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Cyclooxygenase-2 participates in the late phase of airway hyperresponsiveness after ozone exposure in guinea pigs

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Abstract

We examined the role of cyclooxygenase in airway hyperresponsiveness and inflammation after ozone exposure in guinea pigs using a non-selective (indomethacin) and a selective (JTE-522) cyclooxygenase-2 inhibitor. Spontaneously breathing guinea pigs were exposed to ozone (3 ppm) for 2 h after treatment with vehicle, indomethacin (10 mg/kg) or JTE-522 (10 mg/kg). Airway responsiveness to inhaled histamine (PC_{200}) and bronchoalveolar lavage were assessed before, immediately and 5 h after ozone exposure. Ozone caused a significant airway hyperresponsiveness immediately after exposure, which persisted after 5 h. Neither JTE-522 nor indomethacin affected airway hyperresponsiveness immediately after ozone exposure, but significantly attenuated airway hyperresponsiveness 5 h after exposure, suggesting that cyclooxygenase-2 may participate in the late phase of airway hyperresponsiveness but not in the early phase. Ozone caused a significant increase in the concentration of prostaglandin E_2 and thromboxane B_2 in bronchoalveolar lavage fluid immediately after exposure, which decreased to the basal level 5 h after exposure. This increase in prostaglandin E_2 and thromboxane B_2 was significantly inhibited by JTE-522. An expression of cyclooxygenase-2 was detected not only after ozone exposure but also before, and there was no difference in the number of cyclooxygenase-2-positive cells at any time point. An exogenously applied thromboxane A_2 mimetic, U-46619 (10^{-5} M), induced airway hyperresponsiveness 5 h after inhalation, but not immediately or 3 h after inhalation. These data suggest that cyclooxygenase-2 may be constitutively expressed before ozone exposure in guinea pig airway and may synthesize prostaglandin E_2 and thromboxane A_2 transiently under ozone stimulation and that thromboxane A_2 may, in turn, induce the late phase of airway hyperresponsiveness.

Keywords: JTE-522; Cyclooxygenase-2; Prostaglandin E_2; Thromboxane A_2; U-46619; Airway hyperresponsiveness; Ozone; (Guinea pig)

1. Introduction

We previously demonstrated the role of cyclooxygenase metabolites in airway hyperresponsiveness and inflammation after ozone exposure (O'Byrne et al., 1984; Aizawa et al., 1985; Fabbri et al., 1985). The properties of ozone as a potent oxidant and free radical generator result in the initiation of cell damage by peroxidation of lipids in cell membranes (Cross et al., 1976; Mustafa and Tierney, 1978), which causes an increase in the amount of arachidonic acid in the airway (Shimasaki et al., 1976). Cyclooxygenase metabolites such as thromboxane A_2 have been reported to induce airway hyperresponsiveness in dogs (O'Byrne et al., 1984; Aizawa et al., 1985; Fabbri et al., 1985).

Cyclooxygenase has two isoforms (Loll and Garavito, 1994; Belvisi et al., 1997). Cyclooxygenase-1 is a constitutive type, which is expressed in the normal state (Kargman et al., 1995; Mitchell et al., 1995). Cyclooxygenase-2 is an inducible type that is expressed at a high level in the inflammatory response (Maier et al., 1990; Masferrer et al., 1994). Recent studies report the induction of cyclooxygenase-2 in rat lung by cytokines (Liu et al., 1996), and increased expression in polymorphonuclear cells of asthmatic patients (Kuitert et al., 1996). Cyclooxygenase-2 is likely to modulate airway responsiveness in the inflamed airway, such as in asthma.

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In guinea pigs, however, the role of cyclooxygenase metabolites in ozone-induced airway hyperresponsiveness is controversial (Lee and Murlas, 1985; Murlas and Roum, 1985; Murlas et al., 1990). Ozone-induced airway hyperresponsiveness measured 30 min after exposure was attenuated by BW 755C (3-amino-1-(m-(trifluoromethyl) phenyl)-2-pyrazoline, an inhibitor of both lipoxygenase and cyclooxygenase) or FPL 55712 (sodium 7-[3-(4-cetyl - 3hydroxy- 2 -propylphenoxy)-2-hydroxypropoxy]-4-xo-8-propyl-4H-1-benzopyran-2-carboxylate, a leukotriene receptor antagonist), but not by indomethacin. These results suggest that metabolites of arachidonic acids other than cyclooxygenase products may be involved in the development of ozone-induced airway hyperresponsiveness (Lee and Murlas, 1985). It has also been reported that the acute phase of ozone-induced airway hyperresponsiveness is mainly dependent on factors other than prostanoids such as epithelial injury and tachykinin (Mustafa and Tierney, 1978; Murlas and Roum, 1985; Murlas et al., 1990; Nadel, 1992; Koto et al., 1995). In these studies, however, the role of prostanoid was evaluated only immediately after ozone exposure.

The aim of the present study was to determine whether cyclooxygenase plays a role during the time course of ozone-induced airway hyperresponsiveness, and if so, to clarify which isoform of cyclooxygenase is important in this response. The effects of non-selective (indomethacin) and selective (JTE-522) cyclooxygenase-2 inhibitors (Matsushita et al., 1997; Masaki et al., 1998) on airway hyperresponsiveness and the concentrations of prostaglandin E_2 and thromboxane B_2 in bronchoalveolar lavage fluid after ozone exposure were examined. Immunohistochemical investigation was also done on whether ozone caused the expression of cyclooxygenase-2. We found that cyclooxygenase metabolites may participate in airway hyperresponsiveness 5 h after ozone exposure. Although prostanoid have been reported to induce airway hyperresponsiveness in in vivo studies (O'Byrne et al., 1984; Aizawa et al., 1985; Fabbri et al., 1985; Walters et al., 1982), the time course of airway hyperresponsiveness is not fully known. To investigate the time course of prostanoid-induced airway hyperresponsiveness, we studied the effects of inhalation of prostaglandin E_2 and a thromboxane A_2 mimetic (U-46619) on airway responsiveness.

2. Materials and methods

2.1. Study design

2.1.1. Protocol 1

Male Hartley-strain guinea pigs weighing 500–600 g (Kyudo, Kumamoto, Japan) were used. Airway responsiveness to inhaled histamine and bronchoalveolar lavage fluid measurements were done in separate animals to eliminate possible interruptions between the processes. Each measurement was performed in five animals. The measurements were made before, immediately and 5 h after ozone exposure. JTE-522 was used as a cyclooxygenase-2 inhibitor on the basis that it has been reported to inhibit guinea pig cyclooxygenase-2 activity in a concentration-dependent manner (IC_{50} value of 0.64 ± 0.08 μM) (Matsushita et al., 1997). The animals were randomly divided into three groups. Vehicle (0.2 ml/kg 99% ethanol + 0.2 ml saline, p.o.), indomethacin (10 mg/kg, p.o.) or JTE-522 (10 mg/kg, p.o.) was administered 30 min prior to ozone exposure.

Ozone exposure was performed as previously described (Koto et al., 1995; Aizawa et al., 1997). The animals inhaled 3.0 ± 0.1 ppm (mean ± S.D.) of ozone for 2 h while awake and breathing spontaneously in a 24-l plexiglass exposure chamber. Ozone was generated by passing 100% oxygen through an ozonator (Model 0-1-2, Nihon Ozone, Tokyo, Japan) regulated by a variable-voltage supply. The concentration of ozone in the chamber was continuously monitored by an ultraviolet analyzer (Model 1500, Dasibi, Glendale, CA) (Fig. 1A,B).

2.1.2. Protocol 2

To detect the expression of cyclooxygenase-2 immunohistochemically, bronchoalveolar lavage was carried out before, immediately and 5 h after ozone exposure. The streptavidin–biotin immunoperoxidase method was then applied, using anti-cyclooxygenase-2 antibody for inflammatory cells in bronchoalveolar lavage fluid as described below.

2.1.3. Protocol 3

Animals were randomly divided into three groups and made to inhale vehicle (1 ml of 99% ethanol and 9 ml of saline), prostaglandin E_2 (10^{-5} M) or U-46619 (10^{-5} M). Airway responsiveness was measured before, immediately, 3 and 5 h after inhalation (Fig. 1C). Each measurement was done in five animals.

The animals were placed in a 2.8-l plexiglass chamber, which had two small holes in the wall. While awake and breathing spontaneously, the guinea pigs were exposed to aerosolized saline, prostaglandin E_2 or U-46619 for 5 min. Solution concentration was 10^{-5} M (dissolved in 99% ethanol), with each solution (1 ml) dissolved in saline (9 ml). The aerosol was generated by an ultrasonic nebulizer (TUR-3200; Nihon Kohden) connected to one hole. The other hole was connected to a vacuum system, which generated a constant vacuum flow (20 l/min).

2.2. General procedure

2.2.1. Assessment of airway responsiveness

The animals were anesthetized with pentobarbital sodium (50 mg/kg i.p.). They were intubated via a tra-

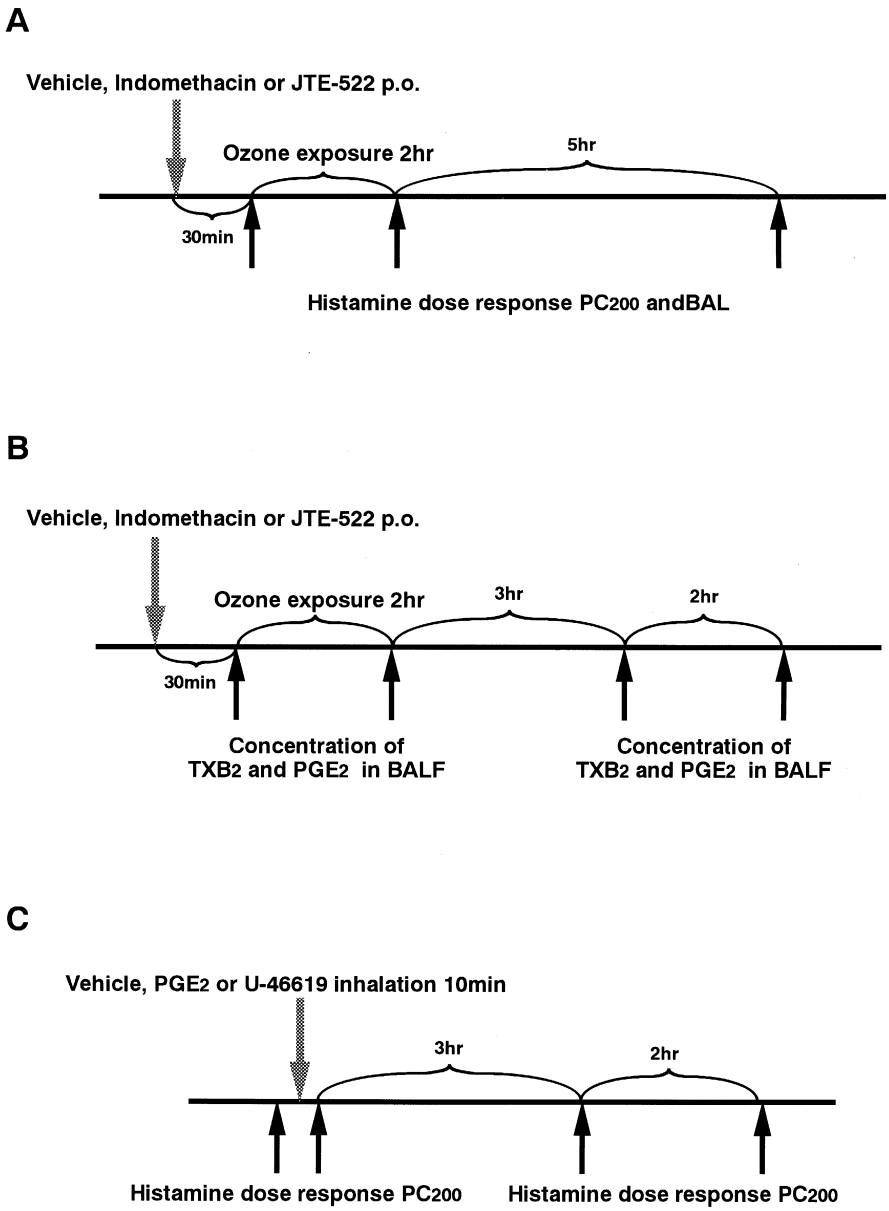

Fig. 1. Study design. (A) Airway responsiveness to inhaled histamine (PC_{200}) and inflammatory cell count in bronchoalveolar lavage fluid were evaluated before, immediately and 5 h after ozone exposure in guinea pigs pretreated with vehicle, indomethacin or JTE-522 30 min before exposure. (B) Concentrations of prostaglandin E_2 and thromboxane B_2 in bronchoalveolar lavage fluid were evaluated before, immediately, 3 and 5 h after ozone exposure in guinea pigs pretreated with vehicle, indomethacin or JTE-522 30 min before exposure. (C) PC_{200} was evaluated before, and immediately, 3 and 5 h after inhalation of vehicle, prostaglandin E_2 or U-46619.

cheostomy and mechanically ventilated with room air using a respirator (Model No. 680, Harvard Apparatus, South Natick, MA, USA) at a constant tidal volume of 7 ml/kg and a rate of 60 breaths/min. To evaluate pleural pressure, a fluid-filled catheter was inserted into the esophagus at a point such that the maximal amplitude of pressure with respiration was obtained. The animals were placed supine in a 2.8-l body plethysmograph. Plethysmograph airflow was measured with a Fleisch pneumotachograph (TV-132T, Nihon Kohden, Tokyo, Japan). Transpulmonary pressure was estimated from the difference between esophageal and airway opening pressure measured at a side hole in the tracheal cannula using a differential pressure transducer (TP-603T, Nihon Kohden). Total pulmonary resistance was calculated from transpulmonary pressure and airflow the method of Amdur and Mead (1958).

Airway responsiveness to histamine was determined by inhalation of histamine (0.017–10 mg/ml) administered via the endotracheal tube. Histamine aerosols (output 1.5 ml/min) were generated with an ultrasonic nebulizer (TUR-3200, Nihon Kohden) placed in line with the ventilator. Concentration–response curves were constructed as

Table 1
Baseline R_L and PC_{200} among the treatment groups

	Vehicle	Indomethacin	JTE-522
R_L ($cmH_2O\ ml^{-1}\ s^{-1}$)			
Before ozone exposure	0.206 ± 0.012	0.221 ± 0.014	0.223 ± 0.018
Immediately after ozone exposure	0.210 ± 0.009	0.212 ± 0.012	0.231 ± 0.013
5 h after ozone exposure	0.212 ± 0.016	0.231 ± 0.014	0.215 ± 0.015
PC_{200} (mg / ml)			
Before ozone exposure	0.766 (GSEM 1.14)	0.797 (GSEM 1.15)	0.680 (GSEM 1.31)
Immediately after ozone exposure	0.066 (GSEM 1.21)	0.093 (GSEM 1.12)	0.049 (GSEM 1.22)
5 h after ozone exposure	0.104 (GSEM 1.45)	0.484 (GSEM 1.29)	0.459 (GSEM 1.14)

follows: saline was given for 15 breaths, and the subsequent total pulmonary resistance value was used as baseline. The histamine aerosol was administered for 15 breaths, separated by 5-min intervals. The concentration of histamine was increased for each series of 15 breaths. Total pulmonary resistance was monitored for 5 min after each nebulization, and the maximum value of total pulmonary resistance was plotted against histamine concentration. To maintain a constant-volume, hyperinflations (three tidal volumes) were obtained between each histamine challenge. Challenge was halted when total pulmonary resistance exceeded 200% of baseline. The concentration of histamine required to produce a 200% increase in total pulmonary resistance (PC_{200}) was calculated by log–linear interpolation from individual animals.

2.2.2. Bronchoalveolar lavage

Bronchoalveolar lavage was assessed before, immediately, 3 and 5 h after ozone exposure in guinea pigs without histamine challenge to prevent histamine or bronchoconstriction from affecting the bronchoalveolar lavage results.

The animals were given a lethal dose of pentobarbital (200 mg/kg i.p.), and the lung was lavaged three times in situ with 0.9% saline under constant hydrostatic pressure (25 cmH_2O) to avoid barotrauma. Approximately 25 ml of saline was introduced at each time, and about 80% of the volume introduced was recovered. The recovery rate of bronchoalveolar lavage fluid in each group was not significantly different.

Indomethacin solution was added to bronchoalveolar lavage fluid to a final concentration of 10^{-5} mol/ml bronchoalveolar lavage fluid to prevent future production of prostanoids. Total cell count was performed under light microscopy using a standard hemocytometer. Following assessment of total cell count, a total of 10^5 cells was suspended in 1 ml 0.9% saline and centrifuged onto glass slides using a centrifugal cell collector ($800 \times g$ for 5 min). After drying in air, the slides were fixed in methanol and the cells were visualized with a modified Wright–Giemsa stain, Diff-Quick for differential cell count, or fixed with paraformaldehyde for immunohistochemical study. Differential cell counts were performed for 500 cells. The results were expressed as cell counts/ml of recovered bronchoalveolar lavage fluid. The remaining supernatant from the bronchoalveolar lavage fluid was stored frozen at −80°C for measurement of thromboxane B_2 and prostaglandin E_2.

2.2.3. Measurement of thromboxane B_2 and prostaglandin E_2

One milliliter of each sample was acidified with 1 N HCl and extracted with a double volume of ethyl acetate by centrifugation. The sample was then evaporated to dryness under a nitrogen stream. The residue was dissolved in benzene–ethyl acetate (60:40). The solution was evaporated by stirring for 30 min, and its supernatant was processed for assay using a radioimmunoassay kit (Daiichi Kagaku, Tokyo, Japan). Samples were briefly incubated with ^{125}I-labeled thromboxane B_2 and ^{125}I-labeled prostaglandin E_2 and were incubated with antiserum for 16 h at 4°C. After this incubation, the antibody-bound fraction was separated by centrifugation. Radioactivity of the antibody-

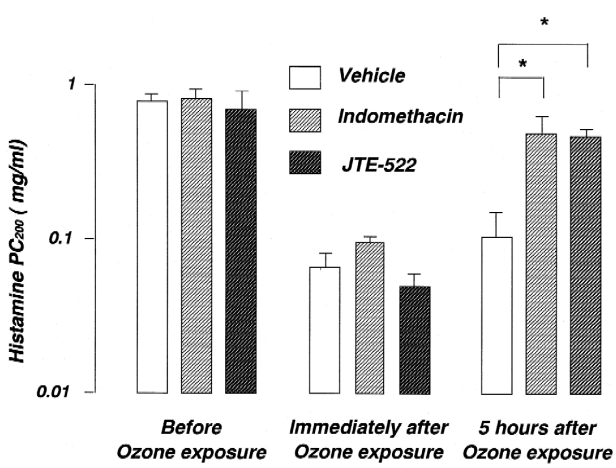

Fig. 2. Effect of indomethacin or JTE-522 on airway responsiveness to histamine. PC_{200} was determined in vehicle-, indomethacin- and JTE-522-treated guinea pigs. Airway responsiveness increased immediately after ozone exposure and remained high for 5 h after exposure. Treatment with indomethacin or JTE-522 significantly inhibited airway hyperresponsiveness 5 h after ozone exposure, but not immediately after exposure. Data are shown as geometric means and GSEM for five animals. $^{*}P < 0.05$.

bound fraction was determined using a gamma scintillation counter (model ARC-950, Aloka, Tokyo, Japan). Sensitivity of this assay is 3.0 pg/ml, and reproducibility, expressed as variance coefficient, is 8.19–9.66% (Matsumoto et al., 1996).

2.2.4. Immunohistochemistry

Expression of cyclooxygenase-2 was assessed by a streptavidin–biotin immunoperoxidase method. After fixing in paraformaldehyde, the slides were rinsed in phosphate-buffered saline. Briefly, after blocking of endogenous peroxidase with 3% hydrogen peroxide followed by incubation with non-immune rabbit serum, the slides were incubated with anti-rat cyclooxygenase-2 mouse monoclonal antibody diluted 1:200 in phosphate-buffered saline dilution buffer. The slides were rinsed in phosphate-buffered saline, incubated sequentially with biotinylated anti-mouse immunoglobulin G (IgG), streptavidin and 3,

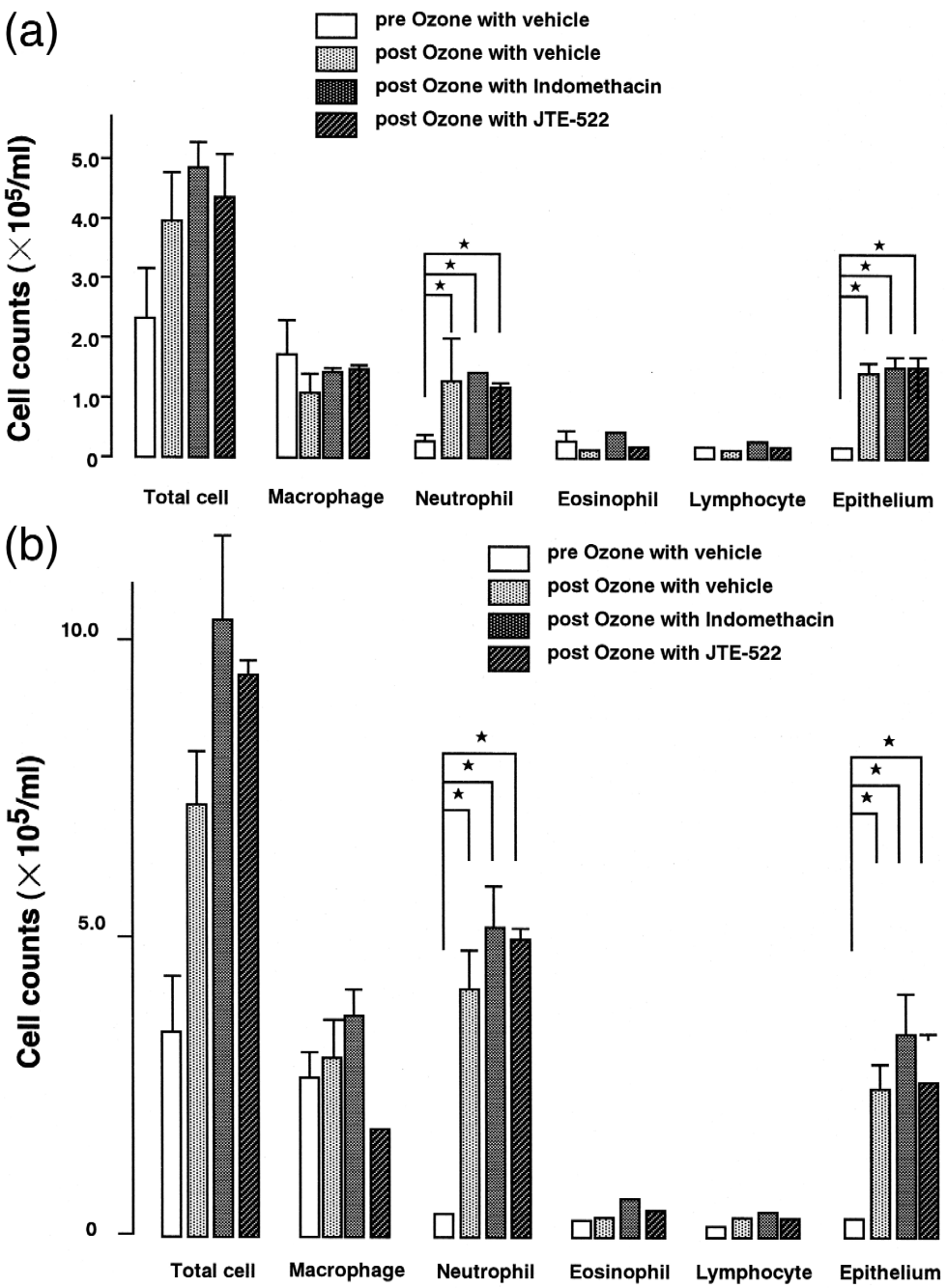

Fig. 3. (a) Differential cell counts in bronchoalveolar lavage fluid immediately after ozone exposure. Neutrophil and epithelial cell counts were significantly increased. Neither indomethacin nor JTE-522 inhibited the increase in these cells. (b) Differential cell counts in bronchoalveolar lavage fluid 5 h after ozone exposure. The increase in neutrophils and epithelial cells continued for 5 h after exposure. Neither indomethacin nor JTE-522 inhibited the increase in these cells. Data are shown as means ± S.E. for five animals. $^{*}P < 0.05$.

3′-diaminobenzidine, and counterstained with Diff-Quick (26).

2.3. Drugs

Histamine diphosphate and indomethacin were obtained from Sigma (St. Louis, MO, USA) and pentobarbital sodium from Abbott Laboratories (North Chicago, IL, USA). JTE-522 (4-(4-cyclohexyl-2-methyloxazol-5-yl)-2-fluorobenzenesulfonamide) was provided by JT (Osaka, Japan). JTE-522 and indomethacin were suspended in 99% ethanol at a concentration of 50 mg/ml, and were diluted with the same volume of saline. Prostaglandin E_2 was provided by Ono Pharmaceutical (Tokyo, Japan). U-46619 ((5*Z*, 9 alpha, 11alpha, 13*E*, 15(*S*))-15-hydroxy-9, (11)-methanoepoxyprosta-5, 13-dienoic acid) and anti-rat cyclooxygenase-2 mouse monoclonal antibody were obtained from Cayman Chemical (Ann Arbor, MI, USA), and an assay kit for streptavidin–biotin immunoperoxidase and 3,3′-diaminobenzidine was obtained from Nichirei (Tokyo, Japan). Diff-Quick was obtained from Baxter (McGaw Park, IL, USA).

2.4. Data analysis

PC_{200} values were expressed as the geometric mean and standard error (GSEM). Other values are expressed as the arithmetic mean and S.E. PC_{200} values were compared by one-way analysis of variance (ANOVA) with the Bonferoni correction. Changes in bronchoalveolar lavage cell counts were compared by the Kruskall–Wallis *H*-test followed by the Mann–Whitney *U*-test. A level of $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Effect of indomethacin or JTE-522 on ozone-induced airway hyperresponsiveness

There were no significant differences in baseline total pulmonary resistance and pre-exposure PC_{200} values between each group (Table 1).

Fig. 2 illustrates histamine PC_{200} before and after ozone exposure. In vehicle-treated animals, ozone caused significant airway hyperresponsiveness immediately after exposure, which lasted for 5 h. Neither indomethacin nor JTE-522 affected the ozone-induced airway hyperresponsiveness immediately after ozone exposure, but did significantly inhibit airway hyperresponsiveness 5 h after ozone exposure to the same extent. There were no significant differences between the indomethacin- and JTE-522-treated groups.

3.2. Effect of indomethacin or JTE-522 on cell counts in bronchoalveolar lavage fluid

The number of neutrophils and epithelial cells was significantly increased immediately after ozone exposure (Fig. 3a) and increased further at 5 h after exposure (Fig. 3b). Neither indomethacin nor JTE-522 affected this increase in the number of neutrophils and epithelial cells.

3.3. Concentrations of thromboxane B_2 and prostaglandin E_2 in bronchoalveolar lavage fluid

In the vehicle-treated animals, concentrations of thromboxane B_2 and prostaglandin E_2 in bronchoalveolar lavage fluid were increased significantly immediately after ozone exposure (Fig 4A,B). The values then decreased 3 h after exposure and returned to baseline at 5 h after exposure.

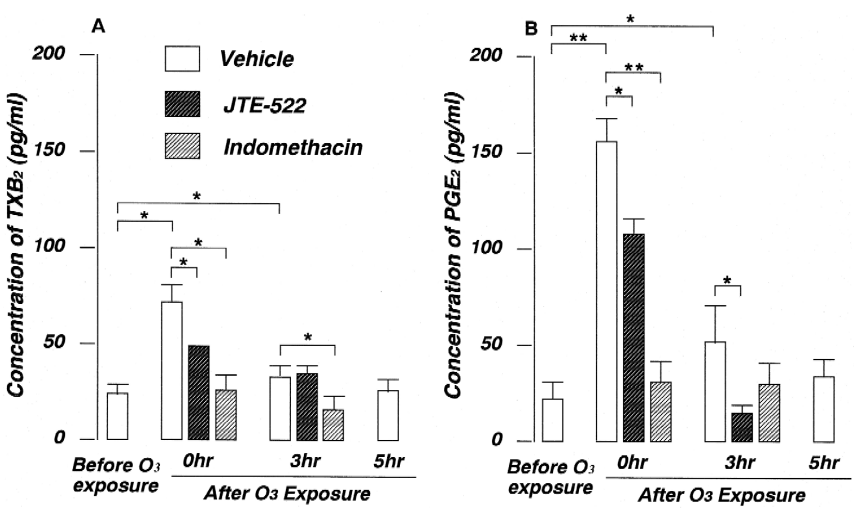

Fig. 4. Concentration of prostaglandin E_2 and thromboxane B_2 in bronchoalveolar lavage fluid after ozone exposure. Concentrations increased significantly immediately after ozone exposure. These increases were lower 3 h after exposure than those immediately after ozone exposure and values returned to the basal level 5 h after exposure. (A) JTE-522 inhibited the increase in thromboxane B_2 partially but significantly immediately after ozone exposure. However, JTE-522 did not inhibit the increase in thromboxane B_2 3 h after exposure. Indomethacin inhibited the increase in thromboxane B_2 significantly almost completely after ozone exposure. (B) JTE-522 inhibited the increase in prostaglandin E_2 partially but significantly immediately after ozone exposure. Further, 3 h after exposure, JTE-522 inhibited the increase in prostaglandin E_2 to the basal level. Indomethacin inhibited the increase in prostaglandin E_2 almost completely after ozone exposure. Data are shown as means ± S.E. for five animals. $^{*}P < 0.05$; $^{**}P < 0.01$.

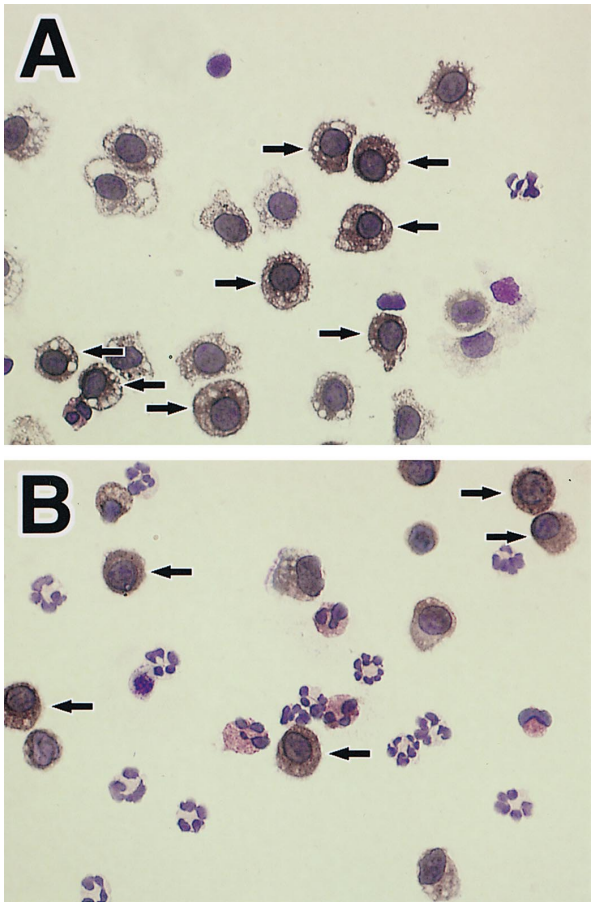

Fig. 5. Expression of cyclooxygenase-2. Cyclooxygenase-2 positive cells appeared red-brown. Expression of cyclooxygenase-2 was detected in alveolar macrophages before (A) and 5 h (B) after ozone exposure, and there was no difference in the number of cyclooxygenase-2-positive cells between the time periods.

JTE-522 inhibited the increase in thromboxane B_2 partially but significantly immediately after ozone exposure but not at 3 or 5 h after exposure (Fig. 4A). JTE-522 inhibited the increase in prostaglandin E_2 significantly immediately and 3 h after exposure (Fig. 4B).

Indomethacin significantly inhibited the increase in thromboxane B_2 and prostaglandin E_2 to the baseline level immediately and 3 h after ozone exposure (Fig. 4A,B).

3.4. Expression of cyclooxygenase-2

Cyclooxygenase-2-positive cells looked red-brown. Expression of cyclooxygenase-2 was detected in alveolar macrophages before, immediately and 5 h after ozone exposure. The number of cyclooxygenase-2-positive cells was the same at all time points (Fig. 5).

Table 2
Baseline R_L among the treatment groups

R_L ($cmH_2O\ ml^{-1}\ s^{-1}$)			
	Vehicle	PGE_2	U-46619
Immediately after inhalation	0.232 ± 0.015	0.220 ± 0.012	0.229 ± 0.014
3 h after inhalation	0.221 ± 0.012	0.209 ± 0.013	0.223 ± 0.012
5 h after inhalation	0.217 ± 0.011	0.222 ± 0.011	0.241 ± 0.012

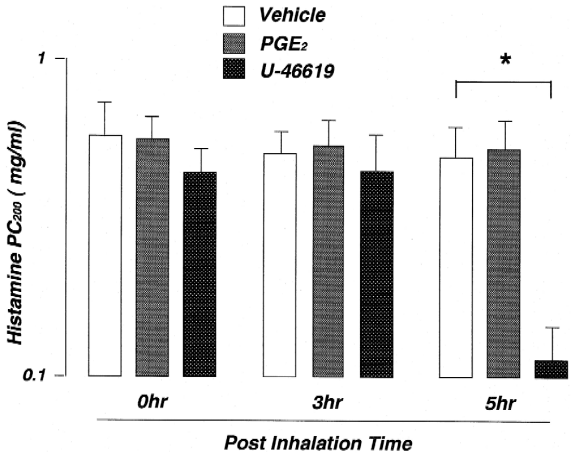

Fig. 6. Effect of exogenous prostaglandin E_2 or U-46619 on airway hyperresponsiveness. Airway responsiveness did not increase after inhalation of prostaglandin E_2. Responsiveness did not increase immediately or 3 h after inhalation of U-46619, but increased significantly 5 h after inhalation. Data are shown as geometric means and GSEM for five animals. $^{*}P < 0.05$.

3.5. Effect of exogenous prostaglandin E_2 or U-46619 on airway hyperresponsiveness

There were no significant differences in baseline total pulmonary resistance between the groups (Table 2). Fig. 6 shows PC_{200} before and after inhalation of prostaglandin E_2 or U-46619. Inhalation of prostaglandin E_2 did not affect airway responsiveness at any time. U-46619 did not affect responsiveness immediately and 3 h after inhalation, but significantly increased responsiveness 5 h after inhalation.

4. Discussion

In the present study, ozone caused airway hyperresponsiveness which lasted at least for 5 h after exposure in guinea pigs. Indomethacin and JTE-522 (a selective cyclooxygenase-2 inhibitor) significantly inhibited the airway hyperresponsiveness observed 5 h after ozone exposure. In contrast, neither agent affected airway hyperresponsiveness immediately after ozone exposure.

These findings indicate that prostanoids play a role in ozone-induced airway hyperresponsiveness even in guinea pigs. Although the role of prostanoids in ozone-induced airway hyperresponsiveness has been demonstrated in dogs (O'Byrne et al., 1984; Aizawa et al., 1985), it is still controversial in guinea pigs (Lee and Murlas, 1985; Murlas and Roum, 1985; Murlas et al., 1990). Results of some previous studies suggested that prostanoids might not have this effect in guinea pigs. It has been reported that the acute phase of ozone-induced airway hyperresponsiveness

depends mainly on factors other than prostanoids such as epithelial injury, tachykinins, leukotrienes (Mustafa and Tierney, 1978; Lee and Murlas, 1985; Murlas and Roum, 1985; Murlas et al., 1990; Nadel, 1992; Koto et al., 1995).

In these previous studies the role of prostanoids was evaluated only immediately after ozone exposure. In the present study, indomethacin or JTE-522 did not prevent airway hyperresponsiveness immediately after exposure, confirming these earlier results, but indomethacin significantly attenuated airway hyperresponsiveness at 5 h after exposure, suggesting that prostanoids may play a role in the late-phase airway hyperresponsiveness induced by ozone. Furthermore, a selective cyclooxygenase-2 inhibitor significantly attenuated this airway hyperresponsiveness to a similar extent as indomethacin, suggesting that cyclooxygenase-2 played a crucial role in these changes.

We used JTE-522 as a selective cyclooxygenase-2 inhibitor in this study. In sheep placenta, JTE-522 inhibited cyclooxygenase-2 (IC_{50}: 0.64 μM) without affecting cyclooxygenase-1 activity at 100 μM and selectivity for cyclooxygenase-1/cyclooxygenase-2 was >156 (Matsushita et al., 1997). In rats, JTE-522 significantly reversed the yeast-induced pyrexic response (ED_{50}: 3.9 mg/kg) (Matsushita et al., 1997).

Cyclooxygenase-2 mRNA was reported to be expressed in the rat lung 4 h after lipopolysaccaride treatment (Liu et al., 1996). It is possible that cyclooxygenase-2 may be induced 5 h after ozone exposure, which may in turn induce the production of prostaglandins and thromboxane A_2 in the airway and that may participate in late-phase airway hyperresponsiveness. For these reasons, we studied changes in thromboxane B_2 and prostaglandin E_2 concentration in bronchoalveolar lavage fluid before and after ozone exposure. Concentrations of both increased significantly immediately after exposure and decreased to near-baseline levels by 5 h after exposure. The immediate and transient increases were partially but significantly inhibited by JTE-522 but they were inhibited by indomethacin to near-baseline levels. The effects of JTE-522 and indomethacin on thromboxane B_2 and prostaglandin E_2 concentration suggested that both cyclooxygenase-1 and cyclooxygenase-2 might participate in the production of these prostanoids immediately after ozone. This time course is inconsistent with the idea that cyclooxygenase-2 is induced by ozone 5 h after exposure. Induction of cyclooxygenase-2 mRNA was demonstrated to occur only several hours after treatment with pro-inflammatory cytokines (Belvisi et al., 1997) and lipopolysaccaride (Liu et al., 1996).

The other possibility is that cyclooxygenase-2 may exist constitutively in guinea pig airway and that metabolites of cyclooxygenase-2 may induce a long-lasting airway hyperresponsiveness. In the present immunohistochemical study, cyclooxygenase-2 expression was detected not only after but also before ozone exposure, with no difference in the number of cyclooxygenase-2-positive cells between any time point. These data indicate that cyclooxygenase-2 exists constitutively in guinea pig airway. Ermert et al. (1998) reported that both cyclooxygenase-1 and cyclooxygenase-2 are constitutively expressed in normal rat lung, and suggested that cyclooxygenase-2 may be not only related to lung inflammation but may also be implicated in regulatory processes under normal conditions. It is plausible that thromboxane A_2 and prostaglandin E_2 had increased rapidly on activation of existing cyclooxygenase-2 with ozone exposure in our model. It is possible that the bronchoalveolar lavage technique itself induced cyclooxygenase-2 in the present study. As cyclooxygenase-2 mRNA was reported to be expressed several hours after stimulation (Belvisi et al., 1997; Liu et al., 1996), there may not have been enough time for cyclooxygenase-2 to be induced by the bronchoalveolar lavage technique, especially in the group measured before ozone exposure. It was strongly suggested that cyclooxygenase-2 might be constitutively expressed before ozone exposure.

The next question is whether a cyclooxygenase-2 metabolite participates in airway hyperresponsiveness 5 h after its increase. To clarify whether thromboxane A_2 and prostaglandin E_2, which are transiently produced by cyclooxygenase-2, can induce airway hyperresponsiveness 5 h after ozone exposure, we examined the time course of airway responsiveness after inhalation of prostaglandin E_2 and that of thromboxane A_2 mimetic, U-46619. Prostaglandin E_2 inhalation had no effect on airway hyperresponsiveness. U-46619 did not affect responsiveness of 3 h after inhalation, but significantly increased responsiveness 5 h after inhalation.

The reason why thromboxane A_2-induced airway hyperresponsiveness occurs so long after thromboxane A_2 inhalation remains unclear. It is possible that thromboxane A_2 induces airway hyperresponsiveness through a second mediator. A study of the relationship between cyclooxygenase, thromboxane A_2 and nitric oxide synthase (Chen et al., 1997) showed that cyclooxygenase inhibitors, a thromboxane A_2 synthase inhibitor and a thromboxane A_2 receptor antagonist inhibited nitric oxide synthase activity by changing cytosolic Ca^{2+} concentration in human platelets, and that this inhibitory effect was reversed by U-46619. It was also reported that the allergen-induced late asthmatic response is closely associated with endogenous nitric oxide (Iijima et al., 1998). In our model, U-46619 may have exerted an effect on a second mediator such as nitric oxide to induce airway hyperresponsiveness 5 h after ozone exposure. These results suggest that thromboxane A_2 may be transiently generated by cyclooxygenase-2 immediately after ozone exposure, and may induce airway hyperresponsiveness a long time after ozone exposure in guinea pigs.

JTE-522 did not completely inhibit the increases in prostaglandin E_2 and thromboxane B_2 concentration immediately after ozone exposure. Cyclooxygenase-1 might also participate in the production of prostaglandin E_2 and

thromboxane A_2. It has been reported that indomethacin, but not the selective cyclooxygenase-2 inhibitor, NS-398, inhibited the acute release of prostanoids induced by lipopolysaccaride in rats (Salvemini et al., 1995). These results suggest that constitutive cyclooxygenase-1 might be responsible for the acute release of prostanoids elicited by lipopolysaccaride in rats. In the present study, ozone may also have caused the activation of cyclooxygenase-1, and prostaglandin E_2 and thromboxane A_2 may be produced partially by cyclooxygenase-1.

In summary, a selective cyclooxygenase-2 inhibitor, JTE-522, inhibited ozone-induced airway hyperresponsiveness 5 h after ozone exposure in guinea pigs but did not inhibit the increase in neutrophils. Concentrations of prostaglandin E_2 and thromboxane B_2 in bronchoalveolar lavage fluid were significantly increased immediately after ozone exposure but decreased to their baseline level 5 h after exposure. JTE-522 inhibited the increase in prostaglandin E_2 and thromboxane B_2 partially but significantly immediately after ozone exposure. Expression of cyclooxygenase-2 was detected before ozone exposure and showed no change after exposure. In addition, exogenously applied U-46619 caused airway hyperresponsiveness 5 h after inhalation. Taken together, these results suggest that cyclooxygenase-2 may exist constitutively in guinea pig airway, and that this cyclooxygenase-2 may produce thromboxane A_2 transiently on ozone stimulation, and this thromboxane A_2 may itself participate in the late phase of airway hyperresponsiveness.

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