Hydrogen peroxide upregulates IL-1β-induced cyclooxygenase-2 expression in human pulmonary epithelial cells

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Abstract

Objective. To investigate the effect of hydrogen peroxide (H₂O₂) on interleukin-1β (IL-1β)-induced-cyclooxygenase-2 (COX-2) expression in human pulmonary epithelial cells (HPECs). Methods. HPEC were grouped into IL-1β group, H₂O₂ group and IL-1β plus H₂O₂ group. COX-2 mRNA expression was determined by reverse transcription PCR, and prostaglandin E2 (PGE2) was detected by ELISA. Results. The COX-2 mRNA expressions induced by 1, 5 and 10 mg/L of IL-1β increased. More PGE2 were released by HPECs treated with 5 mg/L and 10 mg/L of IL-1β. The COX-2 mRNA expression was elevated induced by 0.1, 0.25 and 0.5 mmol/L of both H₂O₂ and IL-1β stimulation compared with IL-β group and with the control group. The concentration of PGE2 released by HPEC in H₂O₂ plus IL-1β group was significantly higher than that in control group and the IL-β group. Conclusion. H₂O₂ upregulates IL-1β-induced COX-2 expression in HPEC at transcriptional level. [Life Science Journal. 2007; 4(4): 33 – 37 ] (ISSN: 1097 – 8135).

Keywords: cyclooxygenase-2; interleukin-1β; hydrogen peroxide; human pulmonary epithelial cells

1 Introduction

Arachidonic acid (AA) and its bioactive derivatives, prostaglandins (PGs), are important mediators of many physiological and pathophysiological processes of human airway[1]. Cyclooxygenase (COX) is a rate-limiting enzyme in the biosynthesis of prostanoids. COX exists in two isoforms. COX-1 is often expressed in many tissues, and is probably responsible for the production of prostaglandins under physiological conditions, maintaining hemostasis[2]. COX-2 is released by many cells, including pulmonary epithelial cell (HPECT) with pro-inflammatory stimuli such as IL-1β, TNF-α, bacterial lipopolysaccharide (LPS)[3]. Furthermore, COX-2 is regarded to be the isoform to produce proinflammatory prostanoids involved in airway inflammation.

Reactive oxygen intermediates (ROIs) play important roles in airway inflammation process[4]. ROIs include radicals, such as superoxide anion (O₂⁻), hydroxyl radical (OH·) and molecules, such as H₂O₂ and ozone. There is increasing evidence that ROIs are not only common injury mediators, but also serve as the second messenger in signal transduction leading to gene expression[5]. It was recently reported that the hydroxyl radical scavengers DMSO, as well as di- and tetramethylthlthiourea, inhibited IL-1β-, TNF-α-, LPS-induced COX-2 expression in rat mesangial cells, indicating ROIs upregulate IL-1β-induced-COX-2 expression[6]. On the other hand, there was an opposite report that ROIs inhibited COX-2 expression induced by IL-1β in cerebral microvascular smooth muscle cells[7]. Up to now there has been not an agreement on how ROIs affect COX-2 expression induced by IL-1β. Furthermore, whether H₂O₂ regulates IL-1β-induced-COX-2 expression in HPEC has been not reported. Therefore we investigated the effect of H₂O₂ on IL-1β-induced-COX-2 expression in HPEC by RT-PCR and ELISA. We demonstrated that H₂O₂ upregulated IL-1β-induced COX-2 expression in HPEC at transcriptional level.

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2 Materials and Methods

2.1 Cell culture

HPEC (CCL-185) were obtained from American Type Culture Collection and cultured in Dulbecco’s Modified Eagle Medium (DMEM) containing 10% FCS and penicillin/streptomycin (100 U/ml) in a humidified gas environment of 95% air and 5% carbon dioxide at 37 ºC. HPEC were subcultured in 0.25% trypsin.

2.2 Cell treatment

HPEC were inoculated at a density of 5 × 10⁴ cells/ml in 25 cm² tissue culture flasks. After reaching confluence, the cells were moved into DMEM with 1% albumin, divided into four groups, and treated respectively as follows:

1. 1, 5 and 10 mg/L IL-1β for 6 hours;
2. 5 mg/L IL-1β for 3, 6, 9 and 12 hours;
3. 0.1, 0.25 and 0.5 mmol/L H₂O₂ for 6 hours;
4. 0.1, 0.25 and 0.5 mmol/L H₂O₂ for 0.5 hour and then 5 mg/L IL-1β for 6 hours.

After the cells had been treated for the required time, the cells were harvested to detect COX-2 mRNA by RT-PCR, and the medium supernatant was collected to determine the concentration of PGE2 by ELISA. For the control cells, only the culture medium was changed without any reagent added.

2.3 RT-PCR

Total RNA was extracted according to kit instructions (Sangon Co., Shanghai). Samples (2 μg) of total RNA with hexametric random primers were heated to 75 ºC for 5 minutes and placed on ice. RNA was then reverse transcribed to cDNA in a 25 μl reaction containing 200 U Moloney murine leukemia virus reverse transcriptase (M-MuLV RT), 40 units of RNAsin, 5 × buffer 5 μl, 10 mmol/L dNTP 2 μl at 42 ºC for 1 hour.

To amplify COX-2 mRNA, specific intron-spanning 20-mer primers were designed on published cDNA sequences obtained from Genebank. The primer sequences were as follows: COX-2 (728 bp) forward: 5'-TGAAACCCACTCCAAACACA-3' and reverse: 5'-TGGAACAACTGCTCATCACC-3'; β-actin (375 bp) forward: 5'-CGTGACATTAAGGAGAAGCTGTGC-3' and reverse: 5'-CTCAGGAGGAGCAATGATCTTGAT-3'.

Each 25 μl reaction mixture consisted of 25 mmol/L MgCl₂, 1.5 μl; 50 mmol/L specific COX-2 primers, 0.5 μl, 50 mmol/L specific β-actin primers 0.5 μl, cDNA 3 μg, Amplitaq DNA polymerase 2.5 U, 10 mmol/L dNTP 0.5 μl, 10 × buffer 2.5 μl. Reaction conditions were as follows: 94 ºC for 5 minutes, 94 ºC for 30 seconds, 55 ºC for 1 minutes, 72 ºC for 2 minutes, 35 cycles, then 72 ºC for 10 minutes.

Products were electrophoresed on a 1.5% agarose gel and evaluated by the Digital Gel Imaging Analyst. The relative values of COX-2 mRNA were calculated using β-actin mRNA as standard.

2.4 Data analysis

All data were expressed as X ± s. Difference between mean values of multiple groups were analyzed by one-way ANOVA with a Newman-Keuls test. P less than 0.05 was considered statistically significant.

3 Results

3.1 COX-2 mRNA expression in HPEC stimulated by different concentrations of IL-1β

COX-2 mRNA expression induced by 1 mg/L, 5 mg/L and 10 mg/L of IL-1β were (143.10 ± 7.16)%, (179.90 ± 9.00)%, and (190.00 ± 9.50)%, respectively, significantly higher than (32.90 ± 1.65)% in the control group (P < 0.05). The COX-2 mRNA expressions induced by 5 mg/L and 10 mg/L IL-1β were significantly higher than the IL-1β group (P < 0.05). The expression of COX-2 mRNA stimulated by IL-1β in HPEC showed time-dependent manner (Figure 1).

3.2 COX-2 mRNA expression in HPEC stimulated by IL-1β at different time

Table 1 showed that the PGE2 released by HPEC treated with 5 mg/L and 10 mg/L of IL-1β were significantly higher than the control group, and higher than the cells treated with 1 mg/L of IL-1β. The concentration of PGE2
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3.4 Effect of \( \text{H}_2\text{O}_2 \) on COX-2 mRNA expression in HPEC.

As shown in Figure 3, the COX-2 mRNA expression in HPEC treated with 0, 0.1, 0.25 and 0.5 mmol/L of \( \text{H}_2\text{O}_2 \) were (69.60 ± 26.82)\%, (68.73 ± 22.42)\%, (68.63 ± 36.26)\%, (62.03 ± 32.12)\%, respectively. These data indicated \( \text{H}_2\text{O}_2 \) didn’t affect COX-2 expression in HPEC (\( P > 0.05 \)).

3.5 COX-2 mRNA expression in HPEC induced by IL-1\( \beta \) and different concentrations of \( \text{H}_2\text{O}_2 \)

Figure 4 showed that the COX-2 mRNA expression induced by 0.1, 0.25 and 0.5 mmol/L of \( \text{H}_2\text{O}_2 \) in the cells pretreated with IL-1\( \beta \) were (149.20 ± 7.46)\%, (189.60 ± 9.48)\%, (239.10 ± 11.96)\%, respectively, significantly higher than (41.60 ± 2.08)\% in the control group and (66.07 ± 3.70)\% in the IL-\( \beta \) group (\( P < 0.05 \)), respectively. The COX-2 mRNA expressions induced by 0.25 mmol/L and 0.5 mmol/L \( \text{H}_2\text{O}_2 \) of cells pretreated with IL-1\( \beta \) were significantly higher than that of treated with 0.1 mmol/L of \( \text{H}_2\text{O}_2 \) plus IL-1\( \beta \). The COX-2 mRNA expression induced by 0.5 mmol/L of \( \text{H}_2\text{O}_2 \) plus IL-1\( \beta \) was significantly higher than that in cells treated with 0.25 mmol/L of \( \text{H}_2\text{O}_2 \) and IL-1\( \beta \). The data also showed \( \text{H}_2\text{O}_2 \) up-regulated IL-1\( \beta \)-induced COX-2 mRNA expression in HPECs in a concentration-dependent manner.

3.6 PGE2 release from HPEC induced by IL-1\( \beta \) and different concentrations of \( \text{H}_2\text{O}_2 \)

Figure 2. Time-dependent effect of IL-1\( \beta \) on COX-2 mRNA expression in HPEC (\( n = 3 \)). *: \( P < 0.05 \), compared with control. #: \( P < 0.05 \), compared with the cell group at 3 hours. ♦: \( P < 0.05 \) compared with the cell group at 6 hours. Δ: \( P < 0.05 \), compared with the cell group at 9 hours.

Table 1 showed that PGE2 released by HPEC treated with 0.1, 0.25 and 0.5 mmol/L of \( \text{H}_2\text{O}_2 \) and 5 mg/L IL-1\( \beta \) were significantly higher than that in the control group. The concentration of PGE2 released by cells treated with 0.25 and 0.5 mmol/L of \( \text{H}_2\text{O}_2 \) and IL-1\( \beta \) was significantly higher than that in the cells treated with IL-\( \beta \) alone. The PGE2 released by cells treated with 0.5 mmol/L of \( \text{H}_2\text{O}_2 \) pretreated with 5 mg/L IL-1\( \beta \) were significantly higher than that in the cells treated with 0.1 and 0.25 mmol/L of \( \text{H}_2\text{O}_2 \) and 5 mg/L IL-1\( \beta \), respectively. These data indicated the release of PGE2 in HPEC induced by IL-1\( \beta \) was concentration-dependent on \( \text{H}_2\text{O}_2 \).

4 Discussion

Airway epithelium injury is one of important manifestations of chronic airway inflammation, which is a critical feature in the pathophysiological processes of many diseases such as chronic obstructive pulmonary disease (COPD) and asthma. Airway epithelium is the layer between the environment and the delicate structures of the lung, acting as the first physical barrier. So it is the target of various proinflammatory agents causing epithelial cells injury, increased secretions of mucus, changes in ciliary and the influx of inflammatory cells. ROIs, a kind of important proinflammatory agent, extensively participate in airway inflammation process\(^{[4,8]}\). For example, ROIs inactivate antiproteinases, damage airspace epithelium, hyperscrete mucus, increase inflammatory mediators in airway of patients with COPD\(^{[4]}\). Prostaglandins (PGs) are extensively involved in phys-
**Figure 3.** Effect of H\(_2\)O\(_2\) on COX-2 mRNA expression in HPEC (n = 3). There was no significant difference in the relative amount of COX-2 mRNA among 4 cell groups. *P > 0.05, respectively.

**Figure 4.** Concentration-dependent effect of H\(_2\)O\(_2\) on IL-1β-induced COX-2 mRNA expression in HPEC (n = 3). *: P < 0.05, vs. control; #: P < 0.05, vs. cells treated with 1 mg/L of IL-1β alone; #: P < 0.05, vs. cells treated with 5 mg/L of IL-1β alone; #: P < 0.05, vs. cells treated with 0.1 mmol/L of H\(_2\)O\(_2\) combined with IL-1β; #: P < 0.05, vs. cells treated with 0.25 mmol/L of H\(_2\)O\(_2\) combined with IL-1β.

**Table 1.** The release of PGE2 induced by various concentrations of IL-1β alone and by H\(_2\)O\(_2\) pretreated with IL-1β in HPEC

<table>
<thead>
<tr>
<th>IL-1β (mg/L)</th>
<th>H(_2)O(_2) (mmol/L)</th>
<th>PGE2 (g/L, × 10(^{-6}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>10.49 ± 0.36</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>15.3 ± 0.22</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>20.86 ± 5.22(^\ddagger)</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>31.16 ± 2.64(^*)(^\ddagger)</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>27.01 ± 5.15(^*)</td>
</tr>
<tr>
<td>5</td>
<td>0.25</td>
<td>32.79 ± 3.01(^*)(^\ddagger)</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>41.13 ± 0.41(^*)(^\ddagger)</td>
</tr>
</tbody>
</table>

*: P < 0.05, vs. control; #: P < 0.05, vs. cells treated with 1 mg/L of IL-1β alone; #: P < 0.05, vs. cells treated with 5 mg/L of IL-1β alone; #: P < 0.05, vs. cells treated with 0.1 mmol/L of H\(_2\)O\(_2\) combined with IL-1β; #: P < 0.05, vs. cells treated with 0.25 mmol/L of H\(_2\)O\(_2\) combined with IL-1β.

Feng et al reported that the hydroxyl radical scavengers DMSO, as well as di- and tetramethylthiourea, inhibited IL-1, TNF-α, and LPS-induced COX-2 expression in rat mesangial cells, suggesting ROIs up-regulated COX-2 expression induced by IL-1β\(^[6]\). In contrast to this result, Fang et al found that the oxygen radical scavenger pyrrolidine dithiocarbamate enhanced IL-1β-induced COX-2 expression in murine cerebral microvascular smooth muscle cells and further draw a conclusion that ROIs may down-regulate COX-2 mRNA expression induced by IL-1β\(^[7]\). In the present research, we demonstrated that IL-1β increased COX-2 mRNA expression in a concentration and time-dependent manner. The increase was further enhanced by H\(_2\)O\(_2\) in a concentration-dependent manner. Our observation is consistent with Feng’s conclusion. These apparently conflicting results might attribute to differences in cell types, cell culture conditions, and the amount or method of stimulation agents.

In the present research, we demonstrated that H\(_2\)O\(_2\) caused a rapid increase of COX-2 mRNA induced by IL-1β, which indicated it involved transcriptional control. Recent study suggested that ROIs might play an important role in inflammation process as mediators of injury and potentially in signal transduction leading to gene expression\(^[6]\). It is well established that nucleotide sequence of 1.8 kb of promoter region of 5'-flanking region of human COX-2 gene contains a TATA box and a number of potential regulatory elements\(^[11]\). Two NF-κB binding sites are present in the COX-2 gene promoter sequence. Many investigations suggest COX-2 gene expression is associated with the activation of NF-κB. In amnion epithelial cell line (WISH), NF-κB and AP-1 are required for COX-2 gene expression\(^[12]\). Nuclear factor IL-6 and NF-κB initiate COX-2 gene expression induced by TNF-α in...
MC3T3-E1 cells. Further study demonstrated that ROIs have been implicated in NF-κB-induced COX-2 expression. ROIs activate NF-κB by the inhibitory subunit (IκB) releasing from NF-κB complex and then permit migration of the active NF-κB dimmer to nucleus, where it can bind to DNA and initiate transcription. In addition, H$_2$O$_2$ has been observed to stimulate degradation of IκB. Therefore, ROIs might initiate transcription through the activation of NF-κB, and finally causes COX-2 expression.

In our research, groups treated by different concentrations of H$_2$O$_2$ weren’t different from the control group, indicating H$_2$O$_2$ didn’t influence the COX-2 expression in HPEC. This opinion was in consistent with the observations of others. Nakamura et al. believed ROIs upregulated COX-2 mRNA expression in bovine luteal cells$^{[13]}$. There was similar finding when newborn rat cardiomyocytes were directly treated by H$_2$O$_2$.$^{[14]}$ On the contrary, Fang et al. reported H$_2$O$_2$ downregulated COX-2 expression.$^{[15]}$ The reason was that infection with adenoviral vectors containing cDNA for human catalase (AdCAT) resulted in induction of COX-2 in cerebral microvascular endothelial cells lysates. Therefore, after direct stimulation, the effect of ROIs on COX-2 expression needs to be assessed in future studies.

The current results showed that H$_2$O$_2$ upregulated COX-2 expression induced by IL-1β in HPEC, and caused PGs release. As a big family, PGs contain a number of members exerting different functions under different situations in vivo. PGD$_2$, PGF$_2$α, TXA$_2$ can cause bronchoconstriction, amplify airway inflammation$^{[16]}$. In contrast, PGE$_2$ is thought to play a role in the down-regulation of allergic inflammation and modulated histamine-induced bronchoconstriction response$^{[17]}$, which means PGE$_2$ is potentially bronchoprotective. These data indicate that H$_2$O$_2$ might participate in airway inflammation through the up-regulation of COX-2 expression to synthesize different PGs.

5 Conclusion

H$_2$O$_2$ upregulates IL-1β-induced COX-2 expression in HPEC at transcriptional level, and further enhanced PGE$_2$ release.

References