Toxicology in Vitro 28 (2014) 700–706

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Effect of hexavalent chromium on proliferation and differentiation to adipocytes of 3T3-L1 fibroblasts

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ARTICLE INFO

Article history:
Received 8 November 2013
Accepted 15 February 2014
Available online 25 February 2014

Keywords:
Hexavalent chromium
3T3-L1 fibroblasts
Proliferation
Adipogenesis
PPAR gamma

ABSTRACT

Heavy metals contamination has become an important risk factor for public health and the environment. Chromium is a frequent industrial contaminant and is also used in orthopaedic joint replacements made from cobalt–chromium-alloy. Since hexavalent chromium (Cr(VI)) was reported as genotoxic and carcinogenic in different mammals, to further evaluate its cytotoxicity, we investigated the effect of this heavy metal in the proliferation and differentiation to adipocytes of 3T3-L1 fibroblasts. These cells, after the addition of a mixture containing insulin, dexamethasone and methylisobutylxanthine, first proliferate, a process known as mitotic clonal expansion (MCE), and then differentiate to adipocytes. In this differentiation process a key transcription factor is induced: peroxisome proliferator-activated receptor gamma (PPAR gamma). We found that treatment of 3T3-L1 fibroblasts with potassium chromate inhibited proliferation in exponentially growing cells and MCE as well as differentiation. A decrease in PPAR gamma content, evaluated by western blot and immunofluorescence, was found in cells differentiated in the presence of chromium. On the other hand, after inhibition of differentiation with chromium, when the metal was removed, differentiation was recovered, which indicates that this may be a reversible effect. We also found an increase in the number of micronucleated cells after treatment with Cr(VI) which is associated with genotoxic effects. According to our results, Cr(VI) is able to inhibit proliferation and differentiation to adipocytes of 3T3-L1 fibroblasts and to increase micronucleated cells, which are all indicative of alterations in cellular physiology and therefore, contributes to further elucidate the cytotoxic effects of this heavy metal.

1. Introduction

Heavy metals contamination has become an important risk factor for public health and the environment. Chromium is a frequent industrial contaminant and is also used in orthopaedic joint replacements made from cobalt–chromium-alloy.

In the last years, public concern about environmental exposure to chromium in soil, tap water and ambient air has been increasing. According to WHO, maximum permitted concentration of chromium in water is 50 μg/l (1 μM). Most surface waters contain between 1 and 10 μg chromium/l, and in USA levels up to 84 μg/l have been found. In general, the chromium content in surface waters reflects the extent of industrial activity[1].

In industrial workers, exposure to chromium particles in the air is associated with lung cancer. Hexavalent chromium (Cr(VI)) is carcinogenic in humans and also in rodents when administered in drinking water as sodium dichromate dihydrate (Witt et al., 2013; Kopec et al., 2012; Salnikow and Zhitkovich, 2008).

Malignancy is associated with an increase in DNA damage and chromosomal aberrations. Cr(VI), has been shown to induce chromosomal aberrations in human fibroblasts *in vitro* and the damage is predominantly aneugenic (Figgitt et al., 2010). The involvement of oxidative stress in the cytotoxic effects of this ion has also been proposed (Kawanishi et al., 2002; Raghunathan et al., 2009).

On the other hand, it has recently been reported that oral administration of potassium dichromate to male rats (1–2 mg/kg) promotes reproductive system toxicity and affects testicular function of adult male rats (Marouani et al., 2012). The toxic effects of lactational Cr(VI) exposure on ovary and uterus in developing female Wistar rats has also been reported (Samuel et al., 2012).

3T3-L1 fibroblasts are a useful tool in the study of adipocyte differentiation. After the addition of a differentiation mixture containing insulin, dexamethasone and 3-isobutyl-1-methylxanthine (MIX), post-confluent 3T3-L1 fibroblasts re-enter the cell cycle. This proliferation step is called mitotic clonal expansion (MCE). MCE precedes the adipogenic gene expression program leading to

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[1] http://www.who.int/water_sanitation_health/dwq/chemicals/chromium.pdf.

http://dx.doi.org/10.1016/j.tiv.2014.02.003
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adipocyte differentiation (Qiu et al., 2001; Martini et al., 2009). In this differentiation process a key transcription factor is induced: peroxisome proliferator-activated receptor gamma (PPAR gamma), which is involved in the induction of proteins associated with the adipocyte phenotype (Farmer, 2006). 3T3-L1 fibroblasts were previously used to evaluate the role of arsenic trioxide, as well as, of a commercial preparation of the herbicide glyphosate, on cell death and differentiation (Wang et al., 2005; Martini et al., 2012).

Since hexavalent chromium was reported as genotoxic and carcinogenic in different mammals, to further evaluate its cytotoxicity, we investigated the effect of this heavy metal on the proliferation and differentiation to adipocytes of 3T3-L1 fibroblasts.

2. Materials and methods

2.1. Chemicals

Dulbecco's Modified Eagle Medium (DMEM), trypsin, insulin, Bodipy 493/503 and Alexa Fluor 568 were obtained from Invitrogen (Carlsbad, CA), 3-isobutyl-1-methylxanthine (MIX) dexamethasone and Hoechst 33258 were purchased from Sigma Chemical Co. (St. Louis, MO). Potassium chromate (K_2CrO_4, CAS 7789-00-6) was purchased from Mallinckrodt (Argentina). 3T3-L1 fibroblasts were obtained from Asociación Banco Argentino de Células (origin: ATCC).

2.2. Cell cultures and treatments

3T3-L1 fibroblasts were cultured in DMEM + 10% fetal bovine serum (FBS) with 100 μg/ml streptomycin, 100 U/ml penicillin and 250 ng/ml fungizone (DMEM + 10% FBS). Mouse embryonic fibroblasts (MEFs) were prepared from CF-1 mouse embryos at day 14 of gestation by culture of small tissue explants in DMEM supplemented with 10% FBS and antibiotics as previously described (Lukas et al., 1995). The outgrowing primary cell population was passaged by trypsinization at a ratio of 1:3–1:4 upon confluency and continuously cultured in DMEM with 10% FBS to favor growth of fibroblastic cells.

2.3. Cell counting in exponentially growing cells

3T3-L1 fibroblasts or MEFs were cultured in 24-well plates until they reached 30–40% confluence. At that moment, some wells were treated for 24, 48 or 72 h with different doses of potassiun chromate, as indicated in each case and others were treated with DMEM + 10% FBS alone (control, C). Potassium chromate stock solution was prepared in DMEM and then it was diluted in cell culture media in order to obtain the final concentrations indicated in each assay. At the end of these treatments, cells were either trypsinized, resuspended in phosphate-buffered saline (PBS) and an aliquot was counted using a Neubauer chamber or incubated for 1 h at 37 °C with filtered MTT solution (5 mg/ml), which was added to each well to reach a final concentration of 1 mg/ml MTT. Afterwards, the supernatants were carefully aspirated and 200 μl of ice-cold ethanol was added to each well to dissolve the crystal product. Absorbance was measured at 570 nm with a plate-reader.

2.4. Differentiation induction of 3T3-L1 fibroblasts or MEFs and Oil-Red-O staining

To induce differentiation, two-days postconfluent 3T3-L1 fibroblasts or MEFs were treated with a differentiation mixture containing 10 μg/ml insulin, 0.5 mM 3-isobutyl-1-methylxanthine (MIX) and 100 nM dexamethasone in DMEM + 10% FBS (DM). Three days after the induction of differentiation, medium was replaced with DMEM + 10% FBS supplemented with 10 μg/ml insulin. Then medium was changed every 2 days with DMEM + 10% FBS. When indicated, potassium chromate was added, as described in each experiment, to obtain the appropriate final concentration. Adipocyte monolayers (usually on day seven) were washed three times with PBS and then fixed for 30 min with 4% formaldehyde in PBS. Oil-Red-O (0.35%) in isopropanol was diluted with water (3:2), filtered, and added to the fixed cell monolayers for 30 min at room temperature. Cells were then washed with water, and the stained triglyceride droplets in the cells were visualized and photographed.

2.5. Western-blot determination

Seven days after induction of differentiation of 3T3-L1 fibroblasts in the absence (DM) or presence of 10 μM potassium chromate (DM + 10 μM Cr(VI)) and untreated (C) cells were harvested in PBS, vortexed and before the addition of Laemmli sample buffer (Laemmli, 1970) an aliquot was obtained for protein determination using the method of Bradford with serum albumin as standard (Bradford, 1976). After SDS-PAGE using equal amount of protein in each lane (5–30 μg), gels were transferred to nitrocellulose membranes, blocked with 5% milk for an hour and then treated overnight with PPAR gamma antibody (from Cell Signaling) followed by HRP-conjugated secondary antibody for 1 h. Finally, chemiluminescence reagent was used for detection.

2.6. Immunofluorescence microscopy for PPAR gamma and lipid detection

Fibroblasts were cultured on coverslips in 24 well-plates and 10 days after induction of differentiation, in the absence (DM) or presence of 10 μM potassium chromate (DM + Cr(VI)) and untreated (C) cells were fixed in 4% formaldehyde, permeabilized in PBS-0.025% Tween 20 and blocked with 5% bovine serum albumin. Then cells were incubated with anti-PPAR gamma antibody followed by Alexa 568-conjugated secondary antibody. Finally the cells were stained with Bodipy 493/503 for visualization of the lipid droplets, and with Hoechst 33258 for visualization of the nuclei.

2.7. Cell counting after MCE

Three days after the addition of DMEM + 10% FBS alone (C), the differentiation mixture without (DM) or with potassium chromate added at the concentrations and time indicated in each case (DM + Cr(VI)), 3T3-L1 cells from 24-well plates were washed with PBS, trypsinized, resuspended in PBS and an aliquot was counted using a Neubauer chamber.

2.8. Statistical analysis

The experiments were carried out three times unless otherwise stated. All data were expressed as mean ± S.E. Statistical analysis was performed by One-Way ANOVA followed by Tukey's post-hoc test, p values below 0.05 were considered significant.

3. Results

3.1. Effect of hexavalent chromium on the proliferation of 3T3-L1 fibroblasts

To evaluate the effect of Cr(VI) in the proliferation of 3T3-L1 fibroblasts, we first tested the effect of the addition of different doses of potassium chromate to exponentially growing cells using MTT colorimetric assay. We found a dose-dependent effect after treatment with Cr(VI) for 24 h, as it is shown in Fig. 1A. Doses of

10 μM Cr(VI) or higher inhibited the increase in cell number while doses of 5 μM or lower not significantly differ from control samples not treated with chromium (C24). The absorbance in MTT assay in cells treated with 25 μM Cr(VI) remained similar to the one obtained at the beginning of the experiment when chromium was added (zero time control, C0) suggesting that proliferation was blocked. At higher doses the absorbance was even lower than that at C0 and some cells were detached from the plates and floated, resulting in a lower number of cells with respect to C0.

When treatments with the metal were extended to 48 h, inhibition was enhanced but a similar dose response was obtained (Fig. 1B). However, when treatment with chromium was extended to 72 h, 5 μM chromium was able to inhibit proliferation and at 10 μM the inhibition was greater than that obtained with shorter treatments and reached a value similar to that of C0 (Fig. 1C). Taking into account the values obtained with 10 μM chromium at 24 and 48 h, this result suggests that the number of cells detached was increasing with the extension of the time of treatment with the metal.

We further analyzed the dose-dependent effect of chromium on 3T3-L1 fibroblasts proliferation by cell counting in Neubauer chamber at 24 and 72 h and found similar results (Fig. 2A and B).

3.2. Effect of hexavalent chromium on the differentiation of 3T3-L1 fibroblasts to adipocytes

Since 10 μM Cr(VI) was able to inhibit proliferation of 3T3-L1 fibroblasts, we wanted to evaluate its effect on the differentiation

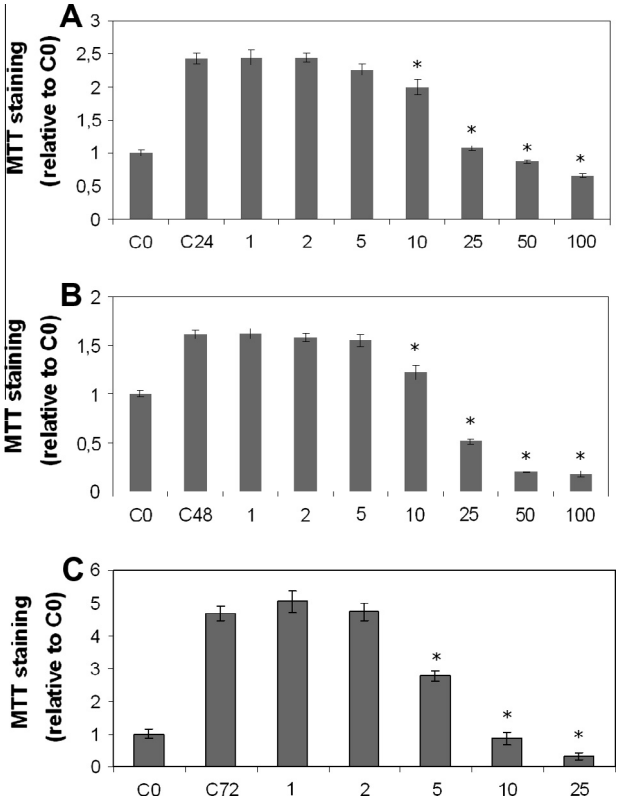

Fig. 1. Dose and time-dependent effect of Cr(VI) on 3T3-L1 fibroblasts proliferation using MTT assay. Cells were cultured in 24-well plates until they reached 30–40% confluence. At that time, two wells were counted as zero time control (C0). Others were treated for 24 (A), 48 (B) or 72 h (C) with medium (C24, C48 and C72 respectively) or different micromolar concentrations of Cr(VI) as indicated in each case. At the end of these treatments, cells were incubated with filtered MTT solution as indicated in methods. Results represent mean ± S.E. of three independent experiments. * Significantly different from C24 (A), C48 (B) or C72 (C), respectively, $p < 0.05$ (ANOVA).

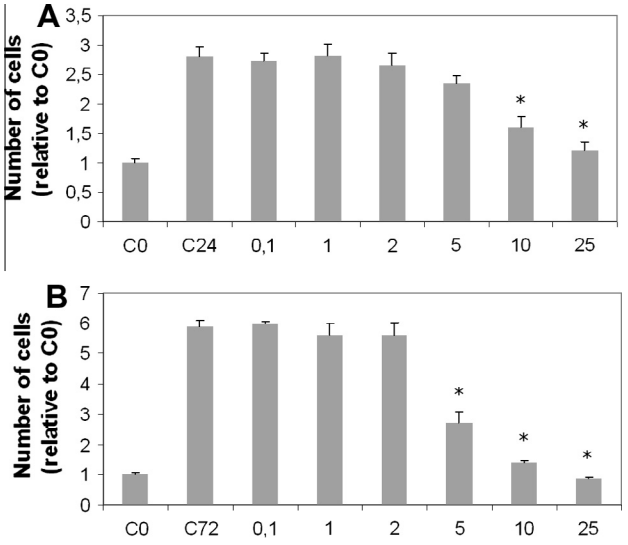

Fig. 2. Dose and time-dependent effect of Cr(VI) on 3T3-L1 fibroblasts proliferation analyzed by cell counting. Cells were cultured in 24-well plates until they reached 30–40% confluence, at that time, two wells were counted as zero time control (C0) and others were treated for 24 (A) or 72 h (B) with medium (C24 and C72, respectively) or different micromolar concentrations of Cr(VI) as indicated in each case. At the end of these treatments, cells were trypsinized and counted in Neubauer chamber as indicated in methods. Results represent mean ± S.E. of three independent experiments. * Significantly different from C24 (A), and C72 (B), $p < 0.05$ (ANOVA).

to adipocytes. Thus, 3T3-L1 fibroblasts were induced to differentiate in the presence of 10 μM chromium until differentiation was complete. After staining cytosolic triglycerides with Oil-Red-O, we found that differentiation was noticeably inhibited (Fig. 3A).

Taking into account that PPAR gamma is a master gene in this differentiation process (Farmer, 2006), we evaluated the amount of this transcription factor by western-blot. As expected, PPAR gamma was increased in samples treated with differentiation mixture with respect to control cells. However, when cells were treated with differentiation mixture in the presence of 10 μM chromium, this increase was inhibited, which is in keeping with the inhibition found in the differentiation of these cells to adipocytes (Fig. 3B).

3.3. Effect of the removal of hexavalent chromium on the differentiation of 3T3-L1 fibroblasts to adipocytes

On the other hand, we wanted to evaluate if 3T3-L1 fibroblasts were able to recover the ability to differentiate after removal of the metal. According to our results, if 10 μM chromium was present until differentiation was reached, usually 7 days after the induction of differentiation, lipid accumulation was severely inhibited as well as PPAR gamma (Fig. 3A and B). When we removed chromium at this point (DM + Cr(VI) 7d) and three days later we compared the differentiation of these cells with cells continuously exposed to chromium for the 10 days (DM + Cr(VI) 10d), we found that cytosolic lipid accumulation and nuclear PPAR gamma content, evaluated by Bodipy 493/503 staining and immunofluorescence respectively, were recovering after removal of the metal (Fig. 4).

Consistently, when 10 μM chromium was only present during the three days of induction with the differentiation mixture and then was removed (DM + Cr(VI) 3d), we found that seven days later, differentiation was similar to the one obtained in cells not treated with chromium (Fig. 4). These results indicate that removal of chromium allows cells to recover differentiation.

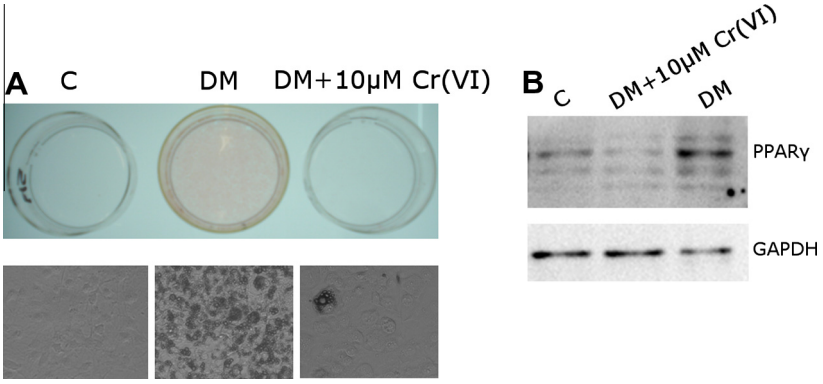

Fig. 3. Effect of Cr(VI) on differentiation of 3T3-L1 fibroblasts. Two days post-confluent cells were treated with: medium alone (C), or with the addition of differentiation mixture (DM), or differentiation mixture plus 10 μM Cr(VI) which was maintained in the medium supplemented with insulin that was added 3 days after induction of differentiation (DM + 10 μM Cr(VI)). Seven days after induction of differentiation: (A) adipocytes were stained with Oil-Red-O and photographed or (B) the amount of PPAR gamma was analyzed by western-blot. Results shown are from a representative experiment repeated three times with similar results.

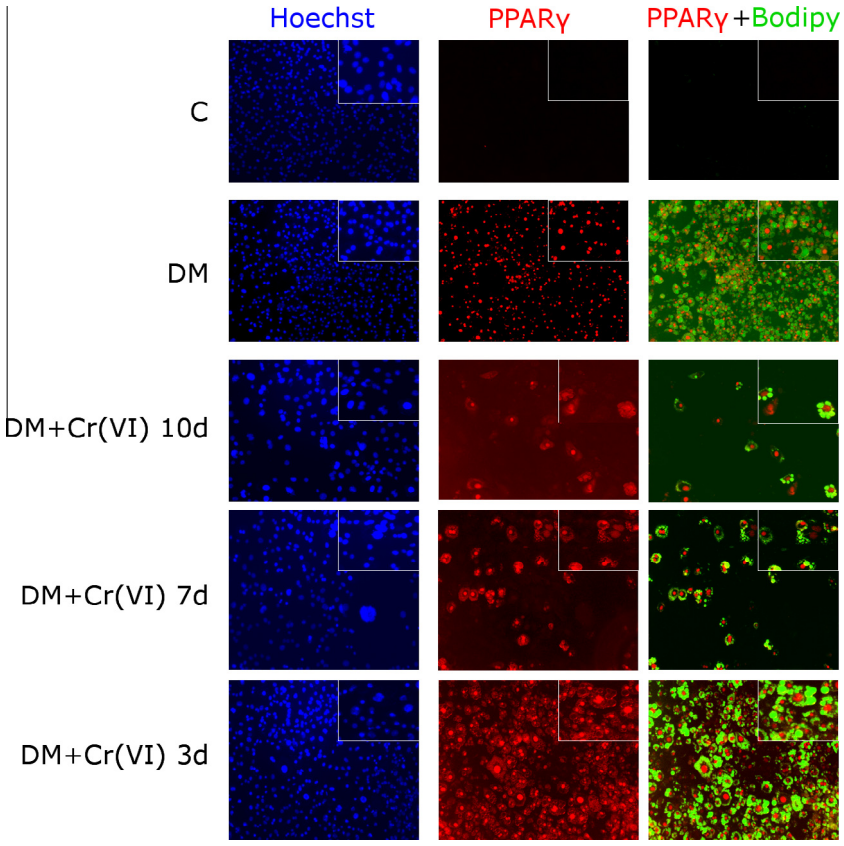

Fig. 4. Effect of the removal of Cr(VI) on the differentiation of 3T3-L1 fibroblasts. Two days post-confluent cells were treated with: medium alone (C), or with the addition of differentiation mixture (DM), or differentiation mixture plus 10 μM Cr(VI). Cr(VI) was maintained for 3 (DM + Cr(VI) 3d), 7 (DM + Cr(VI) 7d) or 10 (DM + Cr(VI) 10d) days after induction. At day 10, the amount of nuclear PPAR gamma was analyzed by immunofluorescence using Alexa 568-conjugated antibody (red), lipid droplets were stained with Bodipy 493/503 (green) and nuclei were stained with Hoechst (blue). Results shown are from a representative experiment repeated three times with similar results. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Effect of hexavalent chromium pretreatment on the differentiation of 3T3-L1 fibroblasts to adipocytes

The effect of the pretreatment with Cr(VI), which was added 24 h prior to the addition of the differentiation mixture was also investigated. In these experiments, doses tested were lower than 10 μM, which almost completely inhibited differentiation. We found that 5 μM chromium, when it was present during the 7 days of differentiation, significantly inhibited cytosolic triglyceride accumulation evaluated by Bodipy 493/503 staining (Fig. 5), although this effect was lower than the one obtained with the dose of 10 μM (Fig. 4). Interestingly, the inhibition obtained with 5 μM chromium was enhanced when pretreatment with the metal for 24 h was also included (Fig. 5).

On the other hand, neither 2 μM nor 1 μM chromium significantly decreased differentiation when they were present along

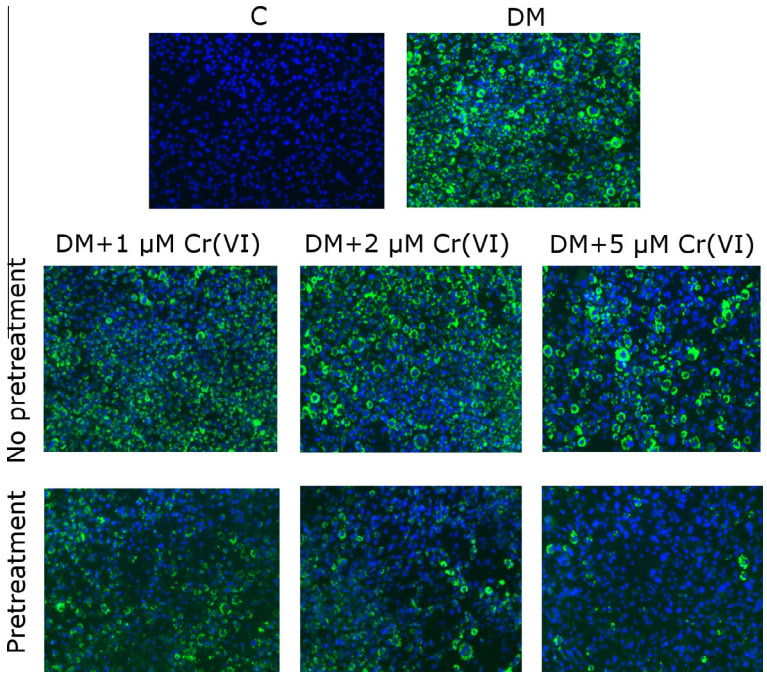

Fig. 5. Effect of Cr(VI) pretreatment on the differentiation of 3T3-L1 fibroblasts. Two days post-confluent cells were treated with: DMEM + 10% FBS (C) or with the addition of differentiation mixture (DM) (upper panel), or differentiation mixture + Cr(VI) at the indicated micromolar concentrations (DM + Cr(VI)) and Cr(VI) was maintained in the medium that was replaced 3 days after induction of differentiation (middle panel). Other cells were pretreated with the different concentrations of Cr(VI) and then were treated with differentiation mixture + Cr(VI) at the same concentration that was used in each pretreatment (lower panel). 7 days after induction of differentiation lipid accumulation was evaluated by Bodipy 493/503 staining (green) and the nuclei were stained with Hoechst (blue) as indicated in methods. Results shown are from a representative experiment repeated three times with similar results. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the differentiation process. However, when pretreatment with 2 μM chromium for 24 h was also included, differentiation was significantly reduced (Fig. 5).

3.5. Effect of hexavalent chromium on mitotic clonal expansion

When post-confluent 3T3-L1 fibroblasts are induced to differentiate, before adipogenesis takes place, the cells re-enter the cell cycle, a process called Mitotic Clonal Expansion (MCE). We investigated the effect of Cr(VI) in MCE. As expected, the addition of differentiation mixture increased cell number, but this increase was almost blocked in the presence of 5 or 10 μM chromium, while concentrations of 2 or 1 μM partially or not significantly inhibited proliferation, respectively (Fig. 6). However, pretreatment with 2 μM chromium for 24 h, significantly enhanced the inhibition obtained. These results further probe the ability of this metal to inhibit proliferation of 3T3-L1 fibroblasts in a dose dependent manner as well as the ability of the pretreatment to enhance the effect.

3.6. Effect of hexavalent chromium on the number of micronucleated cells

Since genotoxic effects of Cr(VI) in human fibroblasts have previously been reported (Figgitt et al., 2010), and an increase in cells with micronuclei is associated with DNA damage, we also investigated the number of micronucleated cells in exponentially growing 3T3-L1 fibroblasts after nuclear staining with Hoechst. We found that treatment of exponentially growing 3T3-L1 fibroblasts for 72 h with doses of 5 or 10 μM Cr(VI), which inhibits proliferation and differentiation, also significantly increased the number of micronucleated cells (Table 1).

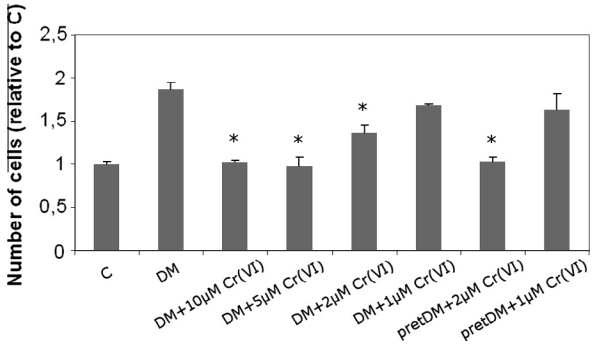

Fig. 6. Effect of Cr(VI) on mitotic clonal expansion. Two days post-confluent 3T3-L1 fibroblasts were treated with: DMEM + 10% FBS (C) or with the addition of differentiation mixture alone (DM), or differentiation mixture + Cr(VI) at the indicated micromolar concentrations (DM + Cr(VI)). Other cells were pretreated with two different concentrations of Cr(VI) (2 and 1 μM), 24 h prior to treatment with the differentiation mixture + Cr(VI) at the same concentration that was used in each pretreatment (pretDM + 2 μM Cr(VI) and pretDM + 1 μM Cr(VI), respectively). After 3 days, cells were counted. Results represent mean ± S.E. of three independent experiments. * Significantly different from DM, $p < 0.05$ (ANOVA).

3.7. Effect of hexavalent chromium on proliferation and differentiation to adipocytes of MEF

To confirm the ability of Cr(VI) to affect preadipocytes ability to proliferate and differentiate, we prepared mouse embryonic fibroblasts (MEFs) to evaluate the effect of this metal on proliferation and differentiation to adipocytes. As it is shown in Fig. 7, Cr(VI) was able to inhibit proliferation of exponentially growing cells and differentiation at similar doses of those found in 3T3-L1 fibroblasts.

Table 1
Effect of hexavalent chromium on the number of micronucleated cells.

Treatment	% Micronucleated cells
C	6.7 ± 0.6
2 μM Cr	9.4 ± 0.2
5 μM Cr	18.3 ± 1.6*
10 μM Cr	24.2 ± 1.2*

3T3-L1 cells were cultured in 24-well plates until they reached 30–40% confluence. At that time cells were treated for 72 h with medium (C) or different concentrations of hexavalent chromium (Cr) as indicated in each case. At the end of these treatments nuclei were stained with Hoechst as indicated in methods and at least 1000 cells were counted and the percentage of micronucleated cells were determined. Results represent mean ± S.E. of two independent experiments.
* Significantly different from C, $p < 0.05$ (ANOVA).

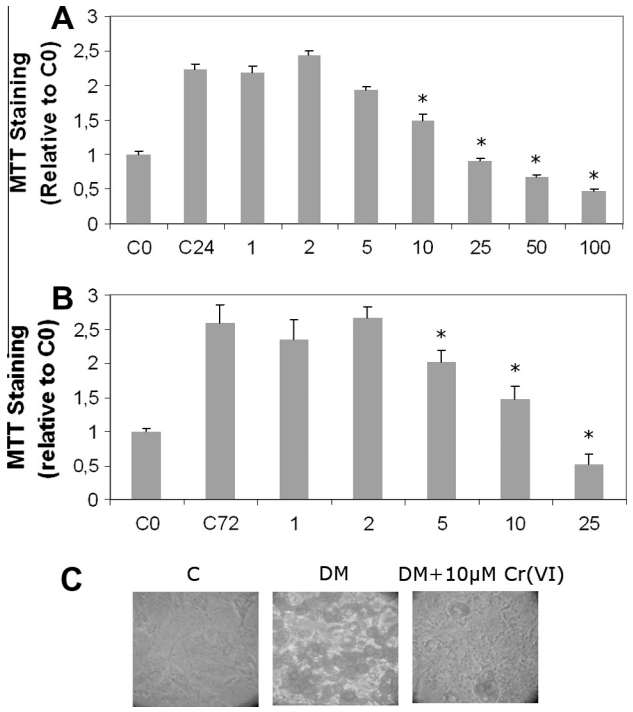

Fig. 7. Effect of Cr(VI) on proliferation and differentiation of MEFs. (A and B) MEFs were cultured in 24-well plates until they reached 50% confluence. At that time, two wells were counted as zero time control (C0). Others were treated for 24 (A), or 72 h (B) with medium (C24 and C72 respectively) or different micromolar concentrations of Cr(VI) as indicated in each case. At the end of these treatments, cells were treated with MTT. Results represent mean ± S.E. of three independent experiments. * Significantly different from C24 (A), or C72 (B), respectively, $p < 0.05$ (ANOVA). (C) Two days post-confluent MEF were treated with: DMEM + 10% FBS alone (C), or with the addition of: differentiation mixture (DM), or differentiation mixture + 10 μM Cr(VI) and Cr(VI) was maintained in the medium that was replaced 3 days after induction of differentiation (DM + 10 μM Cr(VI)). Seven days after induction of differentiation, adipocytes were stained with Oil-Red-O and stained triglyceride droplets in the cells were visualized and photographed as indicated in methods. Results shown are from a representative experiment repeated three times with similar results.

4. Discussion

In this paper we found that hexavalent chromium was able to inhibit proliferation of exponentially growing 3T3-L1 fibroblasts as well as MCE, the proliferation step that precedes differentiation. When exponentially growing cells were treated for 24 o 48 h with doses higher than 25 μM several cells were detached and lost, suggesting an increase in cell death. Moreover, most of the cells that remained attached to the plate were dead, as analyzed by flow cytometry after propidium iodide staining (data not shown).

Herein, we also found that Cr(VI) was able to increase micronucleated cells at doses of 5 and 10 μM in exponentially growing 3T3-L1 fibroblasts which is indicative of genotoxic effects. This is in agreement with a previous report in human fibroblasts (Figgitt et al., 2010) and suggests that DNA damage may be involved in the inhibition of proliferation found in 3T3-L1 fibroblasts.

On the other hand, we found that treatment with Cr(VI) inhibited differentiation of 3T3-L1 fibroblasts to adipocytes which was determined not only by a decrease in cytosolic lipid accumulation but also by a decrease in the amount of PPAR gamma, a transcription factor necessary for adipogenesis (Figs. 3 and 4). In agreement with these results, we also found that Cr(VI) was able to inhibit proliferation of exponentially growing cells and differentiation to adipocytes of MEF, which is another cell model to study adipogenesis.

In addition, we found that when 3T3-L1 fibroblasts were induced to differentiate in the continuous presence of Cr(VI), differentiation was inhibited. However, if the metal was removed three or seven days after the induction, differentiation could be recovered. Interestingly, in both cases the differentiation mixture was removed after three days of induction and was not added later in the replaced medium suggesting that differentiation was arrested upstream of PPAR gamma by the presence of chromium, and resumed after a lag period when the metal was removed. The ability of 3T3-L1 fibroblasts to recover differentiation by removal of an inhibitor of differentiation has previously been reported with two different inhibitors, arsenic trioxide (Wang et al., 2005) and a commercial formulation of the herbicide glyphosate (Martini et al., 2012).

We found that pretreatment with the metal (Fig. 5) or extension of the time of treatment (Fig. 1) were able to enhance its effect or even reduced the dose required.

In spite of the fact that the doses used in this paper (1–10 μM) are higher than those reported in peripheral blood in patients with cobalt–chromium-alloy implants, which are similar to those reported in exposed workers (around 0.2 μM or lower) (Keegan et al., 2008), the presence of higher local doses, depending on the site of entrance of the metal, could not be discarded. Consistently, it was reported that in synovial fluid retrieved at revision arthroplasty from patients with cobalt–chromium-alloy metal-on-metal prostheses, chromium concentration varied from 0.95 to 6.88 μM while in osteoarthritic joints in the absence of a prostheses, concentrations varied from 0.05 to 0.13 μM (Davies et al., 2005). Thus, the concentrations found in synovial fluid in patients with metal implants are similar to the ones used in our experiments.

According to our results, hexavalent chromium is able to inhibit proliferation and differentiation to adipocytes of 3T3-L1 fibroblasts and to increase micronucleated cells, which are all indicative of alterations in physiological processes and therefore contributes to further elucidate the cytotoxic effects of this heavy metal.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

Acknowledgements

This work was supported by research grants from Agencia Nacional de Promoción Científica y Tecnológica and Consejo

Nacional de Investigaciones Científicas y Técnicas of Argentina. The funders had no role in study design, data and analysis, decision to publish, or preparation of the manuscript.

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