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Full Paper

Oxymetazoline Inhibits and Resolves Inflammatory Reactions in Human Neutrophils

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Abstract. The nasal decongestant oxymetazoline (OMZ) exhibits anti-oxidative and anti-inflammatory properties (I. Beck-Speier et al., J Pharmacol Exp Ther. 2006;316:842–851). In a follow up study, we hypothesized that OMZ generates pro-resolving lipoxins being paralleled by production of immune-modulating prostaglandin E_2 (PGE_2) and anti-inflammatory 15(*S*)-hydroxy-eicosatetraenoic acid [15(*S*)-HETE] and depletion of pro-inflammatory leukotriene B_4 (LTB_4). Human neutrophils (PMN) were chosen as the cellular system. The effect of OMZ on these parameters as well as on respiratory burst activity and oxidative stress marker 8-isprostane was analyzed in unstimulated and co-stimulated PMN by ultrafine carbon particles (UCP) or opsonized zymosan (OZ), respectively. In unstimulated cells, OMZ induced formation of PGE_2, 15(*S*)-HETE, and LXA_4. The levels of LTB_4 and 8-isoprostane were not affected, whereas respiratory burst activity was drastically inhibited. In UCP- and OZ-stimulated control cells, all parameters were elevated. Here, OMZ maintained the increased levels of PGE_2, 15(*S*)-HETE, and LXA_4, but substantially suppressed levels of LTB_4 and 8-isoprostane and inhibited the respiratory burst activity. These findings suggest a switch from the pro-inflammatory eicosanoid class LTB_4 to the pro-resolving LXA_4. Since LXA_4 is most relevant in returning inflamed tissue to homeostasis, OMZ is postulated to terminate rhinitis-related inflammation, thus contributing to shortening of disease duration.

Keywords: oxymetazoline, rhinitis, neutrophil, eicosanoid, lipoxin A_4

Introduction

Upper respiratory tract infections (URTIs), which are mainly caused by rhinoviruses, lead to profound economic disprofit due to medical expenses and loss of productivity. Moreover complications of rhinovirus induced URTI, including otitis media (1) and sinusitis (2), as well as exacerbations of lower respiratory tract diseases, for example, asthma (3 – 9) and chronic obstructive pulmonary disease (COPD) (10 – 13), have an enormous impact upon health care.

The symptomatic therapy for an URTI, also known as the common cold, has been effectively carried out by application of the nasal decongestant oxymetazoline (OMZ) for nearly 50 years. OMZ is a sympathomimetic imidazoline derivate with direct alpha-adrenergic activity on vascular smooth muscle cells in the nasal mucosa, leading to vasoconstriction. The onset of action is within seconds (14) and lasts for up to twelve hours (15). In addition to its symptomatic effect, recent studies have also documented anti-oxidative (16, 17) and anti-inflammatory (16, 18) properties of OMZ.

Tüttenberg et al. (18) showed that the anti-inflammatory action of OMZ is partially mediated by the inhibition of pro-inflammatory cytokines (interleukin-1β, tumor necrosis factor-α, interleukin-6, and interleukin-8) as well as reduced T-cell stimulatory capacity of dendritic cells, resulting in a repressed stimulation of T cells. Westerveld et al. (17) showed that OMZ is a potent inhibitor of microsomal lipid peroxidation and a hydroxyl radical scavenger. Furthermore, we have recently reported that OMZ exhibits anti-oxidative and

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anti-inflammatory properties in a cell-free system as well as in a cellular system consisting of canine alveolar macrophages (AM) (16). OMZ was found to clearly inhibit pro-inflammatory reactions such as the respiratory burst and the 5-lipoxygenase (5-LO) pathway with formation of leukotriene B_4 (LTB_4). Besides, this drug had the capability to induce the cyclooxygenase (COX) pathway for formation of immune-modulating prostaglandin E_2 (PGE_2) and the 15-lipoxygenase (15-LO) pathway for production of anti-inflammatory 15(*S*)-hydroxy-eicosatetraenoic acid [15(*S*)-HETE]. These protective processes are known to trigger pathways involving the resolution of inflammation. The resolution of inflammation is an active process with formation of so called "stop signals" such as the newly discovered anti-inflammatory and pro-resolving dual function mediators lipoxin A_4 (LXA_4) and lipoxin B_4 (LXB_4) (19).

In view of the immune-modulating and anti-inflammatory properties of OMZ, we hypothesized that OMZ has the potential to induce the generation of lipoxins that in turn contribute to the resolution phase of inflammation. The resolution phase of inflammation is an important event to return the inflamed tissue to homeostasis and to complete resolution without leaving molecular signs or traces of the leukocyte battle (19). Otherwise chronic inflammatory processes may occur. Because infections by rhinoviruses induce inflammatory reactions with influx of polymorphonuclear neutrophils (PMN) into the nasal tissue (20, 21), these cells serve as a model in this study with OMZ. We suggest that OMZ is able to induce immune-modulating PGE_2 and anti-inflammatory 15-HETE and to inhibit pro-inflammatory LTB_4. Due to the activation and inhibition of these lipid mediators and their corresponding enzymes, a switch of eicosanoids may occur by OMZ, resulting in inhibition of 5-LO with LTB_4 formation and in activation of PGE_2 synthesis and 15(*S*)-HETE generation, leading to production of pro-resolving LXA_4.

Materials and Methods

Materials

Phosphate-buffered saline (PBS) with and without Ca^{2+}/Mg^{2+} was purchased from Biochrome (Berlin, Germany); luminol and zymosan were from Sigma (Deisenhofen, Germany).

Solutions of OMZ and suspensions of ultrafine carbon particles and opsonized zymosan OMZ (Merck, Darmstadt, Germany) was dissolved and diluted in PBS with Ca^{2+}/Mg^{2+}, pH 7, containing 0.1% glucose. Ultrafine carbon particles (UCP) were generated by spark discharging (22) and suspended in distilled water (32 μg/ml) by repeated vortexing and sonification (23). Opsonized zymosan was prepared from zymosan, purified by heating for 30 min at 90°C in 0.9% sterile NaCl solution, and incubated with fresh-frozen human serum in equal-volume portions for 30 min at room temperature according to Allen (24). The washed opsonized zymosan was suspended in PBS (pH 7) containing 0.1% glucose.

Isolation of PMN

PMN were isolated from citrate-anticoagulated venous blood of healthy human volunteers with Polymorphprep (Axis-Shield, Oslo, Norway) according to Beck-Speier et al. (25). Briefly, the isolated layer of PMN was washed with PBS (pH 7.4) without Ca^{2+}/Mg^{2+} to remove the gradient medium. Residual erythrocytes were lysed by treating the cell pellet with distilled water for 30 s. Isotonicity was restored by adding the same volume of 1.8% (w/v) NaCl. The purified cells were resuspended in PBS (pH 7.4) with Ca^{2+}/Mg^{2+} and 0.1% glucose. The viability of the cells was always over 95% as determined by trypan blue exclusion. The cells were identified by microscopic examination of cytospin preparations after May Grünwald Giemsa staining as >99% populations of PMN.

Viability of PMN with OMZ

To prove the effect of OMZ on the viability of the cells, PMN were incubated with various OMZ concentrations (0.1, 0.4, and 1 mM) in PBS (pH 7.4) with Ca^{2+}/Mg^{2+} and 0.1% glucose in the absence of stimulants for 80 min at 37°C. After harvesting the cells by centrifugation ($400 \times g$ for 10 min at room temperature), the cells were resuspended in HEPES buffer, pH 7.4, containing 1 mM EDTA, and aliquots of the cell suspensions were used for determination of the viability by trypan blue exclusion.

Incubation of PMN with OMZ

The effect of OMZ on PMN was determined in the absence or presence of the co-stimulatory agents ultrafine elemental carbon particles (UCP) or opsonized zymosan (OZ). Both stimulants are particulate and are ingested by phagocytic cells, UCP via non-receptor–mediated phagocytosis and OZ via receptor-mediated phagocytosis (16). The following treatments were performed according to our previous study (16): i) to assess the effect of OMZ on unstimulated cells, PMN (0.5×10^6 cells/0.5 ml) were incubated with various concentrations of OMZ (0.1, 0.4, and 1 mM, corresponding to 29.68, 118.72, and 296.8 μg/ml, respectively) in PBS, pH 7.4, with Ca^{2+}/Mg^{2+} and 0.1% glucose for 80 min at 37°C; ii) to measure the effect of OMZ

on stimulated cells, PMN (0.5×10^6 cells/0.5 ml) were incubated in parallel incubations with various concentrations of OMZ (0.1, 0.4, and 1 mM) in PBS, pH 7.4, with Ca^{2+}/Mg^{2+} and 0.1% glucose for 20 min at 37°C and subsequently co-stimulated by UCP (32 μg/ml) or OZ (50 μg/ml) for an additional 60 min. After the incubation procedures, the cells were harvested by centrifugation at $400 \times g$ for 10 min at room temperature. The cells were resuspended in cold HEPES buffer, pH 7.4, containing 1 mM EDTA; homogenized by sonification (3 times, 15 s each time) in ice; and centrifuged at $10{,}000 \times g$ for 15 min at 4°C. Importantly, cells were kept on ice during preparation to avoid activation or degradation processes. The supernatants were taken for analysis of both protein and lipid mediators. Analysis of the lysate is a reliable tool to quantify the eicosanoid production that has to be favored over analysis of extracellular levels (26). The sensitivity of this intracellular approach is supported by normalization of eicosanoid levels to the protein content in the lysate. Analysis of the cellular eicosanoid content appears to be relevant with regard to the autocrine mechanisms of PGE_2, which might trigger both functional and metabolic parameters of PMN.

Determination of protein

Protein was measured at 595 nm in a microtiter plate format by using 5 μl of cell homogenate and 200 μl of 1:5 diluted Biorad solution (Bio-Rad, Munich, Germany) with bovine serum albumin as standard.

Analysis of lipid mediators

For analysis of lipid mediators, the supernatants of the cell homogenates were deproteinized by adding an 8-fold volume of 90% methanol containing 0.5 mM EDTA and 1 mM 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl, pH 7.4 (16). These methanol suspensions were stored at –40°C for 24 h followed by two centrifugation steps at $10{,}000 \times g$ for 20 min at 4°C with a 24-h interval to remove the proteins. Aliquots of the obtained supernatants were dried in a vacuum centrifuge, dissolved in assay buffer, and used for quantification of PGE_2, LTB_4 15(*S*)-HETE, 8-isoprostane, and LXA_4 were measured by their specific enzyme immunoassays (Cayman Chemical) according to the instructions of the manufacturer.

Respiratory burst activity

The respiratory burst activity of human PMN was determined by luminol-dependent chemiluminescence (CL) according to Mishra et al. (27). Briefly, PMN (3×10^4 cells) were preincubated in 0.25 ml PBS, pH 7.4, containing Ca^{2+}/Mg^{2+}, 0.1% glucose, and 0.02 mM luminol for 10 min at 37°C in a chemiluminescence analyzer (Autoluminat LB 953; Berthold Technologies, Bad Wildbad, Germany). CL signals of PMN in the absence and presence of various OMZ concentrations were recorded for 20 min at 37°C. Thereafter, UCP or OZ, respectively, was added, and the CL signals of the cells were monitored for a further 20 min at 37°C.

Statistical analyses

Statistical significance was determined by analysis of variance and the two-sample Student's *t*-test (STATSAK, version 2.12, by G.E. Dallal, 1986; Malden, MA, USA). Changes with $P \leq 0.05$ were considered as significant.

Results

Effect of OMZ on viability of human PMN

The effect of OMZ on the viability of the cells was evaluated by trypan blue exclusion on a percentage basis. As shown in Fig. 1, OMZ did not affect the viability of PMN. The difference of dead and living cells between untreated PMN (0 mM) and OMZ-treated PMN (0.1, 0.4, and 1 mM) was not significant. These data indicate that OMZ exerts no toxic effect on PMN.

Effect of OMZ on generation of eicosanoids and respiratory burst activity

Human PMN were studied for the effect of OMZ in the absence and presence of co-stimulators. The following endpoints were analyzed: a) formation of PGE_2, 15(*S*)-HETE and LXA_4, and b) formation of

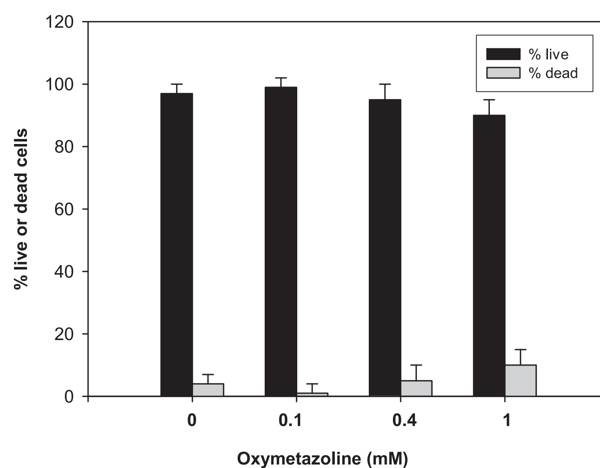

Fig. 1. Effect of OMZ on viability of human PMN. PMN were incubated with various OMZ concentrations (0.1, 0.4, and 1 mM) in PBS, pH 7.4, with Ca^{2+}/Mg^{2+} and 0.1% glucose in the absence of stimulants for 80 min at 37°C. Viability of cells was determined by trypan blue exclusion. Values are given as percentage of live or dead cells (mean ± S.D.) for n = 3 as the number of experiments.

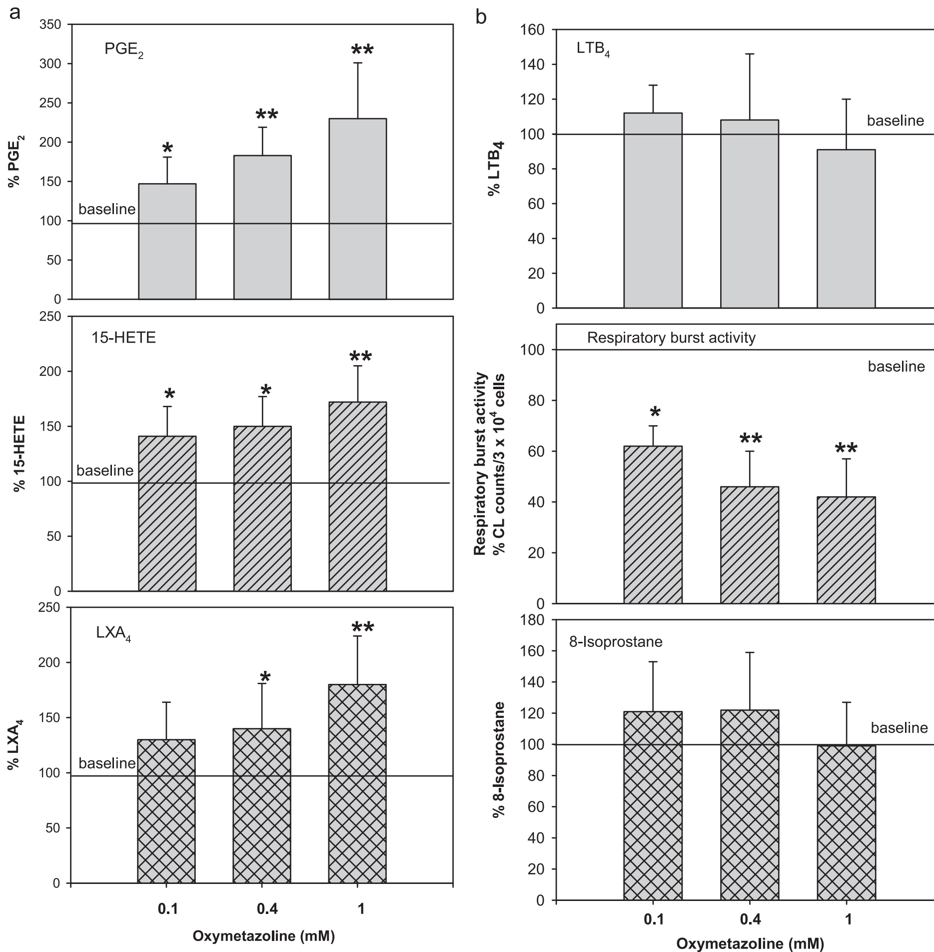

Fig. 2. Effect of OMZ on unstimulated PMN. PMN were incubated with various concentrations of OMZ (0.1, 0.4, and 1 mM) in PBS, pH 7.4, with Ca^{2+}/Mg^{2+} and 0.1% glucose for 80 min at 37°C. Baseline levels were obtained by parallel incubations of the cells without OMZ. The effect of OMZ was measured for (a) generation of immune-modulating PGE_2, anti-inflammatory 15(*S*)-HETE, and pro-resolving mediator LXA_4 and (b) production of pro-inflammatory LTB_4, CL as indicator for respiratory burst activity, and formation of 8-isoprostane as oxidative stress marker. The number of experiments with PMN of different volunteers was n = 7 for all parameters. The data (mean ± S.D.) are given as percentages of the corresponding baseline values. Baselines [100% values (mean ± S.D.) with n = 7] are as follows: PGE_2, 324 ± 114 pg/mg cellular protein; 15(*S*)-HETE, 11007 ± 3930 pg/mg cellular protein; LXA_4, 446 ± 83 pg/mg cellular protein; LTB_4, 476 ± 188 pg/mg cellular protein; respiratory burst activity, 474750 ± 134950 CL counts during 20 min for 3×10^4 cells; 8-isoprostane, 123 ± 50 pg/mg cellular protein. Asterisks indicate changes of parameters in the presence of OMZ (**P*<0.05, ***P*<0.01).

LTB_4, respiratory burst activity, and 8-isoprostane as a marker for lipid peroxidation. These cellular responses were studied under three different conditions.

OMZ in the absence of co-stimulatory agents

At concentrations ranging from 0.1 to 1 mM, OMZ exerted a significant stimulatory effect on formation of PGE_2, 15(*S*)-HETE, and LXA_4 in unstimulated PMN (Fig. 2a), while formation of LTB_4 and the oxidative stress marker 8-isoprostane were not affected by OMZ (Fig. 2b). In contrast, the respiratory burst activity was strongly inhibited by concentrations of 0.1 to 1 mM OMZ to below the baseline level (Fig. 2b).

OMZ in the presence of UCP

UCP were selected as a model stimulant. These particles are known to induce formation of PGE_2, 15(*S*)-HETE, LXA_4, LTB_4, respiratory burst activity, and 8-isoprostane (23). As shown in Fig. 3, UCP applied in the absence of OMZ (UCP control) activated PMN for a significant increase in the level of each of these parameters (P<0.01) in comparison to the respective baseline level. OMZ exerted differential effects on the UCP-induced responses. OMZ did not substantially change the elevated UCP-stimulated levels of PGE_2, 15(*S*)-HETE, and LXA_4 (Fig. 3a). However, the increased levels of UCP-stimulated LTB_4 and 8-isoprostane were drastically suppressed by concentrations of 0.4 to 1 mM OMZ, whereas respiratory burst activity was significantly inhibited by OMZ concentrations of 0.1 to 1 mM (Fig. 3b).

OMZ in the presence of OZ

In addition to the environmental co-stimulant UCP, which is non-physiologic, we also used OZ as a physiologic co-stimulant because of its opsonization with complement components. UCP and OZ are both particulate stimuli, which induce inflammatory and oxidative stress reactions (16). However, OZ with its opsonization elicited stronger biologic responses than UCP, especially concerning LTB_4 and the respiratory burst activity. As shown in Fig. 4, OZ as control stimulant induced the formation of PGE_2, 15(*S*)-HETE, LXA_4, LTB_4, and 8-isoprostane and activated respiratory burst

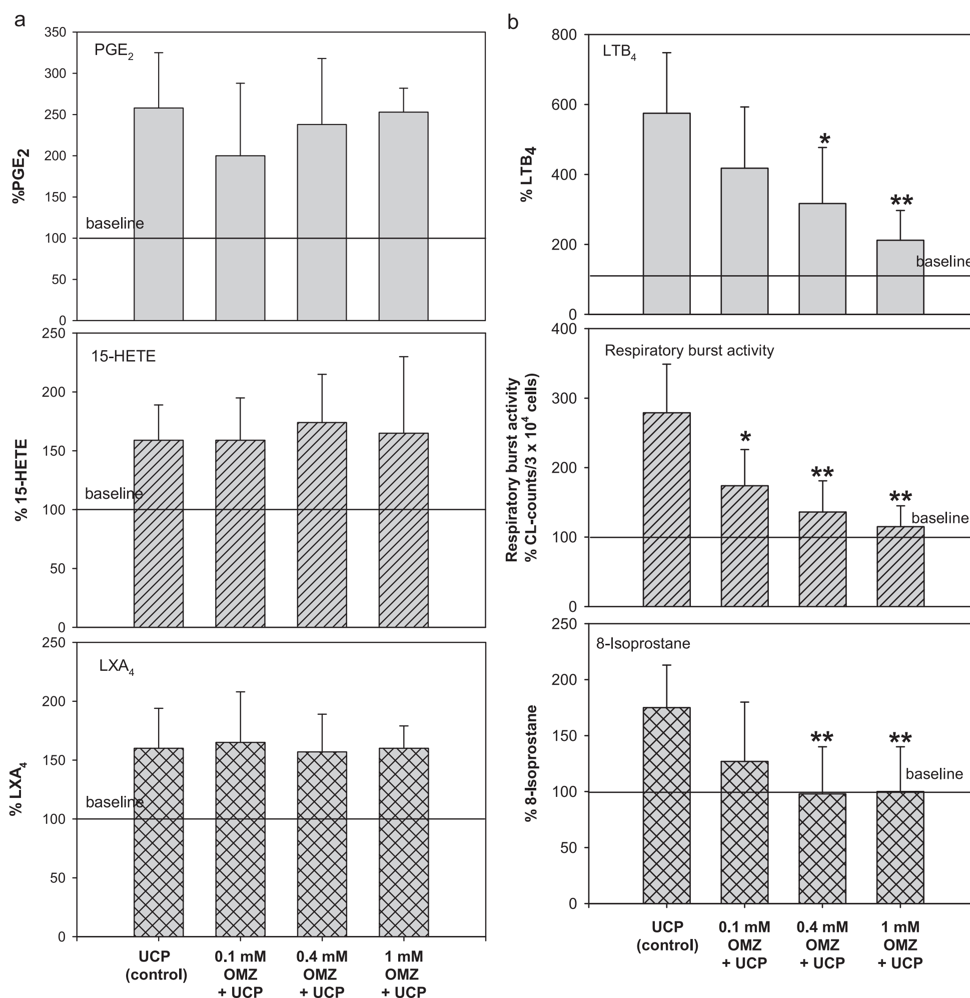

Fig. 3. Effect of OMZ on ultrafine carbon particles (UCP)-induced stimulation of lipid mediators and respiratory burst activity. PMN were preincubated in the absence and presence of OMZ in concentrations ranging from 0.1 to 1 mM for 20 min and subsequently stimulated by UCP for 60 min. The effect was measured for generation of PGE_2, 15(*S*)-HETE, and LXA_4 (a) and production of LTB_4, CL, and 8-isoprostane (b). The number of experiments with PMN of different volunteers was n = 7 for LXA_4 and LTB_4, n = 6 for 15(*S*)-HETE and 8-isoprostane, and n = 5 for PGE_2 and CL. Baseline values were obtained in parallel incubations of the cells in the absence of OMZ and UCP and were the same as those described in Fig. 2. Asterisks indicate a significant suppression of the UCP-induced parameters by OMZ (*$P<0.05$, **$P<0.01$).

activity ($P<0.01$). As already seen with UCP, OMZ exerted different effects on the OZ-induced responses. OMZ essentially did not change OZ-stimulated elevation of PGE_2, 15(*S*)-HETE, and LXA_4 levels (Fig. 4a). However, the increased levels of OZ-stimulated LTB_4, 8-isoprostane, and respiratory burst activity were drastically inhibited by OMZ nearly to baseline levels (Fig. 4b). LTB_4 levels and respiratory burst activity were reduced by OMZ concentrations of 0.4 and 1 mM, whereas the oxidative stress marker 8-isoprostane was inhibited by OMZ concentrations ranging from 0.1 to 1 mM.

Discussion

In a previous study we showed in an in vitro assay that OMZ inhibits the activity of 5-LO, which has pro-inflammatory properties. The activity of 15-LO, showing anti-inflammatory characteristics, is not inhibited. Using canine alveolar macrophages as a cellular model, we recently confirmed that OMZ inhibits the 5-LO pathway, causing a decreased production of LTB_4, and it induces the 15-LO pathway, resulting in an increase of 15(*S*)-HETE generation (16). The present study was designed to investigate the effect of OMZ on the eicosanoid metabolism in human PMN representing the major population of inflammatory cells in the virus-infected nasal tissue (20, 21). Based on our observation that OMZ activates the 15-LO pathway, we postulated that OMZ also induces formation of pro-resolving lipoxins, being the final metabolites of this pathway. These studies were performed both with unstimulated PMN and PMN stimulated by UCP or OZ to simulate inflammatory responses and oxidative stress. Our data demonstrate that OMZ inhibits respiratory burst activity very efficiently, while baseline levels of LTB_4 and 8-isoprostane used as an oxidative stress marker are not affected in unstimulated PMN. However, immune-modulating and anti-inflammatory responses, including the PGE_2 and 15(*S*)-HETE and the pro-resolving mediator LXA_4 production, are significantly increased. In UCP- or OZ-stimulated cells, OMZ

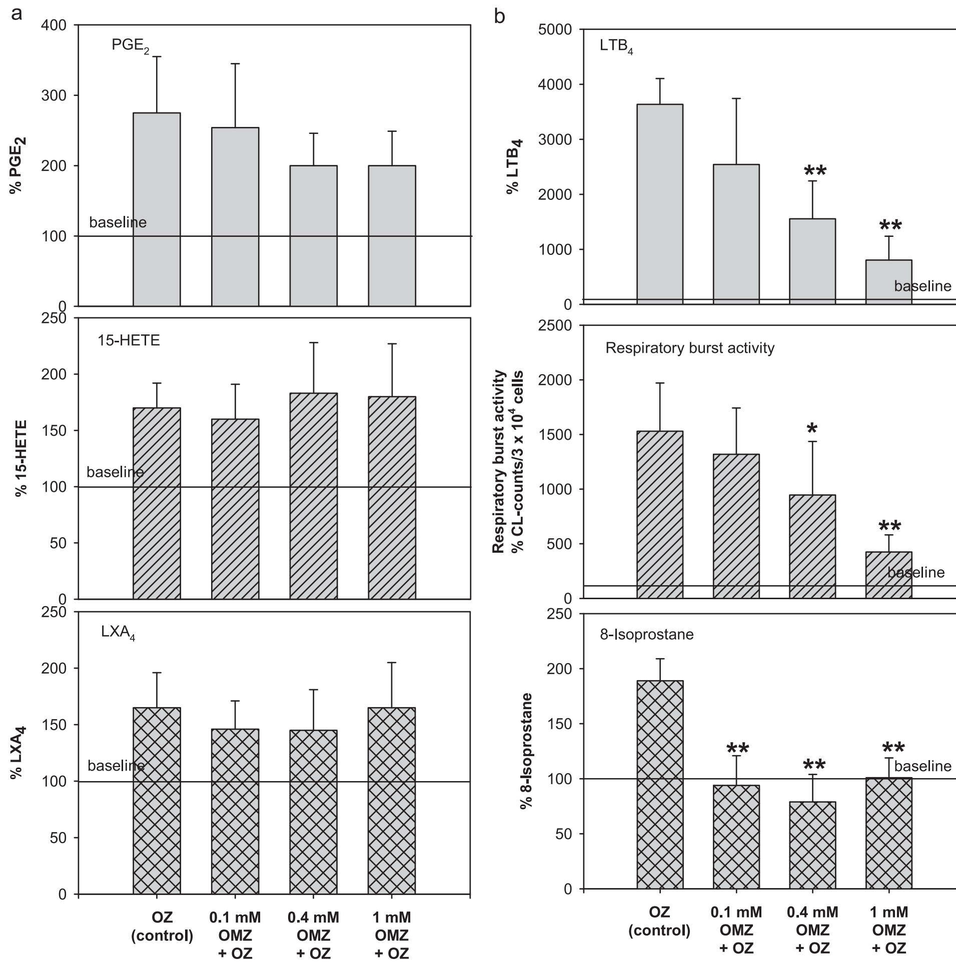

Fig. 4. Effect of OMZ on opsonized zymosan (OZ)-stimulated synthesis of lipid mediators and respiratory burst activity. PMN were preincubated in the absence and presence of OMZ in concentrations ranging from 0.1 to 1 mM for 20 min and subsequently stimulated by OZ for 60 min. The effect was measured for generation of PGE_2, 15(*S*)-HETE, and LXA_4 (a) and production of LTB_4, CL, and 8-isoprostane (b). The number of experiments with PMN of different volunteers was n = 7 for CL, n = 6 for LXA_4, and n = 5 for PGE_2, LTB_4, 15(*S*)-HETE, and 8-isoprostane. Baseline values were obtained in parallel incubations of the cells in the absence of OMZ and OZ and were the same as those described in Fig. 2. Asterisks indicate a significant suppression of the OZ-induced parameters by OMZ (*$P<0.05$, **$P<0.01$).

strongly inhibits the LTB_4 and 8-isoprostane formation and the respiratory burst activity. The increased levels of PGE_2, 15(*S*)-HETE, and LXA_4 reached after stimulation were maintained in the presence of OMZ. We suppose that UCP- and OZ-stimulated cells are at their upper limit for producing PGE_2, 15(*S*)-HETE, and LXA_4, which does not allow a further increase by OMZ under these in vitro conditions. Similar responses regarding PGE_2, 15(*S*)-HETE, LTB_4, respiratory burst activity, and 8-isoprostane were already observed in canine AM (16), which react very similar to human AM (23). The effective concentrations of OMZ for inhibition of pro-inflammatory and oxidative stress reactions in PMN range between 0.1 and 1 mM. These OMZ concentrations have also been shown to be relevant in AM (16). In our previous study we estimated that OMZ in the current product concentration of 1.6 mM (nose sprays for adults and school children) at a dosage volume of 45 μl/puff (28) will be diluted to form a concentration gradient that is in the range of the levels of OMZ used in our experiments (estimated mean value: almost equal to 0.1 mM) (16). Therefore, our present results are of relevance in situ. However, it is still unknown by which receptor(s) OMZ modulates inflammation. Possibly OMZ exerts its effects on lipid mediators via α_1- and/or α_2-adrenoceptors since studies with stably transfected Chinese hamster ovary (CHO) cells have shown that OMZ behaves as a partial agonist of α_{1A}-adrenoceptors (29, 30), and this was also observed for α_{2C}-adrenoceptors (31). In addition, very low or not precisely measurable agonistic activity of OMZ has been observed in transfected CHO cells expressing α_{1D}- (29, 30) or α_{2A}- (32) adrenoceptors.

A viral infection induces a strong inflammation in the nasal tissue leading to an acute rhinitis. Inflammatory mediators play an important role in the pathogenesis of acute rhinitis (20, 21, 33). Nasal secretions become enriched with pro-inflammatory mediators, cytokines and eicosanoids (leukotrienes and prostaglandins), accompanied by an infiltration of PMN (20, 21, 34). LTB_4 is the most potent chemoattractant for PMN and therefore responsible for the neutrophilic infiltration in rhinitis-infected tissue (34, 35).

Data from our in vitro approach demonstrate that

OMZ inhibits the 5-LO–driven production of LTB_4 and the release of reactive oxygen species (ROS), which is paralleled by a substantial increase of PGE_2. Besides having some specific pro-inflammatory properties, PGE_2 was shown in the last decade to exhibit predominantly a regulatory function to control immune responses, for example, by attenuating pro-inflammatory reactions (36). One important target of this cyclooxygenase metabolite is the 5-LO–driven pathway, which is inhibited for leukotriene synthesis and translocation of 5-LO in stimulated PMN (37, 38). Furthermore, PGE_2 was found to up-regulate the expression of 15-LO in PMN (39), which activates the pathway for generation of 15(*S*)-HETE and lipoxins. Exposure of PMN to 15(*S*)-HETE also attenuates levels of LTB_4 substantially after stimulation with Ca^{2+}-ionophore or the FMLP peptide (40). Up-regulation of 15-LO expression and release of 15(*S*)-HETE seem to be a further regulatory mechanism to cope with inflammation (41, 42). There is evidence that 15-LO is expressed in human nasal epithelium to mediate mucociliary differentiation (43). The expression of 15-LO in nasal epithelium may further support our findings about the effect of OMZ on 15(*S*)-HETE formation in PMN. 15(*S*)-HETE is the key metabolite for generation of lipoxins that also involves the action of 5-LO. Lipoxins are primarily engaged in resolution and termination of inflammatory events that return the inflamed tissue to homeostasis. Although PGE_2 initiates a number of responses relevant to inflammation, the central task of this metabolite is to trigger the "lipid mediator class switching" to signal the end of inflammation by activating the transcriptional regulation of 15-LO, resulting in production of LXA_4 (19, 39). Since OMZ induces formation of PGE_2 and 15(*S*)-HETE indicating an activation of 15-LO and reduces LTB_4 production indicating a reduction of 5-LO activity, OMZ is suggested to substantially support this switching of the eicosanoid class from LTB_4 to LXA_4. The importance of PGE_2 induced by OMZ during the inflammatory response in the nasal tissue is emphasized by the study of Han et al. (44), showing that the nasal patency is reduced when the COX activity is inhibited by non-steroidal anti-inflammatory drugs.

Moreover, the respiratory burst activity and the oxidative stress induced by UCP and OZ are inhibited by OMZ. The inhibition of both parameters might be due to the radical scavenger activity of OMZ as proposed by Westerveld et al. (17). OMZ contains a hydroxyl group on its phenyl ring that is able to easily provide a hydrogen atom for reaction with lipid peroxide–related radicals and other reactive oxygen species. Due to its radical scavenger activity, we suggest that OMZ also neutralizes radicals arising from the respiratory burst activity and abolishes UCP- and OZ-induced 8-isoprostane formation. This represents a protective mechanism avoiding excessive release of reactive

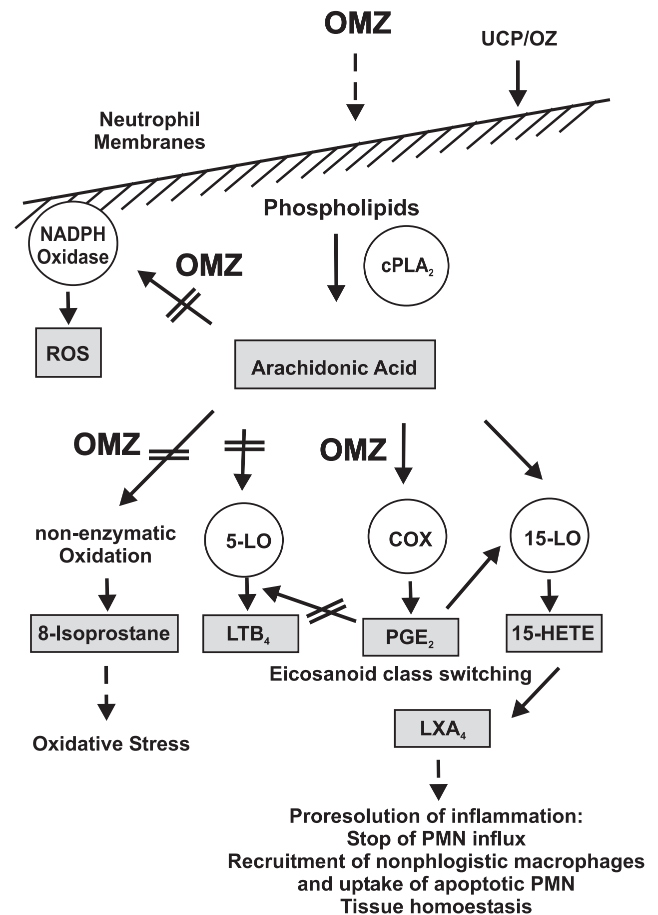

Fig. 5. Oxymetazoline (OMZ) triggered signaling cascade in human neutrophils concerning eicosanoids. According to the effect in alveolar macrophages (16), OMZ interferes with neutrophil membranes by activating cytoplasmatic phospholipase A_2 ($cPLA_2$), which liberates arachidonic acid from membrane phospholipids. Arachidonic acid is further metabolized to immune-modulating PGE_2 by cyclooxygenase (COX) and to anti-inflammatory acting 15(*S*)-HETE by 15-lipoxygenase (15-LO). In contrast to PGE_2 and 15(*S*)-HETE, OMZ inhibits 5-lipoxygenase (5-LO), the initial enzyme of leukotriene synthesis, resulting in reduced production of pro-inflammatory LTB_4. Furthermore, OMZ inhibits respiratory burst activity (NADPH oxidase), leading to diminished production of tissue-damaging reactive oxygen species (ROS), thus intensifying its inhibitory effect on pro-inflammatory reactions. In the presence of environmental or physiologic stimuli such as ultrafine carbon particles (UCP) or opsonized zymosan (OZ), OMZ also inhibits LTB_4 synthesis and respiratory burst activity, whereas PGE_2 and 15(*S*)-HETE production are not affected. In addition, OMZ prevents the formation of the oxidative stress marker 8-isoprostane, which is induced by the oxidative capacity of environmental particles and opsonized zymosan. Due to its inhibition of LTB_4 formation and induction of PGE_2 and 15(*S*)-HETE production, OMZ is able to switch the eicosanoid classes from pro-inflammatory LTB_4 to pro-resolving LXA_4. This highlights the anti-inflammatory and pro-resolving role of OMZ in rhinitis.

oxygen species as a tissue damaging principle.

The elevated levels of LXA_4 triggered by OMZ may also contribute to shortening the duration of the disease (15). In a prospective, multicenter, randomized, placebo-controlled, double-blind parallel group comparison study — OMZ vs. physiological saline solution — rhinitis duration was determined as primary endpoint in common-cold patients (268 Patients at 24 sites). Secondary endpoints were a noticeable relief of nasal congestion, runny nose, stuffy nose, sneezing, impairment of sense of smell and taste, and feeling unwell. The maximum treatment duration was 10 days [1 puff (45 μl of 1.6 mM/0.05% OMZ) in each nostril three times daily]. Symptoms improved significantly with OMZ *versus* the reference group treated with physiological saline solution as early as treatment day 2, and relief was maintained throughout the study period. Duration of rhinitis was significantly reduced by one-third (4 vs. 6 days) with OMZ.

In summary, we propose an OMZ-triggered signaling cascade in PMN as shown in Fig. 5. OMZ seems to be a leading signal that activates production of PGE_2, which then suppresses LTB_4 formation and activates the 15-LO pathway. In a concerted action with PGE_2, the pro-resolving lipid mediator LXA_4 stops further PMN influx, thus terminating the inflammation. Furthermore, LXA_4 stimulates monocyte recruitment for uptake of apoptotic PMN by macrophages (19). LXA_4 is most relevant for its capability to return the inflamed tissue to homeostasis (19). In this regard, the increase and maintenance of LXA_4 levels by OMZ may enhance the termination of rhinitis-related inflammation and thus contribute significantly to shortening the duration of the disease (15).

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