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Efficacy of Ibuprofen and Pentoxifylline in the Treatment of Phosgene-induced Acute Lung Injury

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Phosgene, a highly reactive former warfare gas, is a deep lung irritant which produces adult respiratory distress syndrome (ARDS)-like symptoms following inhalation. Death caused by phosgene involves a latent, 6–24-h, fulminating non-cardiogenic pulmonary edema. The following dose-ranging study was designed to determine the efficacy of a non-steroidal anti-inflammatory drug, ibuprofen (IBU), and a methylxanthine, pentoxifylline (PTX). These drugs were tested singly and in combination to treat phosgene-induced acute lung injury in rats. Ibuprofen, in concentrations of 15–300 mg kg^{-1} (i.p.), was administered to rats 30 min before and 1 h after the start of whole-body exposure to phosgene (80 mg m^{-3} for 20 min). Pentoxifylline, 10–120 mg kg^{-1} (i.p.), was first administered 15 min prior to phosgene exposure and twice more at 45 and 105 min after the start of exposure. Five hours after phosgene inhalation, rats were euthanized, the lungs were removed and wet weight values were determined gravimetrically. Ibuprofen administered alone significantly decreased lung wet weight to body weight ratios compared with controls ($P \leq 0.01$) whereas PTX, at all doses tested alone, did not. In addition, the decrease in lung wet weight to body weight ratio observed with IBU+PTX could be attributed entirely to the dose of IBU employed. This is the first study to show that pre- and post-treatment with IBU can significantly reduce lung edema in rats exposed to phosgene.

INTRODUCTION

Use of therapeutic compounds for the treatment of phosgene-induced lung injury has increased during the past several years.[1–4] The focus of such postexposure therapy has been prevention or amelioration of pulmonary edema, the major life-threatening effect of exposure.[5] Phosgene is a highly reactive and oxidizing chemical intermediate which is used industrially as a chlorinating, dehydrating and carbonyl-forming agent for the production of pharmaceuticals, pesticides and foam rubber products.[6] However, in biological reactions, phosgene is a well-known acylating agent. The annual amount of phosgene used in the USA was estimated to be nearly 1 billion metric tons.[7] Although phosgene is depleted in the chemical reaction, there is the potential for large-scale accidental exposures which, in the past, resulted in a number of chemical worker deaths.[8] The median lethal concentration × time of phosgene exposure (LCt_{50}) in man is approximately 800 ppm × min (3200 mg min m^{-3}) for a 2-min exposure.[9] The permissible exposure limit for phosgene is 0.1 ppm (0.4 mg m^{-3}).[10]

Pentoxifylline (PTX), a methylxanthine derivative, has been shown to be effective for treating peripheral vascular disease due to its capacity to decrease RBC rigidity and increase capillary blood flow.[11] Pentoxifylline has also been shown to inhibit polymorphonuclear leukocyte (PMN) phagocytosis, thereby decreasing superoxide anion production in PMNs and monocytes.[12,13] *In vivo*, PTX improved survival rates in rats following hemorrhagic shock,[14] reduced IL-2-induced acute lung injury in guinea pigs[15] and prevented increased vascular permeability in dogs challenged with endotoxin.[16] The principal mechanism attributed to PTX is that of a phosphodiesterase inhibitor which can increase intracellular cAMP.[11] In addition, it has been suggested that PTX could stimulate synthesis of the vasodilator prostaglandin PGI_2.[17]

Ibuprofen (IBU) is a non-steroidal anti-inflammatory compound which has been used clinically as an antipyretic and to treat rheumatoid arthritis in humans. Ibuprofen has been shown to protect against acute lung injury from smoke inhalation in rabbits,[18] burn injury in sheep[19] and paraquat poisoning in the isolated perfused rat lung model.[20] Ibuprofen decreased oxidant lung injury by decreasing pulmonary edema formation in the isolated perfused rabbit lung after exposure to phosgene.[3]

The purpose of this study was to investigate the efficacy of two clinically available drugs, IBU and PTX. These drugs were administered alone or in combination as pre- and post-treatments against phosgene-induced pulmonary edema in rats. Because of the high risk of potential accidental exposures, prophylactic

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administration may be required to enhance survival from a life-threatening exposure event.

EXPERIMENTAL

Experiments were performed on male Sprague-Dawley rats (Crl:CD BR VAF/Plus) weighing 209–323 g (Charles River Breeding Laboratories, Wilmington, MA). Rats were quarantined for 5 days prior to use and maintained in plastic cages with ground corncob bedding. Rats were maintained at 21–22°C with 40–60% relative humidity and on a 12-h light/dark cycle with no twilight. All rats were provided with commercial rodent rations and had access to water *ad libitum*. Rats in each drug treatment group were exposed to a phosgene LCt_{50} of 80 mg m^{-3} × 20 min (1600 mg min m^{-3} = 400 ppm min).

Exposures were performed in an approved laboratory fume hood. A dose–response test was performed using various phosgene concentrations and a dose chosen that would give at least a doubling of lung wet weight five hours after exposure. Phosgene (10%) mixed in N_2 (Matheson Gas Products, Baltimore, MD) was metered through a Tylan® mass flow controller (Tylan Corp., Torrance, CA) at a rate dependent on the desired concentration. The phosgene–N_2 was mixed with dry, filtered room air and then passed through a Miran infrared spectrometer (Miran 1A, Foxboro Co., Sharon, MA) equipped with a real-time analog output. Concentration versus time graphs were developed and the input concentration was calculated. Rats were whole-body exposed to either phosgene or room air. Exposures occurred in a Plexiglas® chamber (25 cm in height, 28 cm in diameter) with a total capacity of 15.8 l and a flow rate of 20 l min^{-1} followed by a 5-min room air washout also at 20 l min^{-1}. Sham-treated controls were exposed under identical conditions but to 25 min of air. Phosgene from the chamber was passed through a second Miran 1A unit to determine the concentration of phosgene exiting the chamber. Vented gas was passed through M18 and charcoal filters. The coefficient of variation of exposure to phosgene for rats was 2.4 ± 0.2% (mean ± SEM). Euthanasia at the end of 5-h postexposure observation period was by whole-body exposure to 100% carbon dioxide. Necropsy involved abdominal incision, transection of the abdominal aorta and excision of the lungs and trachea up to the larynx. Excess tissue was trimmed away, the lungs were lightly blotted and placed in tared aluminum planchets (4.5 cm diameter) and the wet weight recorded.

Drug administration

Ibuprofen was kindly provided by Upjohn Co., Kalamazoo, MI, as a sterile 50 mg ml^{-1} solution which also contained 6.306 mg ml^{-1} glycine and 4.909 mg ml^{-1} NaCl. Placebo, also supplied by the Upjohn Co., contained 6.306 mg ml^{-1} glycine and water for injection USP. Sodium hydroxide was used to adjust the pH to 7.8–8.0 in both solutions. Pentoxifylline (Trental®), in powder form, was kindly provided by Hoechst-Roussel Pharmaceutical (Sommerville, NJ) and 40 mg ml^{-1} was dissolved in sterile 0.9% NaCl prior to injection USP (Abbott Laboratories, North Chicago, IL). Both drugs were administered intraperitoneally. The maximum volume of fluid given by injection to any animal over the course of an experiment was 3.5 ml. Table 1 shows the dosage combination, time of injections (relative to exposure) and number of rats used in each study. To maintain steady-state plasma drug levels during this study, timing of injections and drug concentrations were determined from human kinetic data published in the *Physicians' Desk Reference*.[21] Plasma $t_{1/2}$ for IBU is 1–2 h and for PTX is 0.5–1 h.[21]

Statistical analysis

Homogeneity of group variances was satisfied in all analyses by Hartley's F-test at a significance level of $P \leq 0.01$.[22] Comparison of lung wet weight to body weight ratio between phosgene-exposed rats and sham-treated controls was performed using Student's *t*-test with a significance level of $P \leq 0.05$. Drug treatment groups were compared using one-way ANOVA and, if significance was determined, Bonferroni's multiple comparison *t*-test was then performed at the $P \leq 0.05$ level. All data are expressed as means ± SEM.

RESULTS

Figure 1 shows the effect of varying doses of IBU on lung wet weight to body weight ratio five hours after start of exposure to phosgene. At 15 and 40 mg/kg IBU was ineffective. However, at 115 and 300 mg/kg,

Table 1. Drug dosages and timing of injections[a]

(a) Ibuprofen dosage (mg kg^{-1})

Rat#	1st (–30 min)	2nd (+60 min)	N
1 (placebo)	0	0 0	9
2	15	7.5	9
3	40	20	9
4	115	58	9
5	300	150	9

(b) Pentoxifylline dosage (mg kg^{-1})

Rat no.	1st (–15 min)	2nd (+45 min)	3rd (+105 min)	N
1 (placebo)	0	0	0	6
2	10	5	5	6
3	27	14	14	6
4	74	37	37	6
5	120	60	60	5

(c) Combined ibuprofen and pentoxifylline dosage (mg kg^{-1})

	Ibuprofen		Pentoxifylline			
Rat no.	1st (–30 min)	2nd (+60 min)	1st (–15 min)	2nd (+45 min)	3rd (+105 min)	N
1 (placebo)	0	0	0	0	0	6
2	75	38	19	10	10	6
3	150	75	37	19	19	6
4	250	125	50	25	25	6

[a]Drugs administered before (–) or after (+) start of exposure.

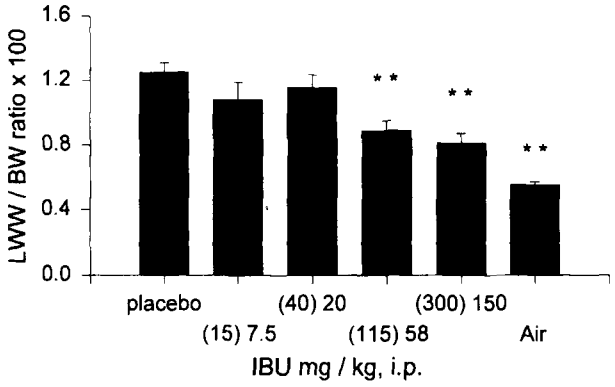

Figure 1. Effect of ibuprofen (IBU) dose-ranging on lung wet weight (LWW) to body weight (BW) ratic. Thirty minutes prior to phosgene exposure rats were administered IBU i.p. in the following concentrations (mg kg^{-1}): group 1, placebo; group 2, 15; group 3, 40; group 4, 115; group 5, 300. Sixty minutes after exposure these same groups were given a second dose of IBU at concentrations of 0, 7.5, 20, 58 and 150 mg kg^{-1}, respectively (see Table 1). Numbers in parentheses represent pretreatment doses. $^{**}P \leq 0.01$ when comapred to group 1.

IBU decreased phosgene-induced lung wet weight to body weight ratio by 40% and 50%, respectively, compared with placebo, $p \leq 0.01$.

In contrast, PTX pre- and post-treatment did not prevent edema formation in rat lung assessed five hours after exposure to phosgene (Figure 2). In fact, at the highest dose of PTX, 120 mg/kg, the lung wet weight to body weight ratio was actually increased by about 20%.

When IBU and PTX were given in combination, lung edema formation was lowered at the two highest doses of each drug (Figure 3) which corresponds to the effective doses for IBU alone. Lung wet weight/body weight ratio decreased by 41%, $p \leq 0.05$; and 71%, $p \leq 0.01$, respectively, compared with placebo.

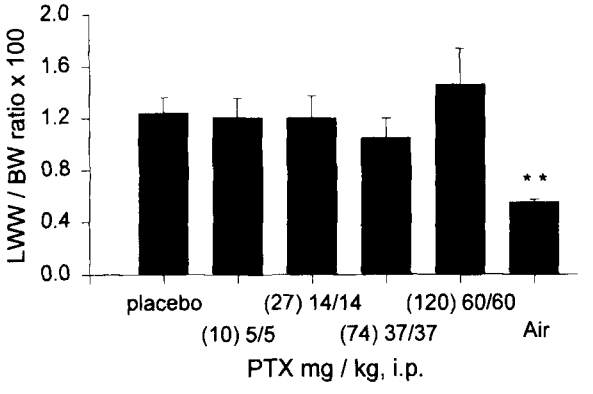

Figure 2. Effect of pentoxifylline (PTX) dose-ranging on lung wet weight (LWW) to body weight (BW) ratio. Fifteen minutes prior to phosgene exposure five groups of rats were administered PTX (i.p.) in the following concentrations (mg kg^{-1}): group 1, placebo; group 2, 10; group 3, 27; group 4, 74; group 5, 120. At both 45 and 105 min after exposure, these same five groups of rats were given a second and third dose of PTX at concentrations of 0, 5, 14, 37 and 60 mg kg^{-1}, respectively (see Table 1). Numbers in parentheses represent pretreatment doses. $^{**}P \leq 0.01$ when compared to group 1.

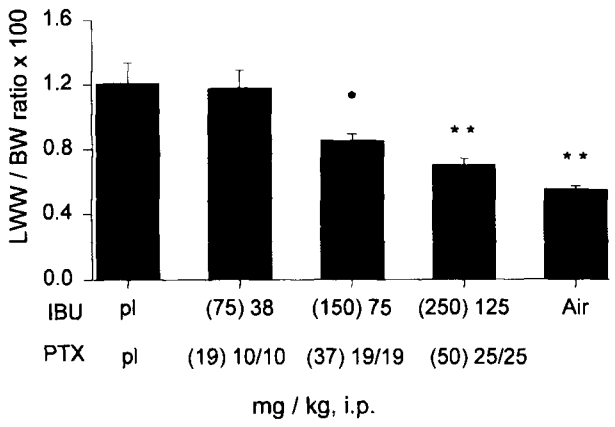

Figure 3. Effect of combined ibuprofen plus pentoxifylline (IBU+PTX) dose-ranging on lung wet weight (LWW) to body weight (BW) ratio. Four groups of rats were administered IBU+PTX. The IBU was injected i.p. 30 min prior to phosgene exposure in the following concentrations (mg kg^{-1}): group 1, placebo (pl); group 2, 75; group 3, 150; group 4, 250. These same groups were injected with PTX 15 min prior to exposure at 0, 19, 37 and 50 mg kg^{-1}, respectively. The IBU treatment, i.p., was then repeated with 0, 38, 75 and 125 mg kg^{-1} 60 min after exposure. Numbers in parentheses represent pretreatment doses. At 45 min after exposure, PTX was injected i.p. in concentrations (mg kg^{-1}) of 0, 10, 19 and 25, respectively, and repeated 105 min after exposure (see Table 1). $^{*}P \leq 0.05$ when compared to group 1; $^{**}P \leq 0.01$ when compared to group 1.

DISCUSSION

Treatment of phosgene-induced acute lung injury has been frustrating largely because of the severe and complicated nature of the injury. Phosgene inhalation can cause ARDS-like symptoms in man.[23] Phosgene is highly reactive and is known to bind to cellular constituents such as -SH, -OH and -NH groups.[24] Moreover, phosgene also causes lipid peroxidation[3,4] and glutathione depletion, increases the production of permeability-enhancing leukotrienes[4] and alters the phospholipid content of lung surfactant.[25] Damage to surfactant may be extremely important in phosgene-induced lung injury since phosgene can form adducts with phospholipid polar heads.[26] If the inhaled concentrations are high enough, phosgene may even cross the air/blood barrier and increase hemolysis.[27,28] As a consequence of these physiological and biochemical events, identifying a single drug to prevent injury or death caused by phosgene inhalation is difficult.

Kennedy *et al.*[3] have found that intravenous administration of IBU in rabbits 10 min after phosgene exposure, followed by IBU given intraperitoneally 2 and 4 h postexposure significantly reduced lung injury. The present study is consistent with these findings and shows that two intraperitoneal injections of IBU, one before and the second 60 min after the start of phosgene exposure, significantly reduce lung edema.

Although PTX treatment has been shown to be effective in ameliorating injury in some models,[29-31] we can speculate that its failure to ameliorate edema in our study may be related to its effect on vascular hemodynamics. Clinically, PTX has been used to treat claudication, a peripheral vascular disease, by increasing microcirculation and thereby reoxygenating tissue.[11]

Increased blood flow in the compromised lung could exacerbate pulmonary edema by increasing circulation to damaged alveoli. We were somewhat surprised by these results since it has been demonstrated that aminophylline, also a methylxanthine, or cAMP protected rabbits from phosgene-induced lung injury.[1,32,33] In combination, the prophylactic effect of IBU+PTX is not surprising and may be attributed exclusively to IBU treatment.

The capacity of IBU to reduce lung wet weight has been linked to the free-radical scavenging and iron chelation mechanisms as outlined by Kennedy *et al.*[3] Increases in vascular pressure, such as those mediated by the vasoconstrictive arachidonic acid metabolite thromboxane B_2, are not produced in response to phosgene poisoning.[2]

We have examined the efficacy of two clinically available drugs, IBU and PTX, on pulmonary edema formation in rats exposed to a lethal concentration of phosgene. Pre- and post-treatment of rats with IBU significantly lowered lung wet weight, whereas PTX did not. This study is the first to demonstrate, in the intact rat inhalation model, that prophylactic treatment with IBU may be beneficial in reducing pulmonary edema in acute phosgene-induced lung injury.

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