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Suppressive activity of fexofenadine hydrochloride on nitric oxide production in-vitro and in-vivo

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Abstract

The aim of this study was to examine the effect of fexofenadine hydrochloride (FEX), a histamine H_1-receptor antagonist, on nitric oxide (NO) production in-vitro and in-vivo. Nasal fibroblasts (5×10^5 cells per mL) were stimulated with 25 ng mL^{-1} tumour necrosis factor-α in the presence of various concentrations of FEX. NO levels in 24-h-culture supernatants were measured by the Griess method and levels of inducible nitric oxide synthase (iNOS) mRNA levels in 12-h-cultured cells were measured by ELISA. FEX at more than 0.5 μg mL^{-1} suppressed NO production from fibroblasts by inhibiting expression of iNOS mRNA. We also examined whether FEX could suppress NO production induced by lipopolysaccharide (LPS) stimulation in-vivo. BALB/c mice were treated with 5.0 mg kg^{-1} LPS i.p. after daily oral doses of FEX, 1.0 mg kg^{-1}, for 1–3 weeks. Plasma was obtained 6 h later and NO levels measured by the Griess method. Expression of iNOS mRNA in lung tissues was measured by ELISA 6 h after LPS injection. Oral administration of FEX for 2 and 3 weeks, but not 1 week, significantly suppressed NO levels in plasma through the inhibition of iNOS mRNA expression, which were enhanced by LPS stimulation. These results suggest that the attenuating effect of FEX on NO production may be of therapeutic benefit in allergic diseases.

Introduction

Allergic rhinitis is associated with nasal mucosal inflammation. There is evidence of accumulation of epithelial mast cell and eosinophils within the nasal wall, tissue eosinophila, T-cell activation and enhanced cytokine expression (Bentley et al 1992; Bousquet et al 1995). Mediators released from activated immune cells, including mast cells, eosinophils and T cells, interact with neural and vascular elements in the nose to induce the symptoms of nasal pruritus, sneezing, rhinorrhoea and obstruction (Howarth 1989). In addition to these effects, mast cells and eosinophils release toxic granules and reactive oxygen species (ROS), which damage the nasal wall (Barnes 1990). Although ROS are accepted to include oxygen ions, free radicals and peroxides, nitric oxide (NO) has attracted attention in airway inflammatory diseases such as allergic rhinitis and asthma (Martin et al 1996; Gratziou et al 2001; Sannohe et al 2003; Redington 2006). Clinical evidence has clearly shown that levels of exhaled NO are increased in patients with airway inflammation, including allergic rhinitis, compared with normal subjects, and that the level of exhaled NO reflects the severity of the disease state (Martin et al 1996; Gratziou et al 2001; Sannohe et al 2003). In this respect, several studies have examined the effects of agents used for the treatment of airway inflammatory diseases, and revealed that corticosteroids could decrease levels of exhaled NO, which was associated with improvements in symptoms and lung function in asthmatic subjects (Redington 2006). It is also reported that the leukotriene receptor antagonist montelukast and the monoclonal anti-IgE antibody omalizumab reduce exhaled NO towards normal levels (Bisgaard et al 1999; Silkoff et al 2004). However, the influence of H_1-receptor antagonists, which are the most important medicines in the treatment of allergic diseases, on NO production remains unclear.

Fexofenadine hydrochloride (FEX), an active metabolite of terfenadine, is a second-generation, non-sedating H_1-receptor antagonist; it is used with remarkable success in the treatment and management of allergic diseases such as allergic rhinitis and atopic dermatitis (Markham & Wagstaff 1998; Casale et al 1999). Although the precise therapeutic mechanisms

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of action of FEX are not fully understood, there is circumstantial evidence that FEX could favourably modify the clinical conditions of the diseases by inhibiting the production of inflammatory mediators such as RANTES (regulated on activation, normal T-cell expressed and secreted), interleukin (IL)-8 and thymus- and activation-regulated chemokine (TARC), among others (Asano et al 2004a, b, c). In the present study we examined whether FEX could also suppress NO production in response to inflammatory stimulation produced by inflammatory cytokines and chemokines in-vitro and in-vivo.

Materials and Methods

Mice

Specific pathogen-free BALB/c male mice, 5 weeks of age, were purchased from Charles River Japan Inc. (Atsugi, Japan) and were maintained in filter (0.2 μm)-barrier cages, and were provided autoclaved food and water ad libitum throughout the experiments to prevent unwanted microbiological infection. All experimental procedures were approved by the Animal Care and Use Committee of Showa University and were carried out in accordance with the guidelines of the Physiological Society of Japan.

Reagents

FEX was kindly donated by Sanofi-Aventis Co. Ltd (Tokyo, Japan) as a pure, preservative-free, water-insoluble powder. For in-vitro use, FEX was dissolved in DMSO at a concentration of 10 mg mL^{-1} and then diluted with minimum essential medium (MEM; Sigma Chemicals, St Louis, MO, USA) supplemented with 5% heat-inactivated bovine serum (MEM-FCS; Irvine, Santa Ana, CA, USA) to give a concentration of 100 μg mL^{-1}. This solution was sterilized by passing through a 0.2 μm filter and stored as a stock solution at 4°C until use. For in-vivo use, FEX was mixed well with 5% tragacanthogum solution at 30 mg mL^{-1} and diluted with normal saline to give a concentration of 5 mg mL^{-1}. Recombinant human tumour necrosis factor (TNF)-α was purchased from Chemicon International Inc. (Temecula, CA, USA), and diluted with MEM-FCS to give a concentration of 50 ng mL^{-1}. Lipopolysaccharide (LPS) derived from *Escherichia coli* (Sigma Chemicals) was dissolved in phosphate buffered saline (PBS) at 1.0 mg mL^{-1}, and sterilized just before use using a 0.2 μm filter.

Cell source

Specimens of nasal polyps were obtained from patients during surgical operations, according to the protocol approved by The Ethics Committee of Showa University. Patients gave written informed consent.

Specimens were cut into small dice (approximately 1 mm in size), washed several times in PBS supplemented with 200 U mL^{-1} penicillin, 200 μg mL^{-1} streptomycin and 5.0 μg mL^{-1} amphotericin B, followed by antibiotic-free RPMI 1640 medium (Sigma Chemicals) supplemented with 10% fetal calf serum (Irvine). Diced specimens were then plated at a density of 10 pieces in 100 mm tissue culture dishes and covered with a cover slip adhered to the dish with sterile petroleum jelly. The dishes were maintained at 37°C in a humidified atmosphere with 5% CO_2. When a monolayer of fibroblast-like cells was found to be confluent, the explanted tissues were removed. The cells were then trypsinized and replated at a concentration of 5×10^5 cells per mL. The medium was changed every 3 days for 2–3 weeks until confluence was reached. Cells were subsequently split 1:2 at confluence and passaged. The cells were characterized (Hirano et al 2003), and used as nasal polyp fibroblasts (NPFs).

Cell culture

Cells from passages 4–7 were washed several times with MEM-FCS, introduced into each well of 24-well culture plates at a concentration of 5×10^5 cells per mL in a volume of 1 mL, in triplicate, and allowed to adhere for 24 h. The plates were then washed twice with MEM-FCS to remove dead and unattached cells. The cells were stimulated with 25 ng mL^{-1} TNF-α in the presence or absence of various concentrations of FEX in a total volume of 2 mL. After 24 h, the culture medium was removed and centrifuged, and the supernatant stored at −40°C. Cells for examination of mRNA expression were cultured in similar manner for 12 h.

LPS treatment

Mice were given intraperitoneal injections of LPS 5 mg kg^{-1}, in a volume not exceeding 0.2 mL (Terao et al 2003).

Treatment of mice with FEX

Mice were given FEX orally by gavage at 1 mg kg^{-1} (the recommended therapeutic dose for humans), once a day for 1–3 weeks in a volume not exceeding 0.3 mL.

Assay for inducible NO synthase (iNOS) mRNA expression

NPFs were stimulated with 25 ng mL^{-1} TNF-α in the absence or presence of various concentrations of FEX for 12 h. NPFs were then homogenized with a tissue homogenizer in an ice-cold water bath. Poly A$^+$ mRNA was separated from homogenates using oligo(dT)-coated magnetic microbeads (Milteny Biotec, Bergische Gladbach, Germany). Inducible NOS mRNA was examined using commercially available ELISA test kits for human iNOS mRNA (R & D Corp., Minneapolis, MN, USA) according to the manufacturer's protocol. To examine iNOS mRNA expression in-vivo, mice were killed by decapitation 6 h after LPS injection (Terao et al 2003). The lungs were removed, and homogenized with a glass tissue homogenizer at 4°C for 60 s. Poly A$^+$ mRNA was separated in a similar manner, and the mRNA expression was examined using ELISA test kits for mouse iNOS mRNA (R & D Corp.).

Preparation of plasma

Blood (1 mL) for cytokine assay was collected by cardiac puncture 2 h after LPS injection, into tubes containing 0.1 mL

heparin (Terao et al 2003). Samples were centrifuged at 4°C for 10 min at 3000 rpm and the plasma collected and stored at –40°C. To prepare protein-free plasma for NO assay, plasma obtained from cardiac puncture 6 h after LPS injection (Terao et al 2003) was filtered through centrifugal ultrafiltration devices (Centricon YM-10; cut-off molecular weight 10 000; Millipore Corp., Bedford, MA, USA) at 5000 *g* for 30 min at 4°C. Protein-free plasma samples were stored at –40°C.

Assay for NO (NO_3^-/NO_2^-)

NO concentration in culture supernatants was measured using a commercially available Griess reagent kit for NO_2^-/NO_3^- assay (Dojindo Co. Ltd, Kumamoto, Japan). The assay was done in duplicate, and the results were expressed as the mean ± s.e. concentration (μM) of triplicate cultures of five different subjects. NO levels in plasma were measured in a similar manner and the results were expressed as the mean ± s.e concentration (μM) of five mice.

Assay for cytokines

IL-1β, IL-6 and TNF-α levels in plasma were measured using commercially available ELISA kits for mouse cytokines (R & D Corp.). The sensitivity of these ELISA test kits for IL-1β, IL-6 and TNF-α was 3.0 pg mL^{-1}, 7.0 pg mL^{-1} and 2.0 pg mL^{-1}, respectively.

Statistical analysis

Statistical differences between control and experimental groups was evaluated by analysis of variance followed by Fisher's partial least-squares deviation test.

Results

Suppression of NO production from nasal polyp fibroblasts by FEX

The first experiments were undertaken to examine the influence of TNF-α stimulation on NO production from NPFs. NPFs were stimulated with various concentrations of TNF-α in triplicate and culture supernatants were collected 24 h later for measurement of NO concentration. As shown in Figure 1, TNF-α stimulation caused a dose-dependent increase in NO production from NPFs, which was first detected at 5 ng mL^{-1} and peaked at 25 ng mL^{-1} TNF-α. The data in Figure 1 also show that the TNF-α concentrations above 30 ng mL^{-1} suppress NO production by NPFs in-vitro.

We then examined the time course of NO production from NPFs in response to stimulation with 25 ng mL^{-1} TNF-α. As shown in Figure 2, NO levels in culture supernatants was significantly increased 24 h after stimulation and plateaued thereafter.

The third set of experiments was designed to examine whether FEX could suppress NO production from NPFs in response to TNF-α stimulation. NPFs were stimulated with 25 ng mL^{-1} TNF-α in the presence or absence of various concentrations of FEX, and culture supernatants were collected 24 h later for NO assay. Stimulation of NPFs with TNF-α caused a substantial production of NO (Figure 3). FEX concentrations below 0.25 μg mL^{-1} did not suppress NO production by NPFs stimulated with TNF-α, whereas concentrations

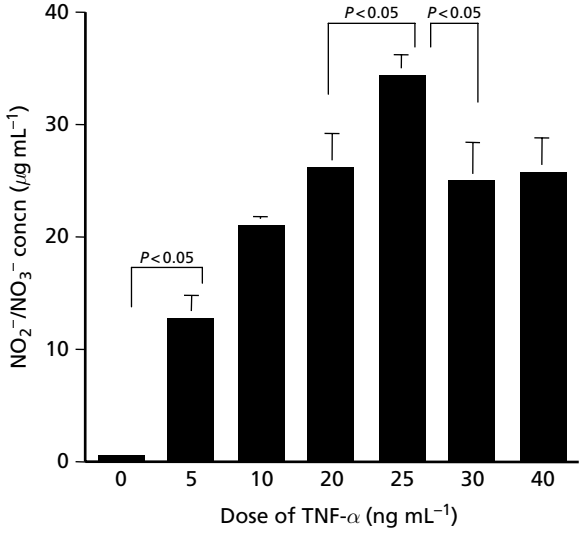

Figure 1 Influence of tumour necrosis factor (TNF)-α stimulation on nitric oxide (NO) production by nasal polyp fibroblasts in-vitro. Cells (5×10^5 cells per mL) were stimulated with 25 ng mL^{-1} TNF-α in the presence of various concentrations of FEX for 24 h. NO (NO_2^-/NO_3^-) levels in culture supernatants were assayed by the Griess method. Data are mean ± s.e.

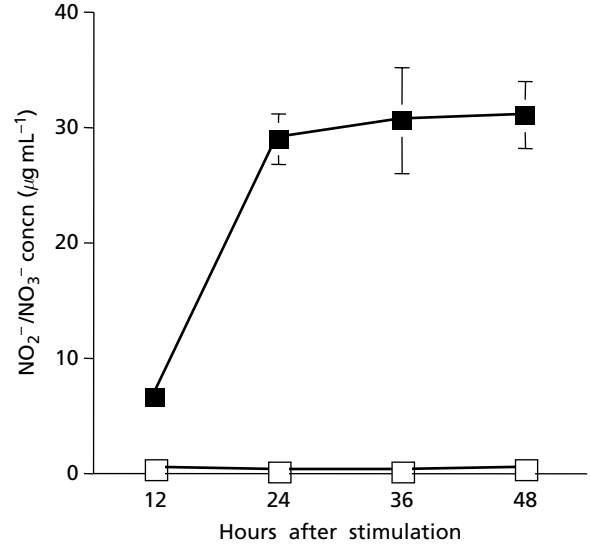

Figure 2 Kinetics of nitric oxide (NO) production from nasal polyp fibroblasts induced by tumour necrosis factor (TNF)-α stimulation in-vitro. Cells (5×10^5 cells per mL) were stimulated with 25 ng mL^{-1} TNF-α for various durations, and NO (NO_2^-/NO_3^-) levels in culture supernatants were assessed by the Griess method. Data are mean ± s.e. White squares: non-stimulated controls; black squares: TNF-α-stimulated cells.

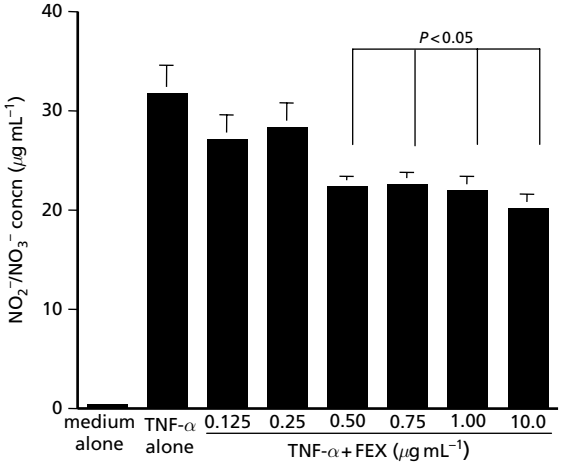

Figure 3 Suppressive activity of fexofenadine hydrochloride (FEX) on nitric oxide (NO) production from nasal polyp fibroblasts in response to tumour necrosis factor (TNF)-α stimulation in-vitro. Cells (5×10^5 cells per mL) were stimulated with 25 ng mL^{-1} TNF-α in the presence of various concentrations of FEX. After 24 h, NO (NO_2^-/NO_3^-) levels in culture supernatants were assayed by the Griess method. Data are mean ± s.e.

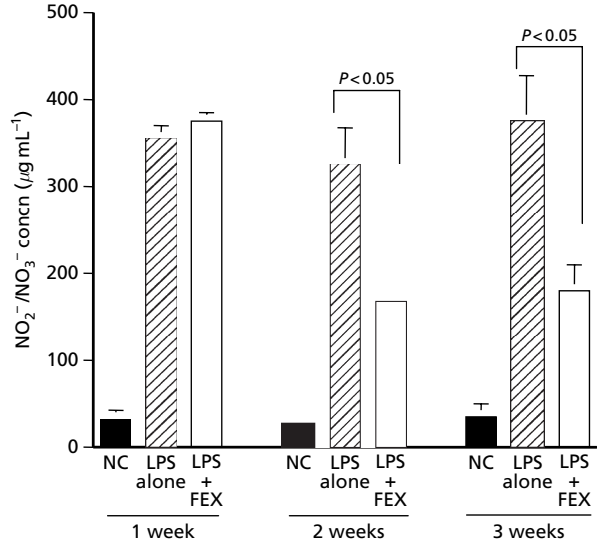

Figure 4 Attenuating effect of fexofenadine hydrochloride (FEX) on nitric oxide (NO) production by lipopolysaccharide (LPS) stimulation in BALB/c mice. BALB/c mice were given FEX 1.0 mg kg^{-1} by gavage once a day for 3 weeks. NO (NO_2^-/NO_3^-) levels in plasma were assayed by the Griess method. NC, treatment with saline. Data are mean ± s.e.

above 0.5 μg mL^{-1} FEX significantly suppressed NO production (Figure 3).

Attenuating effect of FEX on NO production induced by LPS injection in-vivo

This experiment was undertaken to examine whether FEX could inhibit NO production in-vivo. Mice were pretreated orally with FEX (1.0 mg kg^{-1} per day) for 1–3 weeks. These mice received intraperitoneal injection of 5.0 mg kg^{-1} LPS, and plasma NO levels were measured 6 h later. Although pretreatment of mice with FEX for 1 week did not affect NO production induced by LPS injection, FEX did suppress NO production when administered orally to mice for more than 2 weeks: NO levels in plasma obtained from mice pretreated with FEX for 2 and 3 weeks were significantly lower than the appropriate controls (Figure 4). We then examined whether the suppressive effect of FEX on NO production in-vivo is due to a direct suppressive effect of the agent on NO generation, or whether FEX suppressed inflammatory cytokine production stimulated by LPS, resulting in inhibition of NO appearance in plasma. As shown in Table 1, FEX did not suppress inflammatory cytokine production in response to LPS stimulation in-vivo, even when administered to mice for 3 weeks: all cytokine levels in experimental plasma were nearly identical to those in controls.

Table 1 Influence of fexofenadine hydrochloride (FEX) on production of inflammatory cytokines induced by lipopolysaccharide (LPS) injection into BALB/c mice. Mice were treated with FEX, 1 mg kg^{-1} per day for 3 weeks. Cytokine levels were measured 2 h after injection of LPS using ELISA

Group	Cytokine levels (pg mL^{-1}, mean ± s.e.)		
	IL-1β	IL-6	TNF-α
Treated for 1 week			
Control	18.7 ± 2.9	0.3 ± 0.1	25.3 ± 1.7
LPS alone	389.0 ± 12.0	1.9 ± 0.2	2580.5 ± 49.8
LPS + FEX	411.3 ± 15.4*	1.7 ± 0.3*	2630.9 ± 38.6*
Treated for 3 weeks			
Control	19.6 ± 1.8	0.3 ± 0.2	27.8 ± 2.5
LPS alone	383.1 ± 25.7	1.6 ± 0.1	2510.6 ± 140.3
LPS + FEX	355.2 ± 38.5*	1.4 ± 0.2*	2620.5 ± 134.1*

*$P > 0.05$ vs LPS alone.

Influence of FEX on expression of iNOS mRNA in-vitro and in-vivo

NO is generated in response to stimulation with inflammatory cytokines and microbiological molecules in a number of cell types following induction of iNOS. These experiments were done to examine whether FEX suppresses expression of iNOS mRNA to result in inhibition of NO production in-vitro and in-vivo. As shown in Table 2, the internal control GAPDH gene showed intense signals in all samples tested. TNF-α stimulation of NPFs enhanced expression of iNOS mRNA compared with non-stimulated control (Table 2). Although FEX concentrations below 0.25 μg mL^{-1} did not influence expression of iNOS mRNA, 0.5 μg mL^{-1} FEX caused significant inhibition of mRNA expression in response to TNF-α stimulation ($P < 0.05$; Table 2). Treatment of mice with FEX for 2 weeks, but not 1 week, completely suppressed the increase in iNOS mRNA expression induced by LPS injection in mouse lung tissues (Table 3).

Table 2 Influence of fexofenadine hydrochloride (FEX) on expression of inducible nitric oxide synthase (iNOS) in human nasal fibroblasts stimulated with tumour necrosis factor (TNF)-α in-vitro. Nasal fibroblasts (5×10^5 cells per mL) were stimulated with TNF-α in the presence of various concentrations of FEX for 12 h. mRNA levels in cells were measured by ELISA. GAPDH expression was used as control

Group	mRNA levels (amol per 10^5 cells, mean ± s.e.)	
	GAPDH	iNOS
Normal control	8.3 ± 3.0	3.6 ± 1.5
TNF-α alone	8.0 ± 3.6	13.9 ± 5.0
TNF-α + FEX		
125 ng mL^{-1}	8.8 ± 2.6	13.1 ± 4.1
250 ng mL^{-1}	8.3 ± 2.2	16.3 ± 2.0
500 ng mL^{-1}	7.8 ± 3.8	5.2 ± 0.7

Table 3 Influence of fexofenadine hydrochloride (FEX) on expression of inducible nitric oxide synthase (iNOS) in lung tissues from BALB/c mice stimulated with lipopolysaccharide (LPS). Mice were treated with FEX, 1 mg kg^{-1} per day for 3 weeks. mRNA expression was measured in lung tissue taken from the mice 6 h after injection of LPS, using ELISA

Group	mRNA levels (amol per μg RNA, mean ± s.e.)	
	GAPDH	iNOS
Treated for 1 week		
Normal control	416.7 ± 63.5	16.7 ± 3.7
LPS alone	483.3 ± 60.5	317.4 ± 48.2
FEX alone	409.5 ± 59.2	16.9 ± 3.3
LPS + FEX	431.7 ± 41.0	313.6 ± 52.7
Treated for 2 weeks		
Normal control	431.3 ± 89.3	16.2 ± 1.9
LPS alone	458.3 ± 71.7	340.7 ± 31.1
FEX alone	393.3 ± 40.6	15.6 ± 3.0
LPS + FEX	440.0 ± 91.2	23.3 ± 2.8*

*$P < 0.05$ vs LPS alone.

Discussion

FEX, the major active carboxylic metabolite of terfenadine, is an antihistamine with selective peripheral H_1-receptor antagonist activity, and has been approved for relief of the symptoms of allergic rhinitis and atopic dermatitis. Although its effect is mainly described in terms of histamine-receptor antagonist that affects mast cells, the precise therapeutic mechanisms of FEX are not well understood.

Our results clearly show that FEX can inhibit NO production from NFPs in response to TNF-α stimulation in-vitro by suppressing expression of iNOS mRNA. The lowest concentration of the agent that caused significant suppression of NO production was 0.5 μg mL^{-1}, which is lower than therapeutic tissue levels (Simon et al 2001). NO is recognized as one of the important regulators of many cells and tissue functions. NO is produced from various types of cells and tissues (e.g. skeletal muscle, epithelium and fibroblasts) in response to inflammatory stimuli (Taylor & Geller 2001) and plays pivotal roles in the development of airway inflammatory diseases, including allergic rhinitis, as one of the final effector molecules (Kooy et al 1995). Taken together, our results suggest that FEX attenuates airway inflammation by suppressing generation of NO. However, before drawing the conclusion that this activity of FEX underlies one of the therapeutic modes of action of the agent on inflammatory diseases, it is necessary to examine whether FEX could also suppress NO generation in-vivo. Experimental evidence clearly show that administration of LPS to laboratory animals increases the activity of iNOS in cells and tissues, including macrophages and lung tissue, to produce NO (Wadsworth & Koop 2001). This activity of LPS is decreased by treatment of animals with anti-TNF-α antibodies (Harbrecht et al 1994). It is also reported that the half-life of TNF-α is 6–7 min after injection into mice (Beutler et al 1985; Pietersz et al 1998). In the second part of these experiments we therefore used an LPS–mouse system to examine the influence of FEX on NO production in-vivo. The data obtained clearly show that FEX suppressed the production of NO in plasma from mice injected with LPS when mice were treated orally with 1.0 mg kg^{-1} FEX once a day for more than 2 weeks (but not 1 week). Furthermore, this suppressive activity of FEX on NO generation in-vivo is partly due to its inhibitory action on induction of iNOS, but not the secretion of cytokines responsible for enhancement of iNOS expression after inflammatory stimulation.

NO can be generated enzymatically by oxidation of L-arginine to L-citrulline, catalysed by NOS found in a number of cell types (Gaston et al 1994). In human and other mammalian species, three isoforms of NOS have been cloned and characterized. Neuronal NOS (nNOS) and endothelial NOS (eNOS) are constitutively expressed and seem to release relatively small (picomolar) quantities of NO, participating in various physiological process ranging from regulation of vascular tone to development of learning and memory (Kleinert et al 2003). In contrast, iNOS is induced in a number of cell types (e.g. immune cells and fibroblasts) after stimulation by pro-inflammatory cytokines, and produces much higher (nanomolar) levels of NO for long periods (Redington 2006; Kleinert et al 2003). This NO is believed to react extremely rapidly with superoxide to produce the very reactive and toxic peroxynitrite, which can initiate lipid peroxidation on the outer cell membrane and tissue injury (Ferdinandy & Schulz 2001). When NO does not completely form peroxynitrite, the residual NO readily diffuses across the cell membrane and reacts with intracellular superoxide to produce peroxynitrite, which causes DNA damage and apoptotic cell death in inflammatory tissues (Yermilov et al 1995). It has also been found that nitrogen derived from oxidants plays a role in airway diseases as one of the final effector molecules, and suggests the possibility of an important role for peroxynitrite in inflammatory airway diseases (Redington 2006). In addition to harmful effects of NO, this gaseous material can have vasodilatory activities; it is therefore possible that the increased production within airways, and especially the nose, could contribute to both nasal obstruction and plasma protein extravasation (Martin et al 1996). Taken together, our results

strongly suggest that the suppressive activity of FEX on NO generation induced by inflammatory stimulation may underlie the therapeutic mode of action of the agent on inflammatory airway diseases such as allergic rhinitis.

It is reported that induction of excessive iNOS in endothelial cells causes endothelial cell injury and inhibits cellular respiration, which lead to cell dysfunction and cell death (Cuzzocre 2001; Xia & Zweier 2001). It is also observed that iNOS could produce significant amounts of superoxide, which reacts readily with hydrogen to form the most toxic molecule, hydrogen radicals (Xia & Zweier 2001). Furthermore, inhibition of iNOS expression suppresses the production of inflammatory cytokines (TNF-α and IL-1β) as well as matrix metalloproteinases, which are responsible for tissue remodelling in inflammatory diseases (Robinson et al 2006). Our result show the inhibitory effects of FEX on expression of iNOS mRNA, suggesting that the agent causes a decrease in iNOS expression in the cytosol after inflammatory stimulation, inhibiting superoxide generation and preventing tissue injury in allergic rhinitis.

Our results clearly show the inhibitory action of FEX on NO generation through the suppression of iNOS mRNA expression induced by inflammatory stimulation. However, the suppressive mechanisms of FEX on iNOS mRNA expression are not clear at present. Recently, much effort has been directed at defining the signal transduction pathway in expression of iNOS mRNA. In response to stimulation with inflammatory cytokines and bacterial molecules, the iNOS gene undergoes rapid transcriptional activation through binding of stimulatory transcription factors, especially signal transducer and activator of transcription (STAT)-1, activator protein (AP)-1 and nuclear factor (NF)-κB, to its 5′-flanking region (Kristof et al 2005). The c-Jun N-terminal kinase (JNK)/stress-activated protein kinase (SPAK) is also reported to be important in the expression of iNOS mRNA after stimulation by TNF-α and IL-6 (Chan & Riches 1998; Kang et al 2007). Our previous work has clearly shown that FEX can suppress NF-κB, but not AP-1, activation in nasal fibroblasts induced by TNF-α stimulation in-vitro (Asano et al 2004b), suggesting that the suppressive activity of FEX on iNOS mRNA expression is due, at least in part, to the inhibitory effect of the agent on NF-κB activation in-vitro. In addition to these transcription factors, the cyclic AMP (cAMP)/protein kinase A (PKA) pathway has been shown to mediate iNOS expression after LPS stimulation: activation of the cAMP/PKA pathway reduced LPS-induced iNOS expression in murine macrophages (Pang & Hoult 1997) and hepatocytes (Harbrecht et al 2001). The protein kinase C (PKC) pathway is also reported to be involved in LPS-induced iNOS expression, and activation of PKC isoforms, especially PKC-α, diminished iNOS expression in murine macrophages after LPS stimulation (St Denis et al 1998). These reports suggest that FEX affects cAMP/PKA and PKC pathways to suppress expression of iNOS mRNA after LPS stimulation in-vivo. This speculation is supported by the observation that azelastine hydrochloride, an H_1-receptor antagonist, enhances the activity of both cAMP and PKC induced by ionomycin stimulation in mast cells (Abdel-Raheem et al 2005).

Farnesyltransferase has been reported to be an essential enzyme that catalyses the attachment of a farnesyl group from farnesyl pyrophosphate to the cysteine-thiol group of protein C-terminal CAAX consensus sequences (Moores et al 1991; Reiss et al 1991). It is also reported that inhibition of farnesyltransferase activation by a specific inhibitor (SCH 66336) prevents expression of inflammatory mediator mRNA (e.g. cyclo-oxygenase (COX)-2 and matrix metalloproteinases (MMP)-9) in a human leukaemia cell line (THP-1) induced by TNF-α stimulation in-vitro (Na et al 2004). Furthermore, administration of tipifarnib, a specific inhibitor of farnesyltransferase, to mice inhibits the development of inflammatory responses by suppressing expression of mRNA for iNOS, COX-2 and TNF-α and production of these proteins induced by LPS injection (Xue et al 2006). From these reports and our results it may be interpreted that FEX suppresses activation of farnesyltransferase induced by LPS injection and results in inhibition of NO production. Further experiments are required to clarify this point.

Conclusion

Our results strongly suggest that the attenuating effect of FEX on NO production in-vitro and in-vivo constitutes at least part of the therapeutic mode of action of the agent on allergic diseases, including allergic rhinitis.

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