**Procaterol but Not Dexamethasone Protects 16HBE Cells From H₂O₂-Induced Oxidative Stress**

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**Abstract.** Oxidative stress is an important pathophysiological factor of asthma and chronic obstructive pulmonary disease (COPD). We hypothesized that procaterol and dexamethasone might treat inflammation through inhibiting oxidative stress in vitro. This study evaluated procaterol and dexamethasone in the hydrogen peroxide (H₂O₂)-induced immortal human bronchial epithelial cell model of oxidative stress and investigated the underlying mechanisms. Results showed that exposure to 125 μM H₂O₂ for 2 h led to a 50% reduction in the cell viability, significantly increased the percentage of apoptosis, and elevated levels of malondialdehyde and reactive oxygen species. Pretreatment with procaterol (25 – 200 nM) could reduce these effects in a dose-dependent manner. In contrast, pretreatment with dexamethasone (100 nM, 1000 nM) was inefficient. Pretreatment with procaterol plus dexamethasone (100 nM procaterol + 1000 nM dexamethasone) was effective, but the combined effect was not more effective than the sole pretreatment with 100 nM procaterol. The nuclear factor kappa-B (NF-κB) pathway was involved in the pathogenic mechanisms of H₂O₂. Procaterol may indirectly inhibit H₂O₂-induced activation of the NF-κB pathway due to its capability of antioxidation. Glucocorticoids may be not recommended to treat asthma or COPD complicated with severe oxidative stress.

**Keywords:** oxidative stress, procaterol, dexamethasone, nuclear factor kappa-B, hydrogen peroxide

**Introduction**

Asthma and chronic obstructive pulmonary disease (COPD) are both chronic airway inflammatory diseases with high incidence, and COPD has a high mortality rate (1). Oxidative stress is an important pathophysiological factor of asthma and COPD (2). Excess oxidative stress will result in chromatin dysfunction, thereby leading to apoptosis and necrosis. High concentrations of hydrogen peroxide (H₂O₂) can induce the cellular model of oxidative stress (3). The immortal human bronchial epithelial cell line (16HBE) has been widely used in research on asthma and COPD (4, 5). Previously, the H₂O₂-induced 16HBE cell model has been used to evaluate drugs (6).

H₂O₂ stimulation can activate the nuclear factor kappa-B (NF-κB) pathway at the cellular level (7, 8). NF-κB is a key transcriptional factor that coordinates the expression of various inflammatory genes. The NF-κB family includes 5 subunits: RelA (p65), RelB, c-Rel, p50, and p52. NF-κB complexes are retained in the cytosol through binding to the inhibitory protein inhibitory kappa B-α (IκB-α). Upon stimulation, IκB-α is phosphorylated, which results in proteasome-mediated IκB-α degradation (9). The p65 subunit is then phosphorylated, which induces the nuclear transfer of the p65/50 dimer. Finally, the inflammation-related target genes are activated.

The β₂-selective agonists can relieve asthmatic symptoms and elevate the pulmonary function of COPD patients in the clinic (10). Procaterol, a good β₂-selective agonist, has the characteristics of good tolerance and minor side effects. Besides, procaerol can inhibit inflammation both in vivo and in vitro (11). Nevertheless, whether procaterol can treat the cellular models of oxida-
active stress has not been recorded to date.

As a type of glucocorticoids, dexamethasone is good at controlling asthma attacks (12). Although inhaled glucocorticosteroids are recommended for COPD patients under the American College of Physicians guidelines, the GOLD guidelines do not recommend the long-term use of oral glucocorticosteroids for COPD. A meta-analysis involving 14,426 participants also found that inhaled glucocorticosteroids therapy did not affect one-year mortality for COPD (13). Moreover, due to the glucocorticoid resistance (GR) phenomenon, the clinical benefits of corticosteroid therapy in COPD are limited (14). At the cellular level, the pharmacological effects of dexamethasone appear to be controversial when it treats \( \text{H}_2\text{O}_2 \)-induced oxidative stress (15, 16). Thus, whether dexamethasone can relieve \( \text{H}_2\text{O}_2 \)-induced oxidative stress in 16HBE cells is an interesting topic for further study.

In this paper, we hypothesized that procaterol and dexamethasone might have antioxidant functions in vitro and might play a role in anti-inflammation through inhibiting oxidative stress. Here we evaluated the protective effects of procaterol and dexamethasone in the \( \text{H}_2\text{O}_2 \)-induced 16HBE cell model of oxidative stress. Furthermore, we studied the underlying mechanisms for the involvement of the NF-κB pathway.

### Materials and Methods

#### Cell culture

The immortal human bronchial epithelial cell line (16HBE) was obtained from the Cancer Hospital in the Chinese Academy of Medical Sciences. It was maintained in RPMI-1640 medium (M&C Gene Technology) supplemented with 10% fetal calf serum (Gibco, New York, NY, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin (M&C Gene Technology) in a humid atmosphere of 5% \( \text{CO}_2 \) and 95% air at 37°C. 16HBE cells were cultured on dishes (Corning, Tewksbury, MA, USA) for 24 h. After aspiration, the cells were pretreated with various concentrations (25 – 200 nM) of procaterol (Guangdong Otsuka Pharmaceutical Co., Ltd., Shunde, China), or a combination of drugs (100 nM procaterol + 1000 nM dexamethasone) in bronchial epithelial cell medium (ScienceCell Research Laboratories, Carlsbad, CA, USA) for 20 h; and then the bronchial epithelial cell medium was aspirated, followed by exposure to 125 \( \mu \text{M} \text{H}_2\text{O}_2 \) in phosphate-buffered saline (PBS) for another 2 h. The control cells were added with the same medium without \( \text{H}_2\text{O}_2 \) and drugs.

**Viability assay**

16HBE cells in 100 \( \mu \text{l} \) RPMI-1640 medium were seeded at a density of \( 1.5 \times 10^5 \) cells per ml in 96-well plates, and the cell viability was determined by the methyl thiazolyl tetrazolium (MTT) assay. Briefly, the supernatants were aspirated, then 120 \( \mu \text{l} \) of MTT (Sigma, St. Louis, MO, USA) (1 mg/ml in PBS) was added to each well, and the cells were incubated at 37°C for 4 h. The supernatants were carefully aspirated, and 150 \( \mu \text{l} \) of dimethyl sulfoxide (DMSO; Sigma) was added to dissolve the formazan crystals. The optical density (OD) was measured at 570 nm using a microplate reader (BIO-RAD Model 680; Biorad, CA, USA). The cell viability ratio was calculated by the following formula:

\[
\text{Viability ratio} \; (\%) = \frac{(\text{OD}_{\text{treated}})}{(\text{OD}_{\text{control}})} \times 100%
\]

**Intracellular determination of malondialdehyde (MDA) and reactive oxygen species (ROS)**

The MDA concentration in the cells was assayed by MDA detection kits (Jiancheng Bioengineering Institute, Nanjing, China). The methods were based on spectrophotometric measurement of the red color produced by the reaction of thiobarbituric acid with MDA.

The production of ROS was measured using 2',7'-dichlorofluorescin diacetate (DCFH-DA; Beyotime Institute of Biotechnology, Jiangsu, China). DCFH-DA passively enters the cells and is then cleaved by the intracellular esterases to form the non-fluorescent DCFH, which is trapped inside the cell. A reaction with ROS results in oxidation of DCFH to the highly-fluorescent dichlorofluorescin (DCF). Briefly, a stock solution of DCFH-DA (10 mM, DMSO) was stored at –20°C. Cells grown in 96-well plates were washed once with PBS before the addition of DCFH-DA working solution (10 \( \mu \text{M} \), complete medium) for 45 min at 37°C. Cells were then washed three times with PBS. The production of ROS was determined by flow cytometry with the FL1 (flow cytometry channel 1) setting on a FACScan flow cytometer (BD Biosciences, San Jose, CA, USA). Each analysis recorded 10,000 events. Fluorescence was also determined at excitation 485 nm / emission 520 nm using a fluorescence microscope (Olympus IX73 microscope; Olympus Co., Ltd., Tokyo). Pictures were taken using an Olympus DP72 charge-coupled device (Olympus Co.). The optical density of the fluorescence was quantified using ImageJ software (NIH, Bethesda, MD, USA).

**Apoptosis detection by flow cytometry and fluorescence microscope**

16HBE cells in 1 ml RPMI-1640 medium were seeded onto 13-mm-diameter coverslips at a density of \( 1.5 \times 10^5 \) cells per ml in 24-well plates. Annexin V-FITC and propidium iodide (PI) (Keygen Biotech, Nanjing, China) were added to 1 ml RPMI-1640 medium were seeded onto 13-mm-diameter coverslips at a density of \( 1.5 \times 10^5 \) cells per ml in 24-well plates. Annexin V-FITC and propidium iodide (PI) (Keygen Biotech, Nanjing, China)
were applied to differentiate apoptotic or necrotic cells from viable ones. The samples were washed twice with PBS. With the exception of the negative control group, 1 μl Annexin V-FITC (20 μg/ml) and 1 μl PI (20 μg/ml) in 100 μl suspensions was added to each well and incubated for 5 min at room temperature in the dark. After aspiration, cells were washed once with PBS. Flow cytometry was employed to detect FITC and PI fluorescence with FL 1 and FL 2 on a FACSScan flow cytometer (BD Biosciences). FITC and PI fluorescence were also determined using a fluorescence microscope (Olympus IX73 microscope). Pictures were taken using an Olympus DP72 charge-coupled device (Olympus Co.). The optical density of the fluorescence was quantified using the ImageJ software.

**Immunofluorescence staining**

16HBE cells in 1 ml RPMI-1640 medium were seeded onto 13-mm-diameter coverslips in 24-well plates at a density of $1.5 \times 10^5$ cells per ml, followed by the process of pretreatment and H$_2$O$_2$ exposure. After aspiration of the supernatants, cells were washed once in PBS and fixed at cold temperature for 10 min with acetone. After thorough washing with PBS, cells were permeabilized with 200 μl 0.3% Triton X-100 (Sigma) in PBS for 5 min. After washing three times in PBS, cells were incubated in a blocking buffer consisting of 10% goat serum (M&C Gene Technology) and 90% PBS and then incubated overnight in the blocking buffer containing protein-specific primary antibody or without the primary antibody overnight at 4°C. Wells were washed and incubated with the secondary antibody for 1 h at room temperature. After thorough washing, cells were incubated in the 2 μg/ml Hoechst 33342 (M&C Gene Technology) to stain nuclei for 5 min. Single optical sections of cells were obtained using a Leica TCS-NT confocal laser scanning microscope (Leica Microsystems, Heidelberg, Germany). The optical density of the fluorescence was quantified by ImageJ software. Antibodies used were as follows: as primary antibodies, p-NF-κB p65 antibody (Ser 536) (1:50, Santa Cruz Biotechnology) and IκB-α antibody (C-21) (1:50, Santa Cruz Biotechnology) were used; the secondary antibody, and horseradish peroxidase (HRP)-conjugated anti-IgG (1:2000; M&C Gene Technology) was used as the secondary antibody. Actin was used as an internal standard. An enhanced chemiluminescence detection system was applied to detect the target proteins. The optical density of each band was quantified by ImageJ software. Levels of IκB-α were normalized against actin.

**Statistical analysis**

Values are expressed as the mean ± S.D. SPSS 16.0 software was used for statistical analysis. Statistical comparisons between groups were evaluated by ANOVA. Values of $P < 0.05$ were considered statistically significant.

**Results**

**Procaterol but not dexamethasone increased the viability of 16HBE cells**

According to the preliminary MTT assays, not less than 1 μM procaterol or 100 μM dexamethasone had cytotoxicity to 16HBE cells (data not shown). Both the concentrations of procaterol and dexamethasone were avirulent in this study. Exposure to 125 μM of H$_2$O$_2$ for 2 h led to about 50% reduction of the cell viability rate. Pretreatment with procaterol for 20 h elevated the cell viability rate in a dose-dependent manner. Pretreatment with procaterol plus dexamethasone could also significantly increase the cell viability rate, but the combined effect was not better than sole pretreatment with an identical dose of procaterol. Dexamethasone could not increase the cell viability (Fig. 1A). Less than 10 nM procaterol could not significantly increase the viability of the cells (data not shown).

**Procaterol but not dexamethasone decreased the intracellular MDA content**

Compared with the Control group, MDA content was significantly increased in the Model group. Pretreatment with procaterol could reduce the intracellular MDA content in a dose-dependent manner. Pretreatment with procaterol plus dexamethasone could also reduce the MDA content, but the combined effect was not better than the sole pretreatment with the identical dose of procaterol. The sole pretreatment with dexamethasone was inefficient (Fig. 1B).
Procaterol but not dexamethasone attenuated the $H_2O_2$-induced ROS production

The green fluorescence intensity of DCF can reflect the ROS production. As shown in Fig. 2 and Supplementary Fig. 1 (available in the online version only), the ROS production was low in the Control group. Compared with the Control group, the ROS production was significantly increased in the Model group. This implies that exposure to $H_2O_2$ led to a higher intracellular ROS production. Pretreatment with procaterol could reduce the intracellular ROS production in a dose-dependent manner. Pretreatment with procaterol plus dexamethasone could also reduce the ROS production, but the rates in this group were not less compared to the sole pretreatment with the identical dose of procaterol. The sole pretreatment with dexamethasone was ineffective.

Procaterol but not dexamethasone altered the 16HBE cell viability (A) and intracellular MDA levels (B). “P1 – P4” (groups pretreated with various doses of procaterol), “D1 – D2” (groups pretreated with different doses of dexamethasone), and “P3 + D2” (group pretreated with 100 nM procaterol plus 1000 nM dexamethasone). The difference between the “P3 + D2” group and the “P3” group is not significant. Data are shown as the mean ± S.D., n = 6 per group. *P < 0.05, compared with the Control group; **P < 0.05, compared with the Model group.

Procaterol but not dexamethasone attenuated the $H_2O_2$-induced 16HBE cells apoptosis

Cells could be distinguished as follows: viable (no staining), early apoptotic (Annexin V⁺PI⁻), and late apoptotic/necrotic (Annexin V⁺PI⁺) (17). As shown in Fig. 3 and Supplementary Fig. 2 (available in the online version only), the rates of the early apoptotic cells and the late apoptotic/necrotic cells were about 10% and 1% in the Control group. Compared with the Control group, exposure to $H_2O_2$ significantly increased the rates of the early apoptotic cells (about 40%) and the late apoptotic/necrotic cells (about 20%–30%) in the Model group. Pretreatment with procaterol could reduce the rates of both the early apoptotic cells and the late apoptotic/necrotic cells in a dose-dependent manner. Pretreatment with procaterol plus dexamethasone could also reduce the rates, but the rates in this group were not less compared to the sole pretreatment with the identical dose of procaterol. The sole pretreatment with dexamethasone was ineffective.

Procaterol but not dexamethasone altered the NF-κB fluorescence intensity

Immunofluorescence analysis showed that the fluorescence intensity of the phosphorylated NF-κB p65 level (Fig. 4) in the 16HBE cells was markedly increased by exposure to $H_2O_2$, compared with the Control group ($P < 0.05$). The fluorescence intensity of the IκB-α level in 16HBE was significantly reduced in the Model group, compared with the Control group (Fig. 5). Pretreatment with procaterol could reduce the fluorescence intensity of the phosphorylated NF-κB p65 level and increase the fluorescence intensity of the IκB-α level in a dose-dependent manner. Pretreatment with procaterol plus dexamethasone could also reduce the fluorescence intensity of the phosphorylated NF-κB p65 level and increase the fluorescence intensity of the IκB-α level. The sole pretreatment with dexamethasone was inefficient (Figs. 4 and 5).

Procaterol but not dexamethasone increased the IκB-α level in the western blot analysis

$H_2O_2$ exposure significantly induced IκB-α degradation (Fig. 6). Pretreatment with procaterol suppressed the $H_2O_2$-induced IκB-α degradation ($P < 0.05$). Pretreat-
ment with procaterol plus dexamethasone could increase the IκB-α level ($P < 0.05$), but pretreatment with dexamethasone did not increase the IκB-α level ($P > 0.1$).

**Discussion**

Exposure to 125 μM H$_2$O$_2$ for 2 h led to significant oxidative stress, reduction of cell viability, and apoptosis. Pretreatment with procaterol could protect 16HBE cells from H$_2$O$_2$-induced damage in a dose-dependent manner. In contrast, pretreatment with dexamethasone was inefficient. Pretreatment with procaterol plus dexamethasone was effective, but the combined effect was not better than sole pretreatment with the identical dose of procaterol. The NF-κB signal pathway was involved in H$_2$O$_2$-induced cellular stress and the inflammatory reaction. Procaterol might indirectly inhibit H$_2$O$_2$-induced activation of the NF-κB pathway due to its anti-oxidation capability.

In this study, exposure to 125 μM H$_2$O$_2$ for 2 h resulted in a 50% reduction of the cell viability, a significant increase in the percentage of apoptotic and necrotic cells, and elevated levels of MDA and ROS. MDA, as a by-product of lipid peroxidation, has been identified in the past as a marker of oxidative damage (18). The excess accumulation of ROS is termed oxidative stress. The strong oxidation property of H$_2$O$_2$ will induce cellular oxidative stress, thereby leading to apoptosis, necrosis, and reduction of the cellular viability. Consistent with this observation, H$_2$O$_2$ exposure reduces the cellular viability in a dose-dependent manner, increases the intracellular levels of MDA and ROS, and increases the percentage of apoptotic and necrotic cells (19, 20).

For the first time, procaterol (25 – 200 nM) was found in our study to mitigate H$_2$O$_2$-induced oxidative stress, MDA and ROS production, reduction of cell viability, and apoptosis in a dose-dependent manner. The concentrations of procaterol in our study were based on both our preliminary experiments and previously published studies. Concentrations between 10 nM and 100 nM are the most commonly used for procaterol in vitro (21, 22). The concentrations in our study are higher than the
physiological plasma concentrations of procaterol in patients after oral dosing, which are between 1 nM and 10 nM (23, 24). The reasons might be as follows: First, the surroundings in vitro are much simpler than the internal physiological environment. Second, inhibition of H$_2$O$_2$-induced oxidative stress might be the dominant pharmacological effect of procaterol in this study, but the situation is different in the clinic. Similarly, in supratherapeutic concentrations, other β$_2$ selective agonists (fenoterol, salbutamol, terbutaline, clenbuterol, salmeterol, or formoterol) can reduce H$_2$O$_2$-induced oxidative stress and cytotoxicity to human leukocytes, myocardial cells, or human alveolus macrophages (25 – 27). Thus, just like other β$_2$-selective agonists, the supratherapeutic concentrations of procaterol have a good intrinsic scavenger function against ROS. The underlying mechanism might be due to hydroxyl groups of the phenol rings of procaterol’s molecular structure could directly react with ROS produced by H$_2$O$_2$ in the 16HBE cells. However, after the process of medium aspiration, the remnant procaterol (a small amount of 25 – 200 nM) could not offset 125 μM H$_2$O$_2$ in the medium. Therefore, the decrease of oxidative stress by procaterol was mainly caused by the alteration of an intracellular component. We found that procaterol could decrease MDA and ROS production in 16HBE cells. So procaterol might improve the level of intracellular antioxidants or antioxidases. These results imply that high doses of procaterol might be useful for treating asthma or COPD through inhibiting the oxidative stress in addition to dilating the bronchus in vivo. Further studies are needed to determine whether the therapeutic or supratherapeutic concentrations of procaterol can scavenge the high oxidant burden in vivo.

In the present study, dexamethasone could not mitigate H$_2$O$_2$-induced oxidative stress, reduction of the cell viability, and apoptosis. Pretreatment with dexamethasone

![Graph showing early apoptotic cell rates](image-url)

**Fig. 3.** Procaterol but not dexamethasone attenuated the early apoptotic rates of 16HBE cells. Annexin V-FITC and PI was not added in the medium of cells in the negative control group. FL 1 (flow cytometry channel 1) was used to detect Annexin V; FL 2 (flow cytometry channel 2) was used to detect PI. The rates of early apoptotic (Annexin V$^+$PI$^-$) cells are presented. The 16HBE cells were pretreated with various concentrations of procaterol (P1 – P4), different concentrations of dexamethasone (D1 – D2) or a combination of drugs (P3 + D2). The early apoptotic cell rate in the “P3 + D2” group is not less than that in the “P3” group. Data are shown as the mean ± S.D., n = 3 per group. *P < 0.05, compared with the Control group; *P < 0.05, compared with the Model group.
Fig. 4. Procaterol but not dexamethasone reduced the fluorescence intensity of the phosphorylated NF-κB p65 level. Cells in the negative control group were not incubated with the p-NF-κB p65 primary antibody. 16HBE cells were stained with anti-p65 (green) and Hoechst 33342 (blue) and imaged by confocal microscopy. The 16HBE cells were pretreated with various concentrations of procaterol (P1 – P4), different concentrations of dexamethasone (D1 – D2), or a combination of drugs (P3 + D2). Scale bar = 25 μm. Data are shown as the mean ± S.D., n = 6 per group. *P < 0.05, compared with the Control group; #P < 0.05, compared with the Model group.
Fig. 5. Procaterol but not dexamethasone increased the immunofluorescence intensity of the IκB-α level. Cells in the negative control group were not incubated with the IκB-α primary antibody. 16HBE cells were stained with anti-IκB-α (green) and Hoechst 33342 (blue) and imaged by confocal microscopy. The 16HBE cells were pretreated with various concentrations of procaterol (P1 − P4), different concentrations of dexamethasone (D1 − D2), or a combination of drugs (P3 + D2). Scale bar = 25 μm. Data are shown as the mean ± S.D., n = 6 per group. *P < 0.05, compared with the Control group; #P < 0.05, compared with the Model group.
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plus procaterol were effective, but the combined effect was not better than the sole pretreatment with the identical dose of procaterol. The concentrations of dexamethasone in our study were also based on both our preliminary experiments and previously published studies. Concentrations of dexamethasone (10 – 1000 nM) are usually used in vitro (15). However, the mean plasma 50% effective concentration (EC\textsubscript{50}) of dexamethasone for treating asthma patients is only between 1.9 nM and 4.2 nM (28). In a previous study, H\textsubscript{2}O\textsubscript{2}-induced oxidative stress also reduces the effect of dexamethasone on the IL-8 release in the airway epithelium (29). In terms of other cells, although dexamethasone reduces H\textsubscript{2}O\textsubscript{2}-induced macrophages apoptosis in a few studies (15), many more studies found that dexamethasone could not reduce H\textsubscript{2}O\textsubscript{2}-induced oxidative stress and apoptosis (16, 25). Moreover, dexamethasone can directly induce oxidative stress in osteoblasts and hippocampal cells (30). As far as other glucocorticoids are concerned, although fluticasone and budesonide showed protective effects on H\textsubscript{2}O\textsubscript{2}-induced inflammation in alveolar macrophages in a previous study (27), budesonide could not protect 16HBE cells from H\textsubscript{2}O\textsubscript{2}-induced oxidative stress (31), and budesonide had no effects on H\textsubscript{2}O\textsubscript{2} production in vitro (32). Treatment with fluticasone also has no effects on the concentrations of expired H\textsubscript{2}O\textsubscript{2} in patients (33). Furthermore, it is likely that H\textsubscript{2}O\textsubscript{2} induces oxidative stress; thereby it causes the phenomenon of GR. GR is a condition where all or parts of the body cannot respond to glucocorticoids.

Oxidative stress is a causative factor of GR (34). Therefore, the glucocorticoids may be not recommended for the treatment of asthma or COPD complicated with severe oxidative stress.

According to immunofluorescence and western blot analysis, H\textsubscript{2}O\textsubscript{2} exposure increased the phosphorylated NF-\kappaB p65 level in the 16HBE cells, and reduced the I\kappaB-\alpha level. This indicates that the NF-\kappaB pathway is involved in H\textsubscript{2}O\textsubscript{2}-induced cellular stress, and H\textsubscript{2}O\textsubscript{2} exposure leads to the inflammatory reaction. Consistent with the protective effects, procaterol suppressed H\textsubscript{2}O\textsubscript{2}-induced NF-\kappaB activation in a dose-dependent manner; dexamethasone plus procaterol depressed the NF-\kappaB activation to some extent, but the sole pretreatment with dexamethasone did not make a difference. Many studies have reported that H\textsubscript{2}O\textsubscript{2} exposure can stimulate the NF-\kappaB pathway in different cell types, including intestinal epithelial cells, human lens epithelial cells, gastric epithelial cells, and human lung cancer epithelial cells (8, 35 – 37). H\textsubscript{2}O\textsubscript{2} exposure can also activate the NF-\kappaB pathway in the 16HBE cells (6, 7). However, the effect on the NF-\kappaB pathway appears to be cell-specific. In some other cell types, including monocytic cells, astrocytoma, and human umbilical vein endothelial cells, the activation of the NF-\kappaB pathway is insensitive to H\textsubscript{2}O\textsubscript{2} (38). How H\textsubscript{2}O\textsubscript{2} activates NF-\kappaB in the 16HBE cells is potentially important and yet to be investigated.

Nicotinamide adenine dinucleotide phosphate oxidase (NOX) is a key enzyme involved in intracellular ROS
production. NOX can generate intracellular \( \text{H}_2\text{O}_2 \) (39). No study has reported the relationship between NOX and procaterol. \( \text{H}_2\text{O}_2 \) can lead to protein kinase B (Akt) phosphorylation and activate the phosphatidylinositol-3-kinase (PI3-K)/Akt pathway at the cellular level (40, 41). \( \text{H}_2\text{O}_2 \) can also induce the activation of NF-\( \kappa \)B through the PI3-K/Akt pathway (42). Procaterol can elicit an endothelial nitric oxide synthase (eNOS)-dependent relaxation through the PI3-K/Akt pathway in mouse pulmonary artery (43). So procaterol can not inhibit the activation of the PI3-K/Akt pathway. Without the involvement of \( \text{H}_2\text{O}_2 \), procaterol was reported to inhibit the inflammatory reactions through the NF-\( \kappa \)B pathway (22, 44). In contrast, procaterol was also reported to inhibit poly(I:C)-induced inflammation in bronchial epithelial cells via some pathways, but not the NF-\( \kappa \)B pathway (45). In the present study, the NF-\( \kappa \)B signal pathway is involved in \( \text{H}_2\text{O}_2 \)-induced oxidative stress and the inflammatory reaction. The supratherapeutic concentrations of procaterol have a good intrinsic scavenger function against ROS. Therefore, procaterol may directly protect 16HBE cells from \( \text{H}_2\text{O}_2 \)-induced oxidative stress; and it may indirectly inhibit \( \text{H}_2\text{O}_2 \)-induced activation of the NF-\( \kappa \)B pathway due to its antioxidant capability.

Without the involvement of oxidative stress, dexamethasone can inhibit inflammation in bronchial epithelial cells through suppression of the NF-\( \kappa \)B pathway (34, 46). It was also reported that dexamethasone inhibits \( \text{H}_2\text{O}_2 \)-induced inflammation in neutrophils and macrophages through suppressing the NF-\( \kappa \)B pathway (15, 47). However, dexamethasone was also reported to induce apoptosis of rat thymocytes by up-regulating the NF-\( \kappa \)B pathway (48). These paradoxical effects of dexamethasone may be due to the different cell types and culture conditions.

Taken together, exposure to 125 \( \mu \text{M} \) \( \text{H}_2\text{O}_2 \) for 2 h can construct the 16HBE cells model of oxidative stress. The supratherapeutic concentrations of procaterol can scavenge the high oxidant burden induced by \( \text{H}_2\text{O}_2 \) exposure in the 16HBE cells. The NF-\( \kappa \)B pathway may be involved in the pathogenic mechanisms of \( \text{H}_2\text{O}_2 \). Procaterol may indirectly inhibit \( \text{H}_2\text{O}_2 \)-induced activation of NF-\( \kappa \)B pathway due to its capability of antioxidation. The glucocorticoids may be not recommended for the treatment of asthma or COPD complicated with severe oxidative stress.

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**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

**References**

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