

Effect of aerosolized administration of KF19514, a phosphodiesterase 4 inhibitor, on bronchial hyperresponsiveness and airway inflammation induced by antigen inhalation in guinea-pigs

S. MYOU, M. FUJIMURA, K. KURASHIMA, H. TACHIBANA, T. HIROSE and S. NAKAO

The Third Department of Internal Medicine, Kanazawa University School of Medicine, Kanazawa, Ishikawa, Japan

Summary

Background Although phosphodiesterase (PDE) 3 and 4 inhibitors have received much attention for the treatment of bronchial asthma, systemic adverse effects have also been reported.

Objective The purpose of this study was to investigate the effect of inhaled olprinone, a newly developed PDE3 inhibitor, and KF19514, a PDE1 and 4 inhibitor, on antigen-induced airway reactions in guinea-pigs.

Methods Fifteen minutes after inhalation of olprinone (0.1 or 1.0 mg/mL) and KF19514 (0.1 or 0.01 mg/mL), animals were given an antigen challenge. Bronchial hyper-responsiveness and bronchoalveolar lavage fluid cell analysis were performed 24 h after the antigen challenge.

Results Inhalation of olprinone and KF19514 caused a dose-related inhibition of antigen-induced bronchoconstriction. Antigen inhalation significantly increased bronchoconstrictor responses to methacholine, and airway accumulation of neutrophils and eosinophils, 24 h after the antigen challenge. These responses were dose-dependently prevented by KF19514, but not by olprinone.

Conclusion The results indicate that inhaled PDE inhibitors might be useful for treatment of bronchial asthma.

Keywords: antigen challenge, bronchial hyperresponsiveness, airway inflammation, eosinophils, neutrophils, phosphodiesterase inhibitor, KF19514, olprinone, methacholine, guinea-pigs

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Introduction

Cyclic nucleotide phosphodiesterases (PDEs) regulate various physiological responses in many cells and tissues. Some investigators have identified PDE 1, 2, 3, 4 and 5 families in human tracheal [1] and bronchial [2,3] smooth muscles. It has been reported that the selective PDE3 inhibitors have a bronchodilatory effect in asthmatic patients [4,5], and

inhibit antigen-induced bronchoconstriction in guinea pigs [6] and asthmatics [5]. We have also demonstrated that a PDE3 inhibitor, cilostazol, reduces bronchial responsiveness in healthy volunteers [7] and asthmatics [4]. In guinea pigs, PDE4 inhibitors were found to prevent antigen-induced bronchial hyperresponsiveness and inflammatory cell infiltration in the airway, but they did not inhibit allergen-induced early and late phase airway obstruction [8,9].

However, PDE3 inhibitors markedly affect heart rate and blood pressure [10]. At therapeutic doses, cilostazol also produces headache, perhaps as a result of cerebral

Correspondence: S. Myou, The Third Department of Internal Medicine, Kanazawa University School of Medicine, 13–1 Takara-machi, Kanazawa 920–8641, Japan.

vasodilation [7]. In addition, clinical studies conducted with rolipram indicate that the dose-limiting sideeffects of PDE4 inhibitors are likely to be nausea and vomiting [11]. Since the airway and systemic adverse effects cannot be separated, systemic PDE3 and 4 inhibitors may be of little benefit for the treatment of asthma.

Recently, we [12] demonstrated that olprinone, a newly developed PDE3 inhibitor, has a bronchodilator effect in asthmatic patients, although it has been reported that the ultrasonically-nebulized PDE3 inhibitor, milrinone, has no inhibitory effect on histamine- and antigen-induced bronchoconstriction in cats [13], and that direct injection of a PDE3 inhibitor, MKS 492, has no bronchodilatory effect in guinea pigs [14]. On the other hand, it has been reported that nebulized KF19514, a PDE1 and 4 inhibitor, reduces platelet-activating factor (PAF)-induced bronchial hyperresponsiveness and inflammatory cell infiltration in guinea pig airways, although nebulized rolipram, a PDE4 inhibitor, has no inhibitory effect on PAF-induced bronchial hyperresponsiveness [15]. We also demonstrated that inhalation of cilostazol powder and KF19514 solution inhibited histamine- and antigen-induced bronchoconstriction in guinea pigs [16]. However, the effect of inhaled PDE3 or 4 inhibitor on antigen-induced cell accumulation in the airway, and bronchial hyperresponsiveness, has not been investigated. The purpose of this study was to investigate the effect of inhaled olprinone and KF19514 on antigen-induced bronchoconstriction, eosinophil and neutrophil accumulation in the airway, and bronchial hyperresponsiveness to methacholine.

Materials and methods

Chemicals

The following chemicals were used: sodium pentobarbitone (Abbot Laboratories, North Chicago, USA), methacholine (Wako Pure Chemical Ind., Osaka, Japan), KF19514 (5-phenyl-3-(3-pyridyl)methyl-3H-imidazo(4,5-c)(1,8)naphthyridin-4(5H)-one; Kyowa Fermentation, Shizuoka, Japan), olprinone (1,2-dihydro-6-methyl-2-oxo-5-[imidazo(1,2-a)pyridin-6-yl]-3-pyridine carbonitrile hydrochloride monohydrate; Eisai Pharmaceuticals Co., Tokyo, Japan), and ovalbumin (Sigma, St Louis, MO, USA).

Animals

Male, albino, Hartley strain guinea-pigs weighing 300–350 g were obtained from Sankyou Laboratory Service (Toyama, Japan). They were quarantined in the Animal Research Center of Kanazawa University for 1 week before the study. All the animal procedures in this study complied with the standards set out in the Guideline for the Care and

Use of Laboratory Animals on the Takara-machi Campus of Kanazawa University.

Passive sensitization of animals

Guinea-pig homocytotropic antiserum was obtained according to the modified method of Santives *et al.* [17]. Briefly, 500 µg ovalbumin was emulsified in Freund's complete adjuvant and injected intradermally into each guinea pig at multiple sites. Boosting was carried out in the same manner 2 weeks later. Serum was collected from each animal 2 weeks after the booster, pooled, and kept frozen until use. The antibody titre of this serum was 1 : 12 800 at 4 h, 1 : 6400 at 24 h, and 1 : 512 at 7 days, as estimated by passive cutaneous anaphylaxis. Normal guinea-pigs were passively sensitized with 1.0 mL antiserum/kg given intraperitoneally 5 days before the antigen challenge.

Antigen-induced bronchoconstriction

Thirteen minutes before the antigen challenge, animals received diphenhydramine (20 mg/kg, i.p.) as protection against fatal anaphylaxis during the antigen challenge. The PDE3 inhibitor olprinone (0.1 or 1.0 mg/mL dissolved in saline, $n = 7$ in each group) or the PDE4 inhibitor KF-19514 (0.1 or 0.01 mg/mL dissolved in saline, $n = 7$ in each group) was inhaled for 5 min from a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA, USA) operated by compressed air at 5 L/min. The nebulizer output was 0.14 mL/min. Additional animals were included as control groups and received either the inhaled vehicle for olprinone or KF-19514 and inhaled saline instead of the antigen (ovalbumin) challenge (negative control [NC] group), or the vehicle and inhaled ovalbumin (positive control [PC] group) ($n = 7$ in each group).

Animals were placed in a whole body, double chamber plethysmograph to allow measurement of specific airway resistance (sRaw) with a modified version of the method described by Pennock *et al.* [18]. A respiratory mechanics analyser (model PMUA + SAR, Buxco Electronics, Troy, NY, USA) was used to measure the phase shift between nasal and thoracic air flows and compute sRaw on a breath by breath basis. A bias flow of air (20 mL/s) was maintained through the nasal chamber to ensure a constant supply of fresh air to the animals. This flow was subtracted by the respiratory mechanics analyser from the calculations. Fifteen minutes after the administration of olprinone or KF19514, the animals were challenged by exposure for 90 s to an aerosol of ovalbumin, generated from a 10 mg/mL solution in saline by a DeVilbiss 646 nebulizer and passed through the nasal chamber. The sRaw was measured for 5 min before the administration of PDE inhibitors and the antigen challenge.

(baseline measurement), and for 15 min after the antigen challenge.

Antigen-induced bronchial hyperresponsiveness

Antigen-induced bronchial hyperresponsiveness was measured 24 h after the antigen challenge. Guinea pigs were anaesthetized with an intraperitoneal injection of 75 mg/kg sodium pentobarbitone and placed in a supine position. After the trachea was cannulated with a polyethylene tube (outside diameter, 2.5 mm; inside diameter, 2.1 mm), the animals were artificially ventilated with a small animal respirator (Model 1680, Harvard Apparatus Co, Inc, South Natick, MA, USA) adjusted to a tidal volume of 10 mL/kg at a rate of 60 strokes/min. The changes in lung resistance to inflation, the lateral pressure of the tracheal tube (pressure at the airway opening; Pao, cmH₂O), were measured with a pressure transducer (TP-603T, Nihon Koden Kogyo Co, Ltd, Tokyo, Japan) according to the modified method of Konzett and Roessler [19] described by Jones *et al.* [20]. After confirming that the change in Pao following inhalation of histamine and LTC₄ represented the average of the changes in pulmonary resistance (RL) and reciprocal dynamic lung compliance (1/Cdyn) [21], the change in Pao was used as an overall index of bronchial response to bronchoactive agents [22,23]. After completion of all surgical procedures, the animals were over-inflated by two times tidal volume for two breaths by closing the outlet port of the respirator to unify the volume history of the lung. Five minutes after the instillation of artificial respiration when the Pao had stabilized, doubling concentrations of methacholine (25–1600 µg/mL) were given for 20 s with an ultrasonic nebulizer, at intervals of 5 min, until a 200% increase in the Pao was recorded or the dose sequence was completed. The nebulizer generated the aerosol at a rate of 15.2 µL/min during the 20 s period, and 46.4% of the generated aerosol was deposited in the lung, as measured by the radioaerosol technique [24]. The median aerodynamic diameter of the particles of normal saline was $3.59 \pm 1.96 \mu\text{m}$ (mean $\pm$ SD) [25].

Analysis of bronchoalveolar lavage (BAL) cells

After the examination of antigen-induced bronchial hyperresponsiveness, 10 mL sterile saline at 37°C were infused twice through the tracheal cannula. The fluid was recovered manually by means of gentle aspiration with a disposable syringe after each infusion. The total number of cells was determined using a Turk solution. Differential cell counts were performed on cytopspin preparations (Cytospin 2, Shandon, UK) by counting 300 cells after staining with May-Grünwald-Giemsa stain.

Statistical analysis

Bronchial responsiveness to inhaled methacholine was expressed as the dose of methacholine required to provoke a 200% increase (PC₂₀₀) in Pao, and PC₂₀₀ values were logarithmically transformed for analysis and reported as the geometric mean (geometric standard error of the mean [GSEM]). All measurements except PC₂₀₀ were expressed as mean $\pm$ standard error of the mean (SEM). All results were compared by analysis of variance (ANOVA) followed by Fisher's protected least significant difference. Significance was based on a 95% confidence level ($P < 0.05$).

Results

There were no significant changes in sRaw after administration of olprinone or KF19514. The aerosolized antigen caused an acute bronchoconstriction which peaked within 5 min, and olprinone and KF19514 caused a dose-related inhibition of the antigen-induced bronchoconstriction (Fig. 1). Antigen inhalation significantly increased bronchoconstrictor responses to methacholine without residual bronchoconstriction (Fig. 2) and airway accumulation of neutrophils and eosinophils (Fig. 3). These responses were dose-dependently prevented by KF19514, but not by olprinone (Figs 2 and 3).

Discussion

The effect of inhaled PDE3 and 4 inhibitors on antigen-induced early phase bronchoconstriction, airway inflammation and bronchial hyperresponsiveness 24 h after antigen inhalation in guinea-pigs were investigated. The results of the study showed that inhaled olprinone and KF19514 reduced antigen-induced bronchoconstriction, and that inhalation of KF19514, but not olprinone, prevented antigen-induced eosinophil and neutrophil accumulation in BAL fluid and bronchial hyperresponsiveness. Although antigen-induced bronchial hyperresponsiveness was assessed in an anaesthetized model in the present study, it has been demonstrated in an awake model that antigen-induced bronchial hyperresponsiveness is reduced by systemic administration of the PDE4 inhibitor, rolipram, or the dual PDE3/4 inhibitor, ORG 20241, but not the PDE3 inhibitor, CI-930 [6,9].

It has been reported that olprinone inhibits guinea pig heart PDE3 and increases the cyclic AMP contents of isolated guinea pig papillary muscles [26], and that the IC₅₀ values of olprinone, which inhibit PDE1, PDE2, PDE3 and PDE4, are 150, 100, 0.35 and 14 µmol/L, respectively [27]. The maximum dose of olprinone inhaled in this study was 0.70 mg (2.3 µmol). Although olprinone was administered at sufficiently large doses to inhibit antigen-induced bronchoconstriction, olprinone failed to reduce the eosinophil or neutrophil accumulation in BAL fluid, or the

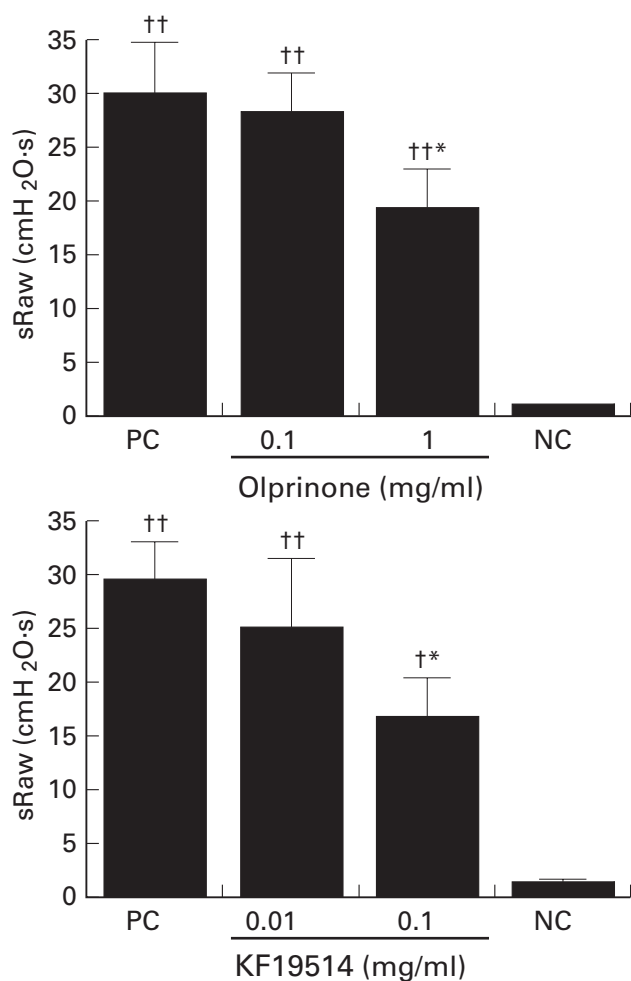


Fig. 1. Effects of inhaled olprinone (a) and KF19514 (b) on antigen-induced early phase airway obstruction in guinea-pigs. Error bars indicate mean $\pm$ SEM. Statistical significance is indicated as follows: † $P < 0.05$ and †† $P < 0.01$ compared with negative control group (NC); * $P < 0.05$ compared with positive control group (PC).

bronchial hyperresponsiveness induced by antigen inhalation, thus making it unlikely that PDE3-mediated mechanisms play an important role in antigen-induced bronchial hyper-responsiveness and airway inflammation.

KF19514 has been reported to inhibit PDE4 and 1, and the IC₅₀ values for PDE1, PDE2, PDE3, PDE4 and PDE5 are 0.27, >10, >10, 0.40 and >10 μ mol/L, respectively [28]. The maximum dose of KF19514 inhaled in this study was 0.07 mg (0.16 μ mol). The PDE1 inhibition by KF19514 does not seem to contribute to the inhibition of inflammatory cell accumulation, because vinpocetine, a selective PDE1 inhibitor, does not inhibit eosinophil accumulation [28,29]. These results suggest that inhalation of a PDE4 inhibitor might reduce antigen-induced bronchial hyper-responsiveness and airway inflammation. However, no

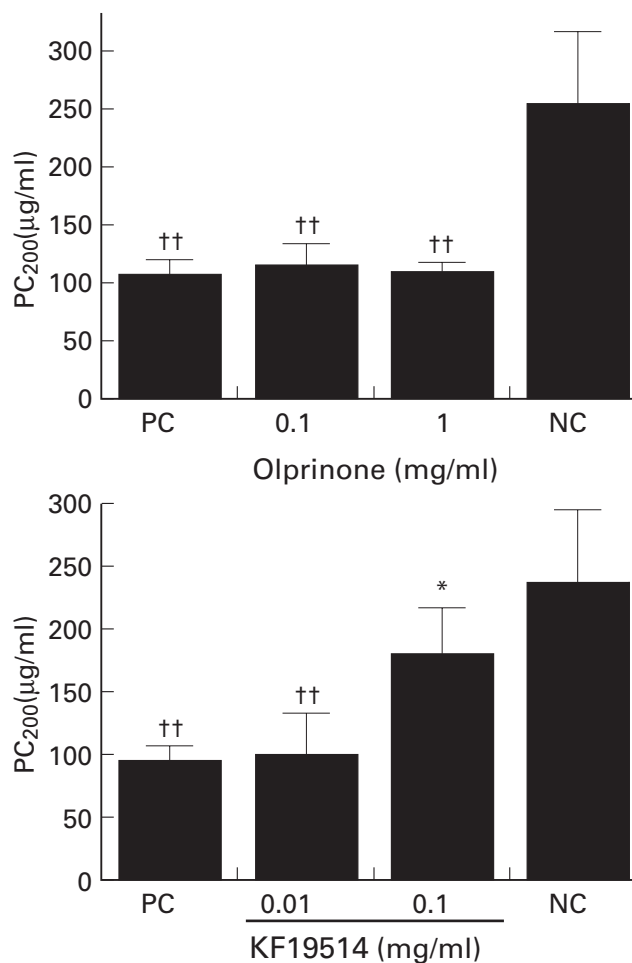


Fig. 2. Effects of inhaled olprinone (a) and KF19514 (b) on bronchial hyperresponsiveness to methacholine 24 h after antigen inhalation in guinea-pigs ($n = 7$ in each group). Error bars indicate mean $\pm$ SEM. PC₂₀₀: provocative concentration of methacholine producing a 200% increase above the baseline, in pressure at the airway opening; PC: positive control; NC: negative control. Statistical significance is indicated as follows: †† $P < 0.01$ compared with the NC group; * $P < 0.05$ compared with the PC group.

data are available on the interaction between inhibitors of PDE1 and PDE4. While it would appear likely that the actions of KF19514 *in vivo* are accounted for by PDE4 inhibition, a contribution of PDE1 inhibition cannot be excluded unless experiments are conducted with selective PDE1 and PDE4 inhibitors alone and in combination. Further studies are required to reach a conclusion.

Airway hyperresponsiveness following antigen challenge occurred at a time when Pao was identical between all groups, thus reflecting a true increase in airway responsiveness and not the results of residual bronchoconstriction from the initiation of methacholine inhalation. The duration of the inhaled effect of KF19514 as a bronchodilator is unclear.

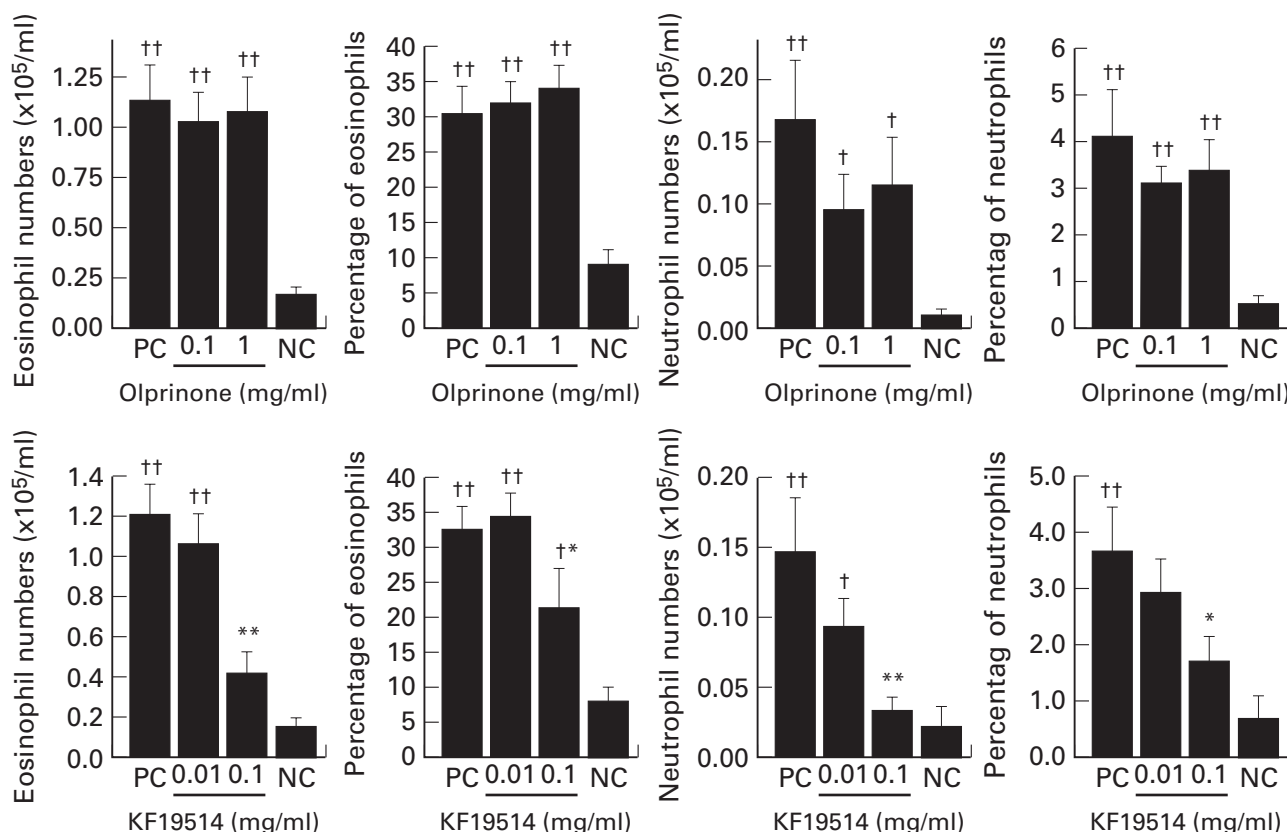


Fig. 3. Effects of inhaled olprinone (a,b,c,d) and KF19514 (e,f,g,h) on eosinophil and neutrophil accumulation in bronchoalveolar lavage fluid 24 h after antigen inhalation in guinea pigs ($n = 7$ in each group). Error bars indicate mean $\pm$ SEM. PC: positive control; NC: negative control. Statistical significance is indicated as follows: $\dagger P < 0.05$ and $\dagger\dagger P < 0.01$ compared with the NC group; $*P < 0.05$ and $**P < 0.01$ compared with the PC group.

Since KF19514 showed no effect on baseline Pao (the initiation of methacholine inhalation), the effect of KF19514 on antigen-induced airway hyperresponsiveness might not result from its bronchodilatory effect.

The means by which PDE4 inhibition reduces airway inflammation and bronchial hyperresponsiveness after the antigen challenge remains unclear, although it is possible to speculate on the nature of certain mechanisms. We have suggested that KF19514 may have an inhibitory effect on antigen-induced mediator release in guinea-pig airway *in vivo* [16], which may be a reason for the inhibitory effect of KF19514 on antigen-induced bronchial hyperresponsiveness. Indeed, thromboxane A₂, which increases bronchial responsiveness, was involved in our experimental model of antigen-induced bronchoconstriction [30]. However, PDE4 inhibitors suppress mast cell mediator release only at very high concentrations [31]. This is unlikely to be a significant action of the drugs *in vivo*. On the other hand, it has been reported that the PDE4 inhibitors, rolipram and Ro 20-1724, markedly inhibit eosinophil infiltration into the guinea pig airways induced by cytokines such as

IL-5 and IL-8 [32], and that rolipram markedly reduces leukotriene B₄-induced polymorphonuclear cell infiltration [33]. These effects of PDE4 inhibitors may be involved in the mechanisms for attenuation of airway inflammation.

In conclusion, it was found that PDE3 and 4 inhibitors reduce antigen-induced bronchoconstriction, and that PDE4 inhibitors attenuate neutrophil and eosinophil infiltration into the airways induced by antigen. These data suggest that inhalation of PDE3 and 4 inhibitors may represent a new approach for the treatment of asthma.

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