Effect of cobalt and chromium ions on human MG-63 osteoblasts in vitro: Morphology, cytotoxicity, and oxidative stress

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Abstract

Recent studies demonstrated that Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions induced cell mortality, TNF-\textgreek{a} secretion, and oxidation of proteins in macrophages. However, little is known about the effects of corrosion products on the osteogenic cells, which have a crucial role in controlling bone remodeling. The aim of the present study was to investigate the effect of Co\textsuperscript{2+} (0–10 ppm) and Cr\textsuperscript{3+} (0–150 ppm) on human MG-63 osteoblast-like cells in terms of cytotoxicity and oxidative stress. Microscopic analysis demonstrated changes in shape, size, and number of cells. Co\textsuperscript{2+} had a greater effect on these parameters than Cr\textsuperscript{3+}. Cell counting showed a significant decrease in the number of MG-63 osteoblasts in a time- and dose-dependent manner, with Co\textsuperscript{2+} more toxic than Cr\textsuperscript{3+}. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) analysis also showed a decreased cellular activity in presence of Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions. Oxidized and nitrated proteins, two markers of oxidative stress, were detected as single bands and revealed time- and dose-dependent protein modifications. We also studied the expression of three antioxidant enzymes. The expression of heme oxygenase-1 was increased by both ions after 24 h, before decreasing gradually thereafter. Glutathione peroxidase expression was also increased in a concentration- and time-dependent manner by both Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions. Co\textsuperscript{2+} decreased catalase expression while Cr\textsuperscript{3+} increased it in a dose- and time-dependent manner. In conclusion, this study demonstrated that Cr\textsuperscript{3+} and Co\textsuperscript{2+} have a cytotoxic effect on MG-63 osteoblasts and have the potential to modify their redox state.

Keywords: Hip replacement prosthesis; Metal ions; Osteoblast; Cytotoxicity; Oxidation of proteins; Antioxidant enzymes

1. Introduction

The success of total hip arthroplasty (THA) has been limited by the production of wear particles [1]. Because of their potential for improved wear performance, there has been a revived interest in metal–metal (MM) bearings, made of cobalt–chromium–molybdenum alloys, as an alternative to the use of conventional metal–polyethylene bearings. However, the most significant concern associated with MM THA is not wear, loosening, or failure, but rather the production of metal particles and ions [2], which can lead to cellular toxicity [3–8], metal hypersensitivity [9–11], and chromosomal changes [12,13]. However, a meta-analysis of several epidemiologic studies has not shown a causal link between MM THA and malignancy [14].

Given the increasing popularity of the use of MM bearings in young patients in whom there will be the potential for a prolonged presence of metal a prolonged
period of metal ions both locally and systemically, there is an urgent need to characterize the cytotoxic effects of such metallic ions. Cytotoxic effect, which may ultimately lead to cell death, can be expressed by noticeable morphological changes, damage to proteins, and modified protein expression. In this regard, increasing evidence is accumulating on the induction of oxidative stress by transition metal ions such as cobalt and chromium [15]. Under normal conditions, a well-balanced action is orchestrated between the formation and the neutralization of reactive oxygen species (ROS). Oxidative stress occurs in the case of an excess of ROS, which can cause irreversible damage to the cell, especially at the lipid, protein, and DNA level [16–19].

When cells are exposed to oxidative stress, they trigger their antioxidant defense system as a compensatory mechanism to protect them from damage induced by ROS [20]. The antioxidant defense of cells is composed of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), and heme oxygenase-1 (HO-1), and of non-enzymatic substances such as vitamins A and E [21]. CAT catalyses the decomposition of two molecules of hydrogen peroxide (H₂O₂) into one molecule each of diatomic oxygen and water [20,21]. GPx uses glutathione (GSH) as a cofactor to reduce hydroperoxide to water molecules and organic hydroperoxide to alcohols [20,21]. Finally, HO-1 also has cytoprotective properties [22] and is the rate-limiting enzyme in the degradation of heme into free iron, bilirubin, and carbon monoxide (Co) [23].

Although previous results demonstrated that Co²⁺ and Cr³⁺ ions induce the oxidation [24] and the nitration [25] of proteins in human U937 macrophages, little is known about the oxidative status of osteoblasts exposed to these metallic ions. In the present study, we used qualitative (microscopy) and quantitative (cell count, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and Western blot) methods to characterize the effect of Co²⁺ and Cr³⁺ ions on human osteoblast-like cells in terms of cytotoxicity and oxidative stress.

2. Materials and method

2.1. Cell culture

MG-63 osteoblast-like cells (American Type Culture Collection, Manassas, VA), originally isolated from a human sarcoma [26], were used for the experiments. MG-63 are relatively immature osteoblasts that have been well characterized and widely used for testing biomaterials [27–29], and show several similarities with isolated human bone-derived cells [30].

MG-63 osteoblasts were grown in 75 cm² flasks with EMEM/EBSS medium supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin, 0.1 mg/ml sodium pyruvate, and 1 μM non-essential amino acids. Cells were cultured at 37°C in a humidified 5% CO₂ atmosphere. At confluence, adherent cells were detached with 0.25% trypsin/0.03% EDTA (Trypsin/EDTA, Sigma-Aldrich, Oakville, ON, Canada), and seeded in 35 × 10 mm dishes at 3 × 10⁵ cells/ml of culture medium. MG-63 osteoblast-like cells were left in culture for 48 h to adhere and recuperate from trypsinisation. Cells were then cultured with 0–100 ppm CO₂ (CoCl₂, Fisher Scientific, Ville St-Laurent, QC, Canada) and 0–150 ppm Cr³⁺ (CrCl₃, Sigma-Aldrich) for 0–72 h in a humidified 5% CO₂ atmosphere. MG-63 osteoblast-like cells without ions were used as negative control. The choice of ion concentrations was based on previous work on the effect of Co²⁺ and Cr³⁺ ions on macrophages in vitro [24,25,31–33]. The concentrations were most likely higher than those in body fluids of patients with MM prostheses where they were usually in the ppb range [34]. On the other hand, studies reported concentrations varying from 2 to 67 ppm Co and 12 to 260 ppm in tissues around revision THAs [35,36]. Moreover, it had been demonstrated that pre-stimulated cells are more sensitive than non-pre-stimulated cells [37,38]. It is therefore possible that osteoblasts in vitro require higher concentrations of a stimulating exogenous agent than cells in vivo since the latter are subject to multiple stimulating factors.

2.2. Microscopy

At the end of the experiments, the medium was removed by aspiration and replaced to observe adherent cells. Pictures were taken using a Zeiss Axiovert 25 microscope with polarized light (Zeiss, North York, Ont.) equipped with a digital Sony DSC-S70 camera. Images are representative of 3 different series of experiments.

For scanning electron microscopy (SEM) analysis, MG-63 cells were seeded on a glass cover slip in 24 well plates at 3 × 10⁵ cells/ml. At the end of incubation, samples were fixed in 2.5% gluteraldehyde in 0.1M sodium cacodylate buffer for 24 h, rinsed, dehydrated in graded alcohol and amyl acetate solutions, and critical-point dried with CO₂ (CPD, Ladd research industries, Burlington, VT). Samples were sputter-coated with a gold–palladium layer and examined using a Hitachi S4700 SEM at an accelerating voltage of 2.0 kV.

2.3. Cell viability

At the end of the incubation, medium was removed by aspiration, washed once with EMEM/EBSS without serum and detached by addition of 0.25% trypsin/0.03% EDTA for 5 min at 37°C. EMEM/EBSS supplemented medium was added to stop the reaction. A sample of each dish was mixed to Trypan blue solution and cells were counted using a haemocytometer.

Cell viability was also assessed by MTT assay (Roche Diagnostics, Laval, QC, Canada). This test is based on the ability of living cells to reduce tetrazolium dye MTT to purple formazan crystals. For this purpose, MG-63 cells were seeded in 96 well plates at 2 × 10⁵ cells/ml. At the end of incubation, MTT solution was added and the microplate was incubated for 4 h at 37°C in a humidified 5% CO₂ atmosphere as indicated by the manufacturer. Liquid was then removed, solubilization solution added, and microplate was shaken for 15 min before reading at 550 nm on an ELISA microplate reader.

2.4. Detection of oxidized proteins

In order to stabilize carbonyl proteins, derivatization was done by incubation of cell extract with dinitrophenylhydrazine (DNPH) following the procedure recommended by the manufacturer (OxyBlot™. Protein oxidation detection kit; Serologicals Corporation, Norcross, GA). Briefly, cells were lysed in 50 μl of caspase assay lysis buffer from BioSource (Nivelles, Belgium), proteins were separated on 4–20% acrylamide gels (SDS-PAGE), and transferred to nitrocellulose membranes. Oxidized proteins were then detected by Western blot using anti-DNP antibody diluted 1:150 in ChemiBlock™/PBS-tween (0.05%) (1:1) (Chemicon International Norcross, GA). Actin (NeoMarkers, Fremont, CA) expression served as an internal control for protein loading. Proteins were detected using NEW Renaissance chemiluminescence reagents (Perkin-Elmer, Woodbridge, Ont.) and analyzed using a VersaDoc image analysis system equipped with a cooled CCD 12 bit camera (Model 3000, Bio-Rad Laboratories, Mississauga, Ont.).

2.5. Detection of nitrated proteins and antioxidant enzymes

At the end of incubations, MG-63 cells were pelleted by centrifugation at 500g for 5 min, lysed in 50 μl of caspase assay lysis buffer, separated on
4–20% acrylamide gels, and transferred to nitrocellulose membranes as for protein oxidation. The presence of nitrated proteins was detected by immunoblotting using anti-nitrotyrosine polyclonal antibody (Cayman Chemical, Ann Arbor, MI) at a concentration of 0.07 μg/ml. Detection of HO-1 (1:1000, Affinity Bioreagents, Golden, CO), GPx (1:10000, Abcam, Cambridge, MA) and CAT (1:30000, Abcam) were also performed by Western blot. Proteins were detected using NEN Renaissance chemiluminescence reagents and analyzed using a VersaDoc image analysis system. For HO-1 and GPX, ChemiGlow™ West chemiluminescence reagents (Alpha Innotech, San Leandro, CA) was used to enhance their signal.

2.6. Statistical analysis

For studies examining cell viability, the data presented were from four or five independent experiments. Each data point was the mean ± standard deviation. Statistical significance was calculated using the Mann–Whitney U non-parametric test. *P* < 0.05 was considered significant.

3. Results

3.1. Microscopic observations

Incubation of MG-63 osteoblast-like cells with Co²⁺ and Cr³⁺ ions did not reveal any specific changes after 24 h when observed by polarized microscopy. However, changes were observed at 72 h in terms of cell number (Fig. 1). While the control showed confluent cells, cells treated with Co²⁺ or Cr³⁺ revealed a decrease in the number of adherent MG-63 osteoblast-like cells. Co²⁺ seemed to be more harmful than Cr³⁺ due to the larger areas of Petri dishes free of cells.

These microscopic observations were correlated with SEM images (Fig. 2). At 24 h, MG-63 cells treated with or without ions, were well attached to glass cover slips and depicted normal shape. However, significant differences in terms of morphology and cell numbers were observed at 72 h. SEM images of the control cells showed a large number of cells with an elongated characteristic shape (fibroblastic phenotype) from 24 to 72 h. On the other hand, Co²⁺ and Cr³⁺ ions increased the cell size, which was especially obvious for Co²⁺ treated cells. Moreover, the intercellular spaces were more important in the presence of Co²⁺ and Cr³⁺ ions, suggesting a decrease in cell number.

3.2. Cell viability

Cell counting was performed to quantify the cytotoxic effect of Co²⁺ and Cr³⁺ ions. Results showed that both ions decreased the number of MG-63 cells in a time- and dose-dependent manner (Figs. 3(A and B)). Indeed, after 24 h, the number of cells was 55% and 80% of control with 10 ppm Co²⁺ and 150 ppm Cr³⁺ ions, respectively. After 72 h, the number of cells decreased to 25% and 65% of control with 10 ppm Co²⁺ and 150 ppm Cr³⁺, respectively. Therefore, cell viability was more severely affected by Co²⁺ than by Cr³⁺.

MTT tests confirmed that the metabolism of MG-63 was affected by the presence of Co²⁺ and Cr³⁺ ions and that Co²⁺ was more harmful than Cr³⁺ (Figs. 3(C and D)). Indeed, MG-63 proliferation, which can be related to cell viability, was decreased by 40% with 10 ppm Co²⁺ and by 16% with 150 ppm Cr³⁺ after 24 h. The maximal cytotoxicity was reached at 72 h with a decrease of 50% with 10 ppm Co²⁺ and 40% with Cr³⁺. The effect of Co²⁺ was time- and concentration-dependent whereas the effect of Cr³⁺ was concentration-dependent only.

3.3. Effect of Co²⁺ and Cr³⁺ ions on the oxidation and the nitration of proteins

Figs. 4–6 showed the effect of Co²⁺ and Cr³⁺ ions on the oxidation and the nitration of proteins in MG-63 osteoblast-like cells. A single 69 ± 2 kDa DNP-derived
protein band was detected for protein oxidation (Fig. 4). When normalized to actin expression, results showed a
time-dependent effect of Co²⁺ and Cr³⁺ with significant
increase at 24 h and a maximal stimulation of 3.4 and 3.9
times the control after 72 h, respectively (Fig. 5). Results
also showed that protein oxidation was concentration-
dependent (Fig. 6). Indeed, significant protein oxidation
was observed at 72 h with 2.5 ppm Co²⁺ (2.0 times the
control) and 25 ppm Cr³⁺ (1.3 times the control) with a
maximal stimulation reaching 3.4 and 3.9 times compared
to the control with 10 ppm Co²⁺ and 150 ppm Cr³⁺,
respectively.

A single band with a molecular weight of 60 ± 2 kDa was
also detected for protein nitration (Fig. 4). When normalized
Fig. 4. Induction of the oxidation and the nitration of proteins in MG-63 osteoblast-like cells. MG-63 osteoblasts were incubated for 48 h without (control) or with 0–10 ppm Co\textsuperscript{2+} or 0–150 ppm Cr\textsuperscript{3+}. Oxidation and nitration of proteins were measured by Western blot as described in Materials and methods. Results are representative of 4 different experiments.

Fig. 5. Effect of Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions on the oxidation and the nitration of proteins in MG-63 osteoblast-like cells. MG-63 cells were incubated for 0–72 h in the presence of 10 ppm Co\textsuperscript{2+} and 150 ppm Cr\textsuperscript{3+}. Protein oxidation (A) and nitration (B) were analyzed by Western blot as showed in Fig. 4. Results are the mean ± standard deviation of 4 experiments.

Fig. 6. Dose–response effect of Co\textsuperscript{2+} and Cr\textsuperscript{3+} on the oxidation and the nitration of proteins in MG-63 osteoblast-like cells. MG-63 cells were incubated for 0–72 h in the presence of 0–10 ppm Co\textsuperscript{2+} and 0–150 ppm Cr\textsuperscript{3+}. Protein oxidation (A–B) and nitration (C–D) were analyzed by Western blot as showed in Fig. 4. Results are the mean ± standard deviation of 4 experiments.
to actin expression, Co$^{2+}$ and Cr$^{3+}$ ions induced the nitration of this 60 kDa band in a concentration- and time-dependent manner (Figs. 5 and 6). On a time scale, the induction of protein nitration appeared to be significant after 24 h with 10 ppm Co$^{2+}$ (1.7 times the control) and 150 ppm Cr$^{3+}$ (1.7 times the control) and maximum at 48 h with 10 ppm Co$^{2+}$ (2.4 times the control) and 150 ppm Cr$^{3+}$ (3.8 times the control) (Fig. 5). Dose-response studies showed a significant increase of protein nitration at 2.5 ppm Co$^{2+}$ (1.4 times the control) and 25 ppm Cr$^{3+}$ (1.5 times the control), reaching a maximum of 2.4 and 3.8 times the control after 48 h with 10 ppm Co$^{2+}$ and 150 ppm Cr$^{3+}$, respectively (Fig. 6).

3.4. Effect of Co$^{2+}$ and Cr$^{3+}$ ions on the expression of antioxidant enzymes

Figs. 7–9 showed the effect of Co$^{2+}$ and Cr$^{3+}$ ions on the expression of antioxidant enzymes in MG-63 osteoblast-like cells. Results demonstrated a single band for CAT, GPx, and HO-1, at 60 ± 2, 92 ± 2, and 32 ± 2 kDa, respectively (Fig. 7). Fig. 8 presented the effect of Co$^{2+}$ and Cr$^{3+}$ ions on the expression of CAT, GPx and HO-1 after 72 h. The effect of metal ions on the expression of all antioxidant enzymes studied was time-dependent. Indeed, CAT expression was down-regulated by Co$^{2+}$ with a significant decrease after 24 h (11% inhibition) and maximal effect at 72 h (36% inhibition). However, Cr$^{3+}$ progressively increased the expression of CAT with a maximal stimulation of 1.7 times the control at 72 h. GPx expression was up-regulated with both ions in a time-dependent manner reaching 2.5 and 1.9 times the control after 72 h with 10 ppm Co$^{2+}$ and 150 ppm Cr$^{3+}$, respectively. Conversely, HO-1 expression reached its maximal stimulation at 24 h with 10 ppm Co$^{2+}$ (6.0 times the control) and 150 ppm Cr$^{3+}$ (2.0 times the control), and gradually decreased over time to reach 2.0 and 1.2 times the control at 72 h with 10 ppm Co$^{2+}$ and 150 ppm Cr$^{3+}$, respectively (Fig. 8).

CAT was down-regulated by 30% in the presence of 10 ppm Co$^{2+}$, while Cr$^{3+}$ up-regulated its expression in a dose-dependent manner, reaching a maximum of 1.6 times the control with 150 ppm Cr$^{3+}$ at 48 h (Fig. 9). Co$^{2+}$ also induced the expression of GPx and HO-1 in a dose-dependent manner with maximal stimulation reaching 3.2 and 1.6 times the control with 10 ppm Co$^{2+}$ at 48 h. GPx and HO-1 expression was less affected by Cr$^{3+}$ with maximal stimulations reaching 1.4 and 1.8 times the control, respectively, after 48 h (Fig. 9).
4. Discussion

The present study shows that Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions induce a time- and dose-dependent cytotoxic effect on MG-63 osteoblast-like cells. The cytotoxic effect is similar to what was observed in J774 macrophages in vitro\cite{7,31,32}, with Co\textsuperscript{2+} being much more toxic than Cr\textsuperscript{3+}. However, MG-63 osteoblasts seem to be more sensitive to Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions compared to J774 macrophages, probably due to their different lineage and cellular activities. The relatively lower toxicity of Cr\textsuperscript{3+} may be due to the fact that Cr\textsuperscript{3+} is not easily ingested by MG-63 cells. In this regard, electron microscopy studies showed that Cr\textsuperscript{3+} forms nanostructures that are internalized by macrophages in specifically localized cytoplasmic areas, suggesting the need for an active pathway such as phagocytosis\cite{39}. Indeed, it has been shown that chromium is more cytotoxic\cite{40} when administered as chromate or dichromate Cr\textsuperscript{6+}, because Cr\textsuperscript{6+} is much more easily taken up by cells\cite{41}. This may also explain why Co\textsuperscript{2+}, which enters more readily into the intracellular environment, is more toxic than Cr\textsuperscript{3+}. Our choice of exposing osteoblasts to Cr\textsuperscript{3+} was based on the instability of Cr\textsuperscript{6+} in solution, making the interpretation of the results more difficult. Therefore, our results probably underestimated the cytotoxic effect of Cr ions on MG-63 osteoblast-like cells.

Few other authors have evaluated the cytotoxic effect of metal ions on osteoblasts. A study on murine MC3T3-E1 osteoblasts also demonstrated a dose-dependent cytotoxic effect of Cr(NO\textsubscript{3})\textsubscript{3} and cobalt chloride (CoCl\textsubscript{2}), with Co\textsuperscript{2+} being more toxic than Cr\textsuperscript{3+}\cite{42}. However, the cytotoxicity was determined after 8 days of incubation. The cytotoxic effect was observed at ion concentrations similar to the present study. Results also corroborate a previous study showing a decrease in cell viability and proliferation of ion-stimulated MG-63 cells at 48 h\cite{43}. Co\textsuperscript{2+} were also more cytotoxic than Cr\textsuperscript{3+}.

Previous studies suggested that oxidative stress might be responsible for the cytotoxic effect of Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions in human U937 macrophages\cite{24,25}. The present study supports the hypothesis that Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions affect the cellular redox state of osteoblasts in vitro. More specifically, the results demonstrated that Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions induce the oxidation and nitration of proteins in a dose- and time-dependent manner. Protein carbonyl is the most commonly measured end product of protein oxidation in biological samples and is known for its deleterious effect on protein function and its implication in several diseases\cite{16,19}. Protein tyrosine nitration has been also demonstrated to be a major biomarker for oxidative stress in various models\cite{44}. In the present study, results show that the induction of protein nitration was maximal after 48 h incubation with both ions, whereas protein oxidation reached its peak intensity at 72 h. This may be explained by the fact that the initial reactive species are different in each case. Results suggest that the induction of oxidative stress may be responsible, at least in part, for the cytotoxic effect of Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions in the MG-63 osteoblast-like cells. These results are in agreement with previous studies showing that Co\textsuperscript{2+}-induced oxidative stress in Chinese hamster ovary (CHO) cells\cite{45} and that Cr\textsuperscript{3+} enhanced the generation of ROS in a concentration-dependent manner in murine macrophages, which was correlated with elevated cytotoxicity\cite{44,46}.

A single oxidized protein band and a single nitrated protein band were detected by Western blot. This is surprising since the production of ROS and RNS leading to oxidative damage is not protein-specific. However, these results are supported by a previous study showing the presence of only one oxidized and one nitrated protein in the serum of smoker and non-smoker lung cancer patients\cite{47}. Also, in the recent moving of molecular medicine beyond genomics to proteomics, it has been clearly demonstrated that the presence of one band on one-dimensional gel electrophoresis may be attributed to several proteins of, sometimes, functional complexes\cite{48,49}. Nevertheless, the nature of the protein(s) remains to be investigated.

![Fig. 9. Dose–response effect of Co\textsuperscript{2+} and Cr\textsuperscript{3+} ions on the expression of anti-oxidant enzymes in MG-63 osteoblast-like cells. MG-63 osteoblasts were incubated for 48 h in the presence of 0–10 ppm Co\textsuperscript{2+} (A) or 0–150 ppm Cr\textsuperscript{3+} (B) ions. Protein expression was performed by Western blot as shown in Fig. 7. Results are the mean ± standard deviation of 4 experiments.](image-url)
Another explanation for cell damage may be related to failure of the active cellular defense mechanisms that remove these reactive species from the cells. Very little is known on the implications of antioxidant enzymes to protect the cell from Co\(^{2+}\) and Cr\(^{3+}\) ions. In the present study, CAT was down-regulated by Co\(^{2+}\) and slightly up-regulated by Cr\(^{3+}\). This may due to the higher toxicity of Co\(^{2+}\) compared to Cr\(^{3+}\). In fact, it has been proposed that under high oxidative stress, the activity of some antioxidant enzymes can decrease, while moderate levels of ROS induce a rise in their activity [50]. The down-regulation of CAT expression by Co\(^{2+}\) is in agreement with the down-regulation of CAT activity reported in vivo in mice [51] and rat [52] liver in response to CoCl\(_2\) loading. The cellular response to Cr\(^{3+}\) seems to be related to the concentration of ions. Indeed, low concentrations of Cr\(^{6+}\)-induced CAT activity in human epithelial cells whereas high Cr\(^{6+}\) decreased CAT activity [53]. Other studies have showed no effect of Cr\(^{6+}\) on CAT mRNA expression in human lung cells [54]. Nevertheless, the weak stimulatory effect of Cr\(^{3+}\) and the inhibitory effect of Co\(^{2+}\) on CAT expression suggest that this enzyme is not particularly protective against the oxidative stress induced by these ions in MG-63 osteoblast-like cells.

Contrary to CAT, GPx expression was slightly, but significantly, increased with both Co\(^{2+}\) and Cr\(^{3+}\) ions. This is in agreement with the induction of GPx expression by cadmium and nickel in CHO-K1 cells [55]. However, in vitro studies have showed that CoCl\(_2\) loading had no significant effect on GPx activity in liver [51,52] while in vitro studies suggested that the effect of Cr\(^{6+}\) on GPx activity depends on the ion concentration and cell type [53,54]. Finally, the inductive effect was slower to what was observed for CAT with significant effect reaching only 48 h after incubation, probably reflecting the different activity of the enzymes. Indeed, GPx is involved in detoxification of metabolites, as well as in the long-term regulation of cell death and cell proliferation [56,57]. Nevertheless, the increase of GPx expression by Co\(^{2+}\) and Cr\(^{3+}\) ions seems insufficient to prevent cell death.

HO-1 is part of the first line of defense against oxidative stress [58]. Also, end products of HO-1, which consists of bilirubin and CO, are able to scavenge oxygen radicals to prevent cell against harmful oxidative stress [58]. The present results revealed that in MG-63 cells, Co\(^{2+}\) and Cr\(^{3+}\) ions induced a rapid stimulation of HO-1 expression that is maximal at 24 h and decreased gradually thereafter. This is in agreement with the stimulation of HO-1 expression by Co\(^{2+}\) in vivo [51,52] and in vitro [45,59], as well as with the effect of Cr\(^{6+}\) in vitro. However, the effect of Cr\(^{3+}\) was less important than the Cr\(^{6+}\) one, probably due to their different toxicity [15,60]. Moreover, a rapid increase of HO-1 expression with a smooth decrease thereafter was also observed in Cr\(^{6+}\)-stimulated human lung cells [54]. This elevated and rapid response of HO-1 to Co\(^{2+}\) and Cr\(^{3+}\) ions may explain the weak effect of these ions on MG-63 cell viability and cell proliferation at 24 h. Also, the stimulation of HO-1 expression was higher at 24 h to what was observed with the other antioxidant enzymes we studied, suggesting an important defensive role of this enzyme at the beginning of the incubation with metallic ions. However, the up-regulation of HO-1 expression is still not enough to protect MG-63 cells against the long-term effects of Co\(^{2+}\) and Cr\(^{3+}\) ions. A possible explanation for this lack of protection from HO-1 may reside in its paradoxical effect. Indeed, it has been reported that over-expression of HO-1 may induced oxidative cell injury in transfected cells [50]. This paradoxical effect of HO-1 remains to be investigated in MG-63-osteoblast like cells.

5. Conclusion

The present study shows that Co\(^{2+}\) (0–10 ppm) and Cr\(^{3+}\) (0–150 ppm) ions induce cytotoxicity and oxidative stress in MG-63 human osteoblast-like cells. However, although the expression of antioxidant enzymes is stimulated by both Co\(^{2+}\) and Cr\(^{3+}\) ions, it seems insufficient to prevent cellular damage that can lead to decreased cell viability and proliferation.

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