

EFFECT OF IPD-1151T (SUPLATAST TOSILATE) ON AIRWAY
HYPERRESPONSIVENESS IN MICEShin-ichi Konno¹⁾, Kazuhito Asano²⁾, Yoshiki Gonokami¹⁾, Masatsugu Kurokawa¹⁾,
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We investigated the effect of IPD-1151T (Suplatast Tosilate, ($\pm$)-[2-[4-(3-ethoxy-2-hydroxypropoxy) phenyl-carbamoyl]-ethyl] dimethylsulfonium p-toluenesulfonate) against allergic bronchoconstriction induced by allergen and methacholine (MCh). BALB/c mice were sensitized by intraperitoneal injection of 0.5 μ g of keyhole limpet hemocyanin mixed with Al(OH)₃. IPD-1151T was administered orally once a day for 5 or 14 days in doses of 10, 30 or 100 mg/kg body weight. Bronchoconstriction was measured 24 hrs after the final drug administration. IPD-1151T inhibited both antigen-mediated allergic bronchoconstriction and induction of bronchial hyperresponsiveness after sensitization or allergen challenge in actively sensitized mice. The degree of inhibition was proportional to the dose and frequency of oral administration of the agent.

Key words: airway hyperresponsiveness — asthma — mice — Stuplatast Tosilate

INTRODUCTION

Bronchial asthma is an inflammatory condition affecting the airway^{1,2)}. Bronchial hyperresponsiveness (BHR) is an important feature of asthma as it reflects the severity of the disease^{3,4)}. The ultimate task in the treatment of asthma is the relief of symptoms to permit a normal life style. The main measures used to achieve this goal include reducing in airflow resistance and shifting in BHR towards the normal range. To accomplish these objectives, several anti-allergic drugs with a bronchodilating activity have been developed and used clinically in the treatment of asthma.

Recently Koda et al.⁵⁾ investigated the immunopharmacological properties of IPD-1151T (Suplatast Tosilate, ($\pm$)-[2-[4-(3-ethoxy-2-hydroxypropoxy) phenyl-carbamoyl]-ethyl] dimethylsulfonium p-toluenesulfonate). An interesting finding in that study was the description of a inhibitory effect of IPD-1151T on inter-

leukin (IL)-4 production from T lymphocytes as well as IgE synthesis by B lymphocytes in response to antigenic stimulation. Since IL-4 and IgE are two compounds known to play an important role in the development of asthma, it is possible that IPD-1151T may manifest bronchodilating properties useful for the treatment and prevention of asthma. To our knowledge, there are no reports describing the influence of IPD-1151T on bronchoconstriction.

We, therefore, investigated the effect of IPD-1151T on allergic airway hyperresponsiveness induced by exposure of keyhole limpet hemocyanin (KLH)-sensitized mice to repetitive antigen and to methacholine (MCh).

MATERIALS AND METHODS

Mice: Five weeks old male BALB/c mice were purchased from Charles River Japan.

Agent: IPD-1151T was kindly supplied by Taiho Seiyaku Co. (Tokyo, Japan) as a pure powder. The drug was dissolved in distilled water at 5.0 mg/ml just before use.

Hapten-protein conjugate: KLH was

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Abbreviations: MCh methacholine; IL interleukin;
KLH keyhole limpet hemocyanin

obtained from Sigma (St. Louis MO). 2,4-dinitrobenzen-sulfonic acid sodium salt (DNBS) was purchased from Tokyo Kasei Corp., Tokyo, Japan. DNBS-KLH conjugate was prepared as previously described^{6,7)}, and referred to as DNP-KLH.

Sensitization: Mice were sensitized by intraperitoneal injection with 5.0 $\mu\text{g}/\text{ml}$ of DNP-KLH mixed with 2 mg of $\text{Al}(\text{OH})_3$ (Wako Pure Chemical Ind., Osaka, Japan) in a total volume of 0.5 ml of saline. Total IgE level were measured before and 14 days after sensitization.

Drug administration: Mice were administered 10, 30, or 100 mg/kg body weight of IPD-1151T orally in a volume not exceeding 0.5 ml. Control mice were orally administered the vehicle only (distilled water). Administration was performed once a day on the following schedules: one group of mice were treated for 5 days from day 9 of sensitization and the others for 14 days from commencement of sensitization (day 0).

Induction of airway hypersensitivity and measurement of airway reactivity: Twenty-four hrs after the final administration of IPD-1151T, mice were anesthetized with intraperitoneal injections of 50 mg/kg body weight pentobarbital sodium (Abbott Lab, North Chicago, IL). When an appropriate plane of anesthesia was induced, the jugular vein was cannulated for administration of 0.5 mg KLH in a volume of 0.1 ml.

The trachea was cannulated and connected to a respiratory (SN-480-3, Shinano Seisakusho, Tokyo) that provided ventilation at a tidal volume of 1.0 ml/100 g body weight and a rate of 100 strokes/min⁸⁾. Under these conditions, we found that the level of arterial oxygen tension ranged between 70 to 80 torr, as measured with a transcutaneous oxygen tension meter (OKV-7101, Nihon Kodan, Tokyo). The respiratory resistance was determined using a modified method of Konzett and Rossler, as described earlier⁸⁾. Briefly, a bypass tube with an opening into the air was placed between the gas supply of the respirator and the endotracheal tube, and

an air flow resistance tube (TV-241T, Nihon Kodan) was connected to the bypass. The volume of air passing through the bypass tube was measured using a differential pressure transducer (TP-602T, Nihon Kodan) connected to the air flow resistance tube and an integrator (EI-601G, Nihon Kodan). All signals were recorded continuously (RTA-1300 recorder, Nihon Kodan). The side arm of the tracheal cannula was connected to a differential transducer to monitor volume of air overflow according to the method of Konzett and Rossler⁹⁾. The results were expressed as mean ($\pm$ SE) volume (ml) of air overflow.

To examine the influence of a bronchoconstrictor agonist on airway hyperresponsiveness 10 min after antigen challenge, mice previously challenged with KLH were injected intravenously with various concentrations of MCh in a volume of 0.1 ml to measure airway reactivity.

Statistics: Statistical significance was determined using Student *t*-test.

RESULTS

The influence of oral administration of IPD-1151T on antigen-induced allergic airway responses in mice is shown in Fig. 1. IgE level were elevated 410 ng/ml from 0 ng/ml 14 days after sensitization. KLH caused a severe allergic bronchoconstriction in sensitized mice. Oral administration of IPD-1151T inhibited allergic bronchoconstriction in a dose-dependent manner. A larger dose and higher frequency of administration produced a higher volume or gradually diminished air overflow. The ED₅₀ or IPD-1151T were 83.7 mg/kg/day for short-term (5 days) and 17.8 mg/kg/day for long-term (14 days) treatment.

To examine the effect of IPD-1151T on bronchial hyperreactivity induced by sensitization or antigen challenge in sensitized mice, we measured bronchial reactivity to MCh in sensitized mice that were administered IPD-1151T for 14

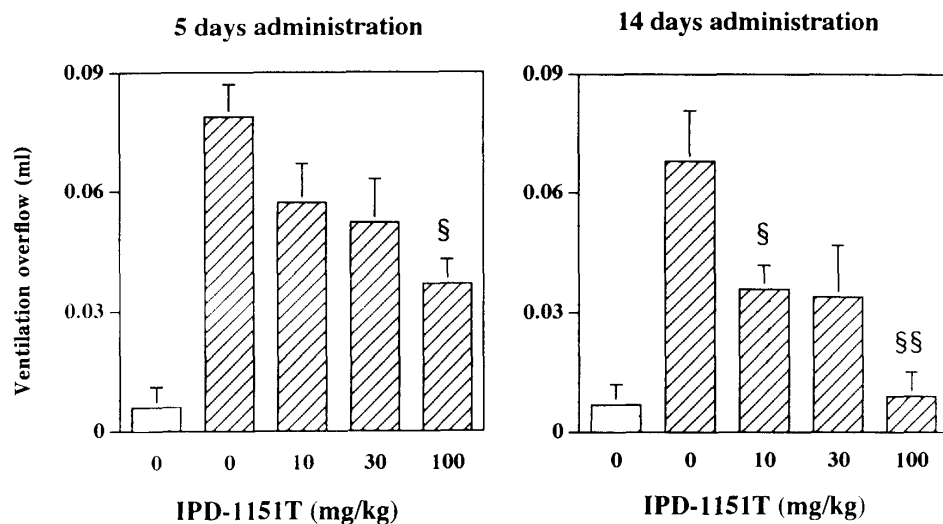


Fig. 1. Effect of IPD-1151T on antigen-induced allergic airway responses in sensitized mice. Each value represents mean $\pm$ S.E. of 5-8 mice. § $p < 0.05$; §§ $p < 0.01$ as compared with IPD-1151T untreated sensitized mice. Open bar: non-sensitized mice, hatched bars: sensitized mice.

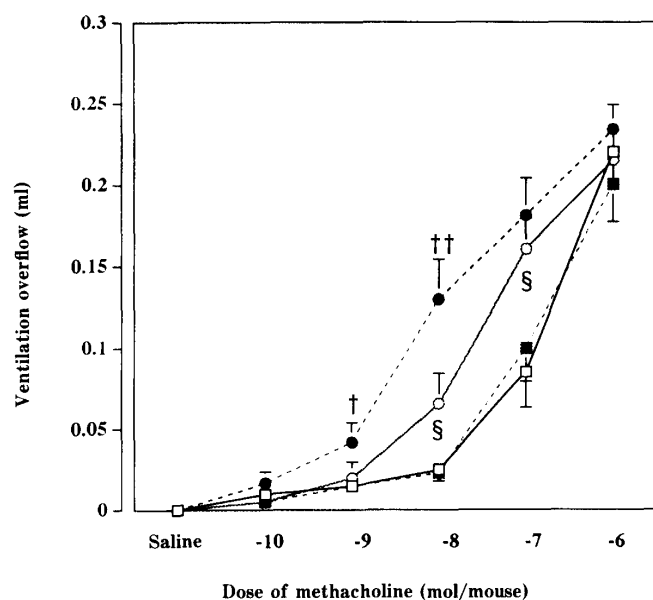


Fig. 2. Influence of sensitization and antigen challenge on bronchial responsiveness to methacholine in mice. Each value represents mean $\pm$ S.E. of 8 mice. § $p < 0.05$; §§ $p < 0.01$, compared with non-sensitized mice before antigen challenge. † $p < 0.05$; †† $p < 0.01$, compared with sensitized mice before antigen challenge. Open squares: non-sensitized mice before antigen challenge, closed squares: non-sensitized mice after antigen challenge, open circles: sensitized mice before antigen challenge, closed circles: sensitized mice after antigen challenge.

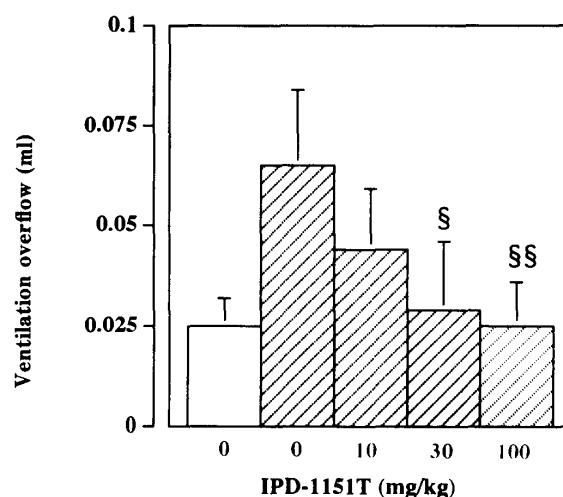


Fig. 3. Effect of IPD-1151T on bronchial hyperresponsiveness to 10^{-8} mol/mouse of methacholine. Each value represents mean $\pm$ S.E. of 8 mice. § $p < 0.05$; §§ $p < 0.01$, compared with no-treated (0 mg of IPD-1151T) sensitized mice. Open bar: non-sensitized mice, hatched bars: sensitized mice.

days after sensitization. Fig. 2 shows that bronchial reactivity to MCh increased in control mice 14 days after sensitization, and that the reactivity was more enhanced by antigen challenge than sensitization. Figs. 3 and 4 show that IPD-1151T inhibited bronchial hyperreactivity induced by sensitization as well as antigen challenge in a dose-dependent fashion. The ED₅₀ of IPD-1151T were 6.2 mg/kg/day and 0.86 mg/

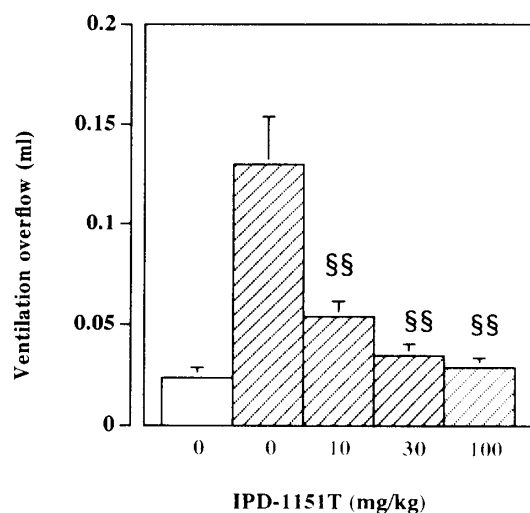


Fig. 4. Effect of IPD-1151T on bronchial hyperresponsiveness to 10^{-8} mol/mouse of methacholine induced by antigen challenge in sensitized mice. Each value represents mean $\pm$ S.E. of 6 mice. §: $p < 0.05$, §§ $p < 0.01$, compared with no-treated (0 mg of IPD-1151T) sensitized mice. Open bar: non-sensitized mice, hatched bars: sensitized mice.

kg/day, respectively.

DISCUSSION

Allergen-induced airway contraction is mainly due to reaction between antigen and allergen specific IgE bounded mast cell. This reaction need specific IgE production from B lymphocytes supposed by sensitized Th2 lymphocytes via cytokin stimulation such as IL-4. Suplatast Tosilate is able to surpress IL-4 production from lymphocytes, and results in inhibition of IgE formation⁵⁾. However, this suppression need more period than 14 days after sensitization⁵⁾, in spit of no significant differences between serum Cmax and AUC when IPD-1151T was admistered for long-term, and those when it was singly administered¹⁰⁾. Furthermore, it is known that IPD-1151T inhibits antigen-mediated histamine release from mast cells, suppresses mast cell induction¹¹⁾. In our study, antigen induced-bronchoconstriction in more surpressed in long-term (14 days) treated mice than in short-term (5

days) treated mice. Based on these earlies results, together with the present findings, we conclude that oral administration of IPD-1151T may inhibit not only histamine release but also IgE production and mast cell induction, thus minimizing the bronchoconstrictive effect of antigenic challenge.

Involvement of the vagal cholinergic nervous system and the release of acetylcholine and serotonin, is considered to be closely associated with the development of airway hyperresponsiveness¹²⁾. It is also generally believed that these humoral factors enhance the level of bronchoconstriction, thus pointing to the importance of examining the influence of agents on allergen-induced airway hyperresponsiveness to humoral factors (acetylcholine and serotonin) when assessing the efficacy of new agents on asthma. On the other hand, it is also reported that IPD-1151T has no direct dilating effect to bronchial smooth muscle via acethylcholine receptor¹³⁾. Therefore, we chose to use sensitization- or antigen-induced increases in airway responsiveness to MCh and examined the influence of IPD-1151T on MCh-mediated allergic airway hyperresponsiveness. As shown in Figs. 3 and 4, IPD-1151T dose-depnedly inhibited airway reactivity to MCh. Allergen-induced increases in airway hyperresponsiveness parallel IgE-mediated allergic reactions within airways and are believed to be due to airway inflammation. There is ample evidence to suggest that platelet activating factor and histamine, released by polymorphonuclear leukocytes (especially mast cells), in response to IgE and specific antigens, are the most important chemical mediators in airway hyperreactivity¹²⁾¹⁴⁾¹⁵⁾. On the other hand, Larsen demonstrated that developing an IgE response to allergen is associated with developing alterations in the nerval control of their airways, and this alteration appears to be sensitized lymphocyte depend¹⁶⁾. Thus, those reports and our results suggest that inhibition of airway hyperresponsiveness may be due to the ability of IPD-1151T to inhibit lymphocyte sensitiza-

tion, IgE production, mast cell differentiation and airway responses to antigen challenge. However, detail mechanisms in the suppressive effect of IPD-1151T on sensitization in mice are still remained to be unclear, more investigations are needed to clarify it.

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IPD-1151T (Suplatast Tosilate) のマウスにおける 気道過敏性に対する作用について

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我々は抗原またはメサコリンによるアレルギー性気道収縮に対する IPD-1151T の影響について検討した。BALB/c マウスを5 μ g の KLH (keyhole limpet hemocyanin) をアラムとともに腹腔内に投与し免疫した。10, 30または100mg/kg の IPD-1151T を5日間または14日間連日経口的に投与した。気道収縮は最後の薬剤投与24時間後に測定した。IPD-1151T は抗原誘発の気道収縮を抑制し、さらに感作後または感作, 抗原投与後の気道過敏性亢進を抑制した。その抑制の程度は薬剤の投与量および投与期間に比例した。
