Calcitonin gene-related peptide protects type II alveolar epithelial cells from hyperoxia-induced DNA damage and cell death

HONGMIN FU, TIESONG ZHANG, RONGWEI HUANG, ZHEN YANG, CHUNMING LIU, MING LI, FANG FANG and FENG XU

1Department of Pediatric Internal Medicine, Children's Hospital, Kunming Medical University, Kunming, Yunnan 650032; 2Pediatric Intensive Care Unit, Children's Hospital, Chongqing Medical University, Chongqing 400014, P.R. China

Received November 27, 2015; Accepted November 25, 2016

DOI: 10.3892/etm.2017.4132

Abstract. Hyperoxia therapy for acute lung injury (ALI) may unexpectedly lead to reactive oxygen species (ROS) production and cause additional ALI. Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide that regulates inflammasome activation. However, the role of CGRP in DNA damage during hyperoxia is unclear. Therefore, the aim of the present study was to investigate the effects of CGRP on DNA damage and the cell death of alveolar epithelial type II cells (AEC II) exposed to 60% oxygen. AEC II were isolated from 19-20 gestational day fetal rats lungs and were exposed to air or to 60% oxygen during treatment with CGRP or the specific CGRP receptor antagonist CGRP8-37. The cells were evaluated using immunofluorescence to examine surfactant protein-C and ROS levels were measured by probing with 2',7'-dichlorofluorescin diacetate. The apoptosis rate and cell cycle of AEC II were analyzed by flow cytometry, and apoptosis was determined by western blotting analysis of activated caspase 3. The DNA damage was confirmed with immunofluorescence of H2AX via high-content analysis. The ROS levels, apoptotic cell number and the expression of γH2AX were markedly increased in the hyperoxia group compared with those in the air group. Concordantly, ROS levels, apoptotic cell number and the expression of γH2AX were significantly lower with a significant arrest of S and G2/M phases in the CGRP8-37 group than in the hyperoxia or CGRP8-37/O2 groups. CGRP was concluded to protect lung epithelium cells against hyperoxic insult, and upregulation of CGRP may be a possible novel therapeutic target to treat hyperoxic lung injury.

Introduction

Acute lung injury (ALI), characterized by severe alveolar damage, results from an acute inflammatory response that leads to edema, neutrophil and macrophage infiltration (1). Development of ALI is a common cause for admission to critical care units, with incidence in intensive care units recently reported to be 10.4% (2). Severe sepsis is the most common risk factor for the development of ALI (2,3). Hyperoxia therapy is a useful part of treatment for patients with acute and chronic cardiovascular and pulmonary diseases (4). However, prolonged exposure to hyperoxia may cause additional deterioration in cases of ALI (5). Acute exposure to hyperoxia induces lung inflammation and injury, leading to impairment in respiratory function (6). Prolonged exposure to high concentrations of oxygen (>50%) may lead to acute or chronic lung damage, which is characterized by dysfunction of alveolar epithelial cells (AEC), repressed proliferation and increased apoptosis and cell death (7,8). Hyperoxic injury is mediated by accumulation of inflammatory factors and direct insult resulting from reactive oxygen species (ROS) (9).

Calcitonin gene-related peptide (CGRP) is a 37 amino acid neuropeptide that is mainly synthesized and distributed in the C fibers of sensory nerves in humans and mammals. It is also the predominant neuromediator to induce vasodilation and neurogenic inflammation (10). However, a previous study indicated that CGRP also serves a role in anti-inflammatory actions and tissue repair, as it decreases interleukin (IL)-8 secretion, suppresses the formation of ROS and induces proliferation in the epithelium (11). ROS are often associated with DNA damage and repair in acute lung injury (12,13). Previous studies reported that a number of drugs reduce the death of lung epithelial cells by blocking DNA damage (14-17). Whether CGRP has the same effects on hyperoxia-injured AEC II is unknown. The present study was designed to evaluate the role of CGRP in a hyperoxic cell model (60% oxygen for 24 h) in AEC II isolated from fetal rats at 19-20 days old, and to investigate whether the mechanism involved DNA damage repair.

Materials and methods

Suppliers of reagents. Reagents were obtained as follows: Trypsin, DNase I, Dulbecco's minimal essential medium/F12
with continuous monitoring on oxygen fraction of air using a Pigeon I oxygen measuring meter (Pigeon Medical Apparatus Co., Ltd., Guangzhou, China).

The experiments were performed in six groups as follows: i) Air group, cells were cultured in the ‘air’ conditions (as above); ii) CGRP/air group, cell medium had 10 \( \mu M \) CGRP added 30 min before culturing in air (as above); iii) CGRP \( \times 10^{-3}/ \) air group, CGRP and 100 \( \mu M \) CGRP added before culturing in air (as above); iv) hyperoxia group, cells were cultured in the ‘hyperoxia’ conditions (as above); v) CGRP/O \( \times 10^{-2} \) group, 10 \( \mu M \) CGRP was added into the medium 30 min before hyperoxia exposure; and vi) CGRP \( \times 10^{-3}/ \) O \( \times 10^{-2} \) group, CGRP and CGRP \( \times 10^{-3} \) were added into the culture medium 30 min before hyperoxia treatment.

**Immunoﬂuorescence assay for SP-C.** AEC II cells were cultured for 15-18 h and treated with CGRP and CGRP \( \times 10^{-3} \) before exposure to air or 60% oxygen, as described above. After 24 h, the slides were removed and rinsed three times with ice-cold PBS. Cells were then ﬁxed with methanol for 15 min at -20°C, rehydrated twice with PBS, then blocked with 1% BSA for 10 min at room temperature. After incubation overnight at 4°C with speciﬁc SP-C (1:500) and \( \gamma \)-H2AX (phospho S139; 1:200) antibodies, the slides were rinsed extensively with PBS, and incubated with a FITC-conjugated secondary antibody (1:1,000), followed by Hoechst 33342 (blue) and PI (red) staining.

**Apoptosis assay and cell cycle analysis.** AEC II were ﬁxed with 70% ethanol and stored at 4°C overnight. Prior to staining, the cell suspension was centrifuged at 500 x g for 5 min, the pellet was washed with PBS and cells were incubated with propidium iodide for 30 min at 4°C in the dark. Cellular apoptosis and cell death were detected by Annexin V and PI staining with an
Annexin V-FITC/PI apoptosis detection kit, as described by the manufacturer. Analysis was performed by flow cytometry and Flowjo software 7.6 (FlowJo LLC, Ashland, OR, USA) was used for acquisition and analysis.

Western blotting. AEC II were incubated with RIPA lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) for 15 min on ice, then centrifuged at 13,000 g for 5 min at 4°C. A total of 20 µg protein from cell lysate was separated by 12% SDS-PAGE. Wet gel system was used to transfer protein samples to a PVDF membrane. Following 5% slim milk blocking at room temperature for 1.5 h, the membrane was incubated with a primary anti-caspase 3 antibody (cat. no. sc-7148; 1:500 dilution; Santa Cruz Biotechnology, Inc.) at 4°C overnight and subsequently incubated with a secondary antibody conjugated to horseradish peroxidase (cat. no. sc-2004; 1:2,000 dilution; Santa Cruz Biotechnology, Inc.). β-actin (cat. no. sc-47778; Santa Cruz Biotechnology, Inc.) was used as an internal control. Protein images were observed with an electro-chemiluminescence solution (Pierce; Thermo Fisher Scientific, Inc.). The experiment was repeated three times.

DNA damage detection by examination of γH2AX immunofluorescence. ACEII cells were seeded at 2x10⁴ cells/well in black 96-well plates with clear, flat bottoms (Costar; Corning Incorporated, Corning, NY, USA). Following treatment, the cells were rinsed with PBS, fixed with 4% formaldehyde in PBS for 15 min at room temperature, and permeabilized with 0.1% Triton X-100 in PBS for 10 min. Nonspecific binding was blocked by incubating the cells with 1% BSA and 0.02% Triton X-100 in PBS for 20 min at room temperature. The cells were sequentially incubated with anti-γH2AX antibody (dilution, 1:500) for 3 h at room temperature, Alexa Flou 488-conjugated anti-rabbit IgG antibody (1:500) at room temperature for 1 h, and Hoechst 33342 (10 µg/ml) for 10 min. The cells were washed 3 times with 0.02% Triton X-100 in PBS for 10 min each time, and were visualized using an ImageXpress Micro Confocal High-Content Imaging System (Molecular Devices, LLC, Sunnyvale, CA, USA). Acquisition and analysis of images, including the number and the total area of γH2AX foci, were measured using the MetaXpress 4.0.0.24 software (Molecular Devices, LLC). Images of stained cells were acquired from the automated fluorescence microscope platform of the ImageXpress using a 40x objective lens.

Statistical analysis. SPSS 19.0 software (IBM SPSS, Armonk, NY, USA) was used for statistical analyses. All data are expressed as the mean ± standard deviation. Group means were compared by analysis of variance with Tukey's tests used for post hoc analyses. P<0.05 was considered to indicate a statistically significant difference.

Results

CGRP reverses the changes in AEC II that are induced by 60% oxygen. SP-C, secreted only by AEC II, is a biomarker of these cells (19). Therefore, AEC II were first plated on slides and the SP-C expression was detected in situ using immunofluorescence (Fig. 1A). SP-C fluorescence in the cytoplasm was markedly decreased in the hyperoxia groups compared with the air group, and treatment with CGRP partially rescued hyperoxia-treated cells, though treatment with CGRP₈₋₁₇ (the CGRP receptor antagonist) did not. The fluorescence intensity did not differ among the three groups cultured in air (Fig. 1A).

Following each treatment, SP-C fluorescence in O₂ groups was markedly lower than that in each respective air group.

In order to evaluate the effects of 60% oxygen to AEC II cells, the levels of ROS in AEC II treated with 60% oxygen and/or CGRP were assessed using 2',7'-dichlorofluorescein diacetate (Fig. 1B). ROS levels were significantly increased following exposure to 60% oxygen for 24 h (Fig. 1B). Administration of 10 µM CGRP prior to hyperoxia significantly inhibited the increase in ROS levels observed in the hyperoxia group (P<0.05; Fig. 1B). To verify these effects, both CGRP and CGRP₈₋₁₇ were applied to competitively block the binding sites, demonstrating that ROS levels return hyperoxia group levels. These data indicated a protective effect of CGRP against hyperoxia insult (Fig. 1B).

CGRP inhibits apoptosis of AEC II that is induced by hyperoxia. A previous study revealed that 95% oxygen could induce AEC II apoptosis (20). To investigate the influence of moderate oxygen on AEC II apoptosis, the proportion of apoptotic cells was detected by flow cytometry. The apoptotic cell number increased in the 60% oxygen groups compared with the air control groups (Fig. 2A and B). The role of CGRP in apoptosis was investigated, revealing that the apoptotic rate significantly decreased in the CGRP₁₀ group compared with the control hyperoxia group (P<0.01; Fig. 2B). Cells treated with high oxygen expressed active caspase-3, which was inhibited by CGRP treatment (Fig. 2C). These findings indicated that hyperoxia triggered apoptosis, which could be inhibited by CGRP.

Analyses of the relationship between double strand breaks (DSB) and apoptosis. As DSB damage induces apoptosis, cellular senescence and pro-inflammatory cytokine production (21), the presence of γH2AX, a marker of DSB and the effects of CGRP were investigated in AEC II. Analysis of AEC II revealed that the hyperoxia group cells contained higher numbers of γH2AX foci and CGRP decreased numbers of γH2AX foci (Fig. 3A). Furthermore, the mean γH2AX fluorescence measured in these cells demonstrated a dose-dependent trend following treatment with different concentrations of CGRP, indicating that DNA fragmentation was occurring during apoptosis (Fig. 3B). These data suggest that DNA damage is directly linked to the induction of apoptosis in AEC II.

CGRP increases the number of AEC II in S and G2/M phases. Alterations to the cell cycle often involve DNA damage and repair (22). For this reason, the effects of CGRP on the cell cycle of cultured AEC II were determined. AEC II treated with CGRP (10 µM), and the proportion of S and G2/M phase cells were detected by flow cytometry to judge the effects on proliferation (Fig. 4). CGRP at a concentration of 10 µM significantly enhanced the proportion of cells in the S- and G2/M-phases, with a consequent reduction of AEC II in G0/G1 (Fig. 4). No significant differences were observed between the levels of proliferative cells in the air control and the air + CGRP group.
Figure 1. Immunofluorescence of SP-C and ROS levels in AEC II treated with CGRP and/or 60% oxygen. (A) AEC II double-labeled with anti-SP-C antibodies/Alexa 488 anti-goat immunoglobulin G. Scale bar, 20 µm. (B) Relative ROS levels in each condition, quantified by dichlorofluorescin fluorescence-activated cell sorting of AEC II. CGRP/CGRP8-37 pre-treated cells were incubated with 60% oxygen for 24 h before ROS measurement (n=3). Values are reported as mean ± standard deviation; P-values were determined by Student’s t-test. *P<0.05 vs. relevant group. SP-C, surfactant protein-C; ROS, reactive oxygen species; CGRP, calcitonin gene-related peptide; AEC II, alveolar epithelial type II cells.

Figure 2. Effects of CGRP on hyperoxia-induced apoptosis in AEC II. (A) Cells were pre-treated with CGRP (10 µM) for 4 h and exposed to 60% oxygen for 24 h, then apoptotic rate was determined by Annexin V/propidium iodide staining and analysed using flow cytometry. (B) Graphical representation of (A), reported as mean ± standard deviation. P-values were determined by Student’s t-test. *P<0.01 vs. relevant group. (C) Cleaved caspase-3, examined by western blot analysis. β-actin was used as a loading control CGRP, calcitonin gene-related peptide; AEC II, alveolar epithelial type II cells.

Figure 3. Hyperoxia-induced γ-H2AX phosphorylation in AEC II in response to CGRP. (A) Visualization of oxidized DNA marker γH2AX by immunofluorescence in response to CGRP and/or 60% oxygen conditions. Nuclei are visualized by counterstaining with Hoescht. (B) Relative quantification of mean fluorescence per cell stained by the DNA damage response protein, γH2AX. All cells were incubated for 24 h in the indicated oxygen conditions before analysis. Values are reported as mean ± standard deviation. CGRP, calcitonin gene-related peptide; AEC II, alveolar epithelial type II cells.
Discussion

The present study reported that fetal AEC II exposure to 60% oxygen for 24 h causes cellular injury by inducing apoptosis, which may be closely associated with the DNA damage repair mechanism; CGRP may reduce the apoptotic rate induced by high oxygen concentration by blocking DNA damage.

The pattern of lung injury is closely correlated with the oxygen concentration and exposure time (23). The model of hyperoxic lung injury, achieved with an oxygen concentration >85%, lasted for over 2 days (24). Lee et al (25) isolated AEC II from the lungs of 95% oxygen-exposed animals and revealed that a sub-lethal exposure time for this cell line was 48 h, whereas 96 h of exposure was invariably lethal. Similarly, Pace et al (26) demonstrated that 80% oxygen exposure for 60 h increased lipid peroxide levels and inflammatory cells in bronchoalveolar lavage. However, most of those studies were conducted on AEC II in vivo or as part of a whole lung homogenate, rather than on cells isolated as a pure population (5,27,28). In the present study, purified AEC II cells were exposed to 60% oxygen for 24 h, and the detrimental effects of moderate oxygen were investigated. As 60% oxygen is more frequently used than 90% in clinical practice, 60% oxygen was used to attain a valid hyperoxic cell model in the current study.

CGRP, mainly expressed in nerve fibers and in the respiratory system, has multiple effects on cells under physiological and pathological conditions. It can serve as a pro-inflammatory factor by stimulating eosinophil migration, contracting human bronchi and effectively dilating human pulmonary vessels in vitro (29). In contrast, other previous reports demonstrated that CGRP suppresses secretion of inflammatory cytokines, such as IL-6, IL-8 and TNFα, from macrophages, suggesting its potential anti-inflammatory properties (30,31). Although CGRP was associated with regulation of inflammatory cytokines, no previous study has related this to DNA damage. The present study indicated that CGRP serves a protective role on hyperoxia-induced AEC II injury by inhibition of oxidative stress and reduction of apoptosis. H2AX expression was also detected, which is a biomarker of DNA damage, particularly for DSB; this revealed increased expression of H2AX in AEC II exposed into 60% oxygen and decreased H2AX expression in the presence of CGRP, which could be reversed by addition of the CGRP receptor antagonist CGRP<sub>8-37</sub>.

In addition, to investigate the relationship between CGRP and DNA damage repair, cell cycle changes were examined. It was revealed that 10 µM CGRP significantly increased the number of AEC II in S/G2, both in air and hyperoxia conditions, and that its antagonist, CGRP<sub>8-37</sub>, significantly attenuated the proliferative effect of CGRP. Previous studies documented that hyperoxia exerts inhibition of cell growth (32,33) which is consistent with the current findings that direct exposure of AEC II to 60% oxygen for 24 h results in an increase of cells in G0/G1 and a decrease of cells in S and G2/M phase. It is hypothesized that high oxygen induced DNA damage, leading to AEC II apoptosis, whilst CGRP reduced DNA damage, enhanced the arrest in S and G2/M phase, promoted cell repair and inhibited apoptosis.

The exact protective mechanisms of CGRP in AEC II under hyperoxia exposure have not been identified in the presently investigated context, and the correlation of CGRP and DNA damage have not been studied. In the present study, CGRP inhibited cell death and DNA damage, which may be indirectly affected by other, associated proteins, rather than direct regulation. A previous study suggested that CGRP binds to receptors expressed on the cell surface of AEC II and activates receptor-coupled G proteins, leading to an induction of intracellular cyclic AMP (cAMP) generation (34). The accumulated cAMP inhibits the accumulation of NF-κB complexes in the nucleus by preventing phosphorylation and degradation of IκB, an NF-κB inhibitor (35). A previous study by the current authors has also demonstrated that high oxygen and CGRP affects constitutive membrane transport of protein kinase C (PKC) α, and observed that the activation of NF-κB in the nucleus. As PKCa and NF-κB appear to serve an important role in apoptosis, it is hypothesized that CGRP inhibits cell damage and apoptosis by activating NF-κB or PKCa associated pathways (36). However, a previous study confirmed that inhibition of NF-κB activity would trigger the protective mechanism of CGRP to confine the inflammatory response (37). Therefore, it is difficult to identify whether

![Figure 4. CGRP effects on the cell cycle arrest induced by hyperoxia. The cell cycle distributions of AEC II treated with CGRP and/or 60% oxygen were determined after 24 h, using flow cytometric analysis. *P<0.05 vs. relevant group. CGRP, calcitonin gene-related peptide; AEC II, alveolar epithelial type II cells.](image)
CGRP inhibited DNA damage via NF-κB, and additional studies are required to understand the mechanism behind this.

In conclusion, the present study demonstrated that exposure to 60% oxygen for 24 h predisposed AEC II to oxidative injury in vitro, including DNA damage and apoptosis; however, exogenous CGRP markedly attenuated hypoxic injury and exerted a cytoprotective effect against hyperoxia insult. This suggests that upregulation of CGRP expression may represent an alternative approach for prevention from hyperoxia-induced lung injury.

Acknowledgements

The present study was supported by the National Natural Science Foundation of China (grant nos. 30470755 and 81260289). Dr Zijie Liu from The First Affiliated Hospital of Kunming Medical University (Kunming, Yunnan, China) is thanked for support in a number of key laboratory techniques.

References

1. Kallet RH and Matthey MA: Hyperoxic acute lung injury. Respir Care 58: 123-141, 2013.
2. Bellantoni G, Laffey JG, Pham T, Fan E, Brochard L, Esteban A, Gattinoni L, van Haren F, Larsson A, McAuley DF, et al: Epidemiology, patterns of care, and mortality for patients with acute respiratory distress syndrome in intensive care units in 50 Countries. JAMA 315: 788-800, 2016.
3. Singh G, Gladdy G, Chandy TT and Sen N: Incidence and outcome of acute lung injury and acute respiratory distress syndrome in the surgical intensive care unit. Indian J Crit Care Med 16: 659-665, 2014.
4. Ward NS, Waxman AB, Homer RJ, Mantell LL, Einarsson O, Du Y and Elias JA: Interleukin-6-induced protection in hyperoxic acute lung injury. Am J Respir Cell Mol Biol 22: 535-542, 2000.
5. Fukumoto J, Fukumoto I, Parthasarathy PT, Cox R, Huynh B, Ramathan GK, Venugopal RB, Allen-Gipson DS, Lockey RF and Kolliputi N: NLRP3 deletion protects from hyperoxia-induced acute lung injury. Am J Physiol Cell Physiol 305: C182-C189, 2013.
6. Crapo JD: Morphologic changes in pulmonary oxygen toxicity. Annu Rev Physiol 48: 721-731, 1986.
7. Durr RA, Dubyabo BA and Thet LA: Repair of chronic hyperoxic lung injury: Changes in lung ultrastructure and matrix. Exp Mol Pathol 47: 219-240, 1987.
8. Wang Y, Feinstein S, Manevich Y, Ho YS and Fisher AB: Lung injury and mortality with hyperoxia are increased in peroxiredoxin 6 gene-targeted mice. Free Radic Biol Med 37: 1764-1743, 2004.
9. Warner BB, Stuart LA, Papes RA and Wispe JR: Functional and outcome of acute lung injury and acute respiratory distress syndrome in the surgical intensive care unit. Indian J Crit Care Med 16: 659-665, 2014.
10. Russo AF: Calcitonin gene-related peptide (CGRP): A new target for migraine. Annu Rev Pharmacol Toxicol 55: 533-552, 2015.
11. Li WJ and Wang TK: Calcitonin gene-related peptide inhibits interleukin-1beta-induced interleukin-8 secretion in human type II alveolar epithelial cells. Acta Pharmacol Sin 27: 1340-1345, 2006.
12. Filomeni G, De Zio D and Cecconii F: Oxidative stress and autophagy: The clash between damage and metabolic needs. Cell Death Differ 22: 377-388, 2015.
13. Bennett MR: Reactive oxygen species and death: Oxidative DNA damage in atherosclerosis. Cir Res 88: 648-650, 2001.
14. Kim SJ, Chen CH, Williams D, Cheng Y, Ridge K, Schumacker PT, Weitzman S, Bohr VA and Kamp DW: Mitochondria-targeted Ogg1 and aconitate-2 prevent oxidant-induced mitochondrial DNA damage in alveolar epithelial cells. J Biol Chem 283: 6165-6176, 2014.
15. Shao L, Perez RE, Gerthoffer WT, Truong WE and Xu D: Heat shock protein 27 protects lung epithelial cells from hyperoxia-induced apoptotic cell death. Pediatr Res 65: 328-333, 2009.
16. Li Y, Teruya K, Katakura Y, Kabayama S, Otsubo K, Morisawa S, Ishii Y, Gude T and Shirahata S: Effect of Reduced Water on the Apoptotic Cell Death Triggered by Oxidative Stress in Pancreatic β-HIT-T15 Cell. In: Animal cell technology meets Genomics. Springer 2: pp121-124, 2005.
17. Thome UH, Davis IC, Nguyen SV, Shelton BJ and Matalson S: Modulation of sodium transport in fetal alveolar epithelial cells by oxygen and corticosterone. Am J Physiol Lung Cell Mol Physiol 284: L376-L384, 2008.
18. Li Z, Fang F and Xu F: Effects of different states of oxidative stress on fetal rat alveolar type II epithelial cells in vitro and ROS-induced changes in Wnt signalling pathway expression. Mol Med Rep 7: 1528-1532, 2013.
19. Li F, He J, Wei J, Cho WC and Liu X: Diversity of epithelial stem cell types in adult lung. Stem Cells Int 2015: 728307, 2015.
20. De Paepe ME, Mao Q, Chao Y, Powell JL, Rubin LP and Sharma S: Hyperoxia-induced apoptosis and Fas/FasL expression in lung epithelial cells. Am J Physiol Lung Cell Mol Physiol 289: L647-L659, 2005.
21. Bajic D, Chae WJ, Croocher J, Eckert KA, Glazer PM, Bothwell AL and Sweeby JB: Interplay between DNA repair and inflammation and the link to cancer. Crit Rev Biochem Mol Biol 49: 116-139, 2014.
22. Branzi D and Foiani M: Regulation of DNA repair throughout the cell cycle. Nat Rev Mol Cell Biol 9: 297-308, 2008.
23. Jankov RP, Luo X, Campbell A, Belcastro R, Cabacungan J, Johnstone L, Frndova H, Lye SJ and Tanswell AK: Fibroblast growth factor receptor-1 and neonatal compensatory lung growth after exposure to 95% oxygen. Am J Respir Crit Care Med 167: 1554-1561, 2003.
24. Rogers LT, Tipple TE, Nelini LD and Welty SE: Differential responses in the lungs of newborn mouse pups exposed to 85% or 95% oxygen. Pediatr Res 65: 33-38, 2009.
25. Lee R, Reddy R, Barsky L, Weinberg K and Driscoll C: Contribution of proliferation and DNA damage repair to alveolar epithelial type II cell recovery from hyperoxia. Am J Physiol Lung Cell Mol Physiol 290: L685-L694, 2006.
26. Pace PW, Yao L, Wilson JX, Possmayer F, Veldhuijzen RA and Lewis JF: The effects of hyperoxia exposure on lung function and pulmonary surfactant in a rat model of acute lung injury. Exp Lung Res 35: 380-398, 2009.
27. Michaelis KA, Agboke F, Liu T, Han K, Muthu M, Galambo CS, Yang G, Dennehy PA and Wright CJ: IκB-mediated NF-κB activation confers protection against hyperoxic lung injury. Am J Respir Cell Mol Biol 289: L647-L659, 2005.
28. Vadivel AK, Alphonse RS, Joncesu L, Machado DS, O'Reilly M, Eaton F, Haromy A, Michelakis ED and Thébaud B: Exogenous hydrogen sulfide (H2S) protects alveolar growth in experimental O2-induced neonatal lung injury. PLoS One 9: e90695, 2014.
29. Springer J, Jeppetti P, Fischer A and Gronenberg DA: Calcitonin gene-related peptide as inflammatory mediator. Pulm Pharmacol Ther 16: 121-130, 2003.
30. Nong YH, Titus RG, Ribeiro JM and Remold HG: Peptides encoded by the calcitonin gene inhibit macrophage function. J Immunol 143: 45-49, 1989.
31. Li W, Wang T, Ma C, Xiong T, Zhu Y and Wang X: Calcitonin gene-related peptide inhibits interleukin-1beta-induced endogenous monocyte chemoattractant protein-1 secretion in type II alveolar epithelial cells. Am J Physiol Cell Physiol 291: C456-C465, 2006.
32. Vettolo PF, Roper JM, Staversky RJ, Wright TW, McGrath-Morrow SA, Maniscalco WM, Finkelstein JN and O'Reilly MA: Type II epithelial cells are critical target for hyperoxia-mediated impairment of postnatal lung development. Am J Physiol Lung Cell Mol Physiol 291: L1101-L1111, 2006.
33. Matalon S: DNA damage and cell cycle checkpoints in hyperoxic lung injury: Braking to facilitate repair. Am J Physiol Lung Cell Mol Physiol 281: L291-L305, 2001.
34. Drissi H, Lasmoles F, Le Mellay V, Marie PJ and Lieberherr M: Activation of phospholipase C-beta1 via Galpach/11 during calcium mobilization by calcitonin gene-related peptide. J Biol Chem 273: 20166-20174, 1998.
35. Brigelius-Flohe R, Banning A, Knü M and Böhl GF: Redox events in interleukin-1 signaling. Arch Biochem Biophys 423: 66-73, 2004.
36. Fu HM, Li L, Wang YJ, Tang CH, Mi HY, Xu F and Kuang FW: The proliferation-promoting effects of calcitonin gene-related peptide on type II alveolar epithelial cell exposed to hyperoxia mediated by protein kinase C alpha pathway. Zhonggou Wei Zhong Bing Ji Ji Yi Xue 22: 263-266, 2010 (In Chinese).
37. Pette J, Piret B, Bonizzii G, Schoonbroodt S, Merville MP, Vanden-Poels S and Bourr Y: Multiple redox regulation in NF-kappaB transcription factor activation. Biol Chem 378: 1237-1245, 1997.