Antioxidants as Novel Agents for Asthma

Seoung Ju Park and Yong Chul Lee*

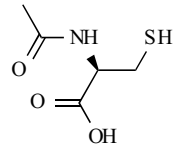
Department of Internal Medicine, Research Center for Allergic Immune Diseases, Chonbuk National University Medical School, Jeonju, South Korea

Abstract: Oxidative stress plays an important role in the pathogenesis of asthma. Recently several investigators have studied the effects of a variety of antioxidants on asthma. Antioxidants, including L-2-oxothiazolidine-4-carboxylic acid, reduce airway inflammation and hyper-responsiveness of asthma and may be novel therapeutic agents for asthma.

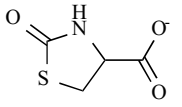
Keywords: Asthma; L-2-oxothiazolidine-4-carboxylic acid; N-acetyl-L-cysteine; NF-κB; oxidative stress; reactive oxygen species; antioxidant.

1. INTRODUCTION

Oxidative stress, defined as exposure to excessive oxidants and/or reduced antioxidant capacity, is directly or indirectly implicated in the pathogenesis of various disease states in humans. For prevention and therapeutic treatment purposes, a growing interest in the role of antioxidants has been expressed over recent decades. The cysteine prodrugs N-acetyl-L-cysteine (NAC) and L-2-oxothiazolidine-4-carboxylic acid (OTC), are two of the most widely investigated antioxidants having shown beneficial effects in disease states in which free radicals have been involved [1-4] (Fig. **1**).



N-acetyl-L-cysteine



L-2-oxothiazolidine-4-carboxylate

Fig. (1). Structure of N-acetyl-L-cysteine (NAC) and L-2-oxothiazolidine-4-carboxylate (OTC).

NAC is a drug with a wide diversity of uses. This diversity is quite unique and is due to its multifaceted chemical properties. Originally, NAC was used to reduce the viscosity and elasticity of mucus, and also as an antidote for acetaminophen poisoning. The acetyl group of NAC functions to speed absorption and distribution on orally ingested cysteine. NAC is deacetylated to cysteine intracellularly and is able to expand antioxidant defenses by increasing intracellular reduced glutathione (GSH) concentration.

*Address correspondence to this author at the Department of Internal Medicine, Chonbuk National University Medical School, 634-18, Keumamdong, Jeonju, 561-712, South Korea.; Tel: 82-63-250-1664; Fax: 82-63-254-1609; E-mail: leeyc@chonbuk.ac.kr

OTC, an analog of 5-oxo-L-proline, is readily transported into the cell where 5-oxo-L-prolinase, a ubiquitous intracellular enzyme, catalyzes ATP-dependent conversion of OTC to S-carboxyl-cysteine. S-carboxyl-cysteine, the initial product of hydrolysis, decarboxylates nonenzymatically to L-cysteine. (Fig. **2**) [5,6]. Both NAC and OTC therefore act as effective cysteine delivery systems for sustainable tissue GSH synthesis and have antioxidant activity.

Asthma is a chronic inflammatory disorder of the airways characterized by an associated increase in airway responsiveness [7]. Although genetic and environmental factors may play a role in the development of asthma, the exact pathogenesis of asthma is not clearly understood. Anti-inflammatory medications are the most effective treatment for asthma. However, subsets of patients with asthma are refractory to treatment or develop significant side effects to the medications. Because of indefinite pathogenesis and no curative treatment, there is a ceaseless research effort into effective asthma drugs. Several new therapeutic strategies for asthma are currently in development based on a better understanding of the complex pathophysiology of the disease.

Oxidative stress is known to play a prominent role in the pathogenesis of various airway disorders such as adult respiratory distress syndrome, cystic fibrosis, idiopathic fibrosis, and chronic obstructive pulmonary disease [8-10]. Oxidative stress occurs also in many allergic and immunologic disorders including bronchial asthma [11]. Therefore, this review focuses primarily on the roles of oxidative stress in asthma and on the effects of antioxidants on the airway inflammation and bronchial hyper-responsiveness.

2. REACTIVE OXYGEN SPECIES (ROS) AND ENDOGENOUS ANTIOXIDANT DEFENSES

ROS is a collective term that includes a large variety of free oxygen radicals (e.g. superoxide anion and hydroxyl radicals) but also derivatives of oxygen that do not contain unpaired electrons (e.g. hydrogen peroxide, hypochlorous acid, peroxynitrite and ozone). Normal metabolic processes in all cells are the major source of ROS and the mitochondria are the major intracellular sites of superoxide generation [12,13]. Activated inflammatory cells

1389-5575/06 $50.00+.00

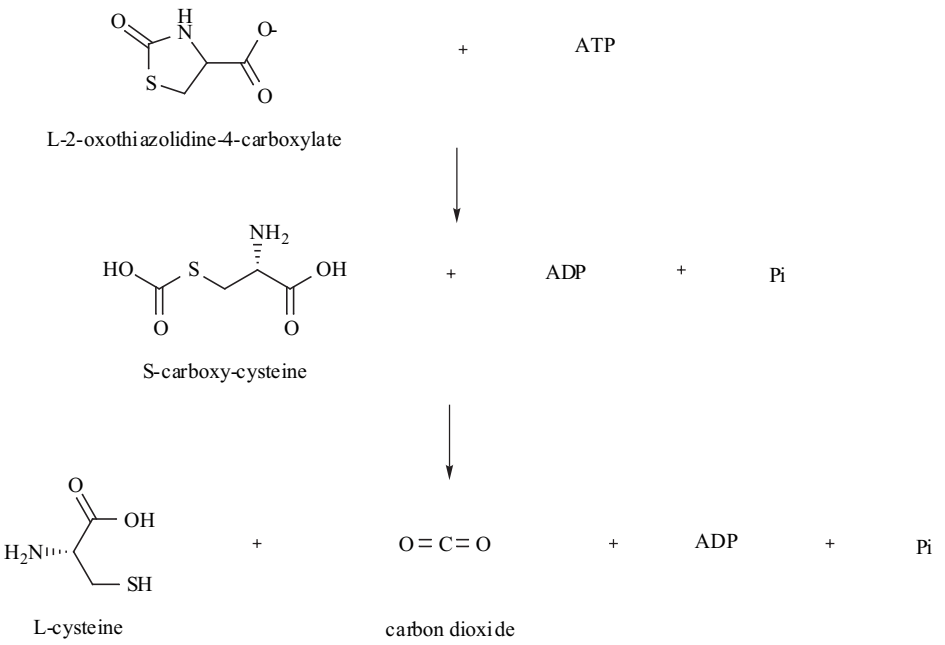


Fig. (2). Conversion of L-2-oxothiazolidine-4-carboxylate to L-cysteine.

(neutrophils, eosinophils, monocytes, and macrophages) produce large amount of ROS (Fig. **3**). These cells are stimulated on encountering inhaled particles, microorganisms, and other mediators, leading to the activation of the membrane-bound NADPH-oxidase complex and the generation of superoxide anion [14-16]. This enzyme complex has also been found to be present in other cell types such as vascular smooth muscle cells and endothelial cells [17,18]. Airway epithelial cells produce both ROS and reactive nitrogen species, and airway fibroblasts are induced to release hydrogen peroxide by cytokines [19,20]. Besides the generation of ROS *via* cellular pathways, formation of ROS in the lungs can also take place by exogenous sources such as ozone, cigarette smoke, ionizing radiation, and other chemicals and dust particles (Fig. **3**) [21].

The primary defense against ROS is endogenous antioxidants, which can be subdivided into enzymatic and

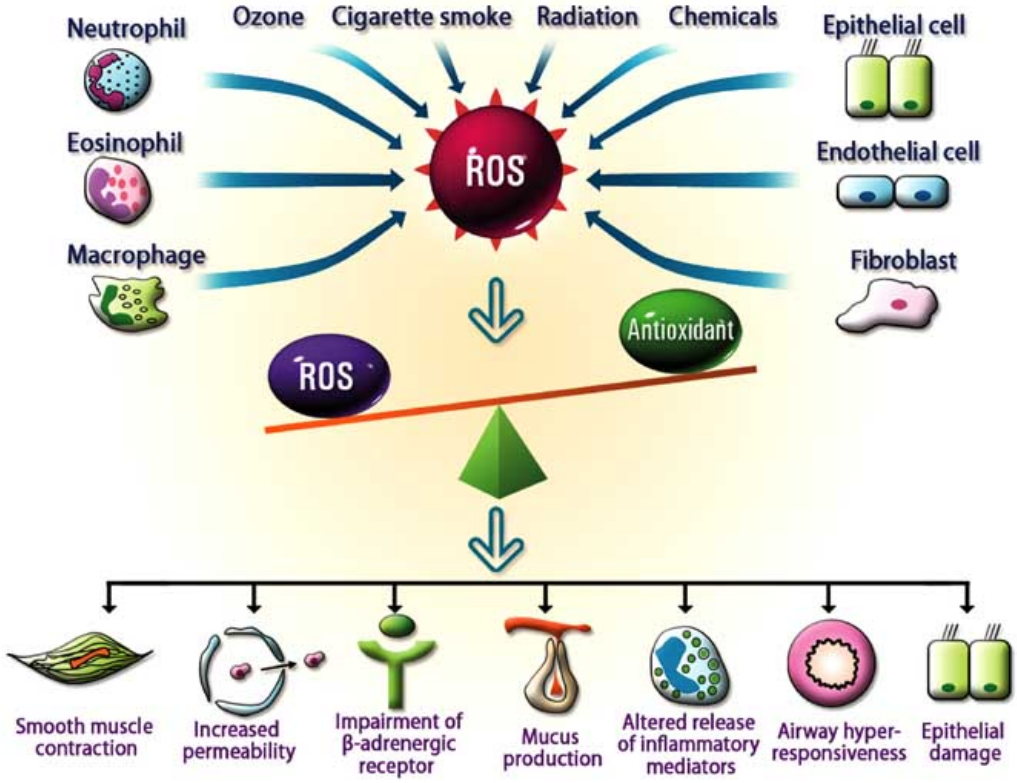


Fig. (3). General overview of sources and effects on airway of ROS.

nonenzymatic categories. The enzymatic antioxidants include the families of superoxide dismutase (SOD), catalase, GSH redox system (GSH-peroxidase and GSH-reductase), and thioredoxin [22-25]. The nonenzymatic antioxidants include low molecular weight compounds such as GSH, ascorbate, urate, α-tocopherol, and high molecular weight species such as albumin.

GSH (L-γ-glutamyl-L-cysteinylglycine), a major component of the cellular antioxidant system, plays an important role in the reduction of ROS. GSH is a substrate for enzymes that catalyze the reactions for detoxification of ROS, and can scavenge peroxynitrite and hydroxyl radicals [26]. Although a glutathione radical ($GS^{\cdot}$) is formed, it is readily neutralized by combining with another glutathione radical to produce oxidized-GSH (GSSG), which is then reduced to GSH by the NADPH-dependent GSH reductase. GSH is particularly abundant in the epithelial lining fluid of the respiratory tract, where its concentration exceeds plasma levels by approximately 100-fold and more than 95% of this GSH is in the reduced form [27,28].

3. OXIDATIVE STRESS IN ASTHMA

Normally, there are sufficient antioxidants in the airways such that the production of a small amount of ROS is inconsequential. If either production of ROS is increased or antioxidants are reduced, the balance of ROS and antioxidants is tipped toward oxidative stress. Because it has been difficult to measure the production or release of ROS in the airways, studies on the effect of oxidative stress in asthma have been started recently by measuring in vitro the production of ROS by cells isolated from blood and/or airways of asthma and by determining levels of antioxidant compounds in the airways [29].

High concentrations of ROS can evaporate from the lining fluid of the airways and can be exhaled with the expired air, and may reflect oxidative stress in the airways. Recently, several studies have shown an increase in hydrogen peroxide and nitric oxide levels in exhaled air from asthmatic patients [30-32]. Furthermore, there is a strong inverse correlation between the hydrogen peroxide content in expired air and forced expiratory volume in one second (FEV_1) and peak expiratory flow rate in asthmatic patients [30]. Airway inflammatory cells are the likely source of these increased ROS production. Most studies using cells isolated from the bronchoalveolar lavage (BAL) fluid of asthmatic patients indicate that these cells spontaneously generate more ROS than control subjects [33-35]. Consistent with the results of studies using expired air, an increase in ROS production by BAL cells is inversely correlated with FEV_1 [35]. Circulating inflammatory cells may also be a source of ROS production. Leukocytes obtained from the blood of asthmatic patients generate more ROS compared to control subjects, and neutrophils and monocytes purified from asthmatic patients are more activated and show enhanced capacity to release ROS [36-38]. Thus, both airway and intravascular inflammatory cells contribute to elevated oxidative stress in asthma. In our recent study, we have measured intracellular generation of ROS in cells from BAL fluid of ovalbumin (OVA)-induced asthmatic mouse using fluorescence microscope, and observed that it is increased after allergen challenge [39].

Evidence for increased oxidative stress in asthma is further provided by the finding of a decreased antioxidant capacity in plasma and BAL fluid of asthmatic patients [9,40]. Most airway antioxidants, such as ascorbate and α-tocopherol, are decreased, and SOD activity is diminished in cells from lavage and brushing samples of patients with asthma [41,42]. In the case of GSH, airway GSH is increased in asthmatic patients, as is the ratio of oxidized to

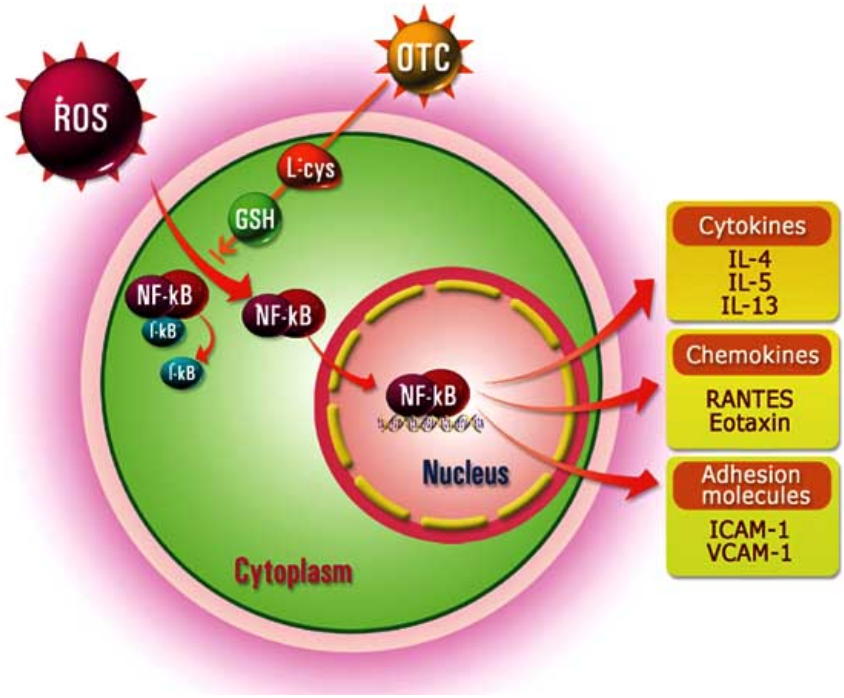


Fig. (4). Schematic diagram of OTC effect on ROS response in asthma.

reduced GSH [41]. This increase in reduced GSH suggests an adaptive response.

Increased oxidative stress can produce many of the features typical of asthma (Fig. **3**). ROS contribute to inducing direct bronchoconstriction and increasing airway hyper-responsiveness to several agonists [43-45]. They can also decrease function and number of β_2-adrenergic receptors in lung tissue and lead to increased permeability of the airways [46,47]. Oxidants induce many degrees of injury to bronchial epithelial cells and decrease numbers and function of epithelial cilia [48,49]. In vitro exposure of structural and inflammatory cells of the lungs to oxidants induces the release of pro-inflammatory mediators including cytokines, chemokines (and their receptors), growth factors, arachidonic acid metabolites, and adhesion molecules (and their ligands) involved in inflammatory cell recruitment in asthma [43-45].

Within the nucleus, oxidants induce changes in gene expression. Oxidative stress can induce activation of nuclear factor κB (NF-κB) and activator protein 1, two pivotal regulators of inflammatory processes [50]. Such activation induces a variety of inflammatory genes that are abnormally expressed in asthma. These genes include cytokines (e.g., IL-4, IL-5, IL-8, IL-9, IL-15, and TNF-α), chemokines (e.g., RANTES, eotaxin, and monocyte chemotactic protein-3), and adhesion molecules (e.g., ICAM-1 and VCAM-1) (Fig. **4**) [51-53]. In our recent study, we have assessed whether these genes are up-regulated by oxidative stress in the OVA-induced asthma model. As expected, expressions of cytokines (IL-4, IL-5, and IL-13), chemokines (RANTES and eotaxin), and adhesion molecules (ICAM-1 and VCAM-1) were increased significantly after allergen challenge in a murine model of asthma [39].

4. ANTIOXIDANT TREATMENT IN ASTHMA

There are two strategies for treating oxidative stress in asthma; reducing exposure to ROS and augmenting antioxidant defenses. Several studies suggest that reducing exposure to environmental oxidants, such as nitrites and ozone, may decrease asthmatic exacerbations through the attenuation of the activity of pulmonary inflammatory cells [54,55]. Because antioxidant therapy which augments antioxidant defense is more useful for therapeutic purpose than the regime of reducing exposure to environmental oxidants, the majority of research performed has been focused on enhancing antioxidants levels. Augmentation of existing antioxidant defenses with catalytic antioxidants might be useful in attenuating asthma. For instance, reduction of airway hyper-responsiveness in guinea pigs by intraperitoneal SOD has been reported [56]. Several studies have demonstrated that the intake of dietary antioxidants influences airway responsiveness to inhaled allergens and irritants [57-59]. For instance, it has been reported that both FEV_1 and FVC are significantly and independently related to mean daily intake of vitamin C, and fresh fruit consumption appears to have a beneficial effect on lung function in children [60,61].

5. GSH AND CYSTEINE

GSH is synthesized from three amino acids in a two-step process, beginning with the combination of cysteine and glutamic acid and ending with the addition of glycine (Fig. **5**). GSH is a vital intra- and extracellular protective antioxidant against oxidative stress and depletion of GSH results in increased susceptibility of the cell to oxidative stress as described previously. Alterations in alveolar and

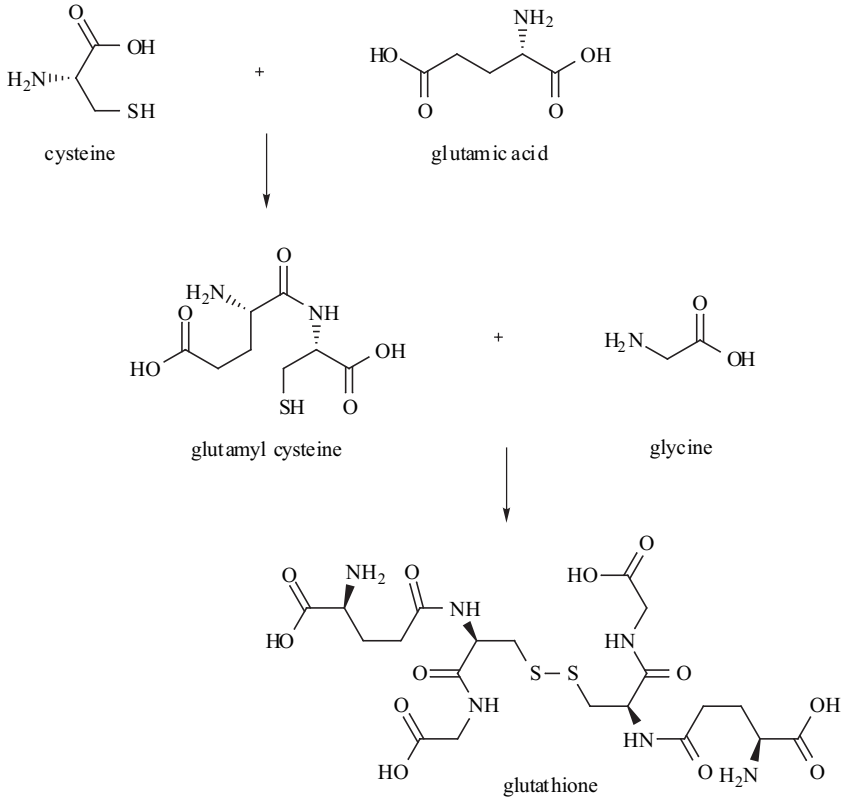


Fig. (5). Glutathione synthesis.

lung GSH metabolism are widely recognized as a central feature of many inflammatory lung diseases such as asthma.

Cysteine is the rate-limiting amino acid for intracellular GSH synthesis [62] and plays an important role as a reducing agent. Besides being a precursor for the synthesis of GSH, it has direct antioxidant property. The thiol (-SH) group of cysteine is capable of interacting with the electrophilic groups of ROS. However, at low concentrations, the primary mechanism of protection by thiol antioxidants is mediated by their pro-GSH property, rather than direct scavenging of ROS [63].

Because of its redox instability, almost all extracellular cysteine is present in the oxidized cystine state [64]. Administration of cysteine is severely restricted because it is rapidly oxidized to cystine and toxic due to the deleterious effect of hydrogen peroxide formed during this oxidation [65]. Therefore, some antioxidants, including NAC and OTC which have the ability to deliver cysteine as a GSH precursor, have been studied.

6. NAC AND OTC IN TREATMENT OF ASTHMA

NAC has antioxidant defense against oxidative stress by increasing intracellular reduced GSH concentration and scavenging of ROS as the cysteine prodrug. NAC can prevent radiation-induced DNA breaks and act as a radio-protectant against many aspects of oxidative damage [66]. Oxidative stress is one of the several stimuli that induce apoptosis in cells, and NAC has an additional positive effect in prevention of apoptic cell death [67]. In addition, NAC treatment has been reported to be beneficial in a number of oxidative stress models, such as systemic sclerosis, HIV infection, hypertension, cardiopulmonary bypass, and ischemia-reperfusion injury [68]. The efficacy of NAC has been investigated in a number of both experimental and clinical studies on pulmonary diseases in which oxidative stress has been involved [1-4]. In asthma, NAC treatment produces beneficial effects in an *in vivo* model of experimental asthma [69]. NAC does not reduce the immediate bronchospasm after antigen exposure but prevents airway hyperreactivity and reduces the eosinophils and Evans blue dye extravasation. NAC inhibits pro-inflammatory NF-κB and tumor necrosis factor-α, and suppresses the inducible form of nitric oxide synthase, inflamatory cytokines, and adhesion molecules, probably by inhibition of NF-κB [70]. In the experimental animal model of asthma, this action mechanism of NAC is well verified [71]. However, although NAC is an effective antioxidant, the bioavailability in humans of NAC given orally is less than 20 % probably due to fast metabolism in the gut wall and the liver [72].

OTC is apparently nontoxic and it has been demonstrated that it is more effective than NAC in replenishing intracellular GSH stores [73,74]. Furthermore, OTC has proven to be effective in elevating lung GSH levels and reducing oxidative lung damage [75]. Recently, our study has revealed that the administration of OTC reduces bronchial inflammation and airway hyper-responsiveness in a mouse model for asthma [39]. The increased ROS generation after allergen challenge is also significantly reduced by the administration of OTC. We have also shown that the amount of increase of NF-κB levels in nuclear protein extracts of lung tissues after allergen inhalation is decreased, and the amount of decrease of NF-κB levels in cytosolic protein extracts of lung tissues is increased by the administration of OTC. Administration of OTC results in significant reduction of NF-κB translocation into nucleus and of expression of adhesion molecules (ICAM-1 and VCAM-1), chemokines (RANTES and eotaxin), and cytokines (IL-4, IL-5, and IL-13). These results indicate that OTC may reduce airway inflammation and hyper-responsiveness through regulation of NF-κB activity (Fig. **4**).

7. CONCLUSION

A significant amount of data showing an increase of oxidative stress in asthma and indicating a potential role of oxidative stress in pathogenesis of the disease has been accumulated over years. Recently, many investigators have studied the effects of a variety of antioxidants to reduce the increased oxidative stress in asthma and so treat the disease. GSH is one of the most important reducing agents against oxidative stress and cysteine is the rate-limiting amino acid for intracellular GSH synthesis. NAC and OTC act as effective cysteine deliverers and are effective in elevating tissue GSH and reducing oxidative lung damage. OTC is more effective than NAC in replenishing intracellular GSH stores and may reduce airway inflammation and hyper-responsiveness of asthma through regulation of NF-κB activity. Therefore, OTC may be a novel recommendable therapeutic agent for bronchial asthma.

ACKNOWLEDGEMENTS

We thank Professor Mie-Jae Im for critical reading of the manuscript. This work was supported by grants from the National Research Laboratory Program and from the Medical Science and Engineering Research Center (R13-2002-038-01004-0) of the Korea Science and Engineering Foundation (to Y. C. L.) and also by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (0412-CR03-0704-0001) (to S. J. P.).

ABBREVIATIONS

BAL	=	Bronchoalveolar lavage
FEV_1	=	Forced expiratory volume in one second
GSH	=	Glutathione
NAC	=	N-acetyl-L-cysteine
NF-κB	=	Nuclear factor κB
OTC	=	L-2-oxothiazolidine-4-carboxylic acid
OVA	=	Ovalbumin
ROS	=	Reactive oxygen species
SOD	=	Superoxide dismutase

REFERENCES

[1] Cotgreave, I.A. *Adv. Pharmacol.*, **1997**, *38*, 205.
[2] Tepel, M.; Van Der Giet, M.; Schwarzfeld, C.; Laufer, U.; Liermann, D.; Zidek, W. *New. Engl. J. Med.*, **2000**, *343*, 180.

[3] Behr, J.; Maier, K.; Degenkolb, B.; Krombach, F.; Vogelmeier, C. *Am. J. Respir. Crit. Care Med.*, **1997**, *156*, 1897.

[4] Repine, J.E.; Bast, A.; Lankhorst, I. *Am. J. Respir. Crit. Care Med.,* **1997**, *156*, 341.

[5] Williamson, J.M.; Meister, A. *Proc. Natl. Acad. Sci. USA*, **1981**, *78*, 936.

[6] Meister, A. *Nutr. Rev.*, **1984**, *42*, 397.

[7] Bousquet, J.; Jeffery, P.K.; Busse, W.W.; Johnson, M.; Vignola, A.M. *Am. J. Respir. Crit. Care Med.,* **2000**, *161*, 1720.

[8] Comhair S.A.; Erzurum S.C. *Am. J. Physiol. Lung Cell. Mol. Physiol.,* **2002**, *283*, L246.

[9] Rahman, I.; Morrison, D.; Donaldson, K.; MacNee, W. *Am. J. Respir. Crit. Care Med*., **1996**, *154*, 1055.

[10] MacNee, W. *Eur. J. Pharmacol.,* **2001***, 429*, 195.

[11] Dworski, R. *Thorax,* **2000**, *55*, S51.

[12] Halliwell B. *Lancet,* **1994**, *344*, 721.

[13] Andreadis, A.A.; Hazen, S.L.; Comhair, S.A.; Erzurum, S.C. *Free Radic. Biol. Med.*, **2003**, *35*, 213.

[14] Conner, E.M.; Grisham, M.B. *Nutrition*, **1996**, *12*, 274.

[15] Babior, B.M. *Blood,* **1999**, *93*, 1464.

[16] Leusen, J.H.; Verhoeven, A.J.; Roos, D. *J. Lab. Clin. Med.,* **1996***, 128*, 461.

[17] Jones, S.A.; O'Donnell, V.B.; Wood, J.D.; Broughton, J.P.; Hughes, E.J.; Jones, O.T.G. *Am. J. Physiol.,* **1996***, 271*, H1626.

[18] Marshall, C.; Mamary, A.J.; Verhoeven, A.J.; Marshall, B.E. *Am. J. Respir. Cell Mol. Biol.*, **1996**, *15*, 633.

[19] Rochelle, L.G.; Fischer, B.M.; Adler, K.B. *Free Radic. Biol. Med.*, **1998**, *24*, 863.

[20] Thannickal, V.J.; Fanburg, B.L. *J. Biol. Chem.*, **1995**, *270*, 30334.

[21] Brunori, M.; Rotilio, G. *Methods Enzymol.*, **1984**, *105*, 22.

[22] Asikainen, T.M.; Raivio, K.O.; Saksela, M.; Kinnula, V.L. *Am. J. Respir. Cell Mol. Biol*., **1998**, *19*, 942.

[23] Cantin, A.M.; Fells, G.A.; Hubbard, R.C.; Crystal, R.G. *J. Clin. Invest.*, **1990**, *86,* 962.

[24] Exner, R.; Wessner, B.; Manhart, N.; Roth, E. *Wien. Klin. Wochenschr*., **2000**, *112*, 610.

[25] Pietarinen-Runtti, P.; Lakari, E.; Raivio, K.O.; Kinnula, V.L. *Am. J. Physiol.*, **2000**, *278*, C118.

[26] Fang, Y.Z.; Yang, S.; Wu, G. *Nutrition*, **2002**, *18*, 872.

[27] Cantin, A.M.; North, S.L; Hubbard, R.C.; Crystal, R.G. *J. Appl. Physiol.*, **1987**, *63*, 152.

[28] van der Vliet, A.; O'Neill, C.A.; Cross, C.E.; Koostra, J.M.; Volz, W.G.; Halliwell, B.; Louie, S. *Am. J. Physiol.*, **1999**, *276*, L289.

[29] Henricks, P.A.; Nijkamp, F.P. *Pulm. Pharmacol. Ther.*, **2001**, *14*, 409.

[30] Antczak, A.; Nowak, D.; Shariati, B.; Król, M.; Piasecka, G.; Kurmanowska, Z. *Eur. Respir. J.*, **1997**, *10*, 1235.

[31] Dohlman, A.W.; Black, H.R.; Royall, J.A. *Am. Rev. Respir. Dis.*, **1993**, 148, 955.

[32] Jöbsis, Q.; Raatgeep, H.C.; Hermans, P.W.M.; De Jongste, J.C. *Eur. Respir. J.,* **1997**, *10*, 519.

[33] Calhoun, W.J.; Bush, R.K. *J. Allergy Clin. Immunol.*, **1990**, *86*, 306.

[34] Calhoun, W.J.; Reed, H.E.; Moest, D.R.; Stevens, C.A. *Am. Rev. Respir. Dis.*, **1992**, *145*, 317.

[35] Jarjour, N.N.; Calhoun, W.J. *J. Lab. Clin. Med.*, **1994**, *123*, 131.

[36] Chanez, P.; Dent, G.; Yukawa, T.; Barnes, P.J.; Chung, K.F. *Eur. Respir. J.*, **1990**, *3*, 1002.

[37] Vachier, I.; Chanez, P.; Le Doucen, C.; Damon, M.; Descomps, B.; Godard, P. *Eur. Respir. J.*, **1994**, *7,* 1585.

[38] Vachier, I.; Damon, M.; Doucen, C.L.; de Paulet, A.C.; Chanez, P.; Michel, F.B.; Godard, P. *Am. Rev. Respir. Dis.*, **1992**, *146*, 1161.

[39] Lee, Y.C.; Lee, K.S.; Park, S.J.; Park, H.S.; Lim, J.S.; Park, K.H.; Im, M.J.; Choi, I.W.; Lee, H.K.; Kim, U.H. *FASEB J*., **2004**, *18*, 1917.

[40] Comhair, S.A.A.; Bhathena, P.R.; Dweik, R.A.; Kavuru, M.; Erzurum, S.C. *Lancet*, **2000**, *355*, 624.

[41] Kelly, F.J.; Mudway, I.; Blomberg, A.; Frew, A.; Sandström, T. *Lancet*, **1999**, *354*, 482.

[42] Smith, L.J.; Shamsuddin, M.; Sporn, P.H.; Denenberg, M.; Anderson, J. *Free Radic. Biol. Med.*, **1997**, *22*, 1301.

[43] Barnes, P.J. *Free Radic. Biol. Med.*, **1990**, *9*, 235.

[44] Barnes, P.J.; Chung, K.F.; Page, C.P. *Pharmacol. Rev.,* **1998**, *50*, 515.

[45] Bowler, R.P.; Crapo, J.D. *J. Allergy Clin. Immunol.*, **2002**, *110*, 349.

[46] Nijkamp, F.P.; Henricks, P.A. *Am. Rev. Respir. Dis.*, **1990**, *141*, S145.

[47] Lei, Y.H.; Barnes, P.J.; Rogers, D.F. *Br. J. Pharmacol.*, **1996**, *117*, 449.

[48] Yukawa, T.; Read, R.C.; Kroegel, C.; Rutman, A.; Chung, K.F.; Wilson, R.; Cole, P.J.; Barnes, P.J. *Am. J. Respir. Cell Mol. Biol.*, **1990**, *2*, 341.

[49] Rusznak, C.; Devalia, J.L.; Sapsford, R.J.; Davies, R.J. *Eur. Respir. J.*, **1996**, *9*, 2298.

[50] Henderson, W.R.Jr.; Chi, E.Y.; Teo, J.L.; Nguyen, C.; Kahn, M. *J. Immunol.*, **2002**, *169*, 5294.

[51] Barnes, P.J.; Adcock, I.M. *Trends Pharmacol. Sci.*, **1997**, *18*, 46.

[52] Stutz, A.M.; Woisetschlager, M. *J. Immunol.*, **1999**, *163*, 4383.

[53] Mori, A.; Kaminuma, O.; Mikami, T.; Inoue, S.; Okumura, Y.; Akiyama, K.; Okudaira, H. *J. Allergy Clin. Immunol.*, **1999**, *103*, S429.

[54] Jorres, R.; Nowak, D.; Magnussen, H. *Am. J. Respir. Crit. Care Med*., **1996**, *153*, 56.

[55] McConnell, R.; Berhane, K.; Gilliland, F.; London, S.J.; Islam, T.; Gauderman, W.J.; Avol, E.; Margolis, H.G.; Peters, J.M. *Lancet*, **2002**, *359*, 386.

[56] Ikuta, N.; Sugyama, S.; Takagi, K.; Satake, T.; Ozawa, T. *Am. Rev. Respir. Dis*., **1992**, *145*, 561.

[57] Bielory, L.; Gandhi, R. *Ann. Allergy*, **1994***, 73*, 89.

[58] Greene L.S. *J. Am. Coll. Nutr.*, **1995**, *14*, 317.

[59] Troisi, R.J.; Willett, W.C.; Weiss, S.T.; Trichopoulos, D.; Rosner, B.; Speizer, F.E. *Am. J. Respir. Crit. Care Med.*, **1995**, *151*, 1401.

[60] Britton, J.R.; Pavord, I.D.; Richards, K.A.; Knox, A.J.; Wisniewski, A.F.; Lewis, S.A.; Tattersfield, A.E.; Weiss, S.T. *Am. J. Respir. Crit. Care Med.,* **1995**, *151*, 1383.

[61] Cook, D.G.; Carey, I.M.; Whincup, P.H.; Papacosta, O.; Chirico, S.; Bruckdorfer, K.R.; Walker, M. *Thorax*, **1997**, *52*, 628.

[62] Lu, S.C. *FASEB J*., **1999**, *13*, 1169.

[63] Kim, Y.G.; Kim, S.K.; Kwon, J.W.; Park, O.J.; Kim, S.G.; Kim, Y.C.; Lee, M.G. *Life Sci*., **2003**, *72*, 1171.

[64] Han, D.; Sen, C.K.; Roy, S.; Kobayashi, M.S.; Tritschler, H.J.; Packer, L. *Am. J. Physiol.*, **1997**, *273*, R1771.

[65] Anderson, M.E.; Meister, A. *Methods Enzymol.,* **1987**, *143*, 313.

[66] Neal, R.; Matthews, R.H.; Lutz, P.; Ercal. N. *Free Radic. Biol. Med.*, **2003**, *34*, 689.

[67] Dringen, R.; Hamprecht, B. *Neurosci. Lett.*, **1999**, *259*, 79.

[68] Atmaca, G. *Yonsei Med. J.*, **2004**, *45*, 776.

[69] Blesa, S.; Cortijo, J.; Martinez-Losa, M.; Mata, M.; Seda, E.; Santangelo, F.; Morcillo, E.J. *Pharmacol. Res.,* **2002**, *45*, 135.

[70] Dekhuijzen, P.N. *Eur. Respir. J.*, **2004**, *23*, 629.

[71] Blesa, S.; Cortijo, J.; Mata, M.; Serrano, A.; Closa, D,; Santangelo, F.; Estrela, J.M.; Suchankova, J.; Morcillo, E.J. *Eur. Respir. J.*, **2003**, *21*, 394.

[72] Borgstrom, L.; Kagedal, B.; Paulsen, O. *Eur. J. Clin. Pharmacol.,* **1986**, *31*, 217.

[73] Williamson, J.M.; Boettcher, B.; Meister, A. *Proc. Natl. Acad. Sci. USA*, **1982**, *79*, 6246.

[74] Mesina, J.E.; Page, R.H.; Hetzel, F.W.; Chopp, M. *Brain Res.*, **1989**, *478*, 181.

[75] Taylor, C.G.; Bauman, P.F.; Sikorski, B.; Bray, T.M. *FASEB J.*, **1992**, *6*, 3101.

Received: July 05, 2005 Revised: August 03, 2005 Accepted: August 04, 2005