Nedocromil sodium reduces cigarette smoke-induced bronchoconstrictor hyperresponsiveness to substance P in the guinea-pig

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ABSTRACT: Acute exposure to cigarette smoke provokes airway hyperresponsiveness to substance P and inactivates neutral endopeptidase (NEP). To determine whether nedocromil sodium can prevent cigarette smoke-induced hyperresponsiveness to substance P, we studied two groups of anaesthetized guinea-pigs.

One group of guinea-pigs was pretreated with aerosolized 0.9% NaCl solution (90 breaths), the other group was pretreated with aerosolized nedocromil sodium (10^-4 M, 90 breaths). In each animal, pretreatment was followed by either exposure to the smoke of one cigarette or exposure to air. After acute exposure to cigarette smoke or to air, we measured the change in total pulmonary resistance (RL) induced by increasing concentrations of aerosolized substance P.

In the absence of nedocromil sodium, the bronchoconstrictor responses to substance P were greater in cigarette smoke-exposed guinea-pigs than in air-exposed animals. Aerosolized nedocromil sodium had no effect on the response to substance P in air-exposed animals, but it reduced cigarette smoke-induced hyperresponsiveness to substance P. The preventive effect on cigarette smoke-induced hyperresponsiveness to substance P was observed at concentrations of aerosolized nedocromil sodium of 3×10^-4, 10^-4, and 3×10^-4 M. In vitro, cigarette smoke solution inhibited NEP activity from lung membrane preparations, but this inhibitory effect was not modified by nedocromil sodium (10^-4 M).

We conclude that aerosolized nedocromil sodium reduces cigarette smoke-induced airway hyperresponsiveness to substance P in vivo. This action of nedocromil sodium is not due to a protective effect on cigarette smoke-induced inactivation of NEP in vitro. Eur Respir J., 1995, 8, 47–52.

Like many other irritants, cigarette smoke is a potent stimulant of airway sensory nerves, which provoke neurogenic inflammation in the airway by releasing endogenous tachykinins [1–3]. We have recently shown in guinea-pigs that exposure to cigarette smoke induces airway hyperresponsiveness to substance P (SP), and that this hyperresponsiveness is associated with inactivation of airway neutral endopeptidase (NEP) [4], a peptidase known to be a major modulator of tachykinin-induced effects in the airway [5]. The effects of cigarette smoke exposure on the response to tachykinins, combined with its stimulating action on the release of tachykinins by sensory nerves, are likely to play a major role in the production of tobacco smoke-induced inflammation in the airways. Drugs that may reduce airway hyperresponsiveness to tachykinins may be important for preventing cigarette smoke-induced airway inflammation.

Nedocromil sodium is known to inhibit the bronchoconstrictor response to antigen [6], cold air [7], exercise [8], adenosine [9], distilled water [10], and hyperosmolar saline [11]. These stimuli are thought to exert their effects largely through inflammatory mediators. In humans, nedocromil sodium is also known to inhibit response to challenges which involve activation of neuronal reflexes, such as sulphur dioxide [12], metabisulphite [13], bradykinin [14], neurokinin A [15], and substance P [9]. Nedocromil sodium does not affect SP-induced smooth muscle contraction in guinea-pig airway in vitro; whereas the drug reduces nonadrenergic noncholinergic-induced bronchoconstrictor response by modulating the release of tachykinins from sensory nerves [16].

The aim of this study was to examine whether nedocromil sodium prevents cigarette smoke-induced increase in the bronchoconstrictor response to SP, and if so, whether this was due to a protective effect of nedocromil sodium on cigarette smoke-induced inactivation of airway NEP.

Methods

Animals

Specific pathogen-free (SPF) male Hartley-outbred guinea-pigs (Charles River France) weighing 320–400 g were used in this study.
Studies in vivo

Measurement of total pulmonary resistance (R_L). Animals were anaesthetized with 80 mg·kg⁻¹ thiopeental sodium given intraperitoneally (i.p.), and placed under a homeothermic blanket (38°C). Every 30 min, additional injections of thiopeental sodium (10 mg·kg⁻¹ i.p.) were given. The level of anaesthesia was monitored on the aspect of the airflow and the transpulmonary pressure signals (see below) during the respiratory cycle. A polyethylene catheter was introduced into the right carotid artery to measure arterial blood gases and pH. Animals were artificially ventilated via a tracheal cannula with a constant-volume respirator (model 683, Harvard Apparatus, South Natick, MA, USA), at a frequency of 90 breaths·min⁻¹.

Tidal volume was adjusted so that mean±SEM arterial blood gases obtained through the catheter placed in the carotid artery were as follows: arterial oxygen tension (Pao₂) 14.1±0.5 kPa (106±3.9 mmHg), arterial carbon dioxide tension (Paco₂) 5.7±0.1 kPa (43±0.9 mmHg) and pH 7.3–7.4. Airflow was monitored continuously using a pneumotachograph (Fleisch No. 000: Richmond, VA, USA) connected to a differential pressure transducer (model DP45, Validyne Engineering Corp., Northridge, CA, USA). Tidal volume was obtained by electrical integration of airflow.

A fluid-filled polyethylene catheter was introduced into the oesophagus to measure oesophageal pressure, as an approximation of pleural pressure. Intrathoracic pressure was measured with a catheter connected to the tracheal cannula. The oesophageal and the tracheal catheters were connected to a differential pressure transducer (model No. 267 AC, Hewlett-Packard Co., Palo Alto, CA, USA) which gave the transpulmonary pressure, defined as the pressure difference between the intrathoracic and the oesophageal pressures. Output signals representing airflow, tidal volume and transpulmonary pressure were amplified using Validyne amplifiers (model CD19 A). These signals were continuously visualized on a computer screen and were recorded on a polygraph recorder (Gould, model ES 1000). Total pulmonary resistance (R_L) was calculated using the method of AMIDUR and MEEAD [17].

Aerosols of drugs (mass median aerodynamic diameter 1.0 µm; geometric standard deviation 1.3) were generated by an ultrasonic nebulizer (model 99, De Vilbiss, Somerset, PA, USA).

Cigarette smoke exposure. Cigarette smoke was collected in a polypropylene syringe at a rate of 1 puff·min⁻¹ (each puff 2 s duration, 30 ml; total of 8–10 puffs per cigarette) to a final butt length of 20 mm. The smoke was then slowly expelled in the inspiratory circuit of the ventilator. Thus, each puff of cigarette smoke was diluted in air by approximately 1/5, and was delivered to the animal during 30 s intervals followed by 30 s with air ventilation.

Effects of nedocromil sodium on cigarette smoke-induced bronchoconstrictor response to SP. To determine the effect of nedocromil sodium on cigarette smoke-induced bronchoconstrictor response to SP, we studied two groups of animals. One group of guinea-pigs received aerosolized 0.9% NaCl solution (90 breaths), followed 5 min later by exposure to the smoke of one cigarette, or exposure to air given in the same conditions as for cigarette smoke exposure. The other group of animals received aerosolized nedocromil sodium (10⁻⁴ M, 90 breaths), followed 5 min later by exposure either to the smoke of one cigarette, or to air given in the same conditions as for cigarette smoke exposure. The bronchoconstrictor response to SP was determined 30 min after exposure to air or to cigarette smoke using the following protocol. After an initial challenge with 0.9% NaCl (15 breaths), we measured the change in R_L induced by increasing concentrations of aerosolized SP (10⁻⁶, 10⁻⁵, 3.2×10⁻⁵, and 10⁻⁴ M; 15 breaths at each concentration). Each successive concentration of SP was delivered after R_L had reached a stable value. Responses were evaluated as the maximum R_L value after each concentration of SP.

Dose-dependent effect of nedocromil sodium on cigarette smoke-induced bronchoconstrictor response to SP. We studied the effect of various concentrations of aerosolized nedocromil sodium on cigarette smoke-induced bronchoconstrictor response to SP. Animals received aerosolized nedocromil sodium at concentrations varying from 10⁻⁶ M to 3×10⁻⁴ M (90 breaths at each concentration) or aerosolized 0.9% NaCl (90 breaths). Pretreatment with nedocromil sodium or 0.9% NaCl was followed 5 min later by exposure to the smoke of one cigarette. We measured R_L in response to increasing concentrations of aerosolized SP (10⁻⁶, 10⁻⁵, 3.2×10⁻⁵, and 10⁻⁴ M; 15 breaths at each concentration) 30 min after being exposed to cigarette smoke. Each concentration of nedocromil sodium was studied in different animals.

In each study described above, administration of pretreatment with 0.9% NaCl or nedocromil sodium at various doses was randomly chosen. Responses were evaluated as maximum R_L level after each dose of SP.

Studies in vitro

Lung membrane preparation. Guinea-pigs were killed by cervical dislocation and exsanguinated. The pulmonary circulation was perfused with a 0.9% NaCl to remove contaminating blood. Lungs were removed and kept on ice until used. Tissues were weighed, then homogenized in 15 vol of ice cold 0.05 M Tris-HCl 0.25 M saccharose buffer (pH 7.4) with a blender (3 times 15 s). The homogenate was centrifuged at 1,000×g for 8 min at 4°C. The resulting supernatant fraction was centrifuged at 45,000×g for 10 min at 4°C to yield a cell membrane pellet which was then resuspended in the Tris-saccharose buffer. The cell membrane fraction was immediately frozen in liquid nitrogen and stored at -80°C, for later determination of NEP activity.

Measurement of NEP-like activity. Determination of NEP activity was performed using the enkephalin degradation method of LLORENS et al. [18]. Briefly, the tissues
were diluted as necessary in assay buffer containing 125 mM NaCl, 50 mM hydroxyethylpiperazine ethane-sulphonic acid (HEPES) buffer, pH 7.4. Duplicate samples of 50 µl of homogenates were incubated at 37°C for 40 min with 1H-Tyr,D-Ala2-leucine enkephalin (20 nM, final volume 100 µl). Phosphoramidon (10^-6 M), a potent inhibitor of NEP [19], was added to parallel duplicate samples. Blanks were obtained by using assay buffer (50 µl) instead of lung membrane preparation under identical experimental conditions. Cleaved 1H-Tyr,D-Ala2-leucine enkephalin was separated chromatographically from uncleaved substrate, and was counted in a scintillation counter. NEP-like activity was assessed by calculating the ratio of cleaved substrate to total 1H-Tyr,D-Ala2-leucine enkephalin under conditions of initial velocity. Substrate degradation inhibited by phosphoramidon 10^-6 M was considered specific for NEP-like activity.

**Preparation of cigarette smoke solution.** A water soluble extract of cigarette smoke (gas-phase) was prepared as follows. The smoke from two cigarettes was collected through a Cambridge filter pad in a polypropylene syringe at a rate of 1 puff·min^-1 (each puff, 2 s duration, 30 ml; total of 8–10 puffs per cigarette) to a final butt length of 20 mm. Each puff of cigarette smoke was bubbled slowly through the assay buffer solution (125 mM NaCl, 50 mM HEPES buffer, pH 7.4) to obtain a concentration of smoke solution of 2 cigarettes·3 ml^-1. The smoke solution was then titrated to pH 7.4. The resulting smoke solutions were kept on ice and were used within 30 min following their preparation.

**Effect of nedocromil sodium on cigarette smoke solution-induced inactivation of NEP-like activity of lung cell membranes.** The cleavage of 1H-Tyr,D-Ala2-leucine enkephalin by lung membrane preparations (50 µl) was determined after a preincubation for 15 min at room temperature with cigarette smoke solution (2 cigarettes per 3 ml) or with assay buffer (25 µl) in the presence or absence of 10^-4 M nedocromil sodium.

**Reagents**

We used commercial cigarettes, Gauloises (Seita, France). Each cigarette contained 16.9 mg tar and 1.31 mg nicotine.

Drugs and chemicals were obtained from the following sources: substance P, phosphoramidon (Peninsula Labs, Merseyside, UK); nedocromil sodium (Fisons Labs Ltd, Loughborough, UK); 1H-Tyr,D-Ala2-leucine enkephalin (CEA, Gif-sur-Yvette, France). All drugs for in vivo studies were prepared in 0.9% NaCl solution on the day of experiment.

**Statistical analysis**

Data are expressed as mean±SEM. Two-way analysis of variance (ANOVA) was used to compare mean values of Rt. for the effects of pretreatment (air exposure and cigarette exposure, with or without nedocromil sodium) and for the effects of various concentrations of nedocromil sodium on the concentration-response curves to SP. We used a multiple contrasts method (Scheffe’s test) to compare the effects of different pretreatments, and the effects of different concentrations of nedocromil sodium on the concentration-response curves to SP. Rt. values before SP challenge and after initial challenge with 0.9% NaCl solution were compared with a one-way ANOVA. Pairwise comparisons between NEP activities in lung membrane preparations incubated with or without cigarette smoke solution in the presence or absence of nedocromil sodium were made by paired t-tests.

**Results**

**Studies in vivo**

**Effects of nedocromil sodium on cigarette smoke-induced bronchoconstrictor response to SP.** Before SP challenge, baseline Rt. values in animal pretreated with aerosolized 0.9% NaCl followed by exposure to air or to cigarette smoke (0.13±0.02 and 0.18±0.02 cmH2O·ml^-1·s, respectively) or in animals pretreated with aerosolized nedocromil sodium (10^-4 M) followed by exposure to air or to cigarette smoke (0.17±0.03 and 0.15±0.02 cmH2O·ml^-1·s, respectively) were not different from one another (p>0.3). After initial challenge with 0.9% NaCl (15 breaths), Rt. values were similar in the different groups of animals (fig. 1) (p>0.3).

After pretreatment with aerosolized 0.9% NaCl, the response to SP was greater in animals that were exposed to smoke exposure than in animals exposed to air (p<0.001). Aerosolized nedocromil sodium by itself had no effect on the response to SP, since after exposure to air the concentration-response relationship to SP were similar in animals pretreated with nedocromil sodium and those pretreated with 0.9% NaCl (p>0.7). As compared to animals pretreated with 0.9% NaCl, pretreatment with nedocromil sodium (10^-4 M) reduced cigarette smoke-induced response to SP (p<0.002). Pretreatment with nedocromil sodium did not completely abolish cigarette smoke-induced hyperresponsiveness to SP, since after pretreatment with nedocromil sodium, the response to SP in animals exposed to cigarette smoke was still slightly greater than in animals exposed to air (p<0.03).

**Dose-dependent effect of nedocromil sodium on cigarette smoke-induced bronchoconstrictor response to SP.** Aerosolized nedocromil sodium at various concentrations (10^-5 to 3×10^-4 M) had no effect on prechallenge Rt. values (p>0.3) and on the Rt. values observed after initial challenge with aerosolized 0.9% NaCl (15 breaths) (p>0.3) (fig. 2).

Aerosolized nedocromil sodium at concentrations equal or superior to 3×10^-5 M induced a shift to the right of the concentration-response relationship to SP in cigarette smoke-exposed animals (p<0.001). However, after nedocromil sodium at concentrations of 3×10^-4, 10^-4, and...
3×10^-5 M the concentration-response relationship to SP were not different from one another (each comparison, p>0.7). Therefore, although the reduction of cigarette smoke-induced hyperresponsiveness to SP was observed with the three highest concentrations of nedocromil sodium, we were unable to actually demonstrate a concentration-dependent effect of this drug.

Discussion

Our results confirm our previous study showing that exposure to cigarette smoke leads to an enhanced bronchoconstrictor response to SP, an effect which was observed as soon as 15 min following smoke exposure [4]. Previous studies have shown that cigarette smoke causes an increase in vascular permeability in airway mucosa, by releasing endogenous tachykinins, as these effects were abolished when animals were pretreated with a tachykinin receptor antagonist, or when nerves were depleted of tachykinins.
by pretreatment with capsaicin [1–3]. In addition to the gas-phase of the smoke, nicotine, another important component of cigarette smoke, causes smooth muscle contraction by releasing SP [20]. Therefore, in addition to inducing neurogenic inflammation, cigarette smoke also provokes hyperresponsiveness to SP. The combination of these two effects is a possible mechanism by which cigarette smoke can exacerbate tachykinin-induced effects; and, thereby, induce an increase in mucus secretion [21], and airway inflammation [22].

Our study showed that nedocromil sodium given by inhalation prior to cigarette smoke exposure reduced the cigarette smoke-induced hyperresponsiveness to SP. The effect of aerosolized nedocromil was observed at concentrations much lower (approximately 50–150 times smaller) than that used in other studies in vivo [23]. Although the protective effect of nedocromil sodium on cigarette smoke-induced hyperresponsiveness to SP was demonstrated at three different concentrations (3 × 10⁻⁴ M, 10⁻⁴ M, and 3 × 10⁻⁵ M) we could not demonstrate a dose-dependent effect of this drug.

The mechanisms whereby nedocromil sodium reduces cigarette smoke-induced hyperresponsiveness is unclear. Indirect evidence suggests that nedocromil sodium does not affect tachykinin receptors on airway smooth muscle, because the drug does not modify the dose–response curve to exogenous SP on tracheal smooth muscle [16]. We cannot exclude the possibility that, in vivo, nedocromil may affect the increase in airway vascular leakage induced by SP that may participate in the bronchoconstrictor effect of the neuropeptide. Such a hypothesis is supported by the finding that in human skin cigarette smoke cromoglycate, a drug close to nedocromil sodium, caused a dose-dependent inhibition of SP and neurokinin B-induced oedema [24]. However, we believe that this hypothesis is unlikely in the guinea-pig airway as nedocromil was shown to have no effect on airway microvascular leakage induced by release of endogenous neuropeptides from capsaicin-sensitive nerves [25].

SP is rapidly degraded by a membrane-bound peptidase, neutral endopeptidase (E.C. 3.4.24.11), that is present in several tissues in the airways, including epithelial cells, smooth muscle, submucosal glands, and nerves [5]. We have previously shown that smoke-induced hyperresponsiveness to SP was likely to be explained by inhibition of airway NEP; additionally, we showed that cigarette smoke-induced inactivation of airway NEP was the consequence of either free radicals present in the smoke itself or the production of free radicals by activation of inflammatory cells induced by cigarette smoke exposure [4] or both. This conclusion was supported by the finding that superoxide dismutase dramatically reduced cigarette smoke-induced hyperresponsiveness to SP in vivo [4]. Previous studies indicated that smoke solutions prepared in a similar manner are capable of generating oxidants for several hours [26, 27], and may exert a direct inhibitory effect on airway NEP activity [4]. Therefore, we examined the possibility that nedocromil sodium could prevent inactivation of airway NEP due to free oxidizing radicals present in gas phase of cigarette smoke solution. Our results showed that nedocromil sodium had no effect on smoke-induced inactivation of NEP in vitro.

Exposure to cigarette smoke has been shown to result in activation of phagocytes [28]. When activated, these phagocytes produce large quantities of free radicals [29]. Nedocromil sodium is known to block activation of several inflammatory cells, such as human granulocytes [30–32], and to inhibit the release of free radicals from activated monocytes and platelets [33]. Nedocromil sodium is also capable of blocking smoke-induced release of neutrophil chemotactic activity from bovine epithelial cells [34]. In vivo studies have also shown nedocromil sodium to inhibit cigarette smoke-induced hyperresponsivity to histamine and the concomitant inflammatory response [23]. Therefore, the protective effect of nedocromil sodium in the present study could be the consequence of reduced influx and/or activation of inflammatory cells in the airway.

In conclusion, we have demonstrated that nedocromil sodium prevents cigarette smoke-induced hyperresponsiveness to SP. By preventing activation of several inflammatory cells, some of which are involved in cigarette smoke-induced inflammation, and by blocking smoke-induced hyperresponsiveness to substance P, nedocromil sodium may be a useful drug to prevent certain aspects of airway inflammation that occur after cigarette smoke exposure.

References


