J Anesth (2005) 19:257–259
DOI 10.1007/s00540-005-0322-4

Journal of
Anesthesia

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Short communications

Effect of cyclooxygenase-2 inhibitor pretreatment on gas exchange after hydrochloric acid aspiration in rats

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Abstract
The present study was carried out to determine the effect of cyclooxygenase-2 (COX-2) inhibitor on acid aspiration-induced lung injury in rats. Rats were allocated into one of four groups. Group H received intratracheal instillation of HCl. Group S received saline intratracheally. Group HC received COX-2 inhibitor (celecoxib) 10 mg/kg intravenously 30 min before intratracheal instillation of HCl. Group C underwent bronchoalveolar lavage (BAL) only. All rats were mechanically ventilated for 30 min before BAL. Arterial blood gas analysis was done immediately before BAL. Groups H, S, and HC were subdivided to each two groups. Groups H-1, S-1, and HC-1 underwent BAL 1 h after instillation, whereas groups H-8, S-8, and HC-8 underwent BAL 8 h after instillation. The BAL fluid was used to measure the prostaglandin E_2 (PGE_2) concentration. Intratracheal HCl resulted in impaired oxygenation. COX-2 inhibitor attenuated the impairment of oxygenation 8 h after instillation but not after 1 h. Intratracheal HCl caused an increase in PGE_2 concentration. COX-2 inhibitor attenuated an increase in PGE_2 concentration 8 h after instillation but not after 1 h. The results show that COX-2 inhibitor attenuates the oxygenation impairment and the increase in alveolar PGE2 concentration during the inflammatory phase of acid aspiration-induced lung injury in rats.

Key words Acid aspiration · Cyclooxygenase-2 · Prostaglandin E_2 · Acute lung injury · Celecoxib

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Acid aspiration-induced lung injury is one of the causes of acute respiratory distress syndrome. Kennedy et al. [1] demonstrated that there was a biphasic injury pattern after acid aspiration. The initial phase consists of acute protein extravasation without marked evidence of inflammation; and the second phase (the inflammatory phase) consists of inflammation resulting from the activation and accumulation of neutrophils.

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Received: July 29, 2004 / Accepted: March 16, 2005

Acid aspiration-induced lung injury is associated with lipid inflammatory mediators, phospholipase A_2 [2], and arachidonic acid (AA) metabolites [3]. Cycloxygenase-2 (COX-2) is responsible for the production of large amounts of prostaglandins (PGs) in the inflammatory response. Alveolar macrophages are a major source of PGs produced by COX-2, and acid aspiration induces significant expression of COX-2 messenger ribonucleic acid (mRNA) in alveolar macrophages [4]. The present study was carried out to determine the phase-related effect of the COX-2 inhibitor in acid aspiration-induced lung injury in vivo in an animal model.

Male Wistar rats (n = 56) weighing 250–350 g were used in this study. The study protocol was approved by the Animal Care Committee of Nagasaki University School of Medicine. Freely fed rats were anesthetized with pentobarbital ($50 mg \cdot kg^{-1}$) administered intraperitoneally and fixed on a heated operating table. The rats were divided into four groups: control (C), HCl (H), saline (S), and HCl after celecoxib (HC). Group S served as the sham operation rats. Group C results served as the baseline. Group HC (n = 16) was given injections of celecoxib $10 mg \cdot kg^{-1}$ via the penile vein 30 min before intratracheal acid instillation. Groups H (n = 16) and S (n = 16) were injected with the same amount of vehicle 30 min before the intratracheal instillation. Group C (n = 8) was injected with celecoxib $10 mg \cdot kg^{-1}$ [5], and 30 min later bronchoalveolar lavage (BAL) was performed with saline (4°C; $30 ml \cdot kg^{-1}$). Groups HC and H underwent tracheal instillation of HCl/saline $1.2 ml \cdot kg^{-1}$ pH 1.25, through a 24-gauge catheter (Angiocath; Becton Dickinson, Sandy, UT, USA) inserted into the trachea via a midline incision, with the rat in a 60° upright position [6]. Group S underwent instillation of saline $1.2 ml \cdot kg^{-1}$ instead of HCl. The trachea and skin were repaired; and groups HC, H, and S were allowed to wake up and breathe spontaneously immediately after the instillation with 50% oxygen in air [6].

Groups H, S, and HC were subdivided into two groups each: H-1 and H-8, S-1 and S-8, and HC-1 and HC-8, respectively. After the induction of anesthesia with pentobarbital $50 mg \cdot kg^{-1}$) administered intraperitoneally, tracheotomy was performed with a 14-gauge catheter and a catheter inserted into the carotid artery immediately before mechanical ventilation. Anesthesia was maintained with pentobarbital ($60 mg \cdot kg^{-1} \cdot h^{-1}$) administered intraperitoneally, and the rats were paralyzed with pancuronium bromide ($0.5 mg \cdot kg^{-1} \cdot h^{-1}$) intramuscularly. The lungs of all the rats were mechanically ventilated with an infant ventilator (IV-100B; Sechrist, Anaheim, CA, USA) under the following ventilator settings [7]: mode was pressure-control; $F_{I_{O_2}}$ 1.0; ventilation frequency $30 \cdot min^{-1}$; peak airway pressure (P_{peak}) 14 cm H_2O; positive end-expiratory pressure (PEEP) 2 cm H_2O; and inspiratory/expiratory ratio 1.0 : 1.8 for 30 min before BAL. Arterial blood gas analysis was performed immediately before BAL in all rats. Groups H-1, S-1, and HC-1 underwent BAL 1 h after instillation. Groups H-8, S-8, and HC-8 underwent BAL 8 h after instillation.

The BAL fluid (BALF) collected by aspiration was analyzed for leukocyte counts, protein concentration, and PGE_2 concentration. Protein concentration in the BALF was measured using the protein analysis kit (Bio-Rad Laboratories, Richmond, CA, USA). The number of leukocytes in BALF was measured by the hemocytometer (Ace Counter FLC-240A; Fukuda Denshi, Tokyo, Japan). The PGE_2 concentration of BALF was measured in duplicate by radioimmunoassay (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Results are presented as means ± SD. Results are evaluated by one-way analysis of variance followed by Fisher's protected least significant difference test, with $P < 0.05$ regarded as significant.

As shown in Table 1, intratracheal instillation of HCl caused increases in Pa_{CO_2} 1 and 8 h after instillation. Intratracheal instillation of HCl resulted in impaired oxygenation. The COX-2 inhibitor improved the oxygenation status 8 h after instillation but not after 1 h. As shown in Table 2, intratracheal instillation of HCl caused increases in the protein concentrations and the number of leukocytes 1 and 8 h after instillation. The COX-2 inhibitor did not attenuate the protein concentration or the number of leukocytes in BALF 1 and 8 h after instillation. Intratracheal instillation of HCl caused an increase in the PGE_2 concentration in BALF. The COX-2 inhibitor attenuated the PGE_2 concentration 8 h after instillation but not after 1 h.

Neutrophil chemotactic activity [8] and alveolar leukocytes [9] increased, reaching a peak 8 h after aspiration. Nishina et al. [10] demonstrated that in-

Table 1. Blood-gas analysis and SBP before BAL and after instillation

Group	Pa_{CO_2} (mmHg)	Pa_{O_2} (mmHg)	SBP (mmHg)
C	42 ± 6	527 ± 61	127 ± 7
S-1	43 ± 4	485 ± 56	111 ± 26
H-1	56 ± 6*,**	252 ± 33*,**	121 ± 16
HC-1	56 ± 6*,**	285 ± 133*,**	119 ± 7
S-8	44 ± 8	555 ± 41	130 ± 20
H-8	60 ± 8*,***	410 ± 44*,***,†	135 ± 14
HC-8	59 ± 12*,***	489 ± 72‡,§	119 ± 8

Values are means ± SD
There were eight rats in each group
C, celecoxib injection/no instillation; S, intratracheal saline; H, intratracheal HCl; HC, celecoxib injection/intratracheal HCl; -1 or -8, hours after instillation; SBP, systolic blood pressure; BAL, bronchoalveolar lavage
* $P < 0.05$, compared with group C; ** $P < 0.05$, compared with group S-1; *** $P < 0.05$, compared with group S-8; † $P < 0.05$, compared with group H-1; ‡ $P < 0.05$, compared with group H-8; § $P < 0.05$, compared with group HC-1

Table 2. BALF assays

Group	Recovery (%)	Protein (mg/ml)	Leukocytes (/μl)	PGE_2 (pg/ml)
C	88 ± 2	0.3 ± 0.1	68 ± 26	96 ± 18
S-1	89 ± 1	0.3 ± 0.1	55 ± 9	101 ± 6
H-1	88 ± 3	0.7 ± 0.3*,**	85 ± 35**	172 ± 19*,**
HC-1	86 ± 3	0.8 ± 0.2*,**	95 ± 32*,**	160 ± 50*,**
S-8	88 ± 2	0.3 ± 0.1	55 ± 18	124 ± 9*
H-8	86 ± 2	0.8 ± 0.2*,***	160 ± 21*,***,†	184 ± 22*,***
HC-8	85 ± 3	1.0 ± 0.1*,***	160 ± 22*,***,‡	121 ± 14*,‡,§

Values are mean ± SD
There were eight rats in each group
BALF, bronchoalveolar lavage fluid; PGE_2, prostaglandin E_2
* $P < 0.05$, compared with group C; ** $P < 0.05$, compared with group S-1; *** $P < 0.05$, compared with group S-8; † $P < 0.05$, compared with group H-1; ‡ $P < 0.05$, compared with group HC-1; § $P < 0.05$, compared with group H-8

creased alveolar leukocytes were involved in increased alveolar polymorphonuclear neutrophils, increased alveolar interleukin-6 (IL-6), increased alveolar IL-8, and an increased histological acute lung injury score after acid aspiration. Kawamae et al. [11] demonstrated that oxygenation decreased, with a peak decrease 1 h after acid instillation in rats. The present results are in accordance with these previous reports regarding two phases in the acid-induced lung injury.

It has been reported that PGE_2 is a mediator that plays a critical role in platelet-activating factor (PAF)-induced pulmonary edema [12]. Because COX-2 inhibitor might reduce accumulation of pulmonary edema, it improved the deteriorated oxygenation during the late phase. In other experimental models of acute lung injury, COX inhibition improved oxygenation, presumably by causing a redistribution of perfusion away from the edematous lung region [13]. Although the mechanisms responsible for this perfusion distribution have not been determined [5], the potent vasoactive effects of eicosanoids are likely to be involved. Induction of the COX-2 protein and PGE_2 synthesis depending on COX-2 occurs within 2–4 h after inflammatory stimulation [14], whereas induction of the COX-1 protein occurs immediately after stimulation regardless of inflammation. COX-2 may play a significant role in the inflammatory phase after acid aspiration.

The present study demonstrated that intratracheal instillation of HCl caused increases in Pa_{CO_2} values after instillation. Kawamae et al. [11] demonstrated that acid instillation increases lung compliance. Because we used pressure-controlled ventilation, an increase in lung compliance resulted in a decrease in respiratory minute volume.

The present study demonstrates that the COX-2 inhibitor does not improve the protein concentration or the number of leukocytes in BALF after acid aspiration. Leukotriene B_4, produced by macrophages and neutrophils in response to inflammatory stimulation, is a potent chemoattractant involved in activating and recruiting neutrophils to the site of injury [15]. A 5-lipoxygenase inhibitor was found to restrain vascular permeability after oxidative stress-induced acute lung injury [16]. Thus, acute protein extravasation and accumulation of neutrophils in the lung could be mediated by 5-lipoxygenase products and macrophage inflammatory protein-2 but not by COX products.

In conclusion, COX-2 inhibitor attenuates impaired oxygenation and increased alveolar PGE_2 concentration during the inflammatory phase of acid aspiration-induced lung injury in rats, suggesting a significant role for COX-2 in the development of acute lung injury.

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