Radioprotective effect on HepG2 cells of low concentrations of cobalt chloride: induction of hypoxia-inducible factor-1 alpha and clearance of reactive oxygen species

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It has been found that low doses of certain toxicants might generate a protective response to cellular damage. Previous data have shown that elevated doses of cobalt (Co) induce injury to cells and organisms or result in radiological combined toxicity. Whether low doses of Co generate a protective effect or not, however, remains controversial. In this study, we investigated the effect and mechanism of action of low dose cobalt chloride (CoCl2, 100 μM) on the viability of irradiated cells. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay was used to observe the radio-sensitivity of HepG2 cells under different pretreatments. The alteration of intracellular DNA damage was further measured using micronucleus (MN) assay. Levels of hypoxia inducible factor-1α (HIF-1α) expression and its target gene, EPO, were monitored by western blot and reverse transcription polymerase chain reaction (RT-PCR), respectively, and intracellular reactive oxygen species (ROS) content was determined by 2′,7′-dichlorofluorescein diacetate (DCFH-DA) probe staining. Our results show that low dose CoCl2 does not influence HepG2 cell viability, but induces the expression of HIF-1α, followed by increased radio-resistance. Additionally, cells treated with HIF-1α siRNA retained a partial refractory response to irradiation concomitant with a marked reduction in intracellular ROS. The change of MN further indicated that the reduction of DNA damage was confirmed with the alteration of ROS. Our results demonstrate that low dose CoCl2 may protect cells against irradiative harm by two mechanisms, namely HIF-1α expression and ROS clearance.

Keywords: cobalt chloride; hypoxia inducible factor-1α; radio-protection; HepG2; reactive oxygen species

INTRODUCTION

Cobalt (Co) is an almost ubiquitous oligoelement in the animal and plant kingdoms, and plays an important role in the maintenance of biological function as a component of vitamin B12 and other cobalamines [1]. Exposure to an excessive amount of Co and its ions (Co (II)) is, however, known to have deleterious effects on the human body by inducing oxidative stress [2, 3]. Elevated levels of Co (II) lead to the accumulation of excessive amounts of reactive oxygen species (ROS), which results in oxidative lesions in DNA and membrane lipid peroxidation, leading to cellular death and dysfunction [4–6]. On the other hand, Co (II) compounds, such as cobalt chloride (CoCl2), induce biochemical and molecular responses similar to those observed under hypoxic conditions [7, 8]. At a molecular level, under hypoxic conditions, hypoxia inducible factor-1 (HIF-1), a heterodimeric protein consisting of the constitutively expressed HIF-1β/ARNT and the highly regulated HIF-1α subunits, is a critical transcriptional factor regulating the expression of many downstream target genes encoding proteins which mediated repair of, and resistance to, physical and chemical damage.

The HIF-1α subunit is hydroxylated by prolyl hydroxylases (PHDs) at proline residues 402 and 564 in the oxygen-dependent degradation domain (ODD). It is then targeted for proteasome-mediated degradation through a protein ubiquitin ligase complex containing the product of the von Hippel Lindau tumor suppressor (pVHL) [9–11]. CoCl2 can mimic hypoxia by stabilizing HIF-1α through...
antagonism of Fe$^{2+}$, which along with oxygen is an essential cofactor [12]. Moreover, it has been shown that cobalt can bind directly to the hydroxylated proline residues within the ODD and inhibit both hydroxylation and the interaction between hydroxylated HIF-1α and pVHL to promote HIF-1α expression [13]. A large number of data indicate that, by activating HIF1α, preliminary exposure to CoCl$_2$ might mimic actual hypoxic conditions, and increase tolerance against damage by other biological and physicochemical factors including ischemia, photodynamic therapy, tert-butyl-hydroperoxide-induced oxidative stress and some chemotherapy drugs [14–16].

As is well known, ionizing radiation (IR), by generating abundant ROS and free radicals, leads to damage of cell and tissue. At low or moderate doses, IR has a wide range of reversible and irreversible harmful effects on cellular DNA, resulting in the appearance of single (SSB) and double (DSB) strand breaks [17, 18]. It is unclear, however, whether CoCl$_2$ regulates the radio-sensitivity of cells via an increase in the refractory response to IR, which is involved in the protection of cells against other harmful factors. According to the work of Gault et al. [19] combination CoCl$_2$ and γ-radiation treatment in the HaCaT cell line results in an additive damage effect, although others observed the opposite effect [20]. In the present study, to evaluate the involvement of Co (II) in the cellular response to IR, we investigated the effect of low concentration CoCl$_2$ pretreatment on the viability of irradiated cells. The content of intracellular ROS and the expression of HIF-1α were then measured to determine possible mechanisms of this effect.

MATERIALS AND METHODS

Cell culture and treatment

The human hepatoma HepG2 cell line (Cell Bank, Chinese Academy of Sciences) was cultured in RPMI-1640 medium (GIBCO BAL, USA) supplemented with 10% fetal bovine serum (FBS) (GIBCO BAL, USA), streptomycin (100 µg/ml) (Sigma-Aldrich, St. Louis, MO, USA) at 37°C in an incubator containing a humid atmosphere of 95% air and 5% CO$_2$ and propagated according to the protocol supplied by the American Type Culture Collection. The cells were treated with 100 µM CoCl$_2$ (Sigma-Aldrich) for 24 h prior to exposure to irradiation.

Irradiation

A Gammacell-40 Exactor with a $^{137}$Cs source (Best Theratronics Ltd, Canada) was used for γ-ray irradiation at a dose rate of 0.8 Gy/min. After the cells were treated with 100 µM CoCl$_2$ for 24 h and then irradiated in 0, 1, 3 and 5 Gy, the cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay [21].

Cell viability assay

The influence of CoCl$_2$ on cell growth was determined using the MTT method. HepG2 cells were seeded in 96-well plates at a density of $5 \times 10^{3}$ cells per well. They were then treated with different concentrations of CoCl$_2$ for 24 h. Furthermore, the medium was replaced with fresh medium allowing cells to be continuously grown up to 72 h. MTT dye was added to a final concentration of 50 mg/ml and cells were subsequently incubated for another 4 h at 37°C. The media containing residual MTT dye was carefully aspirated from each of the wells and 200 µl dimethyl sulphoxide (DMSO) (Sigma-Aldrich) was added to each well to dissolve the reduced formazan dye. The fraction of viable cells was calculated by comparing the optical absorbance of culture exposed to CoCl$_2$ treatment with that of the untreated control.

Treatment of cells with HIF-1α siRNA

Primers were synthesized based on the human HIF-1α cDNA sequence (Genbank No. 22431) as follows: forward and reverse strand (UGUGAGUUCGAUCUCGUAUTT) and (AUCAAGAG CGAACUCACATT); forward and reverse scrambled (sc) strands (UACACCG UUAGCAGA CACCTT) and (GGUGUCUGUAACGGUGUATT), as previously described [22]. Primers were purified then annealed at a final concentration of 20 µM by heating at 95°C for 1 min and incubating at 37°C for 1 h in annealing buffer (potassium acetate, 6 mM Hepes-KOH, pH 7.4 and 0.4 mM magnesium acetate). $5 \times 10^{5}$ HepG2 cells were transiently transfected with 4 µg HIF-1α siRNA using Lipofectamine 2000, according to the manufacturer’s protocol (Invitrogen Life Technologies, USA).

Western blot analysis of HIF-1α expression

Cells were scraped from culture flasks and lysed in lysis buffer containing 10% glycerol, 10 mM Tris-HCl (PH 6.8), 1% sodium dodecyl sulfate (SDS), 5 mM dithiothreitol (DTT) and 1× complete protease inhibitor cocktail (Sigma-Aldrich). The method of Bradford was used to assay concentrations of protein in diverse samples [23]. Protein concentration was measured using an auto multifunction microplate reader. Fifty micrograms of proteins were separated by 8% polyacrylamide-SDS inconsecutive gel electrophoresis. The separated proteins were electro- phoretically transferred to polyvinylidene difluoride membrane. Membranes were blocked with 5% skim milk in Tris-buffered saline (TBS) containing 0.1% Tween 20 at room temperature for 1 h and then incubated with mouse monoclonal HIF-1α antibody (Abcam, USA) at a 1:500 dilution overnight at 4°C, followed by goat anti-mouse IgG for 1 h at room temperature. Signals were detected with enhanced chemiluminescence (ECL plus, Amersham, USA). Microtubule protein (Tubulin, Abcam, USA) at a
1:1000 dilution was used as internal control to observe the changes of HIF-1α bands.

**RNA purification**

Cells were lysed by TRIzol Reagent (Sangon, China) and RNA was extracted according to manufacturer’s instruction (Sangon, China). To avoid genomic DNA contamination, extracted RNA was then purified with the RNeasy kit (Invitrogen, USA). The quantity and quality of RNA was determined by OD measurement at 260 and 280 nm. The integrity of RNA was checked by visual inspection of 28S and 18S rRNAs on an agarose gel.

**RT-PCR**

Two micrograms of RNA were used for cDNA synthesis using Olig-(dt)18 as primer and AMV reverse transcriptase (Invitrogen, USA). The RT reaction was started with 10 min incubation at room temperature and 60 min at 42°C, followed by 10 min at 70°C to terminate the reaction. Subsequently, 2 μl cDNA was used as template for amplification of target cDNAs in a total volume of 25 μl containing 2.5 μl 10× PCR buffer (0.2 M Tris-HCl, pH 8.4, 0.5 M KCl), 0.2 mM dNTP mix, 1.5 mM MgCl2, 0.2 μM of each primer and 1.25 units of Platinum Taq DNA polymerase (Invitrogen, USA). The thermal cycler was set to run at 95°C for 5 min, 30 cycles of 94°C for 1 min, 55°C for 2 min, 72°C for 2 min, with a final extension of 72°C for 10 min. Specific primers for erythropoietin (EPO) were as follows: (upstream: 5'-ATATCAGTCTCCAGACAGCC-3'; downstream: 5'-AGTTGATTGTGCTGGAGTGGAG-3'; 290-bp fragment). PCR products were analyzed by electrophoresis on a 1.2% agarose gel. The specific bands were observed with ethidium bromide and digitally photographed under ultraviolet light, then scanned using the Biosens Gel Documentation System 920 (Shanghai Bio-Tech Co., Ltd, China). Gene expression was calculated as the ratio of mean band density of specific products to that of the internal standard (β-actin).

**Measurement of intracellular ROS**

Digestive cell suspension was incubated with 10 μM of 2′,7′-dichlorofluorescein diacetate (DCFH-DA, Sigma-Aldrich) for 30 min, and then washed three times with PBS for removing redundant DCFH probe. After counting the viable cells, the fluorescence intensities of cells were measured by flow cytometry (Becton-Dickinson) of which the excitation wavelength and the emission wavelength were 488 and 525 nm, respectively.

**Micronucleus assay**

Micronucleus (MN) was measured with the cytokinesis-block technique as a biological end point for the response of mimetic hypoxia to irradiation. Briefly, the cells were exposed to 0.83 μg/ml cytochalasin B (Sigma-Aldrich) for 19–20 h followed by 75 mM KCl hypotonic treatment for 1–3 min and then fixed in situ with methanol:acetic acid (9:1 v/v) for 30 min. Air-dried cells were stained with 5% Giemsa (Sigma-Aldrich) for 10 min. Micronuclei were scored in binucleated cells, and the formation of binucleated cells was measured as the percentage of the total number of cells scored. For each sample, at least 1000 binucleated cells were counted. The MN yield, Y_MN, is the ratio of the number of micronuclei to the number of binucleated cells scored.

**Statistical analysis**

Data are reported as the mean ± SEM of three separate experiments. Statistical significance was measured with the independent sample t test and analysis of variance. A value of P < 0.05 was considered statistically significant.

**RESULTS**

**Selection of nontoxic concentration of CoCl2**

In order to select the experimental concentration of CoCl2, a 24 h dose–response study was conducted by exposing cells to different concentrations of CoCl2. The results of the MTT assay showed no significant decrease in cellular viability after 24 h exposure to CoCl2 concentrations ranging from 12.5 to 100 μM, as shown in Fig. 1. In subsequent studies, the concentrations of CoCl2 were set at 100 μM.

**Variation of HIF-1α expression**

CoCl2 pretreatment resulted in robust induction of intracellular HIF-1α (Fig. 2A and B). Cells treated with 100 μM CoCl2 showed significant increase in HIF-1α expression compared to the control.
CoCl₂, and irradiated in different doses from 1 to 5 Gy, retained elevated levels of HIF-1α. Transfection with HIF-1α-siRNA resulted in loss of HIF-1α expression in cells treated with CoCl₂. There was no statistical difference in levels of HIF-1α between cells treated with HIF-1α-siRNA and normoxic cells. To validate fluctuations in HIF-1α levels, the expression of EPO, the target gene induced by HIF-1 under hypoxic conditions, was evaluated by RT-PCR method. Figure 2C and 2D shows that the trend of EPO expression recapitulated that of HIF-1α.

Effect of irradiation on intracellular ROS levels

After exposure to irradiation, CoCl₂ pretreatment decreased intracellular ROS levels in comparison with normoxic cells. No significant change was observed in ROS levels between mimetic hypoxia cells treated with or without HIF-1α-siRNA (Fig. 3). We conclude that irradiation can induce intracellular ROS levels and that CoCl₂ may partly attenuate this effect.
Viability of irradiated cells

As shown in Fig. 4, as the γ-ray irradiation dose, the viability of the cells pretreated with a low concentration of CoCl₂ was significantly greater compared with untreated cells. After exposure to 3 and 5 Gy irradiation, under mimetic hypoxia, cellular viability was significantly reduced by treatment with HIF-1α-siRNA compared with control cells under mimetic hypoxia; no change was observed under normoxic conditions. Moreover, although the expression of HIF-1α was inhibited, CoCl₂ retained the ability to protect irradiated cells, an effect mediated at least in part by increasing the level of HIF-1α expression.

Change of intracellular micronucleus

MN ratios were determined to evaluate cellular irradiation damage. There was no significant difference in MN ratios between unirradiated cells treated with and without CoCl₂ (Fig. 5). Despite robust increases in MN in all irradiated cells, MN ratios in cells treated with CoCl₂ were reduced compared with untreated cells. In the mimetic hypoxia cells transfected with HIF-1α-siRNA, MN ratios were induced by IR, although this increase was lower compared with the MN ratios in normoxic cells induced by IR. These results suggest that CoCl₂ may reduce the appearance of MN in irradiated cells.

DISCUSSION

Through the Fenton reaction, ionizing radiation generates large amounts of ROS, such as superoxide anions, hydrogen peroxide and hydroxyl radicals, which cause DNA damage, alterations in lipids and destruction of protein structure, eventually resulting in cellular mutation or death [24, 25]. The results of our study also show that levels of ROS in HepG2 cells were increased after exposure to different γ-radiation doses. Concomitant with the elevation of intracellular ROS content, cellular viability was inhibited under the same irradiation conditions. As a consequence, endogenous or exogenous ROS scavengers, as shown in previous studies, may protect cells from the deleterious effects of irradiation [26, 27].

Co (II), traditionally considered a pro-oxidant, has been reported to cause accumulation of ROS in cells and tissue systems [28, 29]. In the present study, we found that ROS content was elevated in cells exposed to low concentrations of CoCl₂, although this failed to reach statistical significance. Surprisingly, when combined with irradiation at low or moderate doses, CoCl₂ could reduce the levels of ROS generated by IR, which was confirmed by an increase in cell viability. Toxicological experiments have shown that CoCl₂ protects cells against chemical insults and blocks injury to biological systems, and that induction of the expression of HIF-1α plays an important role in this effect [30, 31]. Similarly, our results show that like hypoxia, CoCl₂ increases levels of HIF-1α and enhances cellular radio-resistance. In contrast, inhibition of HIF-1α by siRNA decreased the viability of irradiated cells, confirming the importance of HIF-1α in mediating the response to IR. Interestingly, although HIF-1α expression was ablated,
the viability of cells treated with CoCl2 remained higher relative to untreated cells under the same radiation dose. Moreover, no significant difference was observed in ROS levels between HIF-1α-siRNA transfected and untransfected cells at the same radiation dose, implying that since HIF-1α per se does not have reducing capacity, expression of HIF-1α does not affect intracellular ROS. Additionally, ROS content was lower in cells treated with CoCl2 regardless of the presence of HIF-1α-siRNA, demonstrating that the radio-protective effect of CoCl2 correlates with both the expression of HIF-1α protein and the clearance of intracellular ROS. According to studies by Shrivastava et al. [34] and Saxena et al. [33], in the presence of oxidative stress, cobalt supplementation maintains or increases the levels of antioxidant enzyme and glutathione systems, such as superoxide dismutase, glutathione peroxidase, glutathione S-transferase and the reduced glutathione/oxidized glutathione ratio, further attenuating cellular ROS generation. These phenomena have also been reported by earlier studies on other harmful agents [34, 35]. We speculate therefore that administration of CoCl2 at low concentrations activates the cellular antioxidant system, thereby inhibiting the generation of ROS formation by irradiation.

MN are the result of small chromosome fragments that are not incorporated into the daughter nuclei during cell division, and arise as a result of exposure to non- or misrepaired DSBs by various clastogenic agents [36, 37]. It has been suggested that DNA damage by ROS induction leads to the formation of SSBs and DSBs [38, 39]. In the field of radiation biology, the number of radiation-inducing MN is strongly correlative with radiation damage. Due to its reliability and reproducibility, the MN assay is an appropriate biological tool to evaluate in vitro radiosensitivity [40] and, accordingly, we used it to evaluate the scavenging of ROS in irradiated cells in response to CoCl2 pretreatment. There was no statistical change in the number of intracellular MN at nontoxic CoCl2 concentrations. While ratios of MN were significantly increased by IR, CoCl2 pretreatment blocked DNA damage. Furthermore, despite inhibition of HIF-1α expression, MN ratios were lower in irradiated cells pretreated with CoCl2, which negatively correlated with the alteration of intracellular ROS, illustrating that CoCl2 may decrease the DNA damage through scavenging excessive ROS.

Taken together, our results suggest that low CoCl2 concentrations may protect cells against irradiation via a mechanism involving not only induction of HIF-1α expression, but also clearance of intracellular ROS. It will be valuable to assess in future experiments the mechanism of CoCl2 radio-protection in the context of radiation damage by other chemical compounds.

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REFERENCES

1. Karovic O, Tonazzini I, Rebola N et al. Toxic effects of cobalt in primary cultures of mouse astrocytes. Similarities with hypoxia and role of HIF-1 alpha. Biochem Pharmacol 2006;73:694–708.
2. Ponti J, Sabbioni E, Munaro B et al. Genotoxicity and morphological transformation induced by cobalt nanoparticles and cobalt chloride: an in vitro study in Balb/3T3 mouse fibroblasts. Mutagenesis 2009;24:439–45.
3. Hara A, Niwa M, Aoki H et al. A new model of retinal photoreceptor cell degeneration induced by a chemical hypoxia-mimicking agent, cobalt chloride. Brain Res 2006;1109:192–200.
4. Fleury C, Petit A, Mwale F et al. Effect of cobalt and chromium ions on human MG-63 osteoblasts in vitro: morphology, cytotoxicity, and oxidative stress. Biomaterials 2006;27:3351–60.
5. Jung JY, Roh KH, Jeong YJ et al. Estradiol protects PC12 cells against CoCl2-induced apoptosis. Brain Res Bull 2008;76:579–85.
6. Garouel M, Fetoui H, Ayadi Makni F et al. Cobalt chloride induces hepatotoxicity in adult rats and their suckling pups. Exp Toxicol Pathol 2011;63:9–15.
7. Shukla D, Saxena S, Purushothaman J et al. Hypoxic preconditioning with cobalt ameliorates hypobaric hypoxia induced pulmonary edema in rat. Eur J Pharmacol 2011;656:101–9.
8. Borenstein X, Fiszman GL, Bldner A et al. Functional changes in murine mammary cancer cells elicited by CoCl2-induced hypoxia. Nitric Oxide 2010;23:234–41.
9. Cockman ME, Masson N, Mole DR et al. Hypoxia inducible factor-alpha binding and ubiquitylation by the von Hippel-Lindau tumor suppressor protein. J Biol Chem 2000;275:25733–41.
10. Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003;3:721–32.
11. Jantsch J, Chakravortty D, Turza N et al. Hypoxia and hypoxia-inducible factor-1 alpha modulate lipopolysaccharide-induced dendritic cell activation and function. J Immunol 2008;180:697–705.
12. Xi L, Taher M, Yin C et al. Cobalt chloride induces delayed cardiac preconditioning in mice through selective activation of HIF-1α and AP-1 and iNOS signaling. Am J Physiol Heart Circ Physiol 2004;287:H2369–75.
13. Yuan Y, Hilliard G, Ferguson T et al. Cobalt inhibits the interaction between hypoxia-inducible factor-alpha and von Hippel-Lindau protein by direct binding to hypoxia-inducible factor-alpha. J Biol Chem 2003;278:15911–6.
14. Whitlock NA, Agarwal N, Ma JX et al. Hsp27 upregulation by HIF-1 signaling offers protection against retinal ischemia in rats. Invest Ophthalmol Vis Sci 2005; 46:1092–8.
15. Ji Z, Yang G, Shahzidi S et al. Induction of hypoxia-inducible factor-1alpha overexpression by cobalt chloride enhances cellular resistance to photodynamic therapy. Cancer Lett 2006; 244:182–9.
16. Piret JP, Lecoq C, Toffoli S et al. Hypoxia and CoCl2 protect HepG2 cells against serum deprivation- and t-BHP-induced apoptosis: a possible anti-apoptotic role for HIF-1. Exp Cell Res 2004; 295:40–9.
17. Renschler MF. The emerging role of reactive oxygen species in cancer therapy. Eur J Cancer 2004; 40:1934–40.
18. Mishra KP. Cell membrane oxidative damage induced by gamma-radiation and apoptotic sensitivity. J Environ Pathol Toxicol Oncol 2004; 23:61–6.
19. Gault N, Sandre C, Poncy JL et al. Cobalt toxicity: chemical and radiological combined effects on HaCaT keratinocyte cell line. Toxicol In Vitro 2010; 24:92–8.
20. Moon SY, Chang HW, Roh JL et al. Using YC-1 to overcome the radioresistance of hypoxic cancer cells. Oral Oncol 2009; 45:915–19.
21. Bildirici I, Bukulmez O, Ensari A et al. A prospective evaluation of the effect of salpingectomy on endometrial receptivity in cases of women with communicating hydrosalpinges. Hum Reprod 2001; 16:2422–6.
22. Hänze J, Eul BG, Savai R et al. RNA interference for HIF-1α inhibits its downstream signaling and affects cellular proliferation. Biochem Biophys Res Commun 2003; 312:571–7.
23. Kruger NJ. The Bradford method for protein quantitation. Methods Mol Biol 1994; 32:9–15.
24. Leach JK, Van Tuyle G, Lin PS et al. Ionizing radiation-induced, mitochondria-dependent generation of reactive oxygen/nitrogen. Cancer Res 2001; 61:3894–901.
25. Kim GJ, Fiskum GM, Morgan WF. A role for mitochondrial dysfunction in perpetuating radiation-induced genomic instability. Cancer Res 2006; 66:10377–83.
26. Jia D, Koonce NA, Griffin RJ et al. Prevention and mitigation of acute death of mice after abdominal irradiation by the antioxidant N-acetyl-cysteine (NAC). Radiat Res 2010; 173:579–89.
27. Noaman E, Zahran AM, Kamal AM et al. Vitamin E and selenium administration as a modulator of antioxidant defense system: biochemical assessment and modification. Biol Trace Elem Res 2002; 86:55–64.
28. Petit A, Mwale F, Tkaczyk C et al. Induction of protein oxidation by cobalt and chromium ions in human U937 macrophages. Biomaterials 2005; 26:4416–22.
29. Valko M, Rhodes CJ, Moncol J et al. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006; 160:1–40.
30. Dhananjay S, Saxena S, Jayamurthy P et al. Hypoxic preconditioning with cobalt attenuates hypobaric hypoxia induced oxidative damage in rat lung. High Alt Med Biol 2009; 10:57–69.
31. Xing D, Sun X, Li J et al. Hypoxia preconditioning protects corneal stromal cells against induced apoptosis. Exp Eye Res 2006; 82:780–7.
32. Shrivastava K, Shukla D, Bansal A et al. Neuroprotective effect of cobalt chloride on hypobaric hypoxia-induced oxidative stress. Neurochem Int 2008; 52:368–75.
33. Saxena S, Shukla D, Saxena S et al. Hypoxia preconditioning by cobalt chloride enhances endurance performance and protects skeletal muscles from exercise-induced oxidative damage in rats. Acta Physiol (Oxf) 2010; 200:249–63.
34. Gupta S, Athar M, Behari JR et al. Cadmium-mediated induction of cellular defense mechanism: a novel example for the development of adaptive response against a toxicant. Ind Health 1991; 29:1–9.
35. Crawford DR, Davies KJ. Adaptive response and oxidative stress. Environ Health Perspect 1994; 102:25–8.
36. Oliveira NG, Castro M, Rodrigues AS et al. Wortmannin enhances the induction of micronuclei by low and high LET radiation. Mutagenesis 2003; 18:37–44.
37. Sedelnikova OA, Nakamura A, Kovalchuk O et al. DNA double-strand breaks form in bystander cells after microbeam irradiation of three-dimensional human tissue models. Cancer Res 2007; 67:4295–302.
38. Kashino G, Prise KM, Suzuki K et al. Effective suppression of bystander effects by DMSO treatment of irradiated CHO cells. J Radiat Res 2007; 48:327–33.
39. Shao C, Furusawa Y, Kobayashi Y et al. Bystander effect induced by counted high-LET particles in confluent human fibroblasts: a mechanistic study. FASEB J 2003; 17:1422–7.
40. Thierens H, Vral A. The micronucleus assay in radiation accidents. Ann Ist Super Sanita 2009; 45:260–4.