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

Zhigang Yang[1,2], Ping Chen[2,*], Rui Zhou[2], Xudong Xiang[2]

[1]*Department of Respiratory Medicine, Henan Provincial People's Hospital, Zhengzhou, Henan* 450003*, China;* [2]*Department of Respiratory Medicine, The Second Hospital of Xiang Ya Medical College of Central South University, Changsha, Hunan* 410011*, China*

Received September 19, 2007

Abstract

Objective. To investigate the effect of hydrogen peroxide (H_2O_2) 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, H_2O_2 group and IL-1β plus H_2O_2 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 H_2O_2 and IL-1β stimulation compared with IL-β group and with the control group. The concentration of PGE2 released by HPEC in H_2O_2 plus IL-1β group was significantly higher than that in control group and the IL-β group. *Conclusion.* H_2O_2 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 (HPEC) 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 (O^2-), hydroxyl radical ($OH\cdot$) and molecules, such as H_2O_2 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 H_2O_2 regulates IL-1β-induced-COX-2 expression in HPEC has been not reported. Therefore we investigated the effect of H_2O_2 on IL-1β-induced-COX-2 expression in HPEC by RT-PCR and ELISA. We demonstrated that H_2O_2 upregulated IL-1β-induced COX-2 expression in HPEC at transcriptional level.

☆Supported by Social Development Grants of Hunan Province, No. 01SSY2008-38.

*Corresponding author. Email: chenping101@hotmail.com

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 5×10^4 cells/ml in 25 cm^2 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 H_2O_2 for 6 hours;

(4) 0.1, 0.25 and 0.5 mmol/L H_2O_2 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[1] 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 $MgCl_2$, 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 sencods, 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 $\bar{X} \pm s$. 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 (Figure 1).

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

COX-2 mRNA expression induced in HPEC by 5 mg/L IL-1β for 3, 6, 9 and 12 hours were (106.70 ± 5.34)%, (126.30 ± 6.32)%, (161.60 ± 8.08)%, and (149.60 ± 7.48)%, significantly higher than (73.00 ± 3.65)% in the control group ($P < 0.05$). The COX-2 mRNA expression induced by IL-1β for 6 hours, 9 hours and 12 hours were significantly higher than that in the cell group of 3 hours ($P < 0.05$, respectively). The relative amount of COX-2 mRNA expression induced by IL-1β for 9 hours and 12 hours was significantly higher than that in the cell group of 6 hours ($P < 0.05$). The COX-2 mRNA expression induced by IL-1β for 12 hours was significantly lower than that in the cell group of 9 hours ($P < 0.05$). This results showed that the COX-2 mRNA was detectable as early as at 3 hours, peaked at 9 hours and began to drop at 12 hours, which indicated that IL-1β induced COX-2 mRNA expression in a time-dependent manner (Figure 2).

3.3 PGE2 release from HPEC stimulated with different concentrations of IL-1β

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

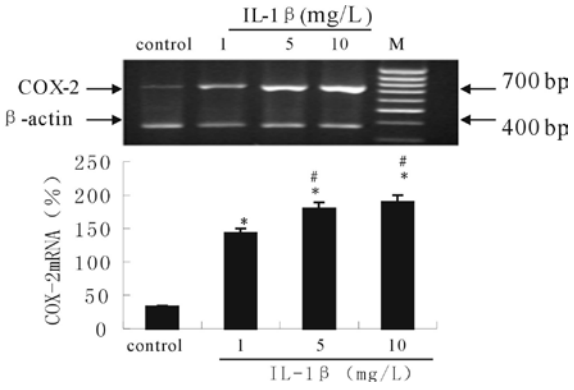

Figure 1. Concentration-dependent effect of IL-1β on COX-2 mRNA expression in HPEC (n = 3). *: $P < 0.05$, compared with control; #: $P < 0.05$, compared with cells treated with 1 mg/L IL-1β.

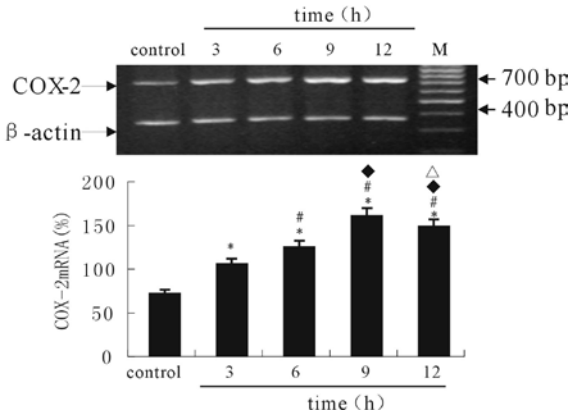

Figure 2. Time-dependent effect of IL-1β on COX-2 mRNA expression in HPEC (n = 3). *: $P < 0.05$, compared with control. #: $P < 0.05$, compared with the cell group at 3 hours. ♦: $P < 0.05$ compared with the cell group at 6 hours, Δ: $P < 0.05$, compared with the cell group at 9 hours.

released by cells treated with 10 mg/L of IL-1β was significantly higher than that in the cells treated with 5 mg/L IL-1β. The PGE2 release induced by IL-1β in HPEC were concentration-dependent.

3.4 Effect of H_2O_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 H_2O_2 were (69.60 ± 26.82)%, (68.73 ± 22.42)%, (68.63 ± 36.26)%, (62.03 ± 32.12)%, respectively. These data indicated H_2O_2 didn't affect COX-2 expression in HPEC ($P > 0.05$).

3.5 COX-2 mRNA expression in HPEC induced by IL-1β and different concentrations of H_2O_2

Figure 4 showed that the COX-2 mRNA expression induced by 0.1, 0.25 and 0.5 mmol/L of H_2O_2 in the cells pretreated with IL-1β 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-β group ($P < 0.05$), respectively. The COX-2 mRNA expressions induced by 0.25 mmol/L and 0.5 mmol/L H_2O_2 of cells pretreated with IL-1β were significantly higher than that of treated with 0.1 mmol/L of H_2O_2 plus IL-1β. The COX-2 mRNA expression induced by 0.5 mmol/L of H_2O_2 plus IL-1β was significantly higher than that in cells treated with 0.25 mmol/L of H_2O_2 and IL-1β. The data also showed H_2O_2 up-regulated IL-1β-induced COX-2 mRNA expression in HPECs in a concentration-dependent manner.

3.6 PGE2 release from HPEC induced by IL-1β and different concentrations of H_2O_2

Table 1 showed that PGE2 released by HPEC treated with 0.1, 0.25 and 0.5 mmol/L of H_2O_2 and 5 mg/L IL-1β 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 H_2O_2 and IL-1β was significantly higher than that in the cells treated with IL-β alone. The PGE2 released by cells treated with 0.5 mmol/L of H_2O_2 pretreated with 5 mg/L IL-1β were significantly higher than that in the cells treated with 0.1 and 0.25 mmol/L of H_2O_2 and 5 mg/L IL-1β, respectively. These data indicated the release of PGE2 in HPEC induced by IL-1β was centration-dependent on H_2O_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 macus, changes in cilliary 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, hypersecrete mucus, increase inflammatory mediators in airway of patients with COPD[4].

Prostaglandins (PGs) are extensively involved in phys-

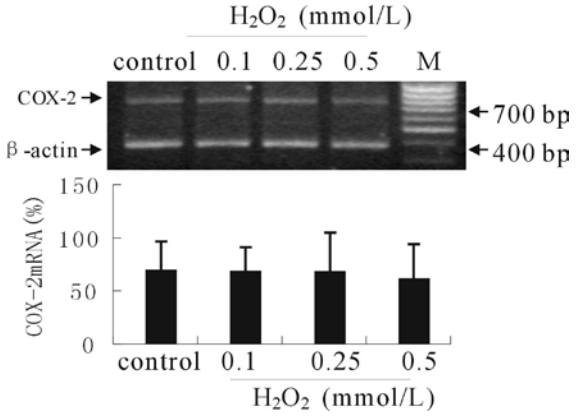

Figure 3. Effect of H_2O_2 on COX-2 mRNA expression in HPEC (n = 3). There was no significant difference in the relative amount of COX-2 mRNA among 4 cell groups. $P > 0.05$, respectively.

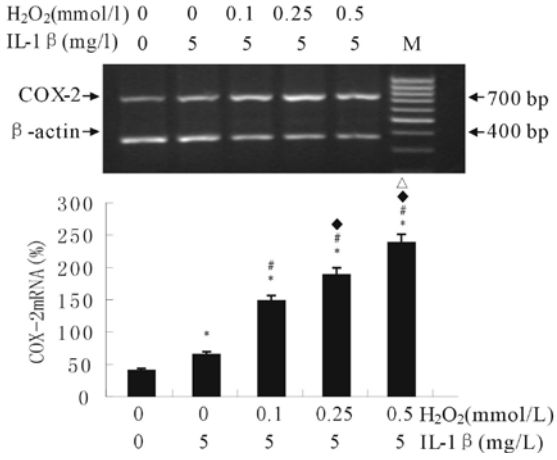

Figure 4. Concentration-dependent effect of H_2O_2 on IL-1β-induced COX-2 mRNA expression in HPEC (n = 3). *: $P < 0.05$, compared with control. #: $P < 0.05$, compared with cells treated with 5 mg/L of IL-1β alone; ♦: $P < 0.05$, compared with cells treated with 0.1 mmol/L of H_2O_2 in the presence of IL-1β; Δ: $P < 0.05$, compared with cells treated with 0.25 mmol/L of H_2O_2 in the presence of IL-1β.

iological and pathophysiological process of airway[1]. COX is a rate-limiting enzyme in the biosynthesis of PGs. COX exists in two isoforms. COX-1 is rutinely expressed whereas COX-2 is expressed when stimulated by various cytokines[3]. IL-1β, is proven to be an important mediator in airway inflammation[9,10]. Recent evidence suggests that increased IL-6, IL-1β, TNF-α and IL-8 have been measured in sputum of patients with COPD, with further increase during exacerbation[10]. Therefore, investigating the effect of ROIs on COX-2 expression induced by IL-1β may be of particular importance in the understanding of pathogenesis of ROIs and the nature of airway inflammation.

Table 1. The release of PGE2 induced by various concentrations of IL-1β alone and by H_2O_2 pretreated with IL-1β in HPEC

IL-1β (mg/L)	H_2O_2 (mmol/L)	PGE2 (g/L, × 10^{-6})
0	0	10.49 ± 0.36
1	0	15.3 ± 0.22
5	0	$20.86 \pm 5.22^{*\#}$
10	0	$31.16 \pm 2.64^{*\#\blacktriangle}$
5	0.1	$27.01 \pm 5.15^{*\blacktriangle}$
5	0.25	$32.79 \pm 3.01^{*\blacktriangle\blacktriangledown}$
5	0.5	$41.13 \pm 0.41^{*\blacktriangle\blacktriangledown\Delta}$

*: $P < 0.05$, *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 0.1mmol/L of H_2O_2 combined with IL-1β; Δ: $P < 0.05$, *vs.* cells treated with 0.25 mmol/L of H_2O_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-regulted 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 expresson 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_2O_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_2O_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 subnit (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_2O_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_2O_2 weren't different from the control group, indicating H_2O_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 leuteal cells[13]. There was similar finding when newborn rat cardiomyocytes were directly treated by H_2O_2[14]. On the contrary, Fang *et al* reported H_2O_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_2O_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 situation *in vivo*. PGD2, PGF2a, TXA2 can cause bronchoconstriction, amplify airway inflammation[16]. In contrast, PGE2 is thought to play a role in the down-regulation of allergic inflammation and modulated histamine-induced bronchoconstriction response[17], which means PGE2 is potentially bronchoprotective. These data indicate that H_2O_2 might participate in airway inflammation through the up-regulation of COX-2 expression to synthize different PGS.

5 Conclusion

H_2O_2 upregulates IL-1β-induced COX-2 expression in HPEC at transcriptional level, and further enhanced PGE2 release.

References

1. Watkins DN, Peroni DJ, Lenzo JC, *et al*. Expression and localization of COX-2 in human airways and cultured airway epithelial cells. Eur Respir J 1999; 13: 999 – 1007.
2. Mitchell JA, Belvisi MG, Akarasereenont P, *et al*. Induction of cyclooxygenase-2 by cytokines in human pulmonary epithelial cells: regulation by dexamethasone. Br J Pharmacol 1994; 113: 1008 – 14.
3. Wu KK. Cyclooxygenase-2 induction: molecular mechanism and pathophysiologic roles. J Lab Clin Med 1996; 128: 242 – 5.
4. Barnes PJ. Reactive oxygen species and airway inflammation. Free Radic Boil Med 1990; 9: 235 – 43.
5. Casola A, Burger N, Liu T, *et al*. Oxidant tone regulates RANTES gene expression in airway epithelial cells infected with respiratory syncytial virus. Role in viral-induced interferon regulatory factor activation. J Biol Chem 2001; 276: 19715 – 22.
6. Feng L, Xia Y, Garcia GE, *et al*. Involvement of reactive oxygen intermediates in cyclooxygenase-2 expression induced by interleukin-1, tumor necrosis factor-alpha, and lipopolysaccharide. J Clin Invest 1995; 95: 1669 – 75.
7. Fang X, Chen P, Moore SA. The oxygen radical scavenger pyrolidine dithiocarbamate enhances interleukin-1β-induced cyclooxygenase-2 expression in cerebral microvascular smooth muscle cells. Microvasc Res 2002; 64: 405 – 13.
8. MacNee W. Oxidants/antioxidants and chronic obstructive pulmonary disease: pathogenesis to therapy. Novartis Found Symp 2001; 234: 169 – 85.
9. Hernandez A, Omini C, Daffonchio L. Interleukin-1 beta: a possible mediator of lung inflammation and airway hyperreactivity. Pharmacol Res 1991; 24: 385 – 93.
10. Chung KF. Cytokines in chronic obstructive pulmonary disease. Eur Respir J Suppl 2001; 34: 50s – 9s.
11. Tazawa R, Xu XM, Wu KK, *et al*. Characterization of the genomic structure, chromosomal location and promoter of human prostaglandin H synthase-2 gene. Biochem Biophys Res Commun 1994; 203: 190 – 9.
12. Allport VC, Slater DM, Newton R, *et al*. NF-KappaB and AP-1 are required for cyclooxygenase 2 gene expression in amnion epithelial cell line (WISH). Mol Hum Report 2000; 6: 561 – 5.
13. Nakamura T, Sakamoto K. Reactive oxygen species upregulates cyclooxygenase-2, P53, and Bax mRNA expression in bovine luteal cells. Biochem Biophys Res Commun 2001; 284: 203 – 10.
14. Adderley SR, Fitzgerald DJ. Oxidative damage of cardiomyocytes is limited by extracellular regulated kinases 1/2-mediated induction of cyclooxygenase-2. J Biol Chem 1999; 234: 5038 – 46.
15. Fang X, Moore AS, Nwankwo OJ, *et al*. Induction of cyclooxygenase-2 by overexpression of the human catalase gene in cerebral microvascular endothelial cells. J Neurochem 2000; 75: 614 – 23.