J Pharmacol Sci **105**, 291 – 297 (2007)

Journal of Pharmacological Sciences
©2007 The Japanese Pharmacological Society

Full Paper

Polycyclic Aromatic Hydrocarbons Aggravate Antigen-Induced Nasal Blockage in Experimental Allergic Rhinitis

Nobuaki Mizutani[1,2], Takeshi Nabe[1,*], Yasuyoshi Ohtani[1], Hai Yan Han[1], Masanori Fujii[1], Shin Yoshino[2], Teruhisa Hirayama[3], and Shigekatsu Kohno[1]

Departments of [1]Pharmacology and [3]Public Health, Kyoto Pharmaceutical University, 5 Nakauchi, Misasagi, Yamashina, Kyoto 607-8414, Japan
[2]Department of Pharmacology, Kobe Pharmaceutical University, 4-19-1 Motoyamakita, Higashinada, Kobe 658-8558, Japan

Received July 4, 2007; Accepted September 28, 2007

Abstract. It has been hypothesized that air pollution has played a role in the increase in allergy prevalence. However, it remains unclear what exact roles are played by polycyclic aromatic hydrocarbons (PAHs), which are encountered in the environment in the form of air pollution, in allergic rhinitis. Thus, we examined whether benzo(a)pyrene (BaP) and 1-nitropyrene (1-NP), representative PAHs, aggravate allergic rhinitis symptoms, using a guinea-pig model. Sensitized animals were repeatedly challenged by inhalation of Japanese cedar pollen once a week. BaP or 1-NP was daily and intranasally administered for 2 weeks (short-term treatment) or for 22 weeks from the time before the sensitization period (long-term treatment). The short-term treatment affected neither nasal blockage nor sneezing induced by antigen. In contrast, the long-term treatment aggravated the antigen-induced nasal blockage that was induced 7 weeks after the start of the treatment with BaP or 1-NP. This aggravation continued during the intranasal treatment with PAH. However, neither sneezing nor Cry j 1-specific IgE antibody production was affected even by the long-term treatment. In conclusion, the long-term treatment with BaP and 1-NP can aggravate allergic rhinitis. The mechanisms underlying this aggravation are not associated with production of Cry j 1-specific IgE.

Keywords: benzo(a)pyrene, 1-nitropyrene, allergic rhinitis, nasal blockage, pollen

Introduction

The prevalence of allergic respiratory diseases such as asthma and rhinitis has increased in recent years, especially in industrialized countries. This increase may be due to changes in environmental factors, including indoor and outdoor air pollution. Indeed, in most countries, people who live in urban areas tend to be more affected by allergic respiratory diseases than people in rural areas (1 – 5). Persistent exposure to air pollution such as traffic emissions, photochemical smog components, and cigarette smoke has often been discussed as one of the factors responsible for this increase.

Polycyclic aromatic hydrocarbons (PAHs) such as benzo(a)pyrene (BaP) and 1-nitropyrene (1-NP), which are encountered in the environment mainly in the form of air pollution, are ubiquitous environmental pollutants found in diesel exhaust particles (DEP) and cigarette smoke (6 – 9). Carcinogenic and mutagenic effects of BaP and 1-NP in various cell types have been well documented (8, 10, 11). However, it remains unclear what exact roles are played by BaP and 1-NP in allergic respiratory diseases including rhinitis.

We previously established an experimental allergic rhinitis model in sensitized guinea pigs, using cedar pollen as the antigen. In that model, we observed development of biphasic nasal blockage with repeated pollen inhalation challenges, immediate sneezing at antigen challenge, and an increase in Cry j 1-specific IgE levels in the sera (12, 13). These results are similar to clinical findings, indicating that the model is suitable for analyzing the pathogenesis of allergic rhinitis.

*Corresponding author. nabe@mb.kyoto-phu.ac.jp
Published online in J-STAGE: November 6, 2007
doi: 10.1254/jphs.FP0071067

In the present study, in order to know whether short- and long-term treatment with PAHs aggravate antigen-induced sneezing and nasal blockage, BaP or 1-NP was daily and intranasally administered for 2 weeks (short-term treatment) or for 22 weeks from the time before the sensitization period (long-term treatment). In the long-term treatment, whether the effects of PAHs are associated with an allergen-specific IgE production were also assessed.

Materials and Methods

Animals

Male 3-week-old Hartley guinea pigs weighing 201 – 250 g were purchased from Japan SLC (Hamamatsu). The animals were housed in an air-conditioned room at 23 ± 1°C and 60 ± 10% humidity, with lights on from 8:00 a.m. to 8:00 p.m. They were fed a standard laboratory diet and given water ad libitum. Sensitization of the animals began 2 weeks after they were purchased. All animal experiments were approved by the Experimental Animal Research Committee at Kyoto Pharmaceutical University.

Reagents

Japanese cedar pollen (*Cryptomeria japonica*) was harvested in Gifu and Shiga prefectures (Japan) in 1998. Lidocaine hydrochloride was obtained from Fujisawa Pharmaceutical Co. (Osaka). BaP and 1-NP were purchased from Wako Pure Chemical Ind. (Osaka) and Sigma Aldrich Chemical Ind. (St. Louis, MO, USA), respectively; they were each dissolved in dimethyl sulfoxide (DMSO).

The $Al(OH)_3$ gel was prepared with 0.25 N NaOH and 0.25 N $Al_2(SO_4)_3$, as described elsewhere (14).

The cedar pollen extract used for sensitization was prepared as described elsewhere (12). Briefly, pollen was suspended in phosphate-buffered saline at 100 mg /ml and kept at 4°C for 18 h with mild stirring. The suspension was then centrifuged (1700 × *g*, 15 min), and the supernatant (which was used as the sensitization antigen) was stored at −80°C until used.

Study design

The protocol followed in this study is shown in Fig. 1. Guinea pigs that had been sensitized with pollen extract plus $Al(OH)_3$ were repeatedly challenged with the pollen once every week. From 1 day after the 18th antigen challenge to the 20th challenge, BaP (100 μg/10 μl per nostril) or 1-NP (10 μg/10 μl per nostril) was daily administered into the bilateral nasal cavities (short-term treatment). On the other hand, in the long-term treatment study, BaP (100 μg/10 μl per nostril) or 1-NP (10 μg /10 μl per nostril) was daily administered into the nostrils from 6 days before the first sensitization to the day of the 20th challenge. On days of sensitization and pollen inhalation challenge, administration of BaP or 1-NP was performed 1 h before the sensitization and 5 min before the pollen challenge.

The animals were divided into 5 groups: 1) non-sensitized-challenged, animals that were not sensitized but challenged once on the day of measurement of specific airway resistance (sRaw); 2) sensitized-challenged-saline; 3) sensitized-challenged-vehicle; 4) sensitized-challenged-BaP; and 5) sensitized-challenged-1-NP, sensitized-challenged animals that were treated with saline, vehicle (DMSO), BaP, or 1-NP.

Sensitization and challenge

As previously described (12), guinea pigs were sensitized twice a day for 7 days, by bilateral intranasal instillation of cedar pollen extracts adsorbed onto $Al(OH)_3$ gel [0.3 μg protein/0.3 mg $Al(OH)_3$], at a volume of 3 μl/nostril. To prevent rapid elimination of the antigen by ciliary movement, the upper airway mucosal surface was anesthetized before each sensitization by administering a 5-min inhalation of a mist of 4% lidocaine hydrochloride solution that was generated by

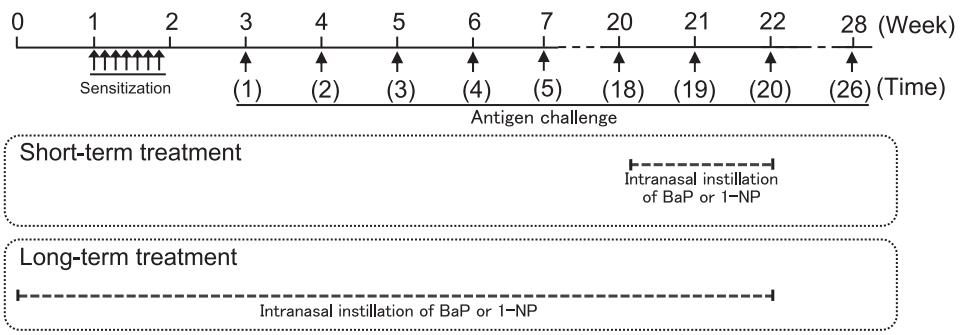


Fig. 1. Schedule for sensitization with cedar pollen extracts and subsequent nasal challenge by inhalation with cedar pollens in the guinea pig and protocol for short- or long-term treatment with BaP or 1-NP. BaP, benzo(a)pyrene; 1-NP, 1-nitropyrene

an ultrasonic nebulizer (NE-U12; Omron, Osaka). The sensitized animals were then intranasally challenged by inhalation of cedar pollen once a week for 26 weeks by using a handmade inhalation apparatus (15).

Measurement of nasal blockage

To evaluate the degree of nasal blockage, specific airway resistance (sRaw) was measured in conscious guinea pigs immediately before and at 1 and 4 h after the 3rd, 5th, 10th, and 20th antigen challenges (long-term treated animals) by using a two-chambered double-flow plethysmograph system (Pulmos-I; M.I.P.S., Osaka) according to the method of Pennock et al. (16). The change in sRaw was expressed as the sRaw value minus the baseline value of sRaw obtained immediately before challenge. At the 13th (long-term treatment study) or 20th (short-term treatment study) antigen challenge, detailed time-course changes in sRaw were measured in BaP- and 1-NP-treated animals. The effects of BaP and 1-NP on early and late phase nasal blockage were expressed as the area under the response curve (AUC) for the changes in sRaw at 0 – 3 h (early phase) and 3 – 10 h (late phase) after challenge.

In order to observe direct irritative action of BaP and 1-NP in the nasal mucosa, we evaluated time course changes in sRaw after instillation of BaP or 1-NP without antigen challenge in sensitized–challenged guinea pigs on day 6 after the 13th antigen challenge.

Determination of sneezing frequency

We counted sneezes from 0 – 1 h after the 13th (long-term treatment study) or 20th (short-term treatment study) pollen inhalation challenge and used the number of sneezes as the sneezing frequency (sneezes per hour).

Measurement of Cry j 1-specific IgE antibody in sera

As previously described (13), Cry j 1-specific IgE antibody was assayed using an enzyme-linked immunosorbent assay (ELISA) kit (Guinea pig IgE ELISA MARUPI; Dainippon Pharmaceutical Co., Osaka), using blood samples collected on day 6 after the 13th inhalation challenge. Because this kit was developed to measure total IgE in guinea pigs, we modified the method provided by the manufacturer to measure Cry j 1-specific IgE. Diluted sera were added to the wells of a microtiter plate pre-coated with anti-guinea-pig IgE antibody, and the plate was then incubated for 1 h at 15°C – 25°C. After washing, 100 ng/ml of biotinylated Cry j 1 (Hayashibara Biochem. Lab., Inc., Okayama) was added at a volume of 100 μl/well, followed by incubation for 1 h at 15°C – 25°C. The plate was washed, and avidin-horseradish peroxidase conjugate (BD PharMingen, San Diego, CA, USA) was then added. Following incubation for 30 min and a subsequent washing, a substrate solution was added, and the enzyme reaction was developed for 30 min at 15°C – 25°C. The reaction was stopped with a stop solution, and absorbance values were measured at 450 nm.

Levels of Cry j 1-IgE in tested sera were expressed in arbitrary units relative to the value of a pooled standard serum obtained from sensitized–challenged guinea pigs. The standard serum was prepared by i.p. injection of the pollen extract [20 μg pollen/20 μg $Al(OH)_3$/ml/time /animal] adsorbed onto $Al(OH)_3$ into naive guinea pigs once a week for a total of 9 times. The sera were harvested 2 weeks after the last sensitization and were combined. The Cry j 1-specific IgE titer of the standard serum was designated as 1000 AU/ml.

Statistical analyses

Statistical analysis was performed by using one-way analysis of variance. If a significant difference was detected, the individual group difference was analyzed by Bonferroni's multiple test. A probability value of $P<0.05$ was considered to indicate statistical significance.

Results

Effects of BaP and 1-NP on pollen-induced nasal blockage

As previously reported (12), in the sensitized–challenged guinea pigs, there were 2 peaks (1 and 4 h after antigen challenge) in the time course of the changes in sRaw at the 13th challenge, indicating that the antigen-induced increase in sRaw consisted of early and late phase nasal blockage responses (Fig. 2). In contrast, in nonsensitized guinea pigs, sRaw did not change after pollen inhalation (Fig. 2).

We investigated the effects of long-term treatment with BaP or 1-NP on early and late phase nasal blockage by measuring sRaw at 1 and 4 h, respectively, after antigen challenges. BaP increased early and late phase nasal blockage at the 5th to 20th antigen challenges, sometimes significantly and sometimes not significantly (Fig. 3). 1-NP increased late phase nasal blockage at the 5th to 10th antigen challenges and increased the early phase at the 20th antigen challenge (Fig. 3). At the 13th challenge, when changes in sRaw were evaluated using AUCs, BaP significantly aggravated the early and late phase nasal blockage, and 1-NP significantly aggravated the late phase nasal blockage (Fig. 4). On the other hand, in the short-term treatment with BaP or 1-NP, neither BaP nor 1-NP affected the antigen-induced biphasic nasal blockage (Fig. 5).

There was no significant difference in baseline sRaw

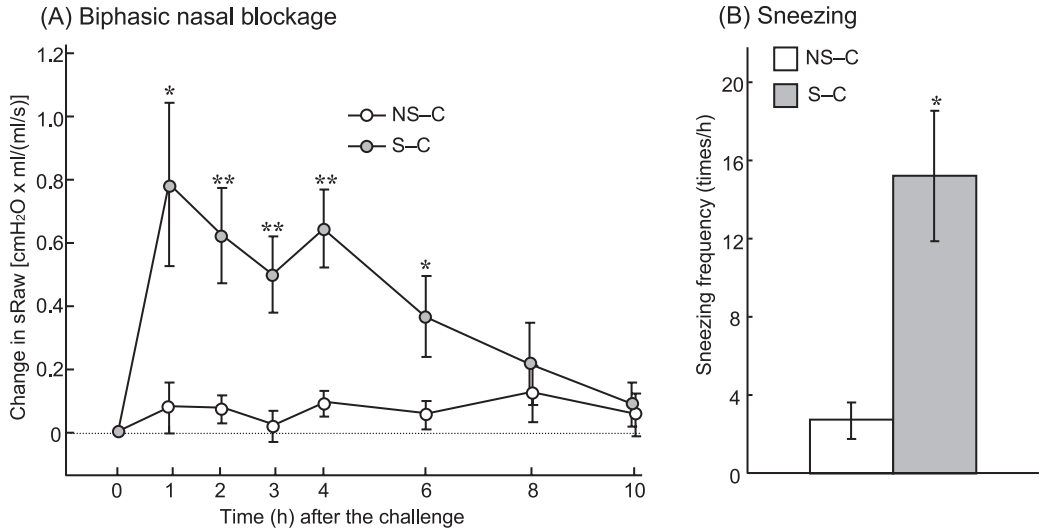


Fig. 2. Time course changes of the biphasic nasal blockage (A) and sneezing frequency (B) induced by the 13th antigen challenge in sensitized guinea pigs. Each point and column represents the mean ± S.E.M. of 10 animals. **P*<0.05 and ***P*<0.01 *vs* NS–C. NS–C, nonsensitized–challenged; S–C, sensitized–challenged.

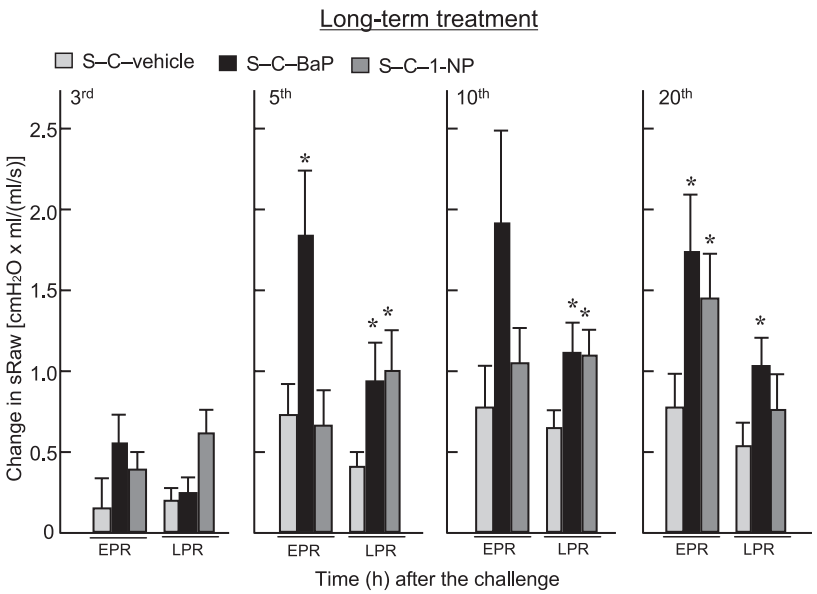


Fig. 3. Time course of effects of long-term treatment with BaP and 1-NP on the biphasic nasal blockage induced by the 3rd to 20th antigen challenges in sensitized guinea pigs. Each column represents the mean ± S.E.M. of 10 animals. **P*<0.05 *vs* S–C–vehicle. S–C, sensitized–challenged; BaP, benzo(a)pyrene; 1-NP, 1-nitropyrene; EPR, early phase response; LPR, late phase response.

at any of the challenges among the 4 sensitized–challenged groups. The mean ± S.E.M. values of baseline sRaw ($cmH_2O \times ml/(ml/s)$) for the 4 sensitized–challenged groups (n = 10) 1 h before the 13th challenge were as follows: sensitized–challenged–saline, 1.78 ± 0.19; sensitized–challenged–vehicle, 1.68 ± 0.14; sensitized–challenged–BaP, 1.73 ± 0.18; sensitized–challenged–1-NP, 1.43 ± 0.15.

Effects of BaP and 1-NP on baseline sRaw

To examine whether BaP or 1-NP has direct irritative action on the nasal mucosa, BaP or 1-NP was intranasally instilled in sensitized guinea pigs 6 days after the 13th antigen challenge, and the time course of sRaw was observed. No significant change in sRaw occurred within 4 h after instillation (Table 1).

Effects of BaP and 1-NP on pollen-induced sneezing

BaP and 1-NP did not affect the frequency of pollen-induced sneezing in short-term and long-term treated animals (Figs. 4 and 5).

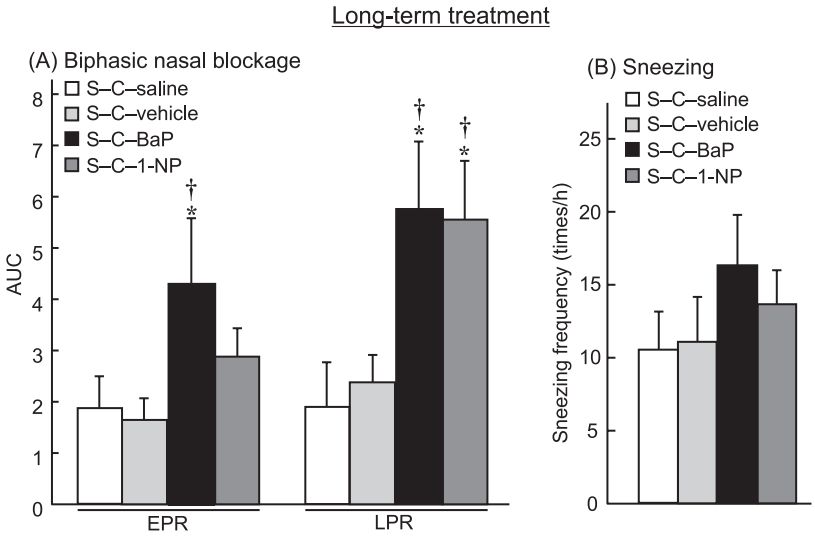


Fig. 4. Effects of long-term treatment with BaP and 1-NP on the biphasic nasal blockage (A) and increase of sneezing frequency (B) induced by the 13th antigen challenge in sensitized guinea pigs. Each column represents the mean ± S.E.M. of 10 animals. **P*<0.05 *vs* S–C–vehicle. †*P*<0.05 *vs* S–C–saline. S–C, sensitized–challenged; BaP, benzo(a)pyrene; 1-NP, 1-nitropyrene; EPR, early phase response; LPR, late phase response.

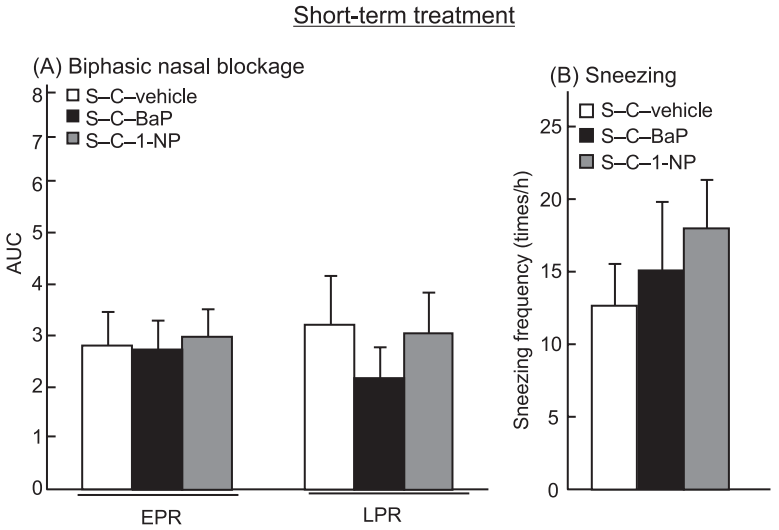


Fig. 5. Effects of short-term treatment with BaP and 1-NP on the biphasic nasal blockage (A) and increase of sneezing frequency (B) induced by the 20th antigen challenge in sensitized guinea pigs. Each column represents the mean ± S.E.M. of 10 animals. S–C, sensitized–challenged; BaP, benzo(a)pyrene; 1-NP, 1-nitropyrene; EPR, early phase response; LPR, late phase response.

Table 1. Time course change in sRaw induced by intranasal instillation of BaP or 1-NP in sensitized guinea pigs

	Change in sRaw [$cmH_2O \times ml/(ml/s)$]				
	10 min	30 min	1 h	2 h	4 h
S–C–vehicle	−0.032 ± 0.058	0.055 ± 0.139	0.027 ± 0.101	0.091 ± 0.140	0.016 ± 0.105
S–C–BaP	0.038 ± 0.133	0.122 ± 0.036	0.105 ± 0.107	0.097 ± 0.058	0.049 ± 0.133
S–C–1-NP	−0.0350 ± 0.046	−0.092 ± 0.079	−0.054 ± 0.045	0.122 ± 0.089	0.056 ± 0.065

This experiment was performed 6 days after the 13th challenge. Each data value represents the mean ± S.E.M. of 10 animals. S–C, sensitized–challenged; BaP, benzo(a)pyrene; 1-NP, 1-nitropyrene.

Effects on Cry j 1-specific IgE production

The level of Cry j 1-specific IgE in sera had increased by 6 days after the 13th antigen challenge (Table 2). IgE was not detected in sera from nonsensitized guinea pigs. Long-term treatment with BaP or 1-NP did not affect IgE production (Table 2).

Resolution of aggravated nasal blockage after cessation of treatment with BaP or 1-NP

Treatment with BaP or 1-NP was stopped after the 20th challenge. Inhalation challenges with pollen once a week were continued until the animals had been challenged 26 times. Table 3 shows the early (1 h) and

Table 2. Effects of BaP and 1-NP on the increase of Cry j 1-specific IgE at the 13th antigen challenge in sensitized guinea pigs

	Amount of IgE (AU/ml)
NS–NC	ND
S–C–vehicle	45.3 ± 19.5
S–C–BaP	49.1 ± 22.0
S–C–1-NP	32.9 ± 11.5

Each data value represents the mean ± S.E.M. of 10 animals. NS–NC, nonsensitized–nonchallenged; S–C, sensitized–challenged; BaP, benzo(a)pyrene; 1-NP, 1-nitropyrene; ND, not detected.

Table 3. Early (1 h) and late phase (4 h) increases in sRaw induced by antigen challenge in sensitized guinea pigs 6 weeks after cessation of administration of BaP or 1-NP

	Change in sRaw [$cmH_2O \times ml/(ml/s)$]	
	1 h	4 h
S–C–vehicle	0.99 ± 0.43	0.58 ± 0.25
S–C–BaP	1.25 ± 0.45	0.44 ± 0.22
S–C–1-NP	0.70 ± 0.26	0.93 ± 0.26

Each data value represents the mean ± S.E.M. of 10 animals. S–C, sensitized–challenged; BaP, benzo(a)pyrene; 1-NP, 1-nitropyrene.

late phase (4 h) increases in nasal blockage induced by the 26th challenge in each group. At the 26th challenge, aggravation of nasal blockage was not observed in the animals that had been treated with BaP or 1-NP.

Discussion

In the present study, when sensitized–challenged guinea pigs were repeatedly treated with intranasal instillation of BaP or 1-NP during the course of sensitization/challenge (long-term treatment), the antigen-induced nasal blockage was potently aggravated. BaP increased the early and late phase nasal blockage at the 5th challenge, and this aggravation continued during the intranasal treatment with BaP until the 20th antigen challenge. 1-NP increased the late phase nasal blockage at the 5th to 10th antigen challenges and increased the early phase only at the 20th challenge. On the other hand, baseline sRaw was not changed by long-term intranasal application of BaP or 1-NP. sRaw did not increase within 4 h after instillation of BaP or 1-NP, indicating that BaP and 1-NP had no direct irritative action on the nasal mucosa. These findings suggest that the aggravation of nasal blockage induced by chronic treatment with BaP or 1-NP is due to augmentation of the allergic response after antigen challenge.

In contrast, short-term (2-week) treatment with BaP or 1-NP, from 1 day after the 18th challenge to the day of the 20th challenge, did not affect antigen-induced biphasic nasal blockage at the 20th antigen challenge in sensitized guinea pigs. This indicates that 2-week treatment with PAHs is not sufficient to augment the allergic response.

On the other hand, it has been reported that the treatment with BaP has a cumulative activity for Th_1/Th_2 cytokine production when mice were intranasally immunized four times with allergen extract in combination with BaP (17). In this murine study, BaP was administered at 50 μg/animal (approximately 2.5 mg/kg) (17). In our guinea-pig study, BaP was intranasally administered at 200 μg/animal (approximately 0.5 mg/kg) because we had aimed to assess the effects of BaP or 1-NP at a relatively low concentration. In addition, lung specimens isolated from non-smokers in Japan were mainly contaminated by BaP and 1-NP, and the amount of BaP existing in the lung of these individuals was approximately 10-fold greater than that of 1-NP (18). From this report, we used 1-NP at 20 μg/animal.

In a previous study, we found that sneezing induced within 1 h after challenge was almost completely suppressed by treatment with anti-histaminic drugs (19). This indicates that histamine released from mast cells immediately after pollen inhalation challenge plays a major role in the occurrence of sneezing. Nevertheless, in the present study, long-term treatment with BaP or 1-NP did not aggravate sneezing. In addition, the allergen-induced increase in Cry j 1-specific IgE was not altered by treatment with BaP or 1-NP. These results strongly suggest that treatment with BaP or 1-NP has no effect on the IgE-mediated mast cell activation in the nasal mucosa, and additionally, they show that the PAH-induced aggravation of allergic nasal blockage in the present model is not related to alteration of anaphylactic mast cell activation.

It is not clear how the long-term treatment with BaP or 1-NP aggravates the antigen-induced nasal blockage. From our previous findings regarding induction mechanisms of the nasal blockage, we can speculate two mechanisms: 1) Because we have demonstrated that cyteinyl leukotrienes (CysLTs) and thronboxane A_2 (TXA_2) are largely involved in the late phase nasal blockage (19, 20), the aggravation of antigen-induced nasal blockage by the long-term treatment with BaP or 1-NP might be due to up-regulation of $CysLT_1$ and/or TP receptors and/or increases in the production of CysLTs and/or TXA_2. 2) Since the nasal blockage in our model is partly but significantly mediated by reactive oxygen species (ROS) (Mizutani et al., unpublished data) and antigen-induced production of ROS can be increased by treatments with DEP in sensitized rats (21), BaP and 1-NP also might up-regulate production of

ROS. We must test these two possibilities in future studies.

To assess whether BaP- or 1-NP-induced aggravation of nasal blockage persists after cessation of treatment with BaP or 1-NP, we stopped the treatment after the 20th challenge and measured the magnitude of pollen-induced nasal blockage at the 26th challenge. Interestingly, the level of nasal blockage returned to the control level. This result suggests that there is a link between the increase in air pollution and the increase in the prevalence of allergic airway diseases, suggesting that reducing air pollution would lower the prevalence of allergic diseases.

In conclusion, when BaP or 1-NP was intranasally administered long-term once a day during repetitive sensitization/challenge, the nasal blockage (but not sneezing) induced by subsequent pollen inhalation challenges was markedly aggravated. This aggravation of allergen-induced nasal blockage was not associated with serum antigen-specific IgE levels.

Acknowledgments

This work was supported by the Kyoto Pharmaceutical University's "High-Tech Research Center" Project, a matching fund subsidy from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, from 2004 to 2008.

References

1 Wjst M, Reitmeir P, Dold S, Wulff A, Nicolai T, von Loeffelholz-Colberg EF, et al. Road traffic and adverse effects on respiratory health in children. Br Med J. 1993;307:596–600.

2 Edwards J, Walters S, Griffiths RK. Hospital admissions for asthma in preschool children: relationship to major roads in Birmingham, United Kingdom. Arch Environ Health. 1994;49:223–227.

3 Braun-Fahrlander C, Gassner M, Grize L, Neu U, Sennhauser FH, Varonier HS, et al. Prevalence of hay fever and allergic sensitization in farmers' children and their peers living in the same rural community. SCARPOL team. Swiss study on childhood allergy and respiratory symptoms with respect to air pollution. Clin Exp Allergy. 1999;29:28–34.

4 Riedler J, Eder W, Oberfeld G, Schrener M. Austrian children living on a farm have less hay fever, asthma and allergic sensitization. Clin Exp Allergy. 2000;30:194–200.

5 Cacciola RR, Sarva M, Polosa R. Adverse respiratory effects and allergic susceptibility in relation to particulate air pollution: flirting with disaster. Allergy. 2002;57:281–286.

6 Rosenkranz HS, McCoy EC, Sanders DR, Butler M, Kiriazides DK, Mermelstein R. Nitropyrenes: isolation, identification, and reduction of mutagenic impurities in carbon black and toners. Science. 1980;209:1039–1043.

7 Scheepers PT, Martens MH, Velders DD, Fijneman P, van Kerkhoven M, Noordhoek J, et al. 1-Nitropyrene as a marker for the mutagenicity of diesel exhaust-derived particulate matter in workplace atmospheres. Environ Mol Mutagen. 1995;25:134–147.

8 Bai F, Nakanishi Y, Takayama K, Pei XH, Tokiwa H, Hara H. Ki-ras mutation and cell proliferation of lung lesions induced by 1-nitropyrene in A/J mice. Mol Carcinog. 1998;22:258–264.

9 Ohura T, Amagai T, Fusaya M, Matsushita H. Polycyclic aromatic hydrocarbons in indoor and outdoor environments and factors affecting their concentrations. Environ Sci Technol. 2004;38:77–83.

10 el-Bayoumy K, Chae YH, Upadhyaya P, Rivenson A, Kurtzke C, Reddy B, et al. Comparative tumorigenicity of Benzo(a)pyrene, 1-nitropyrene and 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine administered by gavage to female CD rats. Carcinogenesis. 1995;16:431–434.

11 Nakanishi Y, Pei XH, Takayama K, Bai F, Izumi M, Kimotsuki K, et al. Polycyclic aromatic hydrocarbon carcinogens increase ubiquitination of p21 protein after the stabilization of p53 and the expression of p21. Am J Respir Cell Mol Biol. 2000;22:747–754.

12 Nabe T, Mizutani N, Shimizu K, Takenaka H, Kohno S. Development of pollen-induced allergic rhinitis with early and late phase nasal blockage in guinea pigs. Inflamm Res. 1998;47:369–374.

13 Nabe T, Kubota K, Terada T, Takenaka H, Kohno S. Effect of oral immunotherapy on nasal blockage in experimental allergic rhinitis. J Pharmacol Sci. 2005;98:380–387.

14 Nabe T, Shinoda N, Yamashita K, Yamada M, Yamamura H, Kohno S. Comparative studies on nebulizers for antigen inhalation in experimental asthma. Allergol Int. 1997;46:261–267.

15 Nabe T, Shimizu K, Mizutani N, Saeki Y, Yamamura H, Takenaka H, et al. A new model of experimental allergic rhinitis using Japanese cedar pollen in guinea pigs. Jpn J Pharmacol. 1997;75:243–251.

16 Pennock BE, Cox CP, Rogers RM, Cain WA, Wells JH. A noninvasive technique for measurement of changes in specific airway resistance. J Appl Physiol. 1979;46:399–406.

17 Kadkhoda K, Pourfathollah AA, Pourpak Z, Kazemnejad A. The cumulative activity of benzo(a)pyrene on systemic immune responses with mite allergen extract after intranasal instillation and ex vivo response to ovalbumin in mice. Toxicol Lett. 2005;157:31–39.

18 Tokiwa H, Sera N, Horikawa K, Nakanishi Y, Shigematsu N. The presence of mutagens/carcinogens in the excised lung and analysis of lung cancer induction. Carcinogenesis. 1993;14:1933–1938.

19 Yamasaki M, Mizutani N, Sasaki K, Nabe T, Matsumoto T, Ashida Y, et al. Involvement of thromboxane A_2 and peptide leukotrienes in early and late phase nasal blockage in a guinea pig model of allergic rhinitis. Inflamm Res. 2001;50:466–473.

20 Mizutani N, Nabe T, Imai A, Sakurai H, Takenaka T, Kohno S. Markedly increased nasal blockage by intranasal leukotriene D_4 in an experimental allergic rhinitis model: contribution of dilated mucosal blood vessels. Jpn J Pharmacol. 2001;86:170–182.

21 Dong CC, Yin XJ, Ma JY, Millecchia L, Wu ZX, Barger MW, et al. Effect of diesel exhaust particles on allergic reactions and airway responsiveness in ovalbumin-sensitized brown Norway rats. Toxicol Sci. 2005;88:202–212.