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Article

The Effects of Asthma Medications on Reactive Oxygen Species Production in Human Monocytes

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Abstract

Asthma is a chronic inflammatory airway disease induced by many environmental factors. The inhalation of allergens and pollutants promote the reactive oxygen species (ROS) production leading to airway inflammation, hyper-responsiveness and remodeling in allergic asthma. The effects of asthma medications on ROS production are unclear. The present study investigated the anti-ROS effects of current asthma medications including inhaled corticosteroid (ICS; budesonide and fluticasone), leukotriene receptor antagonist (LTRA; montelukast), long acting β2 agonists (LABAs; salmeterol and formoterol) and a new extra-LABA (indacaterol). The human monocyte cell line THP-1 cells were pre-treated with different concentrations of the asthma medications at different time-points after hydrogen peroxide (H_2O_2) stimulation. H_2O_2 production was measured with DCFH-DA by flow cytometry. Montelukast, fluticasone and salmeterol suppressed

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H_2O_2-induced ROS production. Indacaterol enhanced H_2O_2-induced ROS production. Budesonide and formoterol alone had no anti-ROS effects, but the combination of these two drugs significantly suppressed H_2O_2-induced ROS production. Different asthma medications have different anti-ROS effects on monocytes. The combination therapy with LABA and ICS seemed not be the only choice for asthma control. Montelukast may be also a good supplemental treatment for the poorly-controlled asthma because of its powerful anti-ROS effects. Our findings provide a novel therapeutic view in asthma.

Keywords: reactive oxygen species (ROS); asthma; montelukast; long-acting β2 agonist (LABA); corticosteroid; monocyte

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Research Manuscript Sections:

1. Introduction

Environmental factors are important for asthma. Many evidences support that the inhalation of aggravating compounds such as allergens and pollutants could increase reactive oxygen species (ROS) production which would lead to oxidative stress-induced damage to the respiratory system in allergic asthma. [1, 2]. Airborne particulate matter (PM) components from fossil fuel combustion can also induce oxidative stress initiated by ROS [3]. Although human lungs have a potent antioxidant system, excessive oxidative and nitrative stress leads to an imbalance of oxidants/antioxidants.

Asthma medications reduce airway inflammation and relief asthma symptoms. leukotriene modifiers, long-acting β2-adrenoreceptor agonists (LABAs) and inhaled corticosteroids (ICS) are common long-term asthma control medications. Leukotriene receptor antagonists (LTRA), such as montelukast, would prevent provoking asthma responses, ameliorate asthma symptoms, improve lung function and reduce β2-agonist use in patients with persistent asthma [4, 5]. β2 adrenoceptor agonists are used widely as bronchodilators in treatment of asthma and have important anti-inflammatory effects on eosinophils and neutrophils [6, 7]. Formoterol and salmeterol are two inhaled long-acting β2-adrenoreceptor agonists (LABAs) widely used for the treatment of asthma and chronic obstructive pulmonary disease (COPD). These two common LABAs were reported to have inhibitory effects on the expression of pro-inflammatory cytokines. Formoterol could suppress lipopolysaccharide (LPS)-induced IL-6 expression in a mouse model [8]. Salmeterol could suppress LPS-induced TNF-α production in THP-1 cells [9] and also reduces the IgE-dependent TNF-α production in human skin mast cells [10]. Indacaterol, an extra-LABA, is new breakthrough for LABA and can be a mono-therapy for COPD, but not for asthma. Indacaterol is able to induce a rapid and long-lasting relaxation of airway smooth muscles via a prolonged activation of β_2-receptors, and provides a persistent bronchodilation due to the prolonged competitive blockade of M_3 muscarinic receptors [11, 12]. Inhaled corticosteroid (ICS) is known to be effective as a maintenance medication in persistent asthma. The clinical usefulness of fixed-dose maintenance therapy with ICS/LABA combination inhalers, such as fluticasone/salmeterol and budesonide/formoterol, have been established though the long-term anti-inflammatory effects of these two inhalers are limited [13].

ROS production is an important pathophysiology of asthma, however, the effects of asthma medications on ROS production is still lacking. Monocytes play an

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important role in asthma pathogenesis. Monocytes of asthmatic patients were shown to produce eminent amounts of reactive nitrogen species, exhibited higher activities of nitric oxide synthase and total free radicals, the feature that was closely related to asthma severity [14]. In the present study, we aimed to investigated whether these common asthma medications would suppress ROS productions on human monocytes. Our findings broaden the knowledge of anti-ROS effects by treatment with common controller medications in asthma.

2. Results

2.1 Montelukast suppressed H_2O_2-induced ROS production in THP-1 cells

H_2O_2 alone could induce ROS production in THP-1 cells at 30-minute, 1-hour and 2-hour time-points (Figure 1A, 1B and 1C). The pre-treatment of montelukast (10^{-6} M~ 10^{-5} M) for 30 minutes significantly suppressed H_2O_2-induced ROS expression in THP-1 cells (Figure 1A). Montelukast suppressed ROS production in THP-1 cells at one hour after H_2O_2 stimulation even at lower concentrations (10^{-7} M and 10^{-5} M) (Figure 1B). Montelukast suppressed ROS production in THP-1 cells at 2-hour time-point after H_2O_2 stimulation at only higher concentration (10^{-5} M) (Figure 1C).

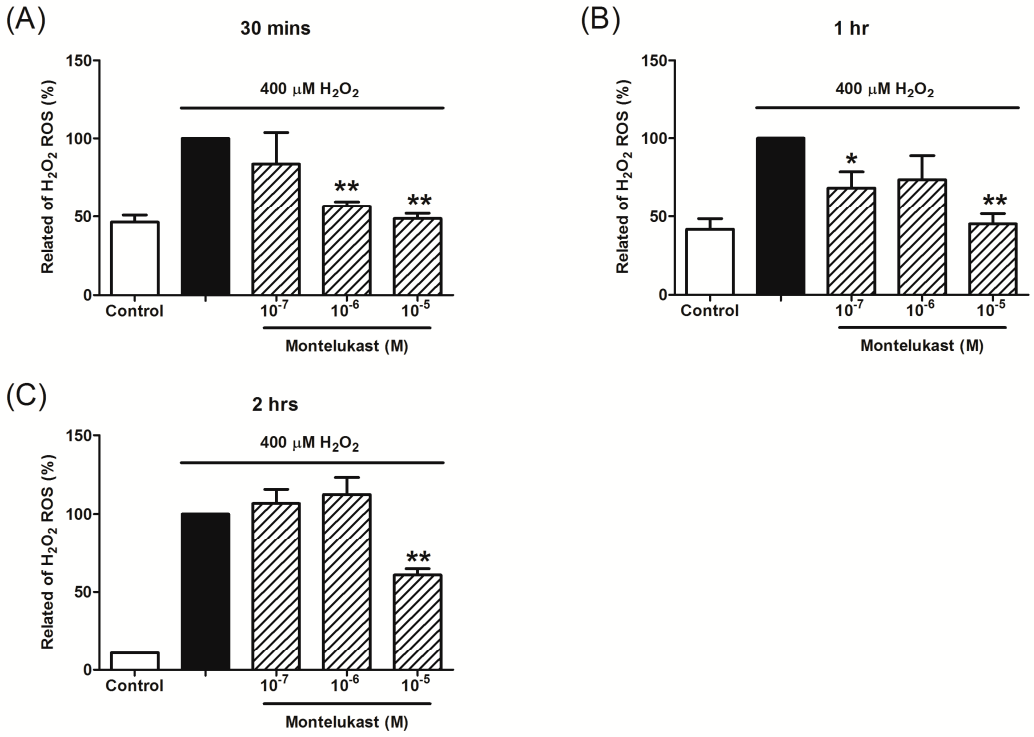

Figure 1. Montelukast suppressed H_2O_2-induced ROS production in THP-1 cells. Montelukast significantly suppressed H_2O_2-induced ROS expression in THP-1 cells at 30-minute (A), 1-hour (B) and 2-hour (C) time-points. (*: $p< 0.05$, **: $p< 0.01$)

2.2 Salmeterol but not formoterol suppressed H_2O_2-induced ROS production in THP-1 cells

Next, we evaluated another common asthma medication LABA's effects on ROS production. Interestingly, salmeterol alone could reduce H_2O_2-induce ROS production in THP-1 cells at only 2-hour time-point, but have no obvious effects at 30-minute and 1-hour time-points (Figure 2A, 2B and 2C). The pre-treatment of another LABA

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formoterol (10^{-9} M~ 10^{-7} M) had no effects on the suppression of ROS production in THP-1 cells at 30-minute, 1-hour and 2-hour time-points (Figure 3A, 3B and 3C).

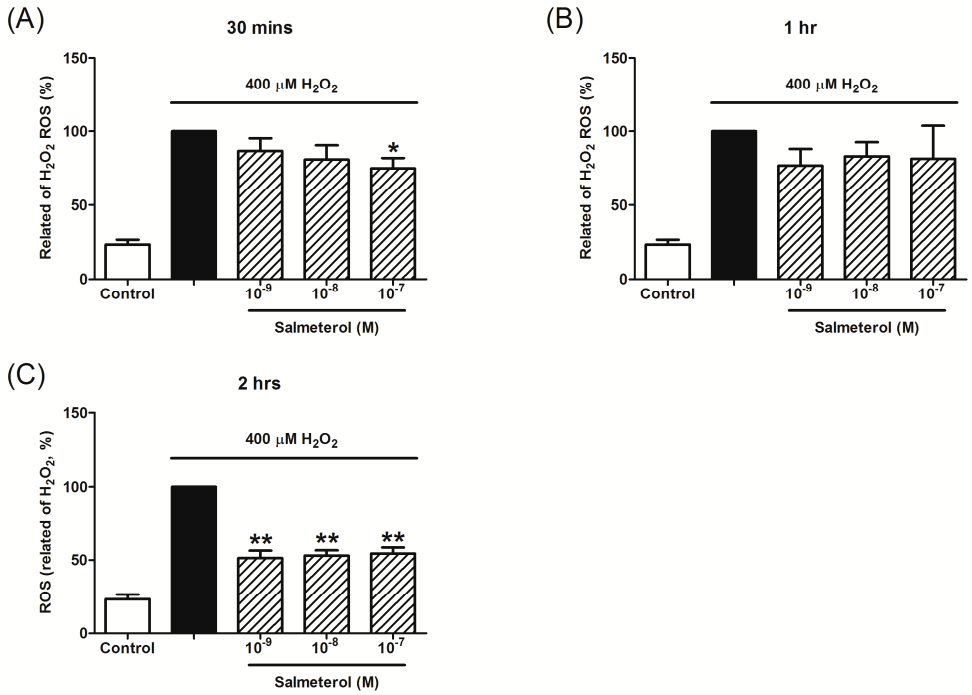

Figure 2. The effects of salmeterol on H_2O_2-induced ROS production in THP-1 cells. (A, B) The higher dose of salmeterol (10^{-7} M) could significantly reduce H_2O_2-induced ROS production in THP-1 cells at 30-minute, but no effects at 1-hour time-point. (C) All three concentrations of salmeterol (10^{-9} M~ 10^{-7} M) could significantly reduce H_2O_2-induced ROS production in THP-1 cells at 2-hour time-point. (*: $p< 0.05$, **: $p< 0.01$)

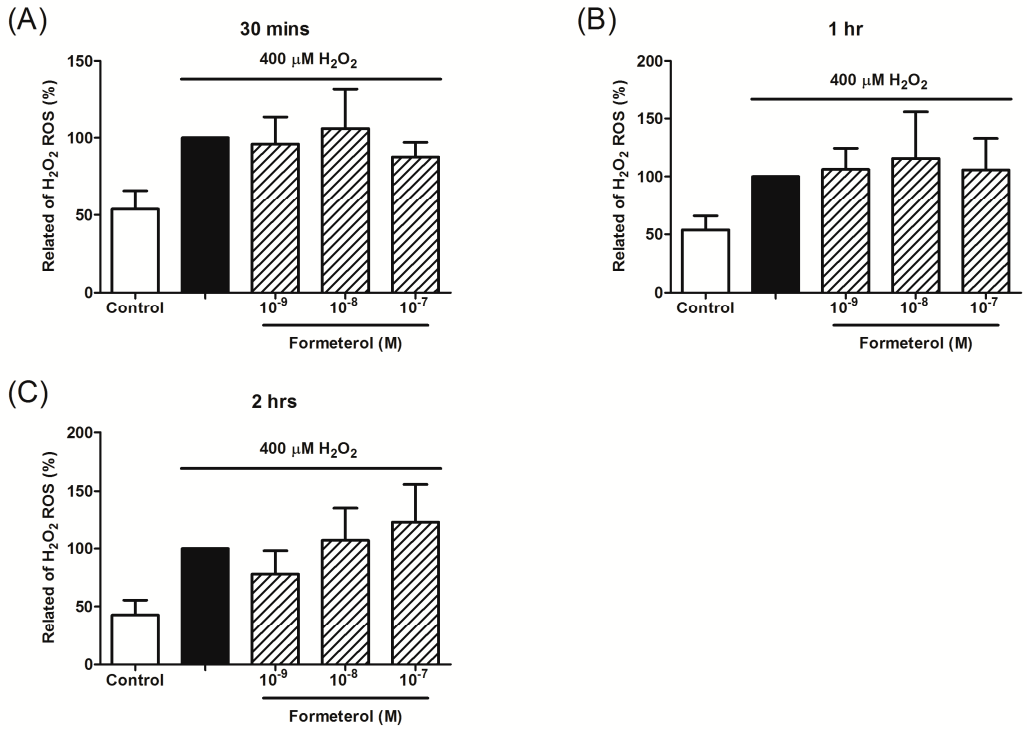

Figure 3. The effects of formoterol on H_2O_2-induced ROS production in THP-1 cells. Formoterol could not significantly suppress H_2O_2-induced ROS expression in

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THP-1 cells at 30-minute (A), 1-hour (B) and 2-hour (C) time-points. (*: $p< 0.05$, **: $p< 0.01$)

2.3 Indacaterol enhanced H_2O_2-induced ROS production in THP-1 cells

We further evaluate extra LABA's effects on ROS production. Interestingly, indacaterol alone enhanced H_2O_2-induce ROS production in THP-1 cells at 30-minute and 1-hour time-points (Figure 4A and 4B). The pre-treatment of indacaterol (10^{-9} M~ 10^{-7} M) had no effects on ROS production in THP-1 cells at 2-hour time-point (Figure 4C).

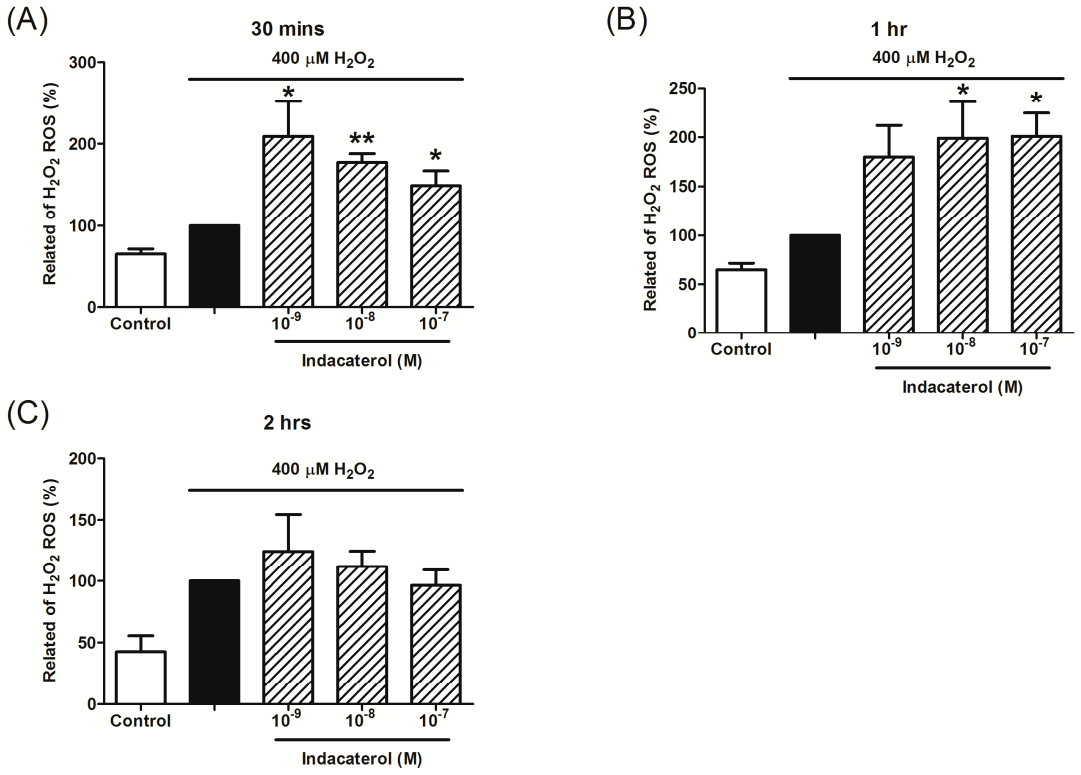

Figure 4. The effects of indacaterol on H_2O_2-induced ROS production in THP-1 cells. Indacaterol could not significantly reduce H_2O_2-induced ROS production in THP-1 cells at 30-minute (A), but significantly enhanced H_2O_2-induced ROS production at 1-hour (B) and 2-hour (C) time-points. (*: $p< 0.05$, **: $p< 0.01$).

2.4 Fluticasone but not budesonide suppressed H_2O_2-induced ROS production in THP-1 cells

We also evaluate another common asthma medication ICS's effects on ROS production. Fluticasone alone could suppress H_2O_2-induce ROS production in THP-1 cells at 2-hour time-point, but have no obvious effects at 30-minute and 1-hour time-points (Figure 5A, 5B and 5C). The pre-treatment of another ICS budesonide (10^{-9} M~ 10^{-7} M) had no effects on the suppression of ROS production in THP-1 cells at 30-minute, 1-hour and 2-hour time-points (Figure 6A, 6B and 6C).

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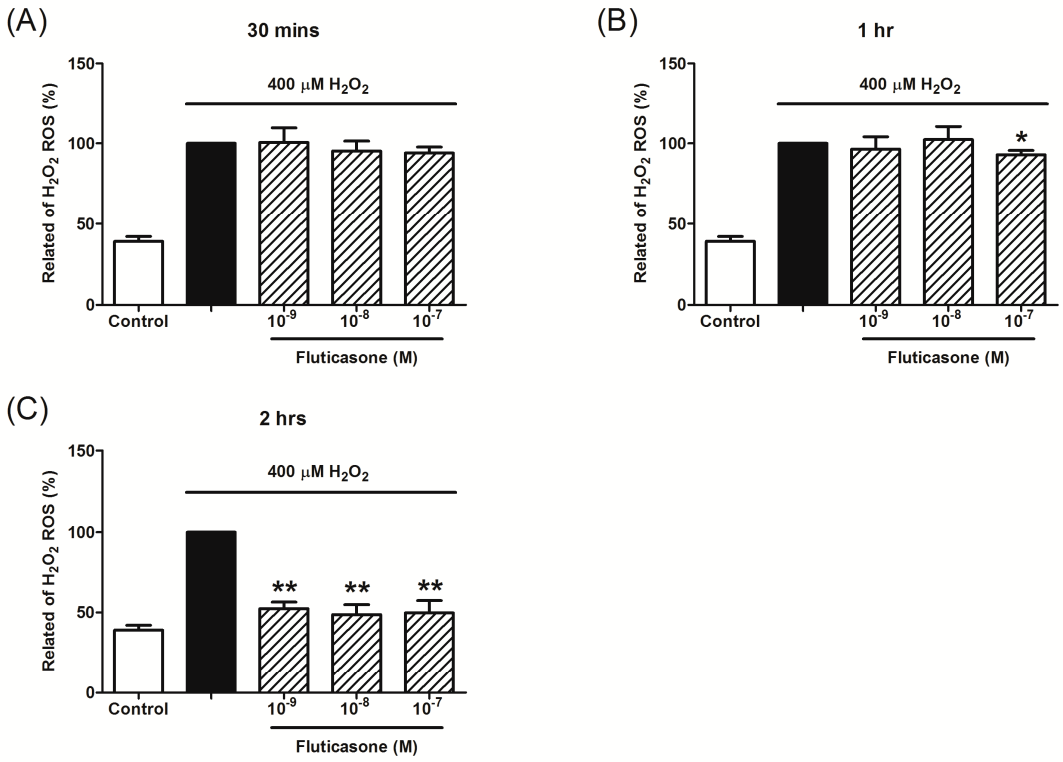

Figure 5. The effects of fluticasone on H_2O_2-induced ROS production in THP-1 cells. (A, B) Fluticasone was not able to significantly reduce H_2O_2-induced ROS production in THP-1 cells at 30-minute, but a higher concentration of salmeterol (10^{-7} M) significantly suppressed H_2O_2-induced ROS production at 1-hour time-point. (C) Fluticasone could significantly reduce H_2O_2-induced ROS production in THP-1 cells at 2-hour time-point. (*: $p< 0.05$, **: $p< 0.01$).

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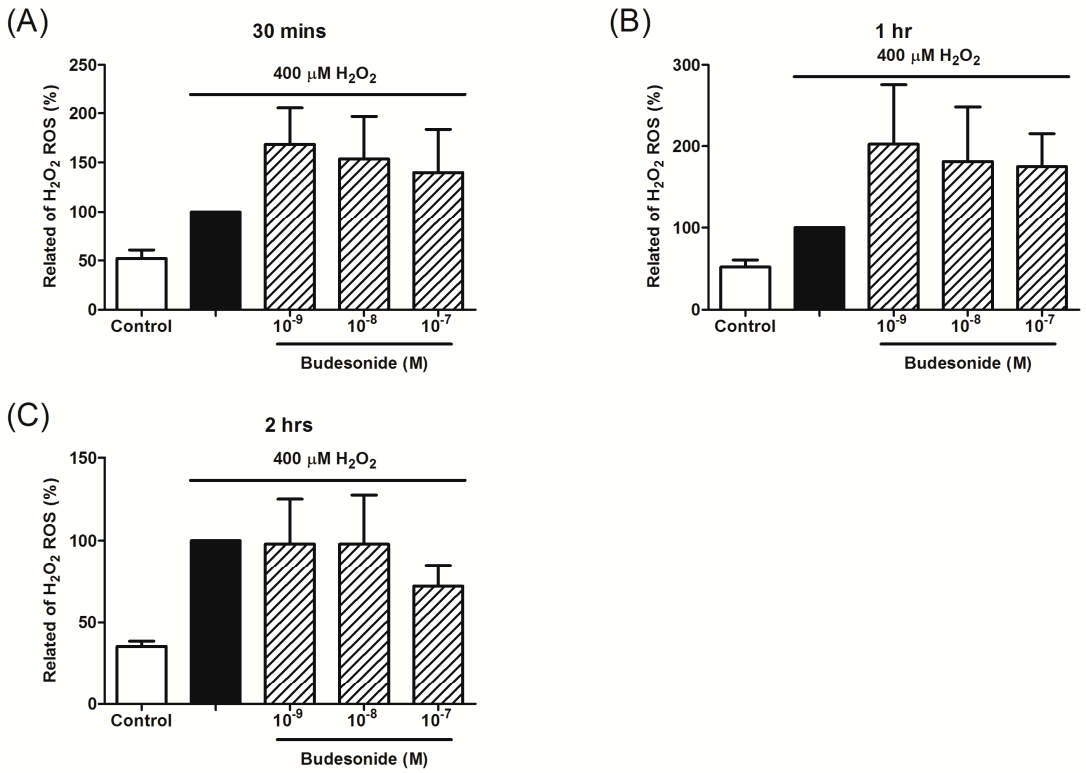

Figure 6. The effects of budesonide on H_2O_2-induced ROS production in THP-1 cells. Budesonide could not significantly reduce H_2O_2-induced ROS production in THP-1 cells at 30-minute(A), 1-hour (B) and 2-hour (C) time-points. (*: $p< 0.05$, **: $p< 0.01$).

2.5 Salmeterol/fluticasone and formoterol/budesonide suppressed H_2O_2-induced ROS production in THP-1 cells

Since different ICS and LABA have diverse effects on ROS production in THP-1 cells. Therefore, we further evaluate the effects of ICS plus LABA on ROS production. Interestingly, salmeterol/fluticasone combination could significantly reduce H_2O_2-induced ROS production in THP-1 cells at 2-hour time-point, but have no obvious effects at 30-minute and 1-hour time-points (Figure 7A, 7B and 7C). The pre-treatment of another ICS and LABA combination formoterol/budesonide also suppressed H_2O_2-induce ROS production in THP-1 cells at 2-hour time-point, but have no obvious effects at 30-minute and 1-hour time-points (Figure 8A, 8B and 8C).

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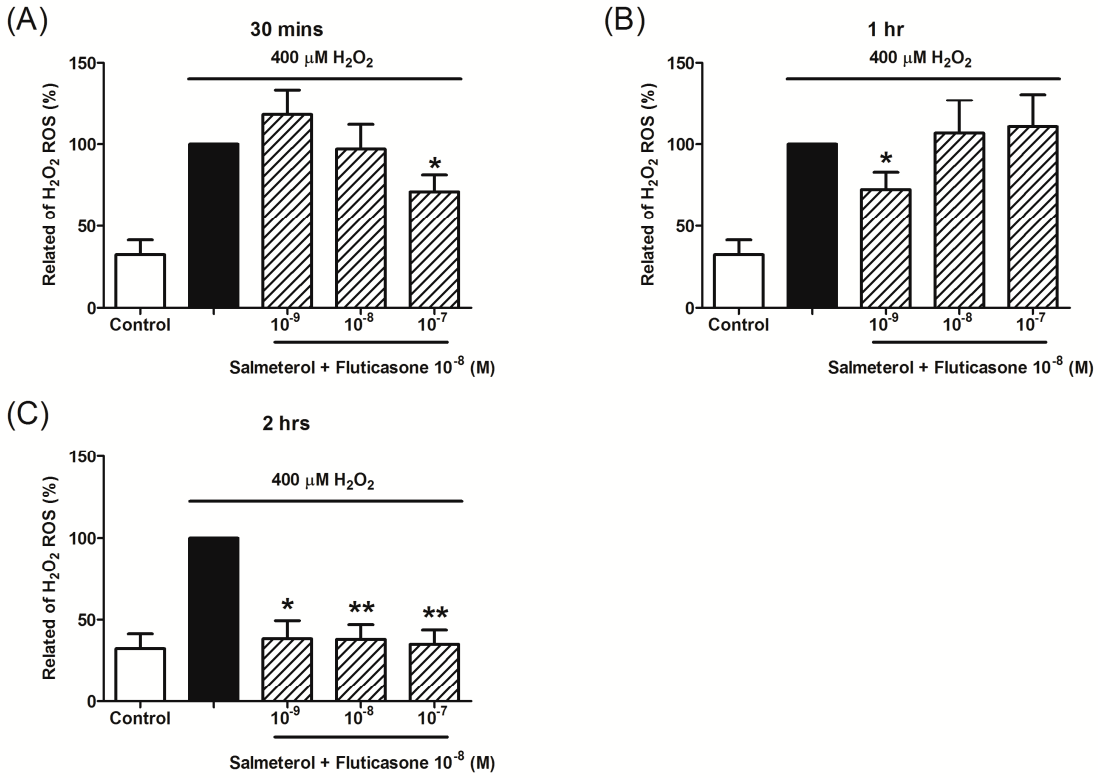

Figure 7. The effects of salmeterol/fluticasone combination on H_2O_2-induced ROS production in THP-1 cells. Salmeterol/fluticasone combination had no obvious effects at 30-minute (A) and 1-hour (B) time-points. (C) The pretreatment of salmeterol/fluticasone combination for 2 hours reduced H_2O_2-induce ROS production in THP-1 cells.

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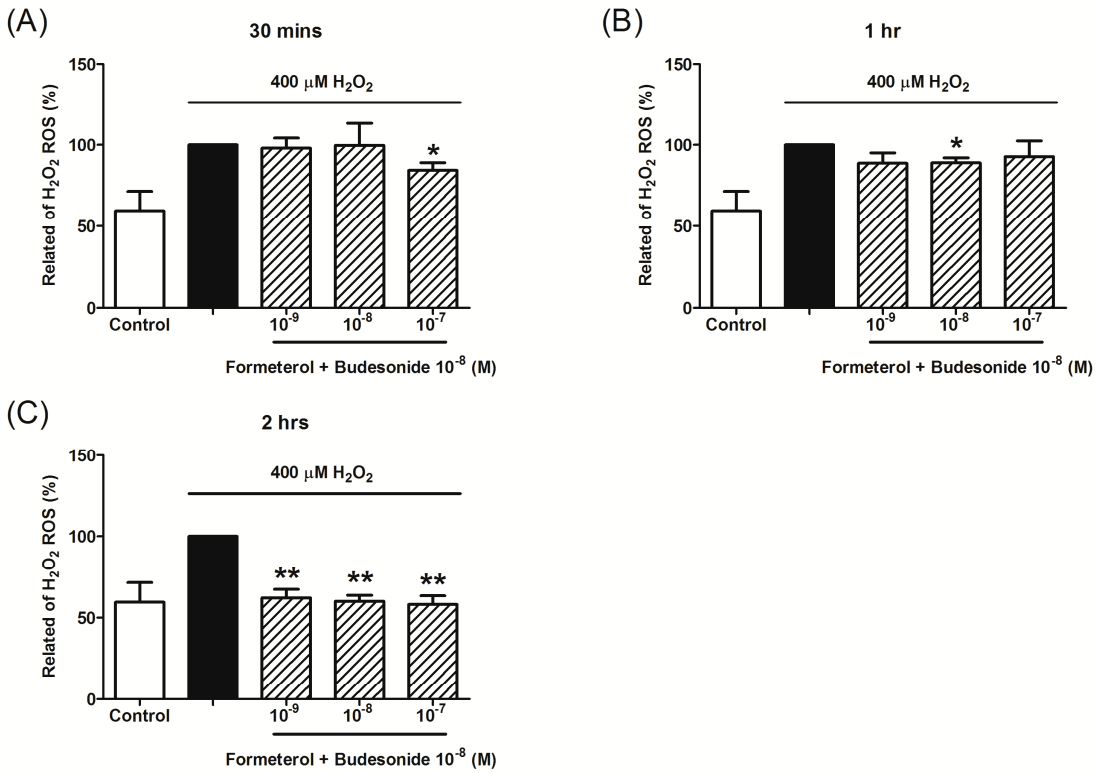

Figure 8. The effects of formoterol/budesonide combination on H_2O_2-induced ROS production in THP-1 cells. (A, B) The pre-treatment of formoterol and budesonide has no obvious effects at 30-minute and 1-hour time-points. (C) The pre-treatment of formoterol and budesonide for 2 hours suppressed H_2O_2-induce ROS production in THP-1 cells.

3. Discussion

Oxidative stress has been determined to be important in the pathogenesis of allergic asthma. Exposure to environmental antigens can stimulate overproduction of ROS that augment bronchial hyper-responsiveness and inflammation [1, 15]. ROS and reactive nitrogen species (RNS), such as hydrogen peroxide and nitric oxide, are important mediators of natural physiological processes. Inflammatory cells which are recruited to the asthmatic airways have an exceptional capacity for producing a variety of highly reactive ROS and RNS that lead to tissue damage and chronic airways inflammation. The useful redox-based therapy to attenuate levels of ROS presents a potential strategy to alleviate oxidative stress-induced airway inflammation in patients with asthma. In the present study, we investigated the anti-ROS effects of several common asthma medications in THP-1 cells. The scheme of our findings is summarized in Figure 9.

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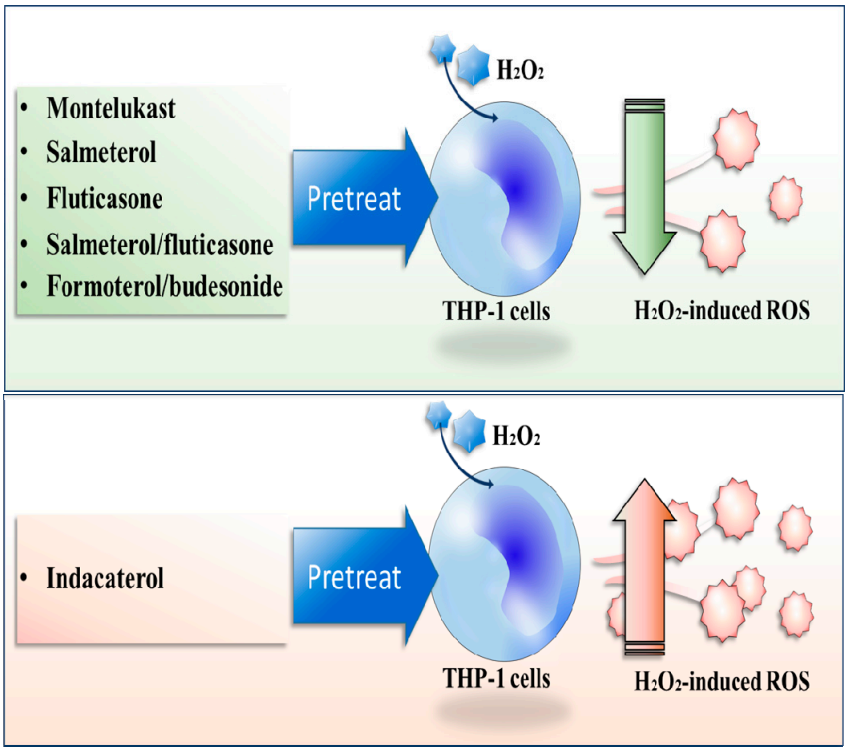

Figure 9: A scheme of anti-ROS effects of asthma medications in monocytes.

According to the Global Initiative for Asthma (GINA) guideline, ICS, LABA and LTRA are the main controllers for asthma patients. Controller medications should have enough anti-inflammation properties because asthma is an airway inflammatory disease. LTRA or ICS monotherapy is the appropriate therapeutic option for the treatment of step 2 asthma. Montelukast and inhaled budesonide provide acceptable asthma control, whereas budesonide inhalation is favored in children aged 2 to 8 years with mild persistent asthma [16]. In our present study, we compare the efficacy of LTRA (montelukast) and ICS (budesonide and fluticasone) in ROS production in monocytes. Montelukast and fluticasone, but not budesonide seem able to obviously decrease ROS production in monocytes. Fluticasone seemed be superior to budesonide in the suppression of ROS production. The cysteinyl leukotrienes LTC4, LTD4, and LTE4 induce many pathophysiological changes in the lungs of asthma patients, including airflow obstruction, mucus secretion and inflammatory cell infiltration. It has been reported that LTD4 stimulation of a Gi/o protein-coupled cysteinyl leukotriene receptor CysLT1-R triggers the transactivation of the epidermal growth factor receptor through the intervention of phosphatidylinostiol-3-OH-kinase and ROS [17]. Montelukast blocks the action of LTD4 on the CysLT1-R in the lungs. Some studies suggest that oral montelukast is not inferior to budesonide in young children with mild persistent asthma in terms of control of symptoms [16, 18]. The suppressive effects of montelukast on ROS production might be through the completion of CysLT1-R binding.

The LABAs are globally used in combination with ICS in moderate-severe adult asthma. However, the LABAs are limited in use in pediatric asthma under the age of five. Some studies showed that salmeterol lacks protective effects on pediatric asthma [19]. In the present study, salmeterol but not formoterol suppressed ROS production in monocyte. indacaterol greatly enhanced H_2O_2-induced ROS. These findings suggest that different LABAs have different properties. Only salmeterol, but not formoterol or indacaterol could suppress ROS production effectively in monocytes.

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Unlike salmeterol, indacaterol does not alter the fluidity of the cell membrane, and its long-lasting bronchodilating effect is due to a high affinity of the lipophilic tail of the molecule for the so-called lipid rafts [20]. The “masking effects” while mono-using LABAs in pediatric patients and “increasing the chance of a severe exacerbation” has been concerned [21]. The monotherapy of LABAs have been labeled with "black box" warnings because of reports of the occurrences of severe asthma exacerbations in some patients with asthma, and even with some associated deaths several years ago [22].

ROS production is elicited by several stimuli, such as immunoglobulins and cytokines [23]. It has been previously reported that eosinophils migrate into tissues and release toxic granule proteins and ROS which lead to tissue damage during the allergic-induced inflammatory process [24]. ICSs pose strongly anti-inflammatory properties in asthma. In the present study, fluticasone but not budesonide has the suppressive effects on ROS production in monocytes. ICS is generally combined with LABA as an asthma controller. The combination of ICS with LABA is the main therapy for moderate-severe asthma. Two fixed combination inhalers (salmeterol/fluticasone and formoterol/budesonide) are increasingly used as a convenient controller in patients with persistent asthma. Our data showed that formoterol/budesonide combination suppressed ROS production in monocytes, even formoterol or budesonide alone has no effects. These findings of anti-ROS effects are compatible with the clinical therapeutic response in asthma.

In the present study, we found that different asthma medications have different anti-ROS effects on monocytes. Both montelukast and the combination of ICS/LABA had anti-ROS effects. The combination therapy with LABA and ICS may not be the only choice for asthma control. Montelukast may be also a good supplemental treatment for the poorly-controlled asthma because of its powerful anti-ROS effects. This study provides a novel therapeutic view in asthma.

4. Materials and Methods

4.1 Cell culture

THP-1 cells (American Type Culture Collection, Rockville, MD, USA) were cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) with 10% fetal bovine serum (Gibco), 100 U/mL penicillin, 100 µg/mL streptomycin and 0.25 µg/mL amphotericin B at 37°C and 5% CO2 in a humidified incubator. The cells were re-suspended in fresh media at a concentration of 1x105/mL, 1mL/well in 24 wells plates or 1x106/mL, 2mL/well in 6 well plates overnight for attachment, and 400 µM of H_2O_2 (Sigma Chemical, MO, USA) was added with or without pretreatment of the cells with montelukast, formoterol, salmeterol, fluticasone or budesonide or in combination of salmeterol/fluticasone and budesonide/formoterol for 30 minutes, one hour and 2 hours. The cells were then collected at 30-minute time-point after H_2O_2 stimulation for ROS production assay.

4.2 Measurement of ROS production

ROS production was measured with DCFH-DA by a flow cytometry [25, 26]. DCFH-DA is cleaved intra-cellularly by nonspecific esterases and turns to highly fluorescent 20, 70-dichlorofluorescin (DCF) upon oxidation by ROS. Following treatment of asthma medications, the medium was aspirated and cells were incubated with 1 mM MDCFH-DA in PBS at 37.8 ° C for 15 min, then cells were washed twice with serum-free medium for flow cytometry assay by immediate DCF detection. The generation of ROS was measured by using DCFH-DA, an oxidation-sensitive

fluorescent probe. Intracellular H_2O_2 or low-molecular-weight peroxides can oxidize DCFH-DA to the highly fluorescent compound DCF. Signals were detected through a 525 nm band-pass filter (FL1 channel) using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA).

4.3 Statistical Analysis

We used SPSS 19th edition for Windows for statistical analysis. Data are presented as means ± SD. Significant differences were assessed using the Mann Whitney test. P values less than 0.05 were considered statistically significant.

Back Matter:

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Author Contributions: MKT and YCL conducted data collection, analysis, interpretation of the data, and writing the first draft of the manuscript. CHL, MYH, MSL and CHK made contributions to the analysis and interpretation of the data. CHH and PLK substantial contributions to the conception, design, and interpretation of the data. All authors reviewed, revised, and approved the manuscript for publication

Conflicts of Interest: The authors declared that no conflict of interest.

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