOS in lungs of animals treated with bleomycin. We then assessed the nitration of protein by nitrotyrosine immunohistochemical staining on tyrosine residues. Lung sections obtained from bleomycin-treated mice revealed a positive staining for nitrotyrosine mostly in the inflammatory cell infiltrate present in the interstitium and also in the alveolar pneumocyte layer (Fig. 4B). In contrast, no staining for nitrotyrosine was found in lungs of bleomycin-treated mice that underwent carnosine treatment. Moreover, mice treated with bleomycin exhibited a substantial increase in lung PARP staining (Fig. 4C), mainly present in inflammatory cells of the interstitium and in the alveolar pneumocyte layer at the nuclear level. Carnosine administration abolished the increased staining for PARP in lung section of bleomycin-treated mice. Finally, we studied total TGF-β1 in lung sections by immunohistochemistry and active TGF-β1 in BAL supernatants by ELISA. Bleomycin induced a remarkable increase of TGF-β1 staining in the alveolar epithelium and in the inflammatory infiltrate.

In contrast, carnosine-treated mice did not exhibit such an increase (Fig. 5A). Similarly, biologically active TGF-β1 levels of the bleomycin-operated animals were significantly reduced in the BAL of the carnosine group (652 ± 38 vs. 158 ± 68 pg/ml; P < 0.01; Fig. 6). There was no iNOS, nitrotyrosine,
PARP, or TGF-β₁ increase in the lungs of sham-operated animals (Figs. 4 and 5). In WT mice, the severe lung injury caused by bleomycin administration was associated with a significant increase of apoptosis, as demonstrated by the marked appearance of dark brown apoptotic cells and intercellular apoptotic fragments (Fig. 5B). Carnosine administration in bleomycin-treated mice strongly diminished immunostaining for TGF-β₁ in the lung of animal treated with bleomycin. B: bleomycin-induced apoptosis was measured by TUNEL-like staining. One week after bleomycin administration, lung tissue demonstrated a marked appearance of dark brown apoptotic cells and intercellular apoptotic fragments. No apoptotic cells or fragments were observed in tissues obtained from bleomycin mice treated with carnosine. Similarly, no apoptotic cells were observed in sections obtained from sham animals.

DISCUSSION

Lung fibrosis is the common endpoint of an heterogeneous group of pathological entities termed interstitial lung diseases, which are characterized by chronic inflammation and progressive fibrosis of the pulmonary interstitium (28). Although the fibrotic process has greatly attracted research efforts, there is nowadays strong evidence that the cellular redox state and the oxidant/antioxidant balance play important roles in the pathogenesis of IPF. Indeed, oxidative stress induces apoptosis of structural cells and upregulates the synthesis of proinflammatory cytokines (45). In BAL fluid and in plasma from patients with IPF, an imbalance between oxidant and antioxidants has been proven (53). Myeloperoxidase, which is a marker of neutrophil activity, is strongly increased in the BAL of these patients (29). Similarly, iNOS, which is the main source of NO during inflammation, and nitrotyrosine, a by-product of peroxynitrite activity, are upregulated in IPF lungs (41, 55).

Intratracheal instillation of the antitumour agent bleomycin is the most commonly used animal model for pulmonary fibrosis. Earlier reports point out that the pathogenesis of bleomycin-induced fibrosis, at least in part, is mediated through the generation of ROS, which cause the peroxidation of membrane lipids and DNA damage (26).
Carnosine is a dipeptide (β-alanyl-L-histidine) discovered nearly 100 years ago. Since then, many functions have been proposed for this compound, including wound healing promoter, ion-chelant agent, antioxidant, and free-radical scavenger (52). Carnosine prevents cellular toxicity in vitro, with a direct anti-peroxidative activity on proteins (31), lipids (50), and DNA bases (38). The antioxidant and metal ion-chelator properties of carnosine have been successfully tested on animal models of stomatitis and duodenal and gastric ulcers and on different ocular disorders (3, 36). Furthermore, carnosine has been proven to affect inflammation directly by modulating cytokine release. In an animal model of diabetes, carnosine reduced IL-6 and TNF-α secretion remains elevated for a prolonged length of time, and it is probable that the extent of inflammation and fibrosis in this model depend on the quantity of active TGF-β1 available (37). Finally, the increase of TGF-β1 mRNA precedes the biosynthesis of type I and type III procollagen in lung fibrosis (32).

Lung edema and fall of body weight were virtually absent, and inflammation was significantly reduced in carnosine-treated animals. Leukocytes recruited into the tissue can contribute to tissue destruction by the production of reactive oxygen metabolites, granule enzymes, and cytokines that further amplify the inflammatory response. These responses are an integral part of the antibacterial defenses. For example, neutrophil-derived myeloperoxidase uses hydrogen peroxide produced by dismutation of superoxide to produce hypochlorous acid, a compound with relevant antibacterial properties (62). On the other hand, ROS can mediate tissue injury. An increased susceptibility to bleomycin has been reported in mice lacking extracellular superoxide dismutase (SOD1), which indicates that superoxide anion radicals play a main role in experimental fibrosis (19). Recently, carnosine and some carnosine derivatives have been shown to scavenge superoxide anion radicals (39) and to chelate copper(II), leading to a complex that shows SOD1-like activity with a catalytic constant equal to that found for native SOD1 (6, 7). Moreover, this natural dipeptide has been shown to protect primary astroglial cell cultures from iNOS-induced oxidative stress by scavenging nitric oxide radicals (12). Superoxide reacts with nitric oxide to generate highly reactive metabolites such as peroxynitrite. This compound is able to oxidize proteins, resulting in...
direct nitration of tyrosine residues. Protein structure and
function can be subsequently altered and enzymatic activity
affected. Proteins containing nitrotyrosine residues have been
detected in different pathologies associated with enhanced
oxidative stress and increased levels of peroxynitrite (30).
Consistent with these data, carnosine reduced the expression of
iNOS and the nitration of tyrosine residues in lung sections.
Nitric oxide mediates vaso- and bronchodilatation, and it is
synthesized from l-arginine by two constitutive forms of NOS,
which are involved in the physiological regulation of airway
function (48). However, iNOS generates much larger quantities
of nitric oxide than the constitutive isoforms, and it is directly
involved in host defense from infections (46) and in various
models of inflammation (16, 61). Exogenous nitric oxide is
able to stimulate in vitro fibroblast proliferation (24), whereas
iNOS upregulation in lung fibroblasts is associated with the
early proliferative response to cytokine stimulation (54). Fi-
nally, the pharmacological inhibition and the genetic disruption
of iNOS have been shown to reduce the development of
inflammatory responses and fibrosis in lung of bleomycin-
treated animals (25).

Nitrotyrosine immunostaining was initially proposed as a
relatively specific marker for the detection of the endogenous
formation of peroxynitrite (4). There is, however, recent evi-
dence that certain other reactions can also induce tyrosine
nitration; e.g., the reaction of nitrite with hypochlorous acid
and the reaction of myeloperoxidase with hydrogen peroxide
can lead to the formation of nitrotyrosine (18). Increased
nitrotyrosine staining is considered, therefore, as an indication
of “increased nitrative stress” rather than a specific marker of
the generation of peroxynitrite. Nevertheless, our results con-
firm previous data on the activity of carnosine on tyrosine
nitration in vitro (22).

We finally tested a novel pathway of inflammation that relies
on the nuclear PARP enzyme activation by superoxide and
peroxynitrite. PARP contributes to the maintenance of
 genomic stability and to the repair of oxidative DNA damage
(5). However, its activity can deplete NAD+ and interfere with
glycolysis and ATP metabolism, ultimately leading to cell
death because of extensive DNA strand breaks (14).

Although the exact role of PARP in human lung fibrosis has
not been investigated, it has been shown that PARP is impli-
cated in experimental fibrosis and that PARP inhibition con-
fers protection from inflammation and fibrosis in different animal
models, including the bleomycin model (60). Indeed, we have
previously shown that PARP is elevated in the lung of bleo-
ymycin-treated mice (25). In this study, we do confirm that
blocynin administration increased PARP levels, whereas car-


Together, our data further support the rationale for antioxi-
dant therapy in interstitial lung diseases.

Similarly to other proposed therapeutic chelating molecules,
carnosine and its derivatives form very stable mono-
and polymeric copper complexes (40, 47), suggesting a dual
action as an antioxidant and also as a chelating agent. For
example, tetrathiomolybdate is a copper-chelating agent (11)
that has been recently proposed for the treatment of cancer (8)
and against bleomycin-induced pulmonary fibrosis in mice
(10). The tetrathiomolybdate metal-chelating affect lowers sys-

temic copper(II) levels, thus inhibiting several proangiogenic
cytokines in cancer (27) and modulating profibrotic and proin-
flammatory cytokines such as TGF-β and TNF-α (9, 23, 42).
Tetrathiomolybdate efficacy in experimental lung fibrosis has
indeed been associated with a significant decrease of systemic
copper level. Considering these results, carnosine may be
considered a potential multifunctional drug with both chelating
and antioxidant activity; these properties may prove useful for
the treatment and the prevention of diseases in which ROS are
thought to play a major role such as the interstitial pathologies
of the lung.

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