

ORIGINAL ARTICLE

Rapid non-genomic effects of glucocorticoids on oxidative stress in a guinea pig model of asthma

FEI LONG, YAN WANG, HUI-HUI QI, XIN ZHOU AND XIAN-QIAO JIN

Department of Respiratory Medicine, First People's Hospital Affiliated to Shanghai Jiao Tong University, Shanghai, China

Rapid non-genomic effects of glucocorticoids on oxidative stress in a guinea pig model of asthmaLONG F, WANG Y, QI H, ZHOU X, JIN X. *Respirology* 2008; **13**: 227–232

Background and objective: Glucocorticoids (GC) may exert therapeutic effects in asthma by a rapid non-genomic mechanism. The lungs of asthmatic patients are exposed to oxidative stress, which is believed to be critical in the pathogenesis of asthma. The aim of this study was to investigate whether GC exert a rapid non-genomic effect on oxidative stress in asthmatic guinea pigs.

Methods: The guinea pig asthma model was used to assess inhibitory effects of budesonide (BUD) on oxidative stress. BAL fluid (BALF), trolox equivalent antioxidant capacity and lung manganese superoxide dismutase (MnSOD) activity were measured by spectrophotometry. Superoxide anion production was measured by cytochrome *c* reduction assay.

Results: Oxidative stress occurred within minutes following antigen challenge and BUD reduced the severity of oxidative stress in asthmatic guinea pigs within 15 min. BUD significantly decreased BALF trolox equivalent antioxidant capacity and lung MnSOD activity, as compared with those of vehicle-treated asthmatic guinea pigs ($P < 0.05$). Additionally, BUD rapidly inhibited *in vitro* superoxide anion production by BALF cells and bronchi harvested from sensitized animals. These rapid effects were not blocked by the GC receptor antagonist RU486 and/or the protein synthesis inhibitor cycloheximide.

Conclusions: BUD reduced oxidative stress in a guinea pig model of asthma by a rapid non-genomic mechanism. These data suggest new mechanisms whereby GC treatments may benefit asthma.

Key words: asthma, budesonide, glucocorticoid, oxidative stress, rapid non-genomic effect.

INTRODUCTION

Currently glucocorticoids (GC) are the most effective treatment for asthma, and inhaled GC have become the first-line treatment for children and adults with persistent asthmatic symptoms. It is generally accepted that the striking effectiveness of GC in asthma is due largely to their genomic anti-inflammatory effects.¹ These genomic effects are characterized by a lag period ranging from 30 min to several hours or even days, suggesting a role in

the longer-term anti-inflammatory effects of GC. However, recent evidence indicates that GC may exert therapeutic effects in asthma through a rapid non-genomic mechanism, immediately after GC exposure or with a very short latency period.^{2,3}

Asthma attacks and experimental antigen challenge are both associated with oxidative stress,^{4,5} which has important consequences for the pathogenesis, severity and treatment of asthma.^{6,7} Reactive oxygen species (ROS) cause lung injury as a result of direct oxidative damage to and shedding of epithelial cells.⁸ Moreover, ROS may contribute to the pathogenesis of asthma by evoking bronchial hyperreactivity,⁹ as well as directly stimulating histamine release from mast cells¹⁰ and mucus secretion from airway epithelial cells.¹¹

Previously, we reported that GC rapidly inhibit superoxide anion (O_2^-) production by macrophages through a rapid non-genomic mechanism.¹² Inflammatory cells are the main source of ROS in asthma and in the present study, we sought to determine

Correspondence: Xian-qiao Jin, Department of Respiratory Medicine, First People's Hospital Affiliated to Shanghai Jiao Tong University, Wujin Road 85, Shanghai 200080, China. Email: jinxianqiao@126.com

Received 18 April 2007; invited to revise 22 May 2007; revised 15 June 2007; accepted 24 June 2007 (Associate Editor: Takahide Nagase).

whether GC exert their rapid therapeutic effects in asthma, in part by decreasing oxidative stress. We investigated the direct effect of inhaled budesonide (BUD), an effective and widely used antiasthma agent, on oxidative stress in a guinea pig model of asthma. Our data show that BUD decreased oxidative stress in this model, possibly through prompt non-genomic mechanisms of action.

METHODS

Animals

All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Hartley guinea pigs (250–300 g, Experimental Animal Center, Shanghai Laboratory Animals Centers of Chinese Academy of Science, Shanghai, China) were used. Animals were housed under controlled temperature conditions (22–26°C), humidity (55 ± 5%) and artificial lighting on a 12 h light–dark cycle (lights on at 9:00 AM), with free access to chow, fresh vegetables and water.

Chemicals and media

Reagents were obtained from Sigma Chemical Co. (St Louis, MO, USA) unless otherwise noted. RU486 was suspended in 1% carboxymethylcellulose and 0.05% Tween-80, and administered orally 20 h and 2 h prior to ovalbumin (OVA) challenge.¹³ BUD for nebulization was suspended in hydroxypropyl methylcellulose (HPMC, viscosity 4000 cps) at 3 mg/mL as recommended by AstraZeneca (Lund, Sweden). All other reagents were analytical grade and obtained from commercial sources.

Sensitization and treatments of animals

Guinea pigs ($n = 60$) were sensitized with 100 mg/kg intraperitoneal (i.p.) plus 100 mg/kg subcutaneous (s.c.) OVA, suspended in saline (20 mg/mL).¹⁴ Two weeks later, they were challenged with an aerosol of OVA in saline (5 mg/mL) and the onset of allergen-elicited cough was observed to verify sensitization. Control animals were treated with isotonic saline instead of OVA.

BUD (3 mg/mL in HPMC vehicle) was administered by inhalation for 5 min, before guinea pigs were challenged with an aerosol of OVA (10 mg/mL in saline) for 25 s (BUD group). Eight OVA-sensitized animals were challenged with normal saline instead of OVA (NC group). Eight OVA-sensitized animals inhaled HPMC before OVA challenge (HPMC group). Eight OVA-sensitized guinea pigs were pretreated with the GC receptor antagonist RU486 (50 mg/kg orally 20 h and 2 h prior to OVA challenge) before BUD inhalation (RU486+BUD group). Another eight OVA-sensitized animals were given RU486 before inhalation of HPMC vehicle (RU486 group).

At the end of the experiments (i.e. 10 min after OVA administration) the animals were anaesthetized with pentobarbital sodium (50 mg/kg i.p.) and underwent thoracotomy. The upper trachea was cannulated and the whole lung was excised and placed in cold phenol red-free Hank's balanced salt solution (HBSS) without Ca^{2+} or Mg^{2+} . The left lung was ligated at the main bronchus, excised and placed in liquid nitrogen for subsequent biochemical analyses. The right lung was used to prepare BAL fluids (BALF) by washing three times with 4 mL of cold (4°C) phenol red-free HBSS without Ca^{2+} , Mg^{2+} , containing 0.5 mM ethylenediaminetetraacetic acid, through the intratracheal cannula. The recovered fluid (75–80% of the injected volume) was centrifuged (100 g, 4°C, 8 min) and the resulting supernatants were frozen at –80°C until use. Cells were washed three times and suspended in phenol red-free HBSS for differential cell count.

BALF trolox equivalent antioxidant capacity assay

The trolox equivalent antioxidant capacity (TEAC) assay was performed as described by Albrecht *et al.*¹⁵

Determination of lung manganese superoxide dismutase activity

Frozen lung samples were homogenized with 10 mM PBS, pH 7.4, sonicated on ice for 1 min and centrifuged at 100 g for 10 min. Supernatants were pre-incubated for 30 min with 2 mmol/L NaCN to inhibit copper-zinc superoxide dismutase activity. The assay is based on superoxide dismutase (SOD)-induced inhibition of the conversion of nitroblue tetrazolium (NBT) to a blue tetrazolium salt, mediated by superoxide radicals generated by xanthine oxidase. The reaction was performed in sodium carbonate buffer, 50 mmol/L, pH 10.1, containing 0.1 mmol/L EDTA, 25 $\mu\text{mol/L}$ NBT, 0.1 mmol/L xanthine and 2 nmol/L xanthine oxidase (Merck, Darmstadt, Germany). The rate of reduction of NBT was monitored at 560 nm. A unit (U) of manganese superoxide dismutase (MnSOD) activity was defined as that required to inhibit the rate of reduction of NBT by 50%. Values were expressed as mU/mg of protein. Protein levels were determined using the bicinchoninic acid (BCA) reagent.

BALF differential cell count

The total cell counts for pooled BALF from each animal in all groups were determined using a Neubauer haemocytometer. Cells were stained with Wright's stain and a minimum of 500 cells (macrophages, eosinophils, neutrophils and lymphocytes) were counted.

Measurement of superoxide anion production by BALF cells

BALF cells harvested from sensitized guinea pigs were washed three times and suspended in phenol red-free

HBSS at a concentration of 1×10^6 cells/mL. The cell suspension was incubated in a 96-well flat-bottomed microtitre plate (200 μ L/well) for 60 min at 37°C in 5% CO₂. Cell viability was determined using Trypan blue dye and was >94%. Cells were then stimulated with phorbol myristate acetate (PMA, 100 ng/mL). O₂⁻ was measured using the cytochrome *c* reduction assay as previously described.¹² BALF cells in HBSS were incubated with BUD for 5 min before addition of PMA and cytochrome *c*.

Experiments were also performed to determine whether pretreatment with RU486 (30 min before BUD, at a final concentration >10 times greater than that of BUD) or cycloheximide (CHX, 10⁻⁴ mol/L, 30 min before BUD) influenced the inhibitory effect of BUD on O₂⁻ production by BALF cells.

Measurement of superoxide anion production by main bronchus tissue rings

Sensitized guinea pigs were anaesthetized with pentobarbital sodium. The lungs were removed rapidly and immersed in Krebs–Henseleit buffer containing (mmol/L): NaCl 118.3, KCl 4.7, MgSO₄ 1.2, CaCl₂ 2.5, KH₂PO₄ 1.2, NaHCO₃ 25.0 and D-(1)-glucose 11.1. The main bronchus was dissected free of loose connective tissue and cut into 2- to 3-mm-thick rings. The rings were mounted in a 96-well plate with Krebs–Henseleit buffer and maintained at 37°C in 5% CO₂. After 30 min of equilibration, O₂⁻ was measured by SOD-inhibited cytochrome *c* reduction as described by Casoni *et al.*¹⁶ Bronchial rings were pretreated with BUD for 5 min before exposure to 10⁻⁴ mol/L acetylcholine (Ach). O₂⁻ production was expressed as nmol/mg wet weight. To determine wet weight, bronchial rings were blotted on filter paper and weighed at the end of the experiments.

Experiments were also performed to determine whether pretreatment with RU486 or CHX (under the conditions described previously) could influence the inhibitory effect of BUD on O₂⁻ production by bronchial rings.

Statistical analysis

Values are presented as mean $\pm$ SEM. Data were analysed using SPSS version 13 for Windows (SPSS Inc., Chicago, IL, USA). One-way ANOVA followed by Dunnett's *t*-test was used to analyse differences between the treated and control groups. A *P*-value less than 0.05 was considered statistically significant.

RESULTS

Rapid effect of BUD on BALF TEAC, lung MnSOD activity and BALF differential cell count

The mean values for BALF TEAC and lung MnSOD activity are presented in Figures 1 and 2, respectively. BALF TEAC and lung MnSOD activity decreased

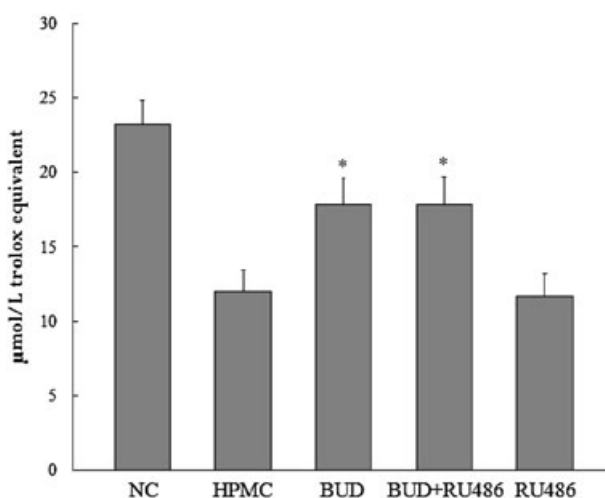


Figure 1 Rapid effect of budesonide (BUD) on BAL fluid trolox equivalent antioxidant capacity of guinea pigs in the different experimental groups. Normal saline control (NC) animals were sensitized with ovalbumin (OVA) and challenged with normal saline; animals in other groups were pretreated with BUD vehicle (hydroxypropyl methylcellulose (HPMC)), BUD, BUD+RU486 or RU486 separately, followed by challenge with OVA. **P* < 0.01 compared with HPMC group (ANOVA followed by Dunnett's *t*-test).

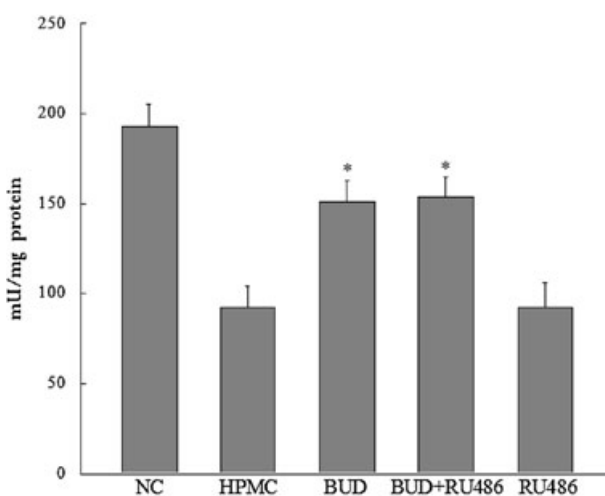


Figure 2 Rapid effect of budesonide (BUD) on manganese superoxide dismutase activity in lung tissues of guinea pigs in the different experimental groups. Treatments and methods were as described for Figure 1. **P* < 0.01 compared with hydroxypropyl methylcellulose (HPMC) group (ANOVA followed by Dunnett's *t*-test). NC, negative control cells.

significantly in the lung tissue of the HPMC group as compared with NC group. BALF TEAC and lung MnSOD activity were significantly higher in the BUD group than in the HPMC group (Figs 1,2).

To assess the possible involvement of the classical intracellular GC receptors in mediating the inhibitory

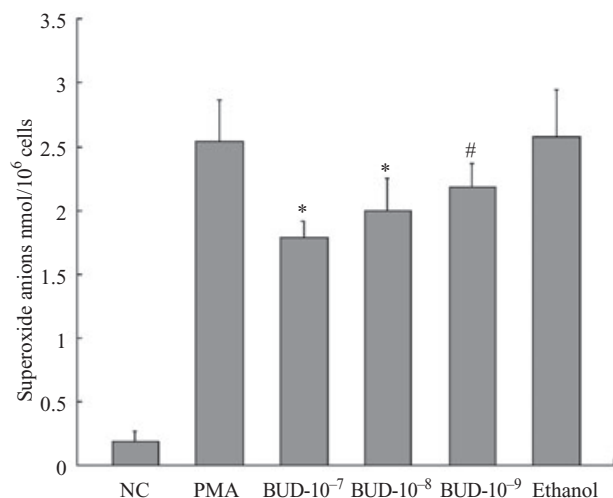


Figure 3 Rapid inhibitory effect of budesonide (BUD) on phorbol myristate acetate (PMA)-stimulated O_2^- production by BAL fluid (BALF) cells. Negative control (NC) cells were incubated in Hank's balanced salt solution alone. BUD was added to BALF cells 5 min before stimulation with PMA. Ethanol (0.05%) had no effect on O_2^- production by BALF cells. * $P < 0.01$ compared with PMA group; # $P < 0.05$ compared with PMA group (ANOVA followed by Dunnett's t -test).

effects of BUD, additional experiments were performed using the GC receptor antagonist RU486. RU486 was administered orally 20 h and 2 h before OVA challenge at 50 mg/kg, which has been shown to completely block the genomic effects of GC.¹⁷ However, the inhibitory effects of BUD were not blocked by RU486, which alone had no effect on BALF TEAC or lung MnSOD activity.

The numbers of total cells, eosinophils, neutrophils, lymphocytes and monocytes in the BALF were not significantly different between all the experimental groups ($P > 0.05$; data not shown). Therefore BUD had no effect on total and differential counts of inflammatory cells in BALF.

Rapid effect of BUD on O_2^- production by BALF cells and main bronchus rings

BALF cells and main bronchus rings were pre-incubated with BUD (4×10^{-2} mol/L stock solution in 100% ethanol) for 5 min before incubation with PMA. O_2^- production by BALF cells (Fig. 3) and main bronchus rings (Fig. 4) was significantly inhibited by BUD at concentrations of 10^{-9} – 10^{-7} mol/L ($P < 0.05$), and the effect was dose dependent. Additionally, 0.05% ethanol did not influence O_2^- production by BALF cells and main bronchus rings.

In order to ascertain that the rapid effect was not mediated by intracellular GC receptors and protein synthesis, RU486 (at a final concentration 10-fold higher than that of BUD) or CHX (10^{-4} mol/L) was added 30 min before BUD. The rapid effects of BUD

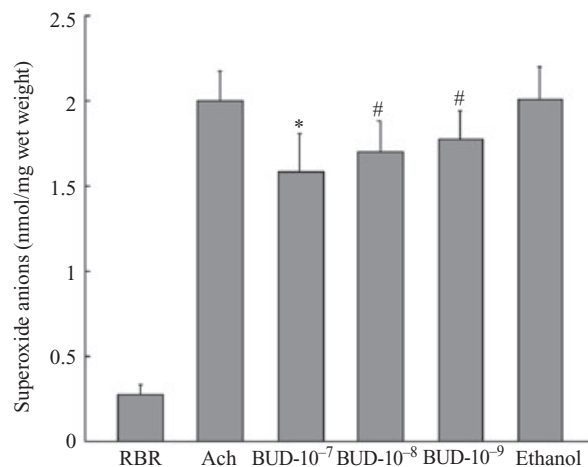


Figure 4 Rapid inhibitory effect of budesonide (BUD) on acetylcholine (Ach)-stimulated O_2^- production by main bronchus rings. BUD was added to bronchial rings 5 min before stimulation with Ach. Resting bronchial rings (RBR) were immersed in Krebs–Henseleit buffer without stimulation with Ach. Ethanol (0.05%) had no effects on O_2^- production by main bronchus rings. * $P < 0.01$ compared with Ach group; # $P < 0.05$ compared with Ach group (ANOVA followed by Dunnett's t -test).

were not blocked by RU486 or CHX ($P > 0.05$). CHX or RU486 alone did not inhibit O_2^- production ($P > 0.05$) (Figs 5,6).

DISCUSSION

It is widely accepted that the striking effectiveness of GC in asthma is due largely to their anti-inflammatory effects, which are characterized by a lag period ranging from 30 min to several hours or even days, and sensitivity to the GC receptor antagonist, RU486, and inhibitors of transcription and translation such as actinomycin D and CHX. However, some studies showed that BUD could rapidly inhibit histamine-induced contraction of main bronchus rings and airway smooth muscle cells through rapid, non-genomic effects.³ Asthma attacks and experimental antigen challenge are both associated with increased release of ROS and rapid loss of antioxidant capacity, resulting in oxidative stress, which plays an important role in the pathogenesis of asthma.^{4,5,7} The results of the present study indicate that inhaled BUD may have a rapid, non-genomic inhibitory effect on oxidative stress in a guinea pig asthma model.

BALF TEAC and lung MnSOD activity were measured to assess the degree of oxidative stress *in vivo*. To determine whether BUD could directly inhibit ROS production by inflammatory cells and bronchial smooth muscle, the effect of BUD on PMA-induced O_2^- production by BALF cells and Ach-induced O_2^- production by bronchial smooth muscles was measured *in vitro*. Inhalation of one clinical dose of BUD (500 µg) results in airway tissue concentrations of

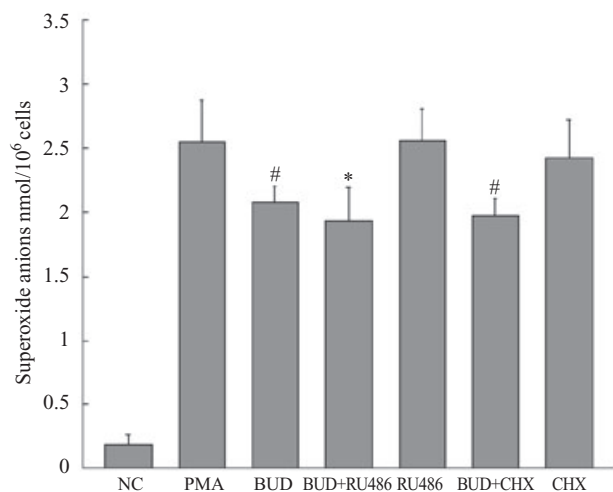


Figure 5 RU486 (10^{-7} mol/L) and cycloheximide (CHX) (10^{-4} mol/L) did not affect the inhibitory action of budesonide (BUD) (10^{-8} mol/L) on phorbol myristate acetate (PMA)-stimulated O_2^- production by BAL fluid (BALF) cells. BALF cells were separately incubated with BUD, BUD+RU486, RU486, BUD+CHX or CHX. RU486 (10^{-7} mol/L) and CHX (10^{-4} mol/L) alone had no effect on O_2^- production by BALF cells. ^{*} $P < 0.01$ compared with PMA group; [#] $P < 0.05$ compared with PMA group (ANOVA followed by Dunnett's *t*-test). NC, normal saline control group.

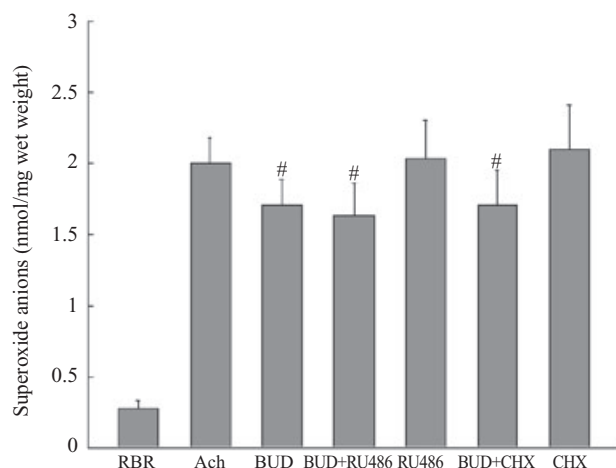


Figure 6 RU486 (10^{-7} mol/L) and cycloheximide (CHX) (10^{-4} mol/L) did not affect the inhibitory action of budesonide (BUD) (10^{-8} mol/L) on acetylcholine (Ach)-stimulated O_2^- production by main bronchus rings. Main bronchus rings were separately incubated with BUD, BUD+RU486, RU486, BUD+CHX or CHX. RU486 (10^{-7} mol/L) and CHX (10^{-4} mol/L) alone had no effect on O_2^- production by main bronchus rings. [#]Significant difference, $P < 0.05$ compared with Ach group (ANOVA followed by Dunnett's *t*-test). RBR, resting bronchial rings.

approximately 10^{-8} – 10^{-7} mol/L,¹⁸ and therefore BUD concentrations of 10^{-9} – 10^{-7} mol/L were used *in vitro*.

BALF TEAC and lung MnSOD activity were markedly higher in the BUD group than in the HPMC

group, and this effect was apparent 15 min after administration of the drug. The results of cell counting showed that the inhibitory effect of BUD on oxidative stress was not caused by a decrease in the number of inflammatory cells, but by a direct inhibition of ROS production by inflammatory cells. BUD also rapidly inhibited *in vitro* O_2^- production by BALF cells and main bronchus rings within 15 min of adding the drug. Moreover, pretreatment of asthmatic guinea pigs, BALF cells and main bronchus rings with the GC receptor antagonist, RU486, or the protein synthesis inhibitor, CHX, did not alter this rapid effect of BUD. Thus, it can be concluded that the effect of BUD was mediated by a rapid non-genomic mechanism rather than by the classical genomic mechanism, which usually has a lag period ranging from 30 min to several hours or even days. BUD rapidly inhibited ROS production within a short time period and may reduce the direct damaging effects of ROS on proteins, lipids and DNA, thereby attenuating airway smooth muscle contraction, airway hyperresponsiveness and mucin secretion.

RU486 is a high-affinity antagonist of the GC receptor that has been demonstrated to be effective in blocking the classical genomic effects of GC.^{17,19} A 10-fold higher concentration of RU486 relative to GC completely blocked the binding of GC to the receptors *in vitro*.²⁰ CHX, a protein synthesis inhibitor, has been shown to block IL-6 synthesis at 1 μ mol/L.²¹ In the present experiments, a CHX concentration of 10^{-4} mol/L was used.

The degree of GC's inhibitory effect was quite small and <25% in most cases. Because O_2^- production and oxidative stress is a complicated process involving multiple cells and signalling molecules, the inhibitory effect of GC may only impact selected populations of cells and signalling molecules, explaining the low degree of the inhibition.

The present findings, taken together with those of others, suggest that besides their classical genomic effects, GC may also exert therapeutic effects in asthma through a non-genomic inhibitory effect on oxidative stress, airway smooth muscle contraction,³ mucin secretion²² and airway mucosal blood flow.²³ Although the exact mechanisms of these actions are not fully understood, the rapid, non-genomic inhibitory effects of GC may be expected to have significant therapeutic implications for asthma treatment.

In conclusion, this study showed that GC have a rapid, non-genomic inhibitory effect on oxidative stress in asthmatic guinea pigs *in vivo*, and on O_2^- production by BALF cells and main bronchus rings *in vitro*. These findings cast new light on the mechanism of action of GC in asthma and may contribute to future developments in the clinical treatment of asthma.

REFERENCES

- Hayashi R, Wada H, Ito K, Adcock IM. Effects of glucocorticoids on gene transcription. *Eur. J. Pharmacol.* 2004; **500**: 51–62.
- Zhou J, Kang ZM, Xie QM, Liu C, Lou SJ *et al*. Rapid nongenomic effects of glucocorticoids on allergic

- asthma reaction in the guinea pig. *J. Endocrinol.* 2003; **177**: R1–4.
- 3 Sun HW, Miao CY, Liu L, Zhou J, Su DF *et al.* Rapid inhibitory effect of glucocorticoids on airway smooth muscle contractions in guinea pigs. *Steroids* 2006; **71**: 154–9.
 - 4 Calhoun WJ, Bush RK. Enhanced reactive oxygen species metabolism of airspace cells and airway inflammation follow antigen challenge in human asthma. *J. Allergy Clin. Immunol.* 1990; **86**: 306–13.
 - 5 Comhair SA, Bhatena PR, Dweik RA, Kavuru M, Erzurum SC. Rapid loss of superoxide dismutase activity during antigen-induced asthmatic response. *Lancet* 2000; **355**: 624.
 - 6 Fujisawa T. Role of oxygen radicals on bronchial asthma. *Curr. Drug Targets Inflamm. Allergy* 2005; **4**: 505–9.
 - 7 Kirkham P, Rahman I. Oxidative stress in asthma and COPD: antioxidants as a therapeutic strategy. *Pharmacol. Ther.* 2006; **111**: 476–94.
 - 8 Hulsmann AR, Raatgeep HR, den Hollander JC, Stijnen T, Saxena PR *et al.* Oxidative epithelial damage produces hyperresponsiveness of human peripheral airways. *Am. J. Respir. Crit. Care Med.* 1994; **149**: 519–25.
 - 9 Sadeghi-Hashjin G, Folkerts G, Henricks PA, Verheyen AK, van der Linde HJ *et al.* Peroxynitrite induces airway hyperresponsiveness in guinea pigs in vitro and in vivo. *Am. J. Respir. Crit. Care Med.* 1996; **153**: 1697–701.
 - 10 Kim JY, Lee KH, Lee BK, Ro JY. Peroxynitrite modulates release of inflammatory mediators from guinea pig lung mast cells activated by antigen-antibody reaction. *Int. Arch. Allergy Immunol.* 2005; **137**: 104–14.
 - 11 Morikawa T, Webber SE, Widdicombe JG. The effect of hydrogen peroxide on smooth muscle tone, mucus secretion and epithelial albumin transport of the ferret trachea in vitro. *Pulm. Pharmacol.* 1991; **4**: 106–13.
 - 12 Long F, Wang YX, Liu L, Zhou J, Cui RY *et al.* Rapid nongenomic inhibitory effects of glucocorticoids on phagocytosis and superoxide anion production by macrophages. *Steroids* 2005; **70**: 55–61.
 - 13 Baccan GC, Oliveira RD, Mantovani B. Stress and immunological phagocytosis: possible nongenomic action of corticosterone. *Life Sci.* 2004; **75**: 1357–68.
 - 14 Masini E, Bani D, Vannacci A, Pierpaoli S, Mannaioni PF *et al.* Reduction of antigen-induced respiratory abnormalities and airway inflammation in sensitized guinea pigs by a superoxide dismutase mimetic. *Free Radic. Biol. Med.* 2005; **39**: 520–31.
 - 15 Albrecht C, Knaapen AM, Becker A, Hohr D, Haberzettl P *et al.* The crucial role of particle surface reactivity in respirable quartz-induced reactive oxygen/nitrogen species formation and APE/Ref-1 induction in rat lung. *Respir. Res.* 2005; **6**: 129.
 - 16 Casoni GL, Chitano P, Pinamonti S, Chicca M, Ciaccia A *et al.* Reducing agents inhibit the contractile response of isolated guinea-pig main bronchi. *Clin. Exp. Allergy* 2003; **33**: 999–1004.
 - 17 Peers SH, Moon D, Flower RJ. Reversal of the anti-inflammatory effects of dexamethasone by the glucocorticoid antagonist RU 38486. *Biochem. Pharmacol.* 1988; **37**: 556–7.
 - 18 Linden M, Brattsand R. Effects of a corticosteroid, budesonide, on alveolar macrophage and blood monocyte secretion of cytokines: differential sensitivity of GM-CSF, IL-1b, and IL-6. *Pulm. Pharmacol.* 1994; **7**: 43–7.
 - 19 Moguilewsky M, Philibert D. RU 38 486 potent anti-glucocorticoid activity correlated with strong binding to the cytosolic glucocorticoid receptor followed by an impaired activation. *J. Steroid Biochem.* 1984; **20**: 271–6.
 - 20 Honer C, Nam K, Fink C, Marshall P, Ksander G *et al.* Glucocorticoid receptor antagonism by cyproterone acetate and RU486. *Mol. Pharmacol.* 2003; **63**: 1012–20.
 - 21 Kirkwood K, Martin T, Andreadis ST, Kim YJ. Chemically modified tetracyclines selectively inhibit IL-6 expression in osteoblasts by decreasing mRNA stability. *Biochem. Pharmacol.* 2003; **66**: 1809–19.
 - 22 Urbach V, Verriere V, Grumbach Y, Bousquet J, Harvey BJ. Rapid anti-secretory effects of glucocorticoids in human airway epithelium. *Steroids* 2006; **71**: 323–8.
 - 23 Kumar SD, Brieva JL, Danta I, Wanner A. Transient effect of inhaled fluticasone on airway mucosal blood flow in subjects with and without asthma. *Am. J. Respir. Crit. Care Med.* 2000; **161**: 918–21.