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Suppression of Perfluoroisobutylene Induced Acute Lung Injury by Pretreatment with Pyrrolidine Dithiocarbamate

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Abstract: Suppression of Perfluoroisobutylene Induced Acute Lung Injury by Pretreatment with Pyrrolidine Dithiocarbamate: Jian ZHAO, *et al*. Beijing Institute of Pharmacology and Toxicology, P.R. China—Perfluoroisobutylene (PFIB) is produced as a main by-product in large quantities by the fluoropolymer industry. As a highly toxic compound, even the case of brief inhalation of PFIB can result in acute lung injury (ALI), pulmonary edema and even death. To test for any preventive or therapeutic effects of pyrrolidine dithiocarbamate (PDTC), a NF-κB activation inhibitor, against PFIB inhalation-induced ALI, mice were exposed in a flow-past exposure system to PFIB and the prophylactic and therapeutic effects of PDTC were studied. The inhibitory effects of PDTC on ALI, the activation of NF-κB, as well as the expression of cytokines (IL-1β and IL-8) after PFIB exposure were evaluated. The results demonstrated that pretreatment with PDTC (120 mg/kg, 30 min before PFIB exposure) could significantly lower the lung coefficient (wet lung-to-body weight ratio, dry lung-to-body weight ratio, water content in the lung, and lung wet-to-dry weight ratio) and protein content in bronchoalveolar lavage fluid (BALF), but no effects of PDTC were found when PDTC was treated after PFIB inhalation, suggesting a preventative effect rather than a therapeutic effect of PDTC. Furthermore, the above preventative effects of PDTC (when given at 30 min before PFIB exposure) on PFIB-induced lung injury were achieved in a dose-dependent manner. In support of these preventive effects of PDTC, our toxicological studies demonstrated that PFIB-inhalation induced a quick activation of NF-κB (0.5 h post PFIB exposure) and expression of IL-1β and IL-8 (0.5 h and 1 h post PFIB exposure, respectively). Pretreatment with PDTC (120 mg/kg, 30 min before PFIB exposure) resulted in a significant inhibitive effect on the activation of NF-κB (0.5 h post PFIB exposure) and expression of IL-1β and IL-8 (1 h post PFIB exposure). The mortality, the extent of lung injury of the mice indexed by lung coefficients, the content of total protein and albumin in BALF, as well as the lung histopathologic changes, were dramatically alleviated in PFIB exposure after pretreatment with PDTC, clearly suggesting that PDTC has a prophylactic role against PFIB inhalation-induced ALI, and that NF-κB activation might play a central role in initiating an acute inflammatory response and in causing injury to the lungs after PFIB inhalation.
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Key words: Perfluoroisobutylene, Pyrrolidine dithiocarbamate, Acute lung injury, NF-κB activation

Perfluoroisobutylene (PFIB) is produced as a main by-product in large quantities by the fluoropolymer industry. As a highly toxic compound, a brief inhalation of PFIB can result in acute lung injury (ALI), pulmonary edema and even death[1)]. No specific antidote or successful therapeutic or preventive measures are currently available. There are no masks effective against PFIB, because PFIB is nonpolar and it cannot be adsorbed by carbon[2)].

Although there is some evidence[3)] that hydrogen fluoride and reactive intermediate species may play a role in the pathogenesis of its toxicity, the mechanisms by which the toxicity of PFIB are mediated have not been completely revealed. What deserves attention is that Lehnert *et al*. observed by electron microscope that the accumulation of blood monocytes and polymorphonuclear cells (PMNs) in the lung's capillary bed was one of the early responses to PFIB inhalation[4)], but unfortunately they didn't clarify the exact significance of this interesting phenomenon in the whole pathologic process of PFIB-induced ALI. Wang *et al*. of our group not only confirmed this phenomenon through the time-course study of myeloperoxidase activity in the lungs of

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mice after PFIB inhalation, but they also revealed that PMNs play a vital role in the pathogenesis of PFIB-induced ALI, in that the mortality and the extent of the lung injury of the mice were dramatically alleviated when the mice were depleted of PMNs by pretreatment of the animals with cyclophosphamide before PFIB exposure[5]. The mechanisms and related pathways of PMN sequestration in the lung induced by PFIB inhalation needs further clarification.

A previous study has shown that cholinolytics (i.e. 3-quinuclidinyl benzilate and anisodamine) might have prophylactic and therapeutic roles in PFIB inhalation induced ALI[6]. These results encouraged us to study methods for preventing ALI induced by PFIB. PMN sequestration and accumulation in the lung is very common in other experimental ALI models such as endotoxin and intestinal ischemia/reperfusion induced ALI models. It might be mediated by several kinds of factors including cytokines, chemokines and adhesion molecules[7, 8], the expression of which depend on the activation and mediation of a very important kind of transcription factor, NF-κB[9, 10]. It is not known whether PFIB inhalation-induced PMN sequestration in the lung is also mediated by the above pathway. Thus, in the current paper, we initiated and tested a working hypothesis, that one role of NF-κB activation, which possibly invokes the increase of proinflammatory cytokines, is to mediate PMN sequestration and accumulation in the lungs of animals after PFIB inhalation, and that pyrrolidine dithiocarbamate (PDTC), a NF-κB activation inhibitor, might have a therapeutic or prophylactic value against PFIB inhalation-induced ALI. A prophylactic profile of PDTC against PFIB inhalation-induced ALI was indeed confirmed.

Materials and Methods

Animal housing and acclimation

Pathogen-free male Kunming mice (18–22 g) were used in this study. The mice were obtained from the Center of Medical Experimental Animals, the Academy of Military Medical Sciences (Beijing, PRC). The animals were housed in quiet, humidified, clean rooms with a light-cycle of 12 h/12 h for a week before use. Food and water were available *ad libitum* except when the mice were in the exposure chamber. All the animal experiments were done in accordance with the Guide for Animal Experimentation of the Chinese Academy of Medical Sciences, Beijing, P. R. China.

Animal exposure to PFIB

PFIB was obtained from the Shanghai Institute of Organic Fluorine Materials at a purity of 98%. The mice were exposed to 130 mg PFIB/m^3 (190 mg/m^3 for mortality determination) for 5 min in a flow-past whole body exposure apparatus, as described in our earlier paper[5].

Characteristics of the effects of PDTC administration at different time-points on PFIB inhalation induced ALI

The mice in PDTC groups (PFIB/PDTC) were intraperitoneally given 120 mg/kg PDTC at 60 min, 30 min or 15 min before PFIB exposure, or at 1 h, 4 h or 10 h post PFIB exposure, while the mice in the PFIB control group (PFIB/saline) received the same volume of saline at the corresponding times. There were 24 mice in each group.

Samples (see below) which were used to evaluate the extent of lung injury were harvested at 24 h post PFIB exposure, when the lesions induced by PFIB inhalation are the most severe according to a previous study of our group[5]. To obtain consistent data, the effects were expressed as relative values, (PFIB/PDTC)/(PFIB/saline), in which the averages of PFIB/saline groups at the same exposure were regarded as 100%.

The dose-effect manner of the preventative effect of PDTC against PFIB inhalation induced ALI

The mice in PDTC groups were given PDTC intraperitoneally at dosages of 120, 60, 30 or 15 mg/kg, at 30 min before PFIB exposure, while the mice in the PFIB control group received the same volume of saline. There were 24 mice in each group.

Samples (see below) which were used to evaluate the extent of lung injury were harvested at 24 h post PFIB exposure. To obtain consistent data, the effects were expressed as relative values, (PFIB/PDTC)/(PFIB/saline), in which the averages of PFIB/saline groups at the same exposure were regarded as 100%.

Lung coefficient

Twelve mice in each group were sacrificed by exsanguination via the abdominal aorta. Lungs were excised, rinsed briefly in saline, blotted, and then weighed to obtain the wet weight. Lungs were then dried in an oven at 80°C for 24 h to obtain the dry weight. The lung coefficients were calculated as follows: wet lung-to-body weight ratio=($mass_{wet\ lung}/mass_{body}$) × 10,000, representing lung edema; dry lung-to-body weight ratio=($mass_{dry\ lung}/mass_{body}$) × 10,000, used to assess lung exudates; water content in the lung=($mass_{wet\ lung}-mass_{dry\ lung}$) $/mass_{wet\ lung}$ × 100; and lung wet-to-dry weight ratio=$mass_{wet\ lung}/mass_{dry\ lung}$ used to compare the lung water content with exudates.

Bronchoalveolar lavage fluid collection and its protein concentration assay

Twelve mice in each group were exsanguinated via the abdominal aorta and their tracheas were exposed. Each animal's trachea was cannulated with a blunt, 18-gauge needle that was secured with a silk ligature. BALF collection was performed with 0.5 m*l* PBS by means of a

1.0 m*l* syringe. The infusion and aspiration of the PBS to each lung were repeated 4 times. The lavage fluid was recovered (average fluid recovery was 0.4 m*l*), and centrifuged at 1,000 × g and 4°C for 10 min. The supernatants were removed and stored at –70°C until the total protein and albumin concentrations were determined by the modified method of Lowry *et al*.[14]. Briefly, 0.1 m*l* BALF was added to 1.9 m*l* Na_2SO_4 (22.2%), and 0.5 m*l* was collected after vortexing as the total protein sample. The remainder was added 0.5 m*l* ether, and the mixture was vortexed and centrifuged (3, 000 rpm) for 5 min at 4°C, before 0.5 m*l* was collected from the underlayer as the albumin sample, and 0.5 m*l* tyrosine (0.2 mg/m*l*) as the standard. Then 9 m*l* NaOH (0.5%) was added to each sample, and 0.3 m*l* Folin phenol was added after standing for 30 min at room temperature (RT). Finally the OD value was recorded at 500 nm after 10 min at RT. The protein concentrations were calculated by the following formulae:

$$C_{\text{total protein}}\ (\text{g}/100\text{m}l) = (OD_{\text{total protein sample}}/OD_{\text{standard}}) \times 6.4;$$
$$C_{\text{albumin protein}}\ (\text{g}/100\text{m}l) = (OD_{\text{albumin sample}}/OD_{\text{standard}}) \times 6.64.$$

Histopathological studies

Mice were administered PDTC (120 mg/kg) or the same volume of saline at 30 min before PFIB exposure and exsanguinated via the abdominal aorta at 24 h post exposure. The thoraxes were opened and the lungs were rapidly removed and immersed in 10% formalin in phosphate-buffered saline for 48 h. After fixation, each left lobe was sliced for sectioning by the method described elsewhere[4]. Paraffin sections, 4 μm thick, were routinely stained with hematoxylin and eosin, and examined by light microscopy.

Determination of the mortality

Mice were administered PDTC (120 mg/kg) or the same volume of saline at 30 min before PFIB exposure, and were observed for 7 d. There were 12 mice in each group. The survival span of each mouse was recorded and the mortality was calculated.

Preparation of nuclear extract

Mice were injected intraperitoneally with 120 mg/kg PDTC (PDTC group) or the same volume of saline (PFIB control group) at 30 min before PFIB exposure and exsanguinated via abdominal aorta transaction at 0.5, 1, 2, 4 and 8 h post PFIB challenge. The lungs were then isolated, rinsed with saline, blotted dry and stored at –80°C.

Nuclear extracts from the lungs were prepared by the method described by Hickenbottom *et al*. with minor modifications[11]. Briefly, frozen lungs were homogenized with cold hypotonic lysis buffer containing 10 mM HEPES, 10 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT and 0.5 mM PMSF. After 15 min on ice, 10% NP-40 was added to the homogenate to a final concentration of 3.125%, and the mixture was vortexed and centrifuged (10, 000 rpm) for 1 min at 4°C. The nuclear pellet was resuspended in ice-cold hypertonic nuclear extract buffer (20 mM HEPES, 10 mM KCl, 420 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 0.5 mM PMSF), incubated on ice for 30 min with intermittent vortexing, and centrifuged (10, 000 rpm) for 5 min at 4°C. The supernatant containing the nuclear extract was collected, divided into aliquots, and stored at –80°C. The protein concentration was determined by the method of Bradford[12].

Electrophoresis mobility shift assay (EMSA)

Activation of the transcriptional factor NF-κB was determined by EMSA analysis. Synthetic DNA sequences (with enhancer motifs underlined) designed by Abraham *et al*. were used as probes in the present study[13]. They were annealed, and formed double-strand DNA probes with single-strand ends consisting of sequences of four thymidines, allowing the ends to be labeled by base pairing with [α-^{32}P]dATP using Sequenase DNA polymerase (USB corporation): κB, 5'-TTTTCGAGCTCGGGACTTT- CCGAGC-3' and 3'-GCTCGAGCCCTGAAAGGCT- CGTTTT-5'.

DNA binding reactions were prepared by the methods described by Hickenbottom *et al*.[11] in a final volume of 20 μl containing 4 μg nuclear extract, 16 fmol ^{32}P-labeled double-stranded NF-κB oligonucleotide, 2 μg poly(dI-dC), 25 mM HEPES (pH 7.9), 0.5 mM EDTA, 0.5 mM DTT, 1% NP-40, 5% glycerol and 50 mM NaCl. After the samples had been incubated at 37°C for 15 min, they were separated on 5% acrylamide gel with TAE running buffer at 10 V/cm. Each gel was then subjected to autoradiography at –80°C for 48 h. The optical density was assessed with the Kodak Digital Science 1D Image Analysis system.

Assay of cytokine in serum

Mice were dosed with PDTC and intoxicated with PFIB in the aforementioned manner. Blood samples were harvested from the infraorbital plexus at 1, 2, 4 and 8 h after PFIB challenge, respectively, and levels of IL-1β and IL-8 in the serum were determined by radioimmunoassay according to the manufacturer's instruction (East Asian Institute of Immunology, General Hospital of PLA, China).

Statistical analysis

Data are shown as the mean ± SEM. Unless particularly indicated, a t-test or one-way analysis of variance followed by Dunnett's test was used to detect differences between groups. Mortality data were obtained from groups of 12 mice and compared by Fisher exact test.

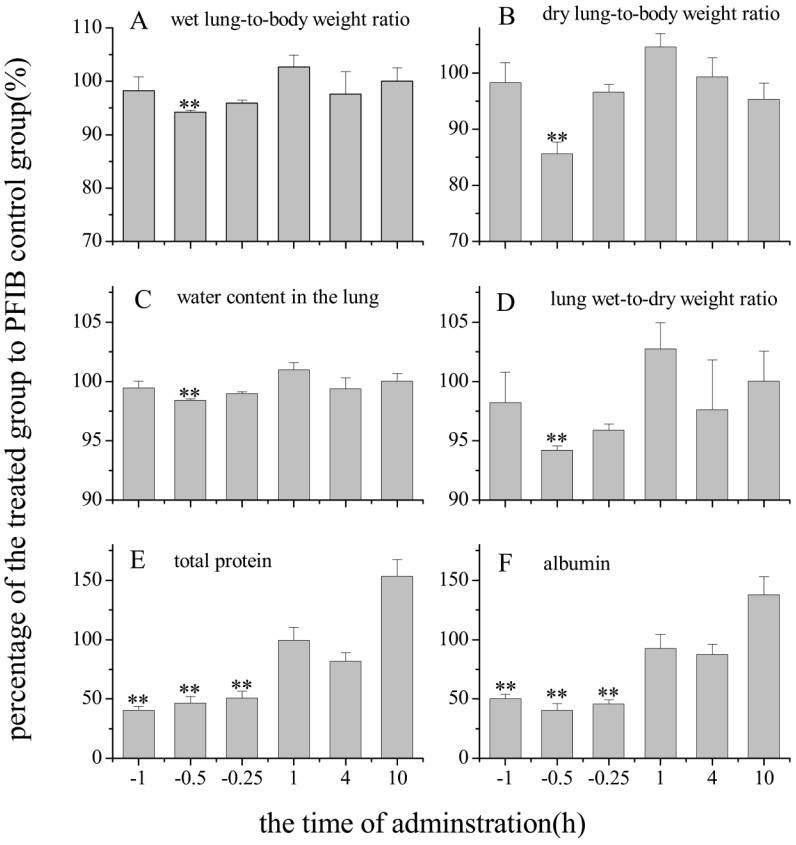

Fig. 1. Relative values of lung index (A~D) and protein concentration in BALF (E&F) at 24 h after exposure to PFIB (130 mg/m^3) for 5 min; the averages of PFIB/saline groups at the same exposure were regarded as 100%. PDTC was administered i.p. at 60 min, 30 min or 15 min before PFIB exposure, or at 1 h, 4 h or 10 h post PFIB exposure. $^{**}p<0.01$ versus PFIB group at the same exposure, n=12.

Results

The dose characteristics of the effects of PDTC administration at different time-points on PFIB inhalation induced ALI

As mentioned in *Materials and Methods*, the values representative of the extent of lung injury were all determined at 24 h post PFIB exposure. It can be discerned from Figs. 1A–D that pretreatment with PDTC (120 mg/kg, 30 min before PFIB exposure) significantly lowered the wet/dry lung-to-body weight ratio, the water content in the lung and lung wet-to-dry weight ratio, while no effects of PDTC were found in other treatment groups, suggesting that a preventative effect rather than a therapeutic effect was achieved with PDTC. There was no difference between the mice treated with PDTC alone and the mice treated with saline.

Figures 1E and F displays the effects of PDTC on the content of protein in BALF. Pretreatments with PDTC (120 mg/kg) at 60 min, 30 min or 15 min before PFIB exposure significantly suppressed the leakage of proteins in the blood to the alveolar spaces, indexed both by the content of total proteins and that of albumin in BALF. These results together with the results of the lung coefficient study, clearly show the preventative effects of PDTC.

The dose-effect manner of the preventative effect of PDTC against PFIB inhalation-induced ALI

To define more clearly the preventative effect of PDTC on PFIB-induced ALI, the dose-response relationship of PDTC dosing at 30 min before PFIB challenge was studied with the dosage beginning at 15 mg/kg and ending at 120 mg/kg. When the results were analyzed by the methods of linear correlation and regression, PDTC was found to decrease dry lung-to-body weight ratio, and the content of total protein and albumin in BALF in a dose-dependent manner (Fig. 2B: y=95.15–0.08 × x, r=–0.989 p=0.011; Fig. 2E: y=42.55–46.91 × $lg(x)$, r=–0.996, p=0.004 ; Fig. 2F: y=88.44–0.39 × x, r=–0.999, p=0.001),

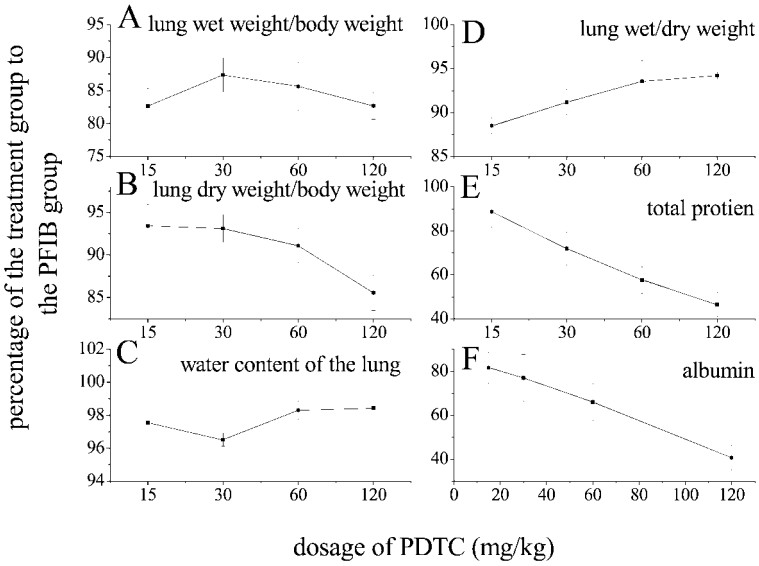

Fig. 2. Relative values of lung index (A~D) and protein concentration in BALF (E&F) at 24 h after exposure to PFIB (130 mg/m^3) for 5 min; the averages of PFIB/saline groups at the same exposure were regarded as 100%. PDTC was administered i.p. at 60 min, 30 min or 15 min before PFIB exposure, or at 1 h, 4 h or 10 h post PFIB exposure. $^{**}p<0.01$ versus PFIB group at the same exposure, n=12.

while lung wet-to-dry weight ratio was dose-dependently increased (Fig. 2D). These results further confirmed the preventative effects of PDTC pretreatment on PFIB induced ALI. There was no difference between the mice treated with PDTC alone and the mice treated with saline.

Effect of PDTC pretreatment on histopathological changes induced by PFIB inhalation

When compared with the normal pulmonary architecture in the control (Fig. 3A) and PDTC groups (Fig. 3B), severe lung damage caused by PFIB inhalation was characterized by diffuse vascular congestion, interstitial and intra-alveolar edema, and the accumulation of abundant inflammatory cells and fibrin in the alveoli. Typical hyaline membrane could also be seen in the PFIB group (Fig. 3C). These PFIB inhalation-induced lung injuries were significantly alleviated by PDTC pretreatment (120 mg/kg, 30 min before PFIB exposure) since the PDTC/PFIB group showed only slight-to-moderate septal thickening, intra-alveolar edema, fibrin deposition and macrophage accumulation in the alveolar spaces and fewer PMNs could be observed in this group (Fig. 3D).

Effect of PDTC pretreatment on PFIB-induced mortality in mice

Table 1 shows the effect of PDTC pretreatment on mice mortality induced by 5-min exposure to 190 mg PFIB/m^3. During the seven days of observation, no mice died or showed any signs of illness in the control or PDTC groups. PDTC pretreatment dramatically decreased the PFIB-induced mortality in mice ($p<0.01$).

NF-κB activity in lungs

Activation of NF-κB in lungs of mice was determined by EMSA at 0.5, 1, 2, 4 and 8 h after PFIB inhalation. Increased activation of NF-κB in lungs was observed at 0.5 h and maintained till 1 h with abrupt decline to the baseline at 2 h after PFIB inhalation. PDTC pretreatment (120 mg/kg, 30 min before PFIB challenge) significantly decreased NF-κB activation at 0.5 h after PFIB inhalation (Figs. 4A and B).

Cytokines in sera

The level of IL-1β in sera dramatically increased at 0.5 h and reached its maximum at 1 h, after which the level quickly returned to the baseline at 2 h post PFIB exposure. The PDTC pretreatment (as mentioned above) reduced serum IL-1β significantly at 1 h post PFIB exposure, after which no differences from the PFIB group were observed (Fig. 5A).

The time course of levels of IL-8 in sera of PFIB-exposed mice exhibited different patterns from that of IL-1β, showing a peak at 1 h, then a following plateau till 4 h post PFIB challenge. Like the changes of serum IL-1β, the PDTC pretreatment inhibited the increase in

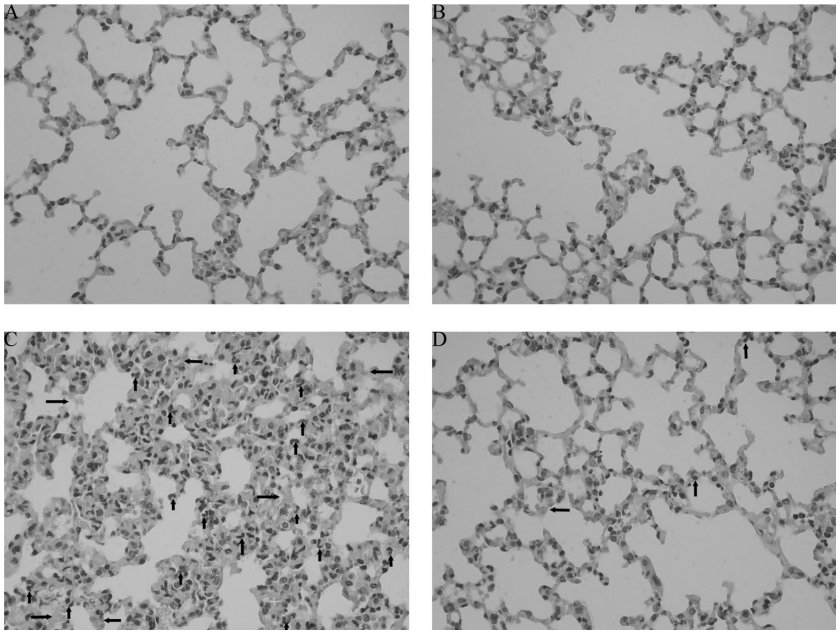

Fig. 3. Light photomicrographs of paraffin-embedded and hematoxylin and eosin-stained sections of representative lungs from mice sacrificed at 20 h post exposure to PFIB (130 mg/m^3 for 5 min). PDTC (120 mg/kg) was administered 30 min before PFIB inhalation. The Control and PDTC groups show normal or essentially normal pulmonary architecture (A&B). The PFIB group shows diffuse interstitial and intra-alveolar edema (→), alveolar hemorrhage (←), hyaline membrane formation, and cellular infiltration composed mainly of PMNs (↑) (C). The PDTC/PFIB group shows modest congestion, interstitial and intra-alveolar edema, and macrophage accumulation (D). Fewer PMNs can be seen. Original magnification: × 40.

Table 1. Effect of PDTC on PFIB-induced mortality in mice

Group	N	Mortality (%)	
Normal	12	0	(0/12)
PDTC	12	0	(0/12)
PDTC/PFIB	12	25%	(3/12)**
PFIB/Saline	12	83%	(10/12)*

The pathogen-free male mice were exposed to PFIB (190 mg/m^3) for 5 min. PDTC (120 mg/kg i.p.) was administered at 30 min before exposure. Mortality was evaluated at 7 d after PFIB exposure. *p<0.01 versus normal group, **p<0.01 versus PFIB group by Fisher's exact test.

serum IL-8 level significantly at 1 h post PFIB exposure, after which no differences from the PFIB group were observed (Fig. 5B).

Discussion

PFIB inhalation-induced ALI and death usually occur in accidents in the fluoropolymer industry and other fire disasters[15, 16]. There are no effective prophylactic or therapeutic drugs or treatments for ALI induced by PFIB inhalation. According to the results of previous experiments in our group, PMN depletion prevents PFIB-induced lung injury[5], suggesting that PMNs play a key role in it. With the working hypothesis that NF-κB activation may be a leading factor that initiates the sequestration of PMNs in pulmonary tissue, the current study used PDTC, a NF-κB inhibitor, in a mouse model of PFIB inhalation-induced ALI, and confirmed its prophylactic role against PFIB inhalation-induced ALI. Pretreatment with PDTC (120 mg/kg, 30 min before PFIB exposure) resulted in reduced mortality, as well as significantly alleviating lung injury as indexed by the lung coefficient, BALF protein leakage and histopathological observations, however no effects of PDTC were found when it was given after PFIB inhalation (Table 1, Figs. 1 and 3).

NF-κB is a protein transcription factor that serves as a critical regulator of a variety of genes, including cytokines and growth factors, adhesion molecules, immunoreceptors and acute-phase proteins[17]. A wide variety of extracellular stimuli can trigger the activation

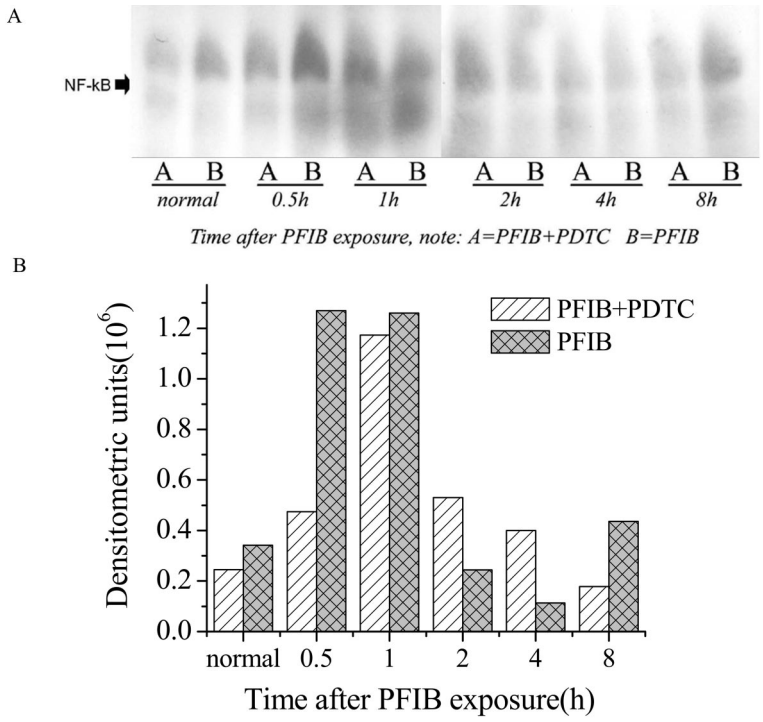

Fig. 4. Effects of PDTC on PFIB-induced activation of NF-κB in lung tissue (Fig. 4A) and densitometric analysis of bands (Fig. 4B). As indicated in *Materials and Methods*, the NF-κB in lung tissue was measured in mice exposed to 130 mg/m^3 of PFIB for 5 min. Evaluation of NF-κB activation by EMSA.

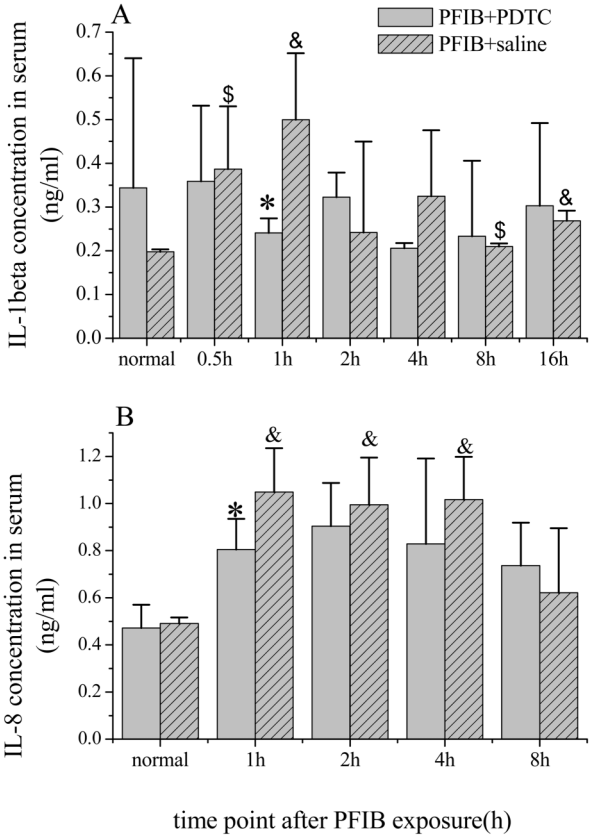

Fig. 5. Effects of PDTC on PFIB-induced changes of IL-1β (A) and IL-8 (B). As indicated in *Materials and Methods*, IL-1β and IL-8 were measured in mice exposed to 130 mg/m^3 of PFIB for 5 min. *p<0.05 versus PFIB; $^{\$}p$<0.05, $^{\&}p$<0.01 versus normal.

of NF-κB, including proinflammatory cytokines (such as TNF-α and IL-1β), bacterial and viral products, UV light and reactive oxygen species (ROS). It appears that the pathways through which different stimuli activate NF-κB ultimately all involve the participation of ROS[18]. In the present study, increased activation of NF-κB occurred at 0.5 h after PFIB exposure, which is similar to the result of ALI induced by LPS or ischemia/reperfusion[19]. As a potent electrophilic agent, PFIB can quickly induce a state of oxidative stress in the lung cells, which may mediate the activation of NF-κB. However, whether NF-κB is activated by PFIB itself or by other derivatives and what the cellular source of the activated NF-κB is in the lung after PFIB inhalation, are still under investigation.

The activation of NF-κB may result in maximal transcription of many cytokines, including TNF-α, IL-1β and IL-8, which are thought to be of great importance

in the generation of acute inflammatory responses[17, 18]. It was observed in our experiments that increased expression of IL-1β and IL-8 occurred at 0.5 h and 1 h post PFIB exposure, respectively, with the maximal expression at 1 h (IL-1β) or high levels of expression (IL-8) till 4 h. Being a typical "early-response cytokine," IL-1β is reported to overlap with many functions of TNF-α, another typical early-response cytokine. They both can have a number of effects on endothelial cells that are germane to microvascular inflammation, including the expression of cell surface adhesion molecules for leukocytes, and production of IL-8, all of which may prime PMNs to the specific area where untoward stimuli exist. The primed PMNs then go into the phase of respiratory burst with release of reactive oxygen metabolites, and induction of phagocytosis, and degranulation of granules containing a number of proteolytic enzymes, resulting in endothelial injury and migration of PMNs into the extravascular space[20]. IL-8 is a potent PMN chemotactic and activating factor. It plays a critical role in ALI by recruiting and activating PMNs[21]. NF-κB activation was decreased with the administration of the specific NF-κB inhibitor, PDTC, before PFIB inhalation, and the levels of IL-1β and IL-8 was decreased. Therefore, we think that the preventative effects of PDTC pretreatment (120 mg/kg, 30 min before PFIB exposure) on PFIB-induced lung injury might be achieved by it inhibiting the activation of NF-κB and the expression of IL-1β and IL-8, resulting in the reduction of PMN sequestration and injury to the lung (Fig. 3C). Detailed studies and the roles of NF-κB activation, IL-1b and IL-8 in the sequestration of PMNs in the lung, are still being performed.

Additionally, our results show that PDTC was only effective when it was administered before PFIB inhalation (Figs. 1, 2 and 3), presumably because NF-κB and proinflammatory cytokines, such as IL-1β and IL-8, had already been activated when PDTC was administered after PFIB inhalation (see Figs. 4 and 5, PFIB group). No difference was found between the group treated with PDTC alone and the group treated with saline, and no toxic responses were found in the study of Bach SP *et al*. even when non-tumor-bearing mice were given PDTC up to 500 mg/kg[22]. These results indicate further investigation of PDTC as a treatment for ALI induced by PFIB should be pursued.

In conclusion, the current study confirmed that PDTC has a prophylactic role against PFIB inhalation-induced ALI and that NF-κB might play a central role in initiating an acute inflammatory response and in causing injury to the lungs after PFIB inhalation. The prophylactic effects of PDTC against PFIB inhalation induced ALI may be achieved by its inhibitory effect on NF-κB activation and it shows potential as a prophylactic drug for those who sent to the locale of PFIB disasters.

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