ELSEVIER

European Journal of Pharmacology 377 (1999) 63–68

ejp

www.elsevier.nl/locate/ejphar

Inhibition of ozone-induced lung neutrophilia and nuclear factor-κB binding activity by vitamin A in rat

Takeshi Hisada, Ian M. Adcock, Yasuyuki Nasuhara, Michael Salmon, Tung-Jung Huang, Peter J. Barnes, K. Fan Chung *

Department of Thoracic Medicine, National Heart and Lung Institute, Imperial College School of Medicine, Dovehouse Street, London, SW3 6LY, UK

Received 25 May 1999; accepted 28 May 1999

Abstract

Vitamin A binds to retinoic acid receptors, which in turn may interact with other transcription factors. We determined its effect (2500 and 5000 IU/kg) on nuclear factor-κB binding activity in the lung, airway inflammation and bronchial hyperresponsiveness in rats exposed to ozone. Ozone (3 ppm, 3 h) caused neutrophil influx into bronchoalveolar lavage fluid ($16.2 \pm 0.8 \times 10^5$ cells/ml, $p < 0.01$) and bronchial hyperresponsiveness ($-\log PC_{200}ACh = 2.54 \pm 0.19$, $p < 0.05$, compared to control animals, respectively). Vitamin A inhibited this neutrophilia dose-dependently together with the increased DNA-binding activity of nuclear factor-κB in lung extracts. Vitamin A did not affect bronchial hyperresponsiveness at both doses. Vitamin A inhibits ozone-induced neutrophilic inflammation through a reduction in nuclear factor-κB DNA binding activity.

Keywords: NF-κB (Nuclear factor-κB); Transcription factor; Asthma; Neutrophilic inflammation; Ozone

1. Introduction

Ozone is an important component of the photochemical oxidative products of air pollution involving substances emitted from automobile engines. Exposure to ozone induces an airway neutrophilic inflammation, airways obstruction, and airway hyperresponsiveness to bronchoconstrictor agents in both humans and animals (Nikula et al., 1988; Hyde et al., 1992; Tsukagoshi et al., 1995; Haddad et al., 1996). Ozone exposure also induces the expression of the C–X–C chemokines, macrophage inflammatory protein-2 (MIP-2) and cytokine-induced neutrophil chemoattractant (CINC) (Haddad et al., 1995, 1996), of the pro-inflammatory cytokines, interleukin-1β and tumor necrosis factor (TNF)-α in the lungs (Pendino et al., 1994). Transcriptional activation of several cytokine genes for C–X–C chemokines including interleukin-8, melanocyte growth stimulatory factor/growth related gene product (MGSA/gro), macrophage inflammatory protein (MIP)-2 and cytokine-induced neutrophil chemoattractant (CINC) appears to be dependent on the binding of transcription factor, nuclear factor-κB (NF-κB), to specific binding site at the 5′-promoter region of those genes (Anisowicz et al., 1991; Mukaida and Matsushima, 1992; Konishi et al., 1993; Haddad et al., 1996). The powerful oxidizing activity of ozone is likely to be responsible for the increase in NF-κB-binding in the lung observed after exposure to ozone (Haddad et al., 1996), because oxidants can rapidly activate NF-κB binding in certain cell lines in vitro (Toledano and Leonard, 1991; Schreck et al., 1991). Such activation of NF-κB is inhibited by corticosteroids through a direct protein-protein interaction between NF-κB and the activated glucocorticoid receptor (Haddad et al., 1996).

Vitamin A is a retinoic acid involved in the differentiation of pulmonary epithelial cells (Jetten et al., 1986) and its actions are mediated through nuclear receptors, including the retinoic acid receptor and retinoid X receptor families (Giguere et al., 1987; Petkovich et al., 1987; Brand et al., 1988; Lehmann et al., 1992; Mangelsdorf et al., 1993). Retinoic acid receptor acts in the form of dimers as DNA binding proteins (Yang et al., 1991), which can activate or repress transcription of specific genes by binding to short stretches of nucleotide sequences, ('enhancers'). The amino acid sequence of the retinoic acid receptor proteins has a strong similarity with those coding

* Corresponding author. Tel.: +44-171-352-8121; fax: +44-171-351-8126; E-mail: f.chung@ic.ac.uk

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00405-7

for steroid hormones, including vitamin D and thyroid hormone receptors. After binding of vitamin A to retinoic acid receptor, the activated receptor interact with other transcription factors through a direct protein-protein interaction or by binding to specific DNA sequences (Pfahl, 1993). Direct protein–protein interactions occur between the transcription factor, activating protein 1 (AP-1) and the retinoic acid receptor, and both corticosteroid and retinoic acid inhibit AP-1 via interaction of their receptors with distinct parts of AP-1 complex (Giguere, 1994). We hypothesized that vitamin A could inhibit increased inflammatory cytokine gene expression by ozone exposure by preventing the NF-κB DNA-binding activity, leading to an inhibition of neutrophil influx into the lungs induced by ozone exposure. We therefore investigated for the first time the effect of vitamin A on NF-κB DNA binding activity in Brown-Norway rats exposed to ozone, and evaluated the effect of vitamin A on ozone-induced neutrophil influx and bronchial hyperresponsiveness.

2. Materials and methods

2.1. Experimental protocol

Virus-free inbred male Brown–Norway rats weighing 250–300 g (Harlan Olac, Bicester, Oxon, UK) were used. They were kept in a special caging system with its own air circulation (Maximizer; Thorens Caging Systems, Hazleton, PA). Seven groups (6–7 animals in each group) of animals were studied. We used all-*trans* retinol palmitate (Sigma, Poole, Dorset, UK) as vitamin A, which was dispersed in 0.15 M NaCl, and 2500 or 5000 IU/kg was injected subcutaneously for three days prior to ozone exposure. After the last injection of vitamin A, animals were exposed to ozone or laboratory air according to the protocol below. The dose and regime for vitamin A administration were partly based on the previous work (Tesoriere et al., 1994). Animals were sacrificed at either 2 or 24 h after cessation of ozone exposure, and bronchoalveolar lavage was performed.

Group 1: Naive rats exposed to filtered laboratory air.

Group 2: Rats pretreated with vitamin A (2500 IU) and exposed to laboratory air.

Group 3: Rats pretreated with vehicle and exposed ozone (3 ppm, 3 h).

Group 4: Rats pretreated with low dose of vitamin A (2500 IU/kg) and exposed ozone (3 ppm, 3 h).

Group 5: Rats pretreated with high dose of vitamin A (5000 IU/kg) and exposed ozone (3 ppm, 3 h).

Group 6: Rats pretreated with vehicle and exposed ozone (3 ppm, 6 h).

Group 7: Rats pretreated with low dose of vitamin A (2500 IU/kg) and exposed ozone (3 ppm, 6 h).

2.2. Ozone exposure

Ozone was generated by passing laboratory air (1 l/min) through a Sander ozonizer (model IV, Sander, Vetze, Germany). The output was diluted with compressed air (10 l/min) controlled by a gas flowmeter (Platon Flow Control, Basingstoke, UK) and fed into a 32-l box made of Perspex. The concentration of ozone was determined by using specific gas sampling tubes (Dragerwerk, Lubeck, Germany) and was maintained at 3 ppm by regular measurement at the output port of the box. Conscious rats were exposed to ozone for 3 or 6 h, and control rats breathed filtered air only.

2.3. Measurement of lung function and airway responsiveness to acetylcholine

Airway responsiveness was measured 18–24 h after a 3- or 6-h exposure to ozone. Rats were anesthetized with an i.p. injection of 2 mg/kg midazolam (Roche Products, Welwyn Garden City, UK) and a s.c. injection of 0.4 mg/kg Hypnorm (Janssen Pharmaceuticals, Wantage, UK), which contains 0.315 mg/ml of fentanyl citrate and 10 mg/ml of fluanisone. A tracheal cannula (1.02-mm outer diameter) was inserted into the lumen of the cervical trachea through a tracheostomy. The animals were connected to a small-animal respirator (Harvard Apparatus, Edenbridge, Kent, UK) and ventilated with 10 ml/kg of air at a rate of 90 strokes/min. Transpulmonary pressure was measured with a pressure transducer (model FCO 40 ± 1000 mm H_2O, Furness Controls, Bexhill, Sussex, UK) with one side attached to an air-filled catheter inserted into the right pleural cavity and the other side attached to a catheter connected to a side port of the intratracheal cannula. Airflow was measured with a pneumotachograph (model F1L, Mercury Electronics, Glasgow, Scotland) connected to a transducer (model FCO 40 ± 20 mm H_2O, Furness Controls). The signals from the transducers were digitized with a 12-bit analog-to-digital board (NB-MIO-16, National Instruments, Austin, TX) connected to a Macintosh II computer (Apple Computer, Cupertino, CA) and analyzed with software (Lab VIEW 2, National Instruments, Austin, TX) that was programmed to measure lung resistance (R_L) according to the method of Von Neergaard and Wirz (1927). Aerosols were generated with an ultrasonic nebulizer (model 2511, PulmoSonic, DeVilbiss, Hazeltown, PA).

The animals were initially injected with propranolol (1 mg/kg i.v.) to inhibit adrenergic effects and suxamethonium (1.5 mg/kg) to stop spontaneous breathing. R_L values after 0.9% NaCl (45 breaths) were used as the baseline value. Increasing half-$\log_{10}$ concentration of acetylcholine were administered by inhalation (45 breaths), with the initial concentration set at 10^{-4} M. The challenge was stopped when an increase in R_L exceeding 200% was obtained. The $-\log_{10}$ of the provocative concentration of

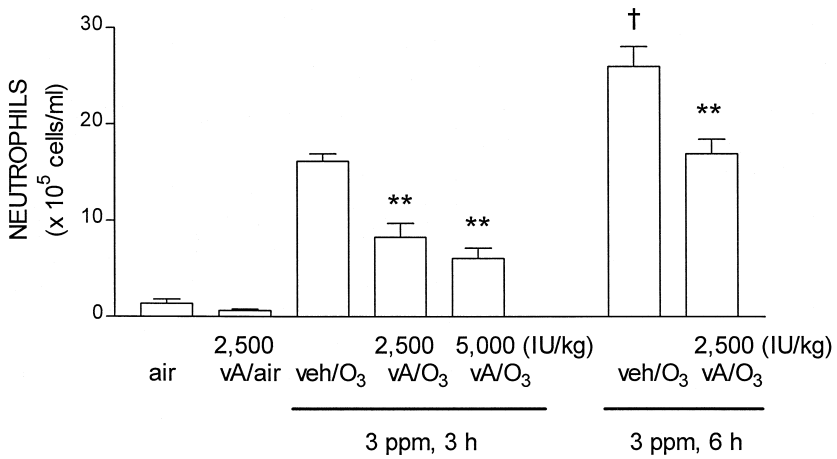

Fig. 1. Effect of vitamin A (vA) on ozone-induced neutrophil influx into bronchoalveolar lavage fluid. Bronchoalveolar lavage was performed 24 h after the cessation of ozone exposure. Vitamin A did not affect neutrophil counts following air exposure. Vitamin A reduced the neutrophil counts induced by ozone (3 ppm, 3 h) with a greater effect at the higher dose of vitamin A (5000 IU/kg). The neutrophilia induced by a longer exposure to ozone (3 ppm, 6 h) was also inhibited by vitamin A (2500 IU/kg). $^{**}P < 0.01$ compared to corresponding ozone-exposed animals. †$P < 0.01$ compared to animals exposed to 3 ppm ozone for 3 h.

acetylcholine producing a 200% increase in R_L ($-\log PC_{200}$ ACh) was calculated from concentration–response curves.

2.4. Bronchoalveolar lavage and cell counting

Rats were administered pentobarbital sodium (200 mg/kg intravenously), and the lungs were lavaged with 2-ml aliquots of saline solution 10 times through the tracheostomy. Lavage fluid was centrifuged (500 g for 10 min at 4°C), and the cell pellet was resuspended in 1 ml of Hank's balanced salt solution. Total cell counts were performed by adding 10 μl of the cell suspension to 90 μl Kimura stain and counting in a Neubauer chamber (American Optical, Southbridge, MA). Differential cell counts were determined from cytospin preparations stained with May-Grünwald stain. Cells were identified as macrophages, neutrophils, eosinophils, lymphocytes, basophils and epithelial cells. Five hundred cells were counted, and the percentage and absolute number of each cell type were calculated.

2.5. Electrophoretic mobility shift assays

Nuclear proteins were extracted from lung tissues using detergent lysis according to a method modified from

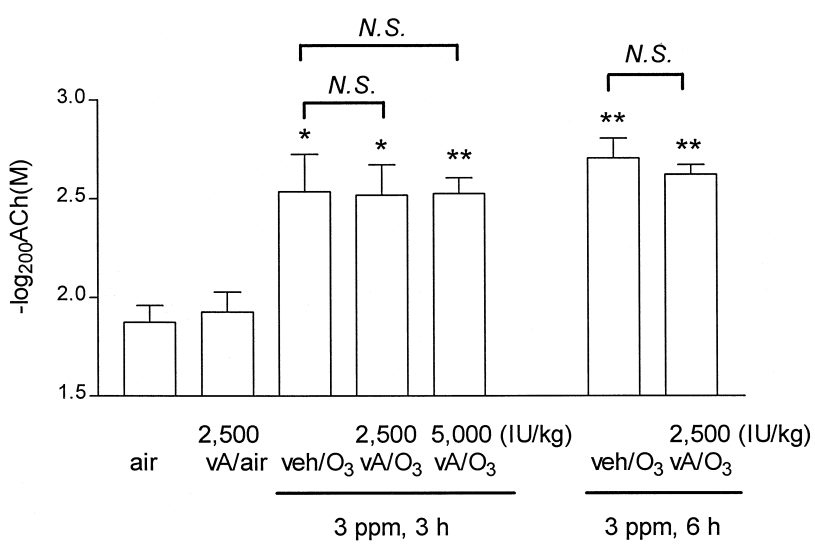

Fig. 2. Effect of vitamin A (vA) on ozone-induced bronchial hyperresponsiveness to acetylcholine, measured as PC_{200}, which is the concentration of acetylcholine needed to increase baseline resistance by 200%, at 24 h after exposure to ozone or air. Vitamin A itself did not affect on bronchial hyperresponsiveness. Ozone (3 ppm for 3 h and 6 h) exposure caused significant bronchial hyperresponsiveness. Vitamin A at both doses did not inhibit bronchial hyperresponsiveness induced by ozone exposure. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to animals exposed to air. N.S.: not significant.

(Gough, 1988). Double-stranded oligonucleotides encoding the consensus target sequence of NF-κB (and flanking regions) (5′-CTCCGGGAATTTCCCTGGC-3′) (Promega, Cambridge, UK) were end-labeled using [γ-^{32}P] dATP and T4 polynucleotide kinase. Ten micrograms of nuclear protein from each sample was incubated with 50,000 cpm of labeled oligonucleotide in 25 μl incubation buffer (4% glycerol, 100 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol, 10 mM Tris–HCl, pH 7.5, 0.8 mg/ml sonicated salmon sperm DNA) for 20 min at 4 C. Protein–DNA complexes were separated on a 6% polyacrylamide gel using 0.25 × Tris–Borate–EDTA running buffer. The retarded bands were detected by autoradiography and quantified by laser densitometry. Specificity was determined by addition of excess unlabelled double-stranded oligonucleotides.

2.6. Data analysis

Data are presented as mean ± S.E.M. PC_{200} ACh data were $\log_{10}$-transformed and reported as geometric mean. Nonparametric analysis of variance (Kruskal–Wallis methods) was used to determine significant variance among the groups. We used Mann–Whitney *U* test to analyze for significant difference between individual groups, and a *P* value < 0.05 was considered significant.

3. Results

3.1. Cell counts in bronchoalveolar lavage fluid

The recovery rates of bronchoalveolar lavage fluid were similar in all groups (approximately 90% of instilled fluid; data not shown). Compared with that for naive animals, the number of neutrophils recovered in bronchoalveolar lavage fluid was increased after 3 ppm ozone exposure for both 3 h ($16.2 \pm 0.8 \times 10^5$ cells/ml) and 6 h ($26.0 \pm 2.1 \times 10^5$ cells/ml). Vitamin A inhibited the neutrophil influx by up to 49% after 3 ppm, 3 h ozone ($P < 0.01$) and 33% after 3 ppm, 6 h ozone ($P < 0.01$), respectively (Fig. 1). The higher dose of vitamin A was more effective than the lower dose of vitamin A in inhibiting the neutrophil influx induced by 3 ppm for 3 h. There was no significant difference between neutrophil counts of animals exposed to air and those of animals pretreated with vitamin A and exposed to air. There were no significant differences in other cell counts (macrophages, lymphocytes, eosinophils and epithelial cells) between groups (data not shown). There was no significant difference in neutrophil influx into bronchoalveolar lavage fluid between air-exposed animals and ozone-exposed animals at 2 h after the cessation of ozone exposure ($1.3 \pm 0.5 \times 10^5$ cells/ml vs. $1.6 \pm 0.3 \times 10^5$ cells/ml).

3.2. Airway responsiveness to acetylcholine

After 24 h, exposure to 3 ppm ozone for 3 h caused significant bronchial hyperresponsiveness to acetylcholine in the groups of animals treated with vehicle as compared with naive animals. Vitamin A alone did not show any effect on bronchial hyperresponsiveness. Both low and high dose vitamin A did not inhibit ozone-induced bronchial hyperresponsiveness. Similar results were obtained with rats exposed to 3 ppm ozone for 6 h (Fig. 2).

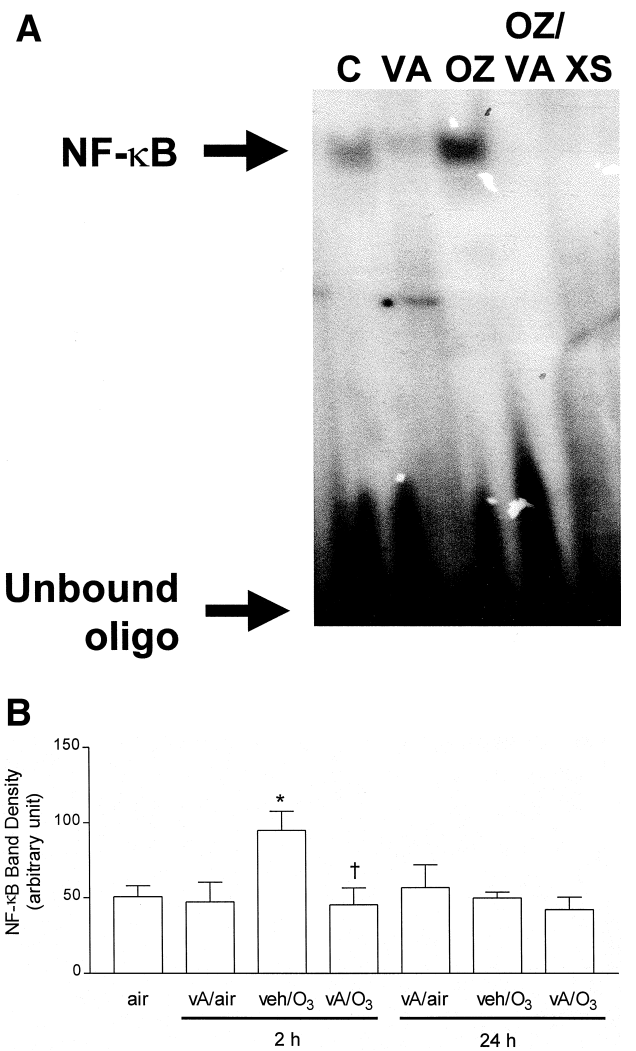

Fig. 3. Effect of ozone and vitamin A on NF-κB-binding activity. The top panel shows an electrophoretic mobility shift assay showing NF-κB-binding activity as a retarded band (arrowed) in 10 μg rat lung cell nuclear proteins from individual animals exposed to filtered air (C) or ozone (OZ; 3 ppm, 3 h) in the presence or absence of vitamin A (VA) (2500 IU/kg) pretreatment at 2 h after air or ozone exposure. Vitamin A inhibited the increase in NFκB binding observed after ozone (OZ/VA). In the presence of excess cold NF-κB oligonucleotide (XS), there was no retarded band, confirming the specificity of binding. The lower panel represents mean densitometric measurement of the retarded NF-κB band at 2 and 24 h after cessation of ozone exposure. This increase observed at 2 h was entirely abrogated by pretreatment with vitamin A. There was no significant effect observed at 24 h. $^*p < 0.05$ compared to air-exposed animals. $\dagger p < 0.05$ compared to vehicle/ozone animals. FP: free probe. $n = 6$ for each group.

3.3. Ozone-induced NF-κB-binding activity

Electrophoretic mobility shift assays on nuclear extracts showed a significant increase in NF-κB DNA-binding activity following ozone exposure (Fig. 3). This activity was maximal at 2 h and returned to basal level at 24 h. Vitamin A alone did not have any effect on the baseline NF-κB DNA-binding activity of air-exposed rats but attenuated this activity in ozone-exposed rats towards control levels at 2 h after exposure to ozone.

4. Discussion

We have shown that ozone (3 ppm for 3 h and 6 h) induces a marked increase in neutrophil counts in bronchoalveolar lavage fluid and NF-κB DNA-binding activity, and that vitamin A is effective in inhibiting ozone-induced neutrophil influx and NF-κB DNA-binding activity. Despite the reduction in ozone-induced neutrophilia, there was no effect on ozone-induced bronchial hyperresponsiveness. Deficiency of vitamin A has been shown to enhance ozone-induced lung neutrophilia (Paquette et al., 1996).

The increased NF-κB binding activity in rat lung was observed by 2 h after cessation of exposure, but not at 24 h. NF-κB is a member of a family of transcription regulatory factors showing a common structural motif for DNA-binding and dimerization. It regulates the transcription of many genes particularly those of cytokines. NF-κB-binding motifs have been described in the proximal promoter regions of cytokine genes such as CINC, MIP-2, TNF-α, interleukin-8, MGSA/GRO, MIP-1α and interleukin-6 genes, and appear to be important in regulating their transcription (Messer et al., 1990 ;Mukaida and Matsushima, 1992; Konishi et al., 1993; Widmer et al., 1993; Ray and Prefontaine, 1994). CINC is a C–X–C chemokine cloned in the rat, and has potent neutrophil chemoattractant activities (Watanabe et al., 1991; Iida et al., 1992). With the identification and cloning of the CINC gene, an NF-κB motif has been described in its 5′-upstream promoter region, and no other *cis*-acting regulatory elements were identified up to the 1 kilobases upstream region that has been cloned (Konishi et al., 1993). We have previously shown that CINC mRNA expression is closely linked to NF-κB DNA-binding activity following ozone exposure in Brown-Norway rats (Haddad et al., 1996). This induction of NF-κB activity is likely to be the direct oxidant effect of ozone as has been shown in vitro studies in various cell types particularly airway epithelial cells (Schreck et al., 1991; Adcock et al., 1994). The mechanism of this effect is likely to be through an increase breakdown of I-κB, which keeps NF-κB in an inactive state (Schreck et al., 1992).

The induction of CINC expression following exposure may be responsible for ozone-induced neutrophilia because an anti-CINC antibody inhibited ozone-induced neutrophilia (Koto et al., 1997). In addition, corticosteroids reduced the expression of CINC mRNA, together with NF-κB binding activity, following ozone exposure (Haddad et al., 1996), suggesting the close link between NF-κB binding activity and CINC expression. Furthermore, ozone-induced lung neutrophilia was also inhibited by corticosteroid (Haddad et al., 1996). This effect is likely to occur through the activation of glucocorticoid receptor, which binds to NF-κB through a protein-protein interaction, leading to a repression of expression of CINC and other cytokines. Retinoic acid receptors also belong to the nuclear receptor supergene family, and acts in a similar manner as the activated glucocorticoid receptor. They can either interact with other transcription factors or bind to their own specific DNA sequences (Pfahl, 1993). Both corticosteroids and retinoic acid inhibit AP-1 binding activity by a direct protein–protein interaction with distinct parts of the AP-1 complex (Yang-Yen et al., 1990; Schule et al., 1991). In in vitro studies of a human lung epithelial cell line (A549), we have found that vitamin A can inhibit AP-1 and NF-κB activation induced by interleukin-1β (Adcock et al., unpublished). Therefore, it is likely that the effect of vitamin A in inhibiting ozone-induced neutrophilic inflammation is through the prevention of NF-κB binding to DNA through a direct protein–protein interaction with NF-κB.

Although vitamin A blocked ozone-induced lung neutrophilia, it had no effect on ozone-induced bronchial hyperresponsiveness. This temporal dissociation between the presence of neutrophils and the induction of bronchial hyperresponsiveness has also been observed in our previous study in which an anti-CINC antibody inhibited ozone-induced lung neutrophilia but not bronchial hyperresponsiveness. Our data indicates that neutrophils are not important in the pathogenesis of bronchial hyperresponsiveness in this ozone-exposure model. By contrast, bronchial hyperresponsiveness can be inhibited by antioxidants (Tsukagoshi et al., 1995), while ozone-induced neutrophilia is not (Salmon et al., 1998). Thus, bronchial hyperresponsiveness may be induced by mechanism related to oxidant injury, such as epithelial damage. Vitamin A may not possess a direct anti-oxidant effect in our model.

Acknowledgements

We thank Prof. M. Mori for his help and suggestion with this work.

References

Adcock, I.M., Brown, C.R., Kwon, O., Barnes, P.J., 1994. Oxidative stress induces NFκB DNA binding and inducible NOS mRNA in human epithelial cells. Biochem. Biophys. Res. Commun. 199, 1518–1524.

Anisowicz, A., Messineo, M., Lee, S.W., Sager, R., 1991. A NF-κB-like

transcription factor mediates IL-1/TNF-α induction of gro in human fibroblasts. J. Immunol. 147, 520–527.

Brand, N., Petkovich, M., Krust, A., Chambon, P., de The, H., Marchio, A., Tiollais, P., Dejean, A., 1988. Identification of a second human retinoic acid receptor. Nature 332, 850–853.

Giguere, V., 1994. Retinoic acid receptors and cellular retinoid binding proteins: complex interplay in retinoid signaling. Endocr. Rev. 15, 61–79.

Giguere, V., Ong, E.S., Segui, P., Evans, R.M., 1987. Identification of a receptor for the morphogen retinoic acid. Nature 330, 624–629.

Gough, N.M., 1988. Rapid and quantitative preparation of cytoplasmic RNA from small numbers of cells. Anal. Biochem. 173, 93–97.

Haddad, E.B., Salmon, M., Sun, J., Liu, S., Das, A., Adcock, I., Barnes, P.J., Chung, K.F., 1995. Dexamethasone inhibits ozone-induced gene expression of macrophage inflammatory protein-2 in rat lung. FEBS Lett. 363, 285–288.

Haddad, E.-B., Salmon, M., Koto, H., Barnes, P.J., Adcock, I., Chung, K.F., 1996. Ozone induction of cytokine-induced neutrophil chemoattractant (CINC) and nuclear factor-kB in rat lung: inhibition by corticosteroids. FEBS Lett. 379, 265–270.

Hyde, D.M., Hubbard, W.C., Wong, V., Wu, R., Pinkerton, K., Plopper, C.G., 1992. Ozone-induced acute tracheobronchial epithelial injury: relationship to granulocyte emigration in the lung. Am. J. Respir. Cell Mol. Biol. 6, 481–497.

Iida, M., Watanabe, K., Tsurufuji, M., Takaishi, K., Iizuka, Y., Tsurufuji, S., 1992. Level of neutrophil chemotactic factor CINC/gro, a member of the interleukin-8 family, associated with lipopolysaccharide-induced inflammation in rats. Infect. Immun. 60, 1268–1272.

Jetten, A.M., Rearick, J.I., Smits, H.L., 1986. Regulation of differentiation of airway epithelial cells by retinoids. Biochem. Soc. Trans. 14, 930–933.

Konishi, K., Takata, Y., Yamamoto, M., Yomogida, K., Watanabe, K., Tsurufuji, S., Fukioka, M., 1993. Structure of the gene encoding rat neutrophil chemoattractant. Gro. Gene 126, 285–286.

Koto, H., Salmon, M., Haddad, E., Huang, J.T., Zagorski, J., Chung, K.F., 1997. Role of anti-cytokine-induced neutrophil chemoattractant (CINC) in ozone-induced airway inflammation and hyperresponsiveness. Am. J. Resp. Crit. Care Med. 156, 234–237.

Lehmann, J.M., Jong, L., Fanjul, A., Cameron, J.F., Lu, X.P., Haefner, P., Dawson, M.I., Pfahl, M., 1992. Retinoids selective for retinoid X receptor response pathways. Science 258, 1944–1946.

Mangelsdorf, D.J., Kliewer, S.A., Kakizuka, A., Umesono, K., Evans, R.M., 1993. Retinoid receptors. Recent Prog. Horm. Res. 48, 99–121.

Messer, G., Weiss, E.H., Baeuerle, P.A., 1990. Tumor necrosis factor beta (TNF-beta) induces binding of the NF-κB transcription factor to a high-affinity κB element in the TNF-beta promoter. Cytokine 2, 389–397.

Mukaida, N., Matsushima, K., 1992. Regulation of IL-8 production and the characteristics of the receptor for IL-8. Cytokine 4, 41–53.

Nikula, K.J., Wilson, D.W., Giri, S.N., Plopper, C.G., Dungworth, D.L., 1988. The response of the rat tracheal epithelium to ozone exposure. Injury, adaptation, and repair. Am. J. Pathol. 131, 373–384.

Paquette, N.C., Zhang, L.Y., Ellis, W.A., Scott, A.L., Kleeberger, S.R., 1996. Vitamin A deficiency enhances ozone-induced lung injury. Am. J. Physiol. 270, L475–L482.

Pendino, K.J., Shuler, R.L., Laskin, J.D., Laskin, D.L., 1994. Enhanced production of linterleukin-1, tumor necrosis factor-α, and fibronectin by rat lung phagocytes following inhalation of a pulmonary irritant. Am. J. Respir. Cell Mol. Biol. 11, 279–286.

Petkovich, M., Brand, N.J., Krust, A., Chambon, P., 1987. A human retinoic acid receptor which belongs to the family of nuclear receptors. Nature 330, 444–450.

Pfahl, M., 1993. Nuclear receptor/AP-1 interaction. Endocr. Rev. 14, 651–658.

Ray, A., Prefontaine, K.E., 1994. Physical association and functional antagonism between p65 subunit of transcription factor NF-κB and the glucocorticoid receptor. Proc. Natl. Acad. Sci. U.S.A. 91, 752–756.

Salmon, M., Koto, H., Lynch, O.T., Haddad, E., Lamb, N., Quinlan, G.J., Barnes, P.J., Chung, K.F., 1998. Proliferation of airway epithelium after ozone exposure: effect of apocynin and dexamethasone. Am. J. Respir. Crit. Care Med. 157, 970–977.

Schreck, R., Rieber, P., Baeuerle, P.A., 1991. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-κB transcription factor and HIV-1. EMBO J. 110, 2247–2258.

Schreck, R., Albermann, K., Baeuerle, P.A., 1992. Nuclear factor κB: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). Free Radic. Res. Commun. 17, 221–240.

Schule, R., Rangarajan, P., Yang, N., Kliewer, S., Ransone, L.J., Bolado, J., Verma, I.M., Evans, R.M., 1991. Retinoic acid is a negative regulator of AP-1-responsive genes. Proc. Natl. Acad. Sci. U.S.A. 88, 6092–6096.

Tesoriere, L., Ciaccio, M., Valenza, M., Bongiorno, A., Maresi, E., Albiero, R., Livrea, M.A., 1994. Effect of vitamin A administration on resistance of rat heart against doxorubicin-induced cardiotoxicity and lethality. J. Pharmacol. Exp. Ther. 269, 430–436.

Toledano, M.B., Leonard, W.J., 1991. Modulation of transcription factor NF-kB binding activity by oxidation-reduction in-vitro. Proc. Natl. Acad. Sci. U.S.A. 88, 4328–4332.

Tsukagoshi, H., Haddad, E.-B., Sun, J., Barnes, P.J., Chung, K.F., 1995. Ozone-induced airway hyperresponsiveness: role of superoxide anions. NEP and BK receptors. J. Appl. Physiol. 78, 1015–1022.

Von Neergaard, K., Wirz, K., 1927. Die messung der stromungswideerstande in den atemwegen des menschen, insbesondere bei asthma und emphysem. Z. Klin. Med. 105, 51–82.

Watanabe, K., Koizumi, F., Kurashige, Y., Tsurufuji, S., Nakagawa, H., 1991. Rat CINC, a member of the interleukin-8 family, is a neutrophil-specific chemoattractant in vivo. Exp. Mol. Pathol. 55, 30–37.

Widmer, U., Manogue, K.R., Cerami, A., Sherry, B., 1993. Genomic cloning and promoter analysis of macrophage inflammatory protein (MIP)-2, MIP-1α, and MIP-1β, members of the chemokine superfamily of proinflammatory cytokines. J. Immunol. 150, 4996–5012.

Yang, N., Schule, R., Mangelsdorf, D.J., Evans, R.M., 1991. Characterization of DNA binding and retinoic acid binding properties of retinoic acid receptor. Proc. Natl. Acad. Sci. U.S.A. 88, 3559–3563.

Yang-Yen, H.-F., Chambard, J.-C., Sun, Y.-L., Smeal, T., Schmidt, T.J., Drovin, J., Karin, M., 1990. Transcriptional interference between C-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein–protein interaction. Cell 62, 1205–1215.