OZONE EXAGGERATES NASAL ALLERGIC INFLAMMATION

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Abstract
A double-blind randomised trail design was used to address the effect of ambient ozone on the nasal airways and to evaluate the effects of ozone on allergic mucosa. Ten grass pollen allergic rhinitics were exposed for 2 hours at rest on 2 separate occasions to 400 ppb ozone and filtered air respectively. The exposure to 400 ppb ozone and filtered air was performed prior to the grass pollen season and again during the season. Baseline nasal lavage in which histamine, eosinophil cationic protein (ECP), myeloperoxidase (MPO), total proteins, and albumin were measured and neutrophils, eosinophils and epithelial cells were counted, was made immediately prior to exposure (-120 min). After 2h of exposure to ozone/filtered air repeated measurements were performed at 0, 30, 60, 120, 240, 360 minutes post exposure.

Significant increases were observed when exposed to ozone versus filtered air during the pollen season for histamine (AUC1; p = 0.05), MPO (AUC2; p = 0.05), ECP (AUC2; p = 0.008), total proteins (AUC, p = 0.02; AUC1, p = 0.007; AUC2, p = 0.05), and albumin (AUC, p = 0.007; AUC1, p = 0.02; AUC2, p = 0.005). There was also a significant increase in the total protein level (AUC, p = 0.05; AUC1, p = 0.02; AUC2 p = 0.05) and albumin (AUC, p = 0.03; AUC1, p = 0.03; AUC2, p = 0.04) after ozone exposure versus air out of season. Significant increase of the neutrophils (p = 0.01 and p = 0.007) in the nasal lavage fluid (NLF) at time points 0 min and 360 min respectively were observed, while eosinophils and epithelial cells significantly increased only at time point 360 min (p = 0.02 and p = 0.02 respectively) all of them after ozone exposure versus filtered air during the season. Neutrophils also significantly increased in the NLF at time point 0 min and 360 min (p = 0.03 and p = 0.01) while epithelial cells increased only at time point 360 min (p = 0.01) after ozone exposure versus filtered air out of season. We can conclude that ozone induces neutrophil and eosinophil recruitment into the nose and this is accompanied by activation, as evidenced by release of MPO and ECP in NAL. Pre-existing allergic mucosal inflammation during the pollen season, exaggerates the response to ozone, particularly in relationship to the recruitment of eosinophils and neutrophils 6h following exposure.

Key words: mediators, allergic rhinitis, ozone.

Introduction
Ozone is a photochemical oxidant pollutant formed as a result of a series of complex chemical reactions taking place in the atmosphere, involving oxides of nitrogen, sunlight and volatile organic compounds. It is well documented that ozone can produce adverse respiratory health effects in both animals and humans [1–3]. Much of the research involving humans has been on the effects of ozone on the lower respiratory tract. However, humans are predominantly nose breathers, and the cells lining the nasal passages are the first to come in contact with an air pollutant [4]. Approximately 40% of inhaled ozone is taken up in the nasal passages in healthy human subjects [5], raising concern that toxicity caused by ozone may also occur in the upper airways.

Ozone has been shown to induce inflammatory changes in the upper airways of both normal and allergic subjects. Graham and Koren [6, 7] observed that ozone exposure was associated with increased neutrophil content of NLF from nonallergic individuals, indicating that ozone also induces nasal inflammation. The same authors presented data on soluble mediators in the NLF in which they demonstrated that ozone caused a tryptase increase, indicating mast cell release, immediately
postexposure. The albumin level was elevated only 18h postexposure, indicating an elevated epithelial permeability [8]. Field studies performed by Frischer and colleagues [9] reveal increased amounts of eosinophil cationic protein (ECP) and myeloperoxidase (MPO) in NLF obtained from both allergic and normal children on days during which ambient ozone levels were increased.

When asymptomatic subjects with allergic rhinitis were exposed to 500 ppb ozone for 2h, significant increases in neutrophils, eosinophils, mononuclear cells, albumin, and histamine were observed in the NLF [10]. Thus, allergic rhinitis patients may have a partly different nasal response to ozone compared with healthy subjects [11].

We examined whether short-term exposure to ambient concentrations of ozone induced the release of the inflammatory mediators or infiltration of leukocytes into the NLF of allergic rhinitis patients during or out of the allergic season.

Methods
Ten allergic rhinitis subjects with skin reactivity to grass pollen, who were otherwise healthy, non-smoking, 5 males and 5 females, between the ages of 19 and 43 (27.9 ± 2.1 years of age, mean ± SEM) were recruited into this study. A battery of 10 standard antigens was employed, although only persons sensitive to grass pollen extract were enrolled.

Study design
A double-blind randomised trial was used to evaluate the effects of ozone on allergic mucosa. Ten grass pollen allergic rhinitics were exposed for 2h at rest on 2 separate occasions to 400 ppb ozone and filtered air, during the season and out of the season respectively. Baseline measurements of histamine, ECP, MPO, total proteins, albumin, neutrophils, eosinophils and epithelial cells were performed in the nasal lavage immediately prior to a 2h exposure (-120 min). Repeated measurements were performed at 0, 30, 60, 120, 240, and 360 minutes post-exposure for the mediators and at 0 and 360 minutes for the cell counts.

Ozone exposure
Exposures were carried out in a randomised single-blinded controlled fashion using a purpose-built ozone exposure system in our laboratory. Ozone was produced using a generator (Trigged Ozone System, Glasgow, Model NVF 1/20 DE, manufactured by Ordup Maskin, Copenhagen, Denmark) capable of producing 5–30 mg/h of ozone. The gas was delivered via a mask fitted with a one-way valve to prevent the mixture of inspired and expired air. Ozone concentration was measured every 20 seconds at the mask by means of a probe connected to a UV photometric ozone analyser (Model 427, Rotork, Oxon, UK) with a precision of 20 ppb and a linearity of ± 10 ppb. A standard RS232 output cable was used to link the analyser to a computer in order to display the concentration on the computer screen every 20 seconds. These values were averaged during the challenge period (399 ± 9 ppb, mean ± SD for the 10 subjects who completed the study).

We used hospital compressed air as a source to generate ozone/filtered air. In order to ensure that all the air used was free from contamination, a series of filters was incorporated into the system before this air was fed into the generator to produce ozone. The concentration of ozone could be adjusted by regulating the flow of ozone into the generator together with the flow of filtered air into a mixing chamber fitted between the ozone generator and the mask. Using a humidifier (Fisher and Paykel Clinical Humidifier, Model MR 730, New Zealand) in the system a relative humidity of 40–60% and temperature of 25°C was achieved at the mask. The minute ventilation was measured using pneumotachographs incorporated in the breathing circuit. The pressure drop across the stainless steel mesh of the pneumotachograph was transmitted to a pressure transducer via a pair of tubes. A bipolar analogue signal is then converted to a digital value which is transmitted via a dedicated link to the computer. The computer programme then averages this value over a sampling window of 0.5 seconds.

Nasal Lavage Technique
The nasal lavage technique employed was modified from procedures described previously by Naclerio and co-workers [12]. Four lavages with 5 ml 0.9% NaCl at 37°C in each nostril were performed to reduce possible resting mediator levels to a stable baseline. The lavage fluid from both nostrils was pooled and immediately put on ice. The combined sample was centrifuged at 1500 xg for 10 min at 4°C. The supernatant was stored in adequate fractions at -80°C until analysed.

The remaining cell pellet was resuspended in 0.2 ml RPMI cell culture media containing 0.1% (wt/vol) bovine serum albumin (Sigma Chemical, Poole, UK). Albumin was added to ensure that cells would adhere to the microscope glass for differential staining. A 100 µl aliquot was separated for cytocentrifuge preparation using a Shandon cytocentrifuge device (Shandon Southern Instruments, Runcorn, UK). The slides were air dried, and differential cell counts were made after staining with rapid Giemsa stain (HemaGurr, BDH, Poole, UK). We counted the total number of each cell type on the slide and calculated the number of cells/ml.
Histamine Assay
A spectrofluorometric assay of histamine in microtitre wells coated with a glass fibre matrix was performed [13, 14]. The limit of detection of this assay is approximately 5 ng/ml.

Measurement of total protein and albumin concentrations
Total protein concentrations were measured in 0.05 ml replicates of nasal lavage fluid by the method of Bradford [15] using Coomassie Blue G-250 reagent as the indicator. Absorbance was read at 595 nm and concentrations were derived from a standard curve constructed with bovine serum albumin by interpolation. The lower limit of total protein detectable by this method is 5 μg/ml with a coefficient of variation for repeated measurements of 1.2%. Albumin concentrations were measured by rocket immunoelectrophoresis using the method of Weeke [16]. Three microlitre of NLF were introduced into wells cut in agarose gel and subjected to electrophoresis (4 V/cm) for 14h in a "window" of agarose containing 1.18 μl/ml of rabbit anti-human antibody. Immunoprecipitates were stained with Coomassie Brilliant Blue and albumin concentrations derived from standard curves constructed with human albumin by interpolation. The lower limit of albumin detectable by this method is 1 μg/ml with a coefficient of variation for repeated measurements of 5%.

ECP and MPO measurement
Commercial assays were used for measurements of ECP by fluorometric enzyme immunoassay (FEIA), and MPO by radioimmunoassay (both from Pharmacia, Upsalla, Sweden). The sensitivities of the assays were 2 μl/l, and 8 μg/ml respectively.

Data analysis
Statistical analyses were performed using SPSS 7.0. All measurements at each time point were adjusted from the baseline and summarised using the area under the response curve for the early response (-120 to 120 min; AUC1), late response (120 to 360 min; AUC2) and total response (-120 to 360 min; AUC) as appropriate. The resulting areas for all four visits were compared using the non-parametric Friedman's two-way ANOVA procedure. If significant results were obtained using the Friedman two-way ANOVA procedure, the Wilcoxon matched-pairs signed-rank test was used to investigate the pre-planned comparisons of interest; 0 min versus baseline, 360 min versus baseline. P < 0.05 was used as the level of significance. Correlation between mediators and cells at 360 min were investigated using the Spearman rank correlation coefficient.

Results
The study was designed for the patients to be exposed to either 400 ppb ozone or filtered air on two separate occasions (two weeks apart) during the grass pollen season in the UK, and the same procedure was repeated out of the season. Thus, for all patients four sets of data were collected. Using the non-parametric Friedman’s two-way ANOVA procedure we analysed the area under the response curve for the early response (-120 to 120 min; AUC1), late response (120 to 360 min; AUC2) and total response (-120 to 360 min; AUC) for all four visits. There were significant results for ECP for the late response (AUC2, p = 0.02), total proteins for the total, early and late response (AUC, p = 0.02; AUC1, p = 0.003; AUC2, p = 0.02) and albumin for the total, early and late response (AUC, p = 0.05; AUC1, p = 0.02 and AUC2, p = 0.008) respectively. Of the pre-planned pair-wise comparison of interest the following were shown to be significant using Wilcoxon matched-pairs signed-rank test for: ECP (AUC2, p = 0.008) Fig. 1a, histamine (AUC1, p = 0.05) Fig. 1b, MPO (AUC2, p = 0.05) Fig. 2, total proteins (AUC, p = 0.02; AUC1, p = 0.007; AUC2, p = 0.05) Fig. 3a, and albumin (AUC, p = 0.007; AUC1, p = 0.02; AUC2, p = 0.005) Fig. 3b, all when ozone exposure was compared with air, during the pollen season. There was also a significant increase in the total protein level (AUC, p = 0.05; AUC1, p = 0.02; AUC2 p = 0.05) Fig. 4a, and albumin (AUC, p = 0.03; AUC1, p = 0.03; AUC2, p = 0.04) Fig. 4b after ozone exposure versus air out of season indicating increased vascular permeability as an intrinsic ozone effect. No other combinations showed any significant difference. Histamine was significantly increased only during the early response, ECP and MPO only during the late phase and total proteins and albumin during the whole period including the early and late response phases. The pollen count was similar during the two exposure days in the season and does not
account for the differences between compared parameters during the season. The only difference was that the patients were exposed to ozone, which probably exaggerates the ongoing allergic inflammation. This effect was not seen when ozone was given to the patients out of the season except for total proteins and albumin level, indicating that the combination of ozone and allergen contribute to the significant increase in the inflammatory parameters. Intragroup comparison using Friedman’s two-way ANOVA analyses revealed significant results only for total proteins and albumin during ozone exposure both in the season (p = 0.007 and p = 0.01 respectively) and out of season (p = 0.03 and p = 0.007 respectively). When Wilcoxon’s matched-pairs signed-rank test was used for the pre-planned pairs we found a significant increase of total proteins immediately (p = 0.02) and 6h after the ozone exposure (p = 0.01) in comparison to baseline during the season as well as out of season (p = 0.01 at time point 0 min) and (p = 0.04 at time point 360 min).

Similarly, for albumin there was a significant increase immediately (p = 0.007) and 6h after ozone exposure (p = 0.01) in comparison to baseline during the season as well as out of season (p = 0.01 and p = 0.02 respectively).

Friedman two-way ANOVA analysis of the cells for all four visits revealed significant differences for neutrophils (p = 0.01 and p = 0.002), eosinophils (p = ns, p = 0.03) and epithelial cells (p = ns, and p = 0.006) at time point 0 min and 360 min respectively. Wilcoxon’s matched-pairs signed-rank test showed a significant increase of the neutrophils (p = 0.01 and p = 0.007) Fig. 5a in the NLF at time points 0 min and 360 min respectively, while eosinophils and epithelial cells significantly increased only at time point 360 min (p = 0.02 Fig. 6 and p = 0.02 Fig. 7a respectively) all of them after ozone exposure versus filtered air during the season. Neutrophils also significantly increased in the NLF at time point 0 min and 360 min (p = 0.03 and p = 0.01) Fig. 5b while epithelial cells increased only at time point 360 min (p = 0.01) Fig. 7b after ozone exposure versus filtered air out of season. There was no significant change in the number of eosinophils out of the pollen season.

We found a significant correlation between the MPO level and number of neutrophils, both adjusted from the baseline at time point 360 min (r = 0.73, p = 0.02) Fig. 8 after ozone exposure in the season. Similarly, there was a significant correlation between the ECP level and number of eosinophils, both adjusted from baseline at time point 360 min (r = 0.84, p = 0.002) Fig. 9 again after ozone exposure in the season.

Figure 1a and 1b – Change of ECP and histamine from baseline in NAL during allergic season
Ozone exaggerates nasal allergic inflammation

**Figure 2** – Change of MPO from baseline in NAL during allergic season

**Figure 3a and 3b** – Change of total proteins and albumin from baseline in NAL during allergic season
Figure 4a and 4b – Change of total proteins and albumin from baseline in NAL out of season

Figure 5a and 5b – Change neutrophils from baseline in NAL during and out of allergic season
Ozone exaggerates nasal allergic inflammation

Figure 6 – Change of eosinophils from baseline in NAL during allergic season

Figure 7a and 7b – Change of epithelial cells from baseline in NAL during and out of season
Discussion

Our results show a statistically significant increase in histamine, ECP, MPO, albumin and total proteins as well as increased number of neutrophils, eosinophils and epithelial cells in the NLF after ozone exposure in comparison to air exposure during the pollen season. When ozone was given to the patients out of season, there was a significant increase only in total proteins, albumin, neutrophils and epithelial cells in the NLF in comparison to air exposure day.

We specifically examined the effect of 400 ppb ozone on nasal inflammatory responses in allergic rhinitics at rest for 2h during and out of the grass pollen season. The level and duration of ozone exposure were selected because they are the same as those that have induced neutrophil influx into the nasal tissues of normal subjects in other studies [17]. Although the level of ozone used was relatively high (400 ppb) exposure was only for 2h and occurred without exercise, unlike a study of normal subjects who underwent heavy exercise [8]. Each subject underwent both an ozone and filtered air exposure during and out of grass pollen season. This approach allowed for examination of the intrinsic effect of ozone on nasal inflammation (out of season) as well as the effect of ozone on the ongoing allergic inflammation in the nasal mucosa (during the season).

We found that total proteins and albumin concentrations were significantly increased after the ozone exposure out of season indicating an increased vascular permeability as an intrinsic ozone effect. Similarly, an even higher significant increase was seen when patients were exposed to ozone during the season. The ongoing allergic reaction has probably contributed to the higher increase in the level of total proteins and albumin. The intragroup comparison has shown that total protein and albumin concentrations are significantly higher immediately and 6h after the ozone exposure during and out of season in comparison to baseline values. It has been demonstrated in another study that albumin level was elevated in the NLF only 18h post-exposure indicating increased epithelial permeability in the nose [6, 18]. Koren and associates demonstrated increases in total proteins, albumin, and IgG in BAL fluid following ozone exposure in humans, suggesting increased vascular permeability in the lower respiratory tract [8]. Bascom and colleagues have reported that immediately following 500 ppb ozone for 4h allergic patients had an increased level of albumin, neutrophils, eosinophils and mononuclear cells in the NLF [10].

In our study we found a significantly increased concentration of histamine in the early phase when the patients were exposed to ozone during the season but not out of season. Bascom and colleagues have also shown a small but significant increase in nasal lavage histamine occurring with ozone exposure [10]. This could indicate a mast-cell activation, hence histamine is stored in mast cell granule. During the allergic season the mast cells are activated, thus a combination of ozone and allergen could contribute to the histamine increase in the NLF after ozone exposure. Ozone itself did
not change the histamine level out of season. Histamine was increased in NLF in the early phase when an increase of total proteins and albumin was also seen. Why histamine was not increased during the late phase response is not entirely clear, but one of the reasons could be that an increase in the vascular permeability allows more histaminases to enter the NLF and degrade histamine. The patients also remained indoors until the last NLF was taken being deprived of contact with the allergen. Another possibility is that increased fluid at the nasal airway surface caused by even greater increase in the vascular permeability during the late phase response could have diluted the histamine concentration at the airway surface in the later NLFs. Graham and Koren demonstrated that ozone caused a tryptase increase, indicating mast cell release, immediately postexposure [8].

We also demonstrated a significant increase in ECP concentration in the late phase when patients were exposed to ozone during the season. Ozone itself did not significantly change the ECP level out of season. ECP is a granule constituent of eosinophils, and although its presence in NLF could result from ex vivo eosinophil degranulation, elevated ECP levels in NLF probably represent in vivo eosinophil activation. The number of eosinophils in the NLF was significantly increased during the late phase when patients were exposed to ozone during the season. There was also a significant correlation between the ECP concentration and eosinophil number at time point 360 min after ozone exposure in the season. During the season there is a combined action of ozone and allergen contributing to greater plasma exudation especially in the late phase. ECP, which is released by eosinophils during the allergen season, may be moved to the airway surface by combined ozone and allergen exudation of plasma that floods the lamina propria before entering the airway lumen through unidirectional paracellular epithelial routes [19].

MPO was significantly increased in the late phase response when patients were exposed to ozone during the season but not out of the season. Field studies performed by Frischer and colleagues [9] reveal increased amounts of MPO in NLF obtained from both allergic and normal children on days during which ambient ozone levels were increased. We were unable to demonstrate increased level of MPO in the NLF during ozone exposure out of the season. Out of the season there was a smaller influx of neutrophils in the NLF (p = 0.03) in comparison to the neutrophil influx during the season (p = 0.003). The reason could be the much larger influx of neutrophils during ozone exposure in the season (p = 0.003) versus out of season (p = 0.03). Maybe the number of neutrophils was not high enough to produce detectable quantities of MPO in the NLF out of season. This could also explain why MPO was increased in the late phase only. Neutrophils increased from baseline after ozone exposure to a greater extent in the late phase (p = 0.007 at time point 360 min) than in the early phase (p = 0.03 at time point 0 min) during the season. There was also a significant correlation between the MPO concentration and neutrophil number at time point 360 min after ozone exposure in the season.

Out of the season, we also demonstrated a significant influx of neutrophils in the early (p = 0.03) and late phase (p = 0.01) after ozone exposure in comparison to filtered air. Bascom and colleagues reported an influx predominantly of neutrophils in the NLF of allergic rhinitis patients exposed to ozone [10]. Graham and Koren found an increased neutrophil number in the NLF immediately after ozone exposure which continued to increase at the 18-h postexposure [6]. McBride and colleagues found that short-term exposure to an ambient concentration of ozone induces upper airway inflammation in subjects with asthma [3]. A significant increase was observed in the number of neutrophils and epithelial cells recovered in the NLF of these patients 7 to 10 min after exposure to 240 ppb ozone and neutrophil levels were also significantly higher 24h after ozone exposure [3].

Ozone exposure did not significantly change the number of eosinophils out of season. But, when patients were exposed to ozone during the season, a significantly higher number of eosinophils were found in the NLF 6 hours after exposure in comparison to air (p = 0.02). There was no change in the number of eosinophils immediately after ozone exposure during the season. Similarly, Graham and co-workers did not find an ozone-induced increase in eosinophils to the nasal mucosa of the normals [6] as did the persons with allergic rhinitis studied by Boscom and co-workers [10], indicating that eosinophil influx to the nasal airways is a unique response of allergic subjects to ozone exposure [7].

Our study also suggested that sloughing of epithelial cells occurred only 6h after ozone exposure during (p = 0.02) and out of season (p = 0.01) in comparison to filtered air. When compared to baseline values, the number of epithelial cells significantly increased 6h after the exposure to ozone.
increasing epithelial injury. It may be that the epidermal injury due to eosinophil-derived mediators, ozone could exacerbate allergic illness by increasing in this study provides evidence that, in humans, ozone can exacerbate allergic illness by increasing epithelial injury. The observation of epithelial sloughing in this study provides evidence that, in humans, ozone can exacerbate allergic illness by increasing epithelial injury due to eosinophil-derived mediators. We found that allergen during the naturally occurring grass pollen season enhanced ozone-induced inflammation. Eosinophil influx, ECP and MPO release were only increased in persons exposed to ozone during the season when compared with values obtained with air exposure. Such a relationship is important because susceptible individuals may encounter allergen before ozone exposure or undergo simultaneous exposure to ozone and allergen since the grass pollen season and the peak ambient ozone levels coincide.

REFERENCES

Целта на оваа двојно-слепа рацономизирана студија е да се евалуира ефектот на озонот врз назалните патишта, особено врз алергски променетата слушокожа. Во оваа студија, десет пациенти со алергиски ринитис алергички на полен од трева, беа изложени, за време од 2 часа во миривање, на 400 ррб (делови од милијардата) озон и филтриран воздух во два различни денови. Експозицијата на 400 ррб озон и филтриран воздух беше изведена пред почетокот на поленската сезона и се повтори за време на поленската сезона на трева. Непосредно пред експозицијата (-120 мин.), било на озон или филтриран воздух, кај сите пациенти беше направена назална лаважа во која беа мериени базалните концентрации на хистаминот, еозинофилни протеини (ECP), миело пероксидазата (MPO), тоталните протеини и албуминот и беше одредуван бројот на неутрофилите, еозинофилите и епителните клетки.

По двохасовната експозиција било на озон или филтриран воздух, повторно кај сите пациенти беше направена назална лаважа, во која беа одредувани нивото на сите, претходно спомнати, медијатори и клетки, и тоа 0 мин., 30, 60, 120, 240 и 360 мин. по завршувањето на експозицијата. Притоа беше регистрирано сингификсантно зголемување на концентрацијата на хистаминот (AUC1; p = 0.05), MPO (AUC2; p = 0.05), ECP (AUC2; p = 0.008), тоталните протеини (AUC; p = 0.02, AUC1; p = 0.007, AUC2; p = 0.05) и албуминот (AUC; p = 0.007, AUC1; p = 0.02, AUC; p = 0.005), кога пациентите беа експонирани на озон во споредба со филтриран воздух за време на поленската сезона.

Исто така беше регистрирано сингификсантно зголемување на нивото на тоталните протеини (AUC; p = 0.05, AUC1; p = 0.02, AUC2; p = 0.05) и албуминот (AUC; p = 0.03, AUC1; p = 0.03, AUC2; p = 0.04) по експозицијата на пациентите на озон наспроти филтриран воздух надвор од поленската сезона.

Бројот на неутрофилите во назалниот лават беше регистрирано сингификсантно зголемување на бројот на неутрофилите непосредно по (0 мин.) p = 0.01 и 360 мин. по експозицијата на озон наспроти филтриран воздух за време на поленската сезона, додека пак бројот на еозинофилите и епителните клетки значително се зголемија единствено 360 мин. по експозицијата на озон.

Бројот на неутрофилите во назалниот лават сингификсантно се зголеми непосредно по (0 мин.) p = 0.03 и 360 мин. по експозицијата (p = 0.01) на озон наспроти филтриран воздух во период надвор од поленската сезона, додека пак епителните клетки значително се зголемија само 360 мин по експозицијата (p = 0.01).

Може да заклучиме дека експозицијата на озон доведува до регулатиране и активирање на неутрофилите и еозинофилите во назалната мукоза што се доведува со значителното зголемување на концентрацијата на MPO и ECP во назалниот лават. Постоечката алергиска инфламација за време на поленската сезона го зголемува ефектот на озонот што се согледува во поголемото инфлукусе на неутрофили и еозинофил во лаватот 6 часа после експозицијата на озон за време на поленската сезона.

Ключни зборови: медијатори, алергиски ринитис, озон.