CdCl$_2$ has zero-order kinetic cellular influx and induces cytotoxicity and genotoxicity at low concentrations in human leukocytes *in vitro*

Vanessa Fontoura dos Santos[1], Elvio Adílio Serpa[1], Fabiana Ernestina Barcellos da Silva[1], Érico Marlon de Moraes Flores[2], Michel Mansur Machado[1], Luís Flávio Souza de Oliveira[1]*

[1]Graduate Program in Pharmaceutical Sciences, Universidade Federal do Pampa (UNIPAMPA), Uruguaiana, BR 472 Km 592, Caixa Postal 118, CEP 97500-970, Brazil.

*E-mail: tcheluisoliveira@gmail.com

[2]Chemistry Department, Universidade Federal de Santa Maria (UFSM), Santa Maria, Av. Roraima, 1000-Camobi, Santa Maria - RS, CEP 97105-900, Brazil.

Summary

Cadmium (Cd^2+) is a nonessential heavy metal that possesses a high capacity of bioaccumulation and exhibits toxic characteristics even at low concentrations. This study evaluated the genotoxicity and cytotoxicity in human leukocytes in vitro after exposure to a lower range of Cd^2+concentration (1-25 μg/mL) using an unprecedented strategy by correlating between intracellular Cd^2+ levels after exposure and cellular damage. Results demonstrated that Cd^2+exposure from 5 to 25 μg/mL significantly increased the unviability of leukocytes, as well as the DNA damage, which was dose-dependent. The intracellular Cd^2+ levels in leukocytes ranged from 9.85 to 94.38 pg/cell, and cytotoxicity and genotoxicity were induced at a concentration of 24.22 pg/cell. The relationship between exposure concentration and intracellular Cd^2+ levels suggests that its influx occurs in human leukocytes under zero-order kinetics.

Keywords: Cadmium, cellular damage, DNA damage, defense cells.

Resumen

El CdCl$_2$ tiene un influjo celular cinético de orden cero e induce citotoxicidad y genotoxicidad a bajas concentraciones en leucocitos humanos *in vitro*

El cadmio (Cd^2+) es un metal pesado no esencial que posee una alta capacidad de bioacumulación y presenta características tóxicas incluso en bajas concentraciones. Este estudio evaluó la genotoxicidad y la citotoxicidad en leucocitos humanos *in vitro* después de la exposición a un rango inferior de concentración de Cd^2+ (1-25 μg / mL) mediante una estrategia sin precedentes al correlacionar los niveles intracelulares de Cd^2+ después de la exposición y el daño celular. Los resultados demostraron que la exposición a Cd^2+ de 5 a 25 μg/mL aumentó significativamente la inviabilidad de los leucocitos, así como el daño en el ADN, que era dependiente de la dosis. Los niveles intracelulares de Cd^2+ en leucocitos oscilaron entre 9,85 y 94,38 pg/célula, y se indujo la citotoxicidad y la genotoxicidad a una concentración de 24,22 pg/célula. La relación entre la concentración de la exposición y los niveles intracelulares de Cd^2+ sugiere que su influjo se produce en leucocitos humanos bajo una cinética de orden cero.

Palabras clave: Cadmio, daño celular, daño en el ADN, células de defensa.

Introduction

Cadmium (Cd) is a heavy metal belonging to class II-B of the periodic table [1] that, even at low concentrations, has toxic effects [2, 3]. In addition, Cd is a widely distributed pollutant in several sources as industrial waste, plastics and odontologic materials [2], cigarette smoke [4], forest fires [3], pigments [5], and fertilizers [6] and thus causes food and water contamination [7, 8].

Cd^2+ bioaccumulates primarily in the liver, kidneys, bones, and pancreas [9, 10], which ultimately results in hepatic and renal dysfunctions, as well as osteoporosis and pancreatic cancer [11].

The mechanism by which Cd^2+ induces cellular injuries appears to be supported, at least in part, by apoptotic pathways [3, 12]. Furthermore, the potential injury caused by Cd^2+ appears to be associated with our own ability to induce protein denaturation and oxidative stress and decrease the enzymatic activity [13-15].

However, only a few studies have evaluated the genotoxicity in human cells exposed to lower concentrations of Cd. Herein, we report the *in vitro* minimal Cd^{2+} concentrations that could cause genotoxicity and cytotoxicity in human leukocytes. These screening experiments were performed at concentrations ranging from 1 to 25 μg/mL, which are significantly lower than those cited in the literature. We also determined the intracellular Cd^{2+} levels after cellular exposure to understand the relationship with the observed cell damage and also to elucidate the kinetic entrance of Cd^{2+} into human leukocytes under experimentally controlled conditions.

Materials and methods

Ethical aspects

The experimental protocols performed in this study, including the collection of venous blood samples, were submitted to and approved by the Universidade Federal de Santa Maria (RS, Brazil) Research Ethics Committee under the registration number 0089.0.243.000-07.

Material preparation

The study material was prepared as previously described [16]. Briefly, the material that was used for *in vitro* assays was new and disposable to avoid Cd^{2+} contamination. Control samples of the reagents were tested to identify metal contamination. Before use, all glassware was immersed in 20% aqueous HNO$_3$ (v/v) for 24 h and subsequently rinsed with deionized water.

Both the comet and cell viability assays were performed in triplicate, while six replicates were analyzed to quantify the intracellular Cd^{2+} content. The following screening strategy was performed for all assays: (1) negative control–adjusted leukocyte suspension (LS) (10^4 cells/mm^3) in phosphate-buffered saline (PBS; pH 7.4); (2) positive control–LS plus 4 mM H$_2$O$_2$; tests–composed of LS with either 1, 2.5, 5, 10, 15, or 25 μg/mL Cd^{2+} (CdCl$_2$ analytical grade, Merck®, Darmstadt, Germany). This concentration range was chosen based on specialized literature data that related the metal concentration with cellular or DNA damage [17-19]. All sample groups were incubated for 1 h at room temperature (25°C) under slow stirring of which the direction was inverted every 10 min. Thereafter, the abovementioned tests were performed, as described below, with the exception of the intracellular Cd^{2+} quantification. In this case, the samples were additionally washed five times with PBS (pH 7.4) and centrifuged at 2000 rpm for 10 min at room temperature.

Intracellular Cd²⁺ Content (Inductively Coupled Plasma Mass Spectrometry-ICP-MS)

The intracellular Cd^{2+} concentration in the leukocytes was quantified using ICP-MS analysis. The samples were adequately diluted in 5% HNO_3 (v/v) by the factor required to obtain within the concentration range of the calibration curve and analyzed by ICP-MS (PerkinElmer SCIEX, Model Elan DRC II, Thornhill, Canada) to determine the extracted Cd content. A certified reference material was used for the measurements, which resulted in consistent concentrations (98%–101%). Special care was taken concerning the material and the treatment of the samples (human leukocytes).

The water used was previously distilled and deionized in an ion-exchange column and purified using a Milli-Q system (Millipore®, Billerica, USA) with a resistivity of 18.2 MΩ/cm. The reagent used for the decomposition of LS (HNO_3, 65%, 1.4 kg/L, Merck®, Darmstadt, Germany) was of redistilled analytical grade (model duoPUR distillation Subboiling Distillation System, Milestone®, Sorisole, Italy).

Argon (99.999 % purity, White Martins, Brazil) was used for plasma generation. Hydrochloric acid solution was prepared by diluting the concentrated acid in water. Sodium tetrahydroborate ($NaBH_4$, PA, minimum purity of 97%, Nuclear®, Brazil) solution was prepared by dissolving the solid reagent in a 0.1% (w/v) NaOH solution (minimum purity of 99%, Vetec®, Brazil). Cd^{2+} was added from a stock solution of $CdCl_2$ (1000 mg/L). All materials were decontaminated through immersion in an aqueous HNO_3 20% (v/v) solution for 24 h and subsequently rinsed with distilled/deionized water.

The leukocyte decomposition was done by the wet method in an open system (block digester-Tecnal, model TE 040/25 for 40 quartz tubes, aluminum body, and thermostat). The samples were weighed and transferred to glass tubes (2.5 × 40 cm), followed by addition of 4 mL of concentrated HNO_3 and 1 mL of 1 N HCl. The temperature was increased to 50 °C for 20 min and 90°C for 4 h and 30 min. Glass funnels were placed on the upper end of the pipes for the efficiency of the digestion procedure. After cooling to room temperature, the solutions were transferred to graduated polypropylene vials, and the volume was adjusted to 25 mL using ultra-purified water. The samples were adequately diluted in 5% HNO_3 (v/v) by the factor required to obtain within the concentration range of the calibration curve and analyzed by ICP-MS.

The intracellular Cd^{2+} content was calculated, considering the number of 10^4 cells/mm³ from LS, taking into account the Cd^{2+} concentration to which the cells were exposed and the value determined by the ICP-MS analysis for the same, which was assumed as the content of Cd^{2+} taken up by cells. The data obtained from the replicates were taken into account to generate the data of their respective groups.

Cytotoxicity assessment

The cytotoxicity of Cd^{2+} was evaluated by a cell viability test using the trypan blue method to determine the loss of membrane integrity [20]. Initially, the cells were exposed to the previously established Cd^{2+} concentration. After the incubation, 100 μL of LS was mixed for 8 min with 100 μL of 0.4% trypan blue solution. The cell viability was determined microscopically (400 × magnification) in a Neubauer chamber, and the following two categories of cells were scored: (1) living cells that appeared uncolored or light blue and (2) dead cells with a blue color. For this assay, 300 cells were counted for each sample analyzed.

Genotoxicity Assessment

The alkaline comet assay (single cell gel electrophoresis assay) was performed to evaluate genotoxicity according to a previously described methodology [16] and in accordance with the comet assay guidelines [21]. The microscope slide analysis was performed, counting 100 cells per slide and classifying them based on damage levels from 0 (no damage) to 4 (maximum damage). The average obtained for each treatment allowed calculating the respective damage rate, considering the DNA damage scale from 0 (100 cells×0) to 4 (100 cells×4) as proposed earlier [22].

Statistical analysis

All data obtained from the tests carried out in this study were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test, wherever appropriate, considering $p < 0.05$ for statistical significance.

Results

Evaluation of Intracellular Cd Content in Human Leukocytes

One of the challenges in studies on metal toxicology is to determine the quantity of the heavy metal that is capable of entering into the cytoplasm from the cell exposed to the metal. This issue is also applicable to Cd^{2+} and needs to be elucidated. Furthermore, it is crucial to understand if there is a constant cell entry percentage or if this entry is disproportional considering the different concentrations of exposure. Figure 1 shows an increasing intracellular Cd^{2+} concentration in human leukocytes exposed to CdCl$_2$ at concentrations ranging from 1 to 25 μg/mL, which occurred in a concentration-dependent manner ($p < 0.001$).
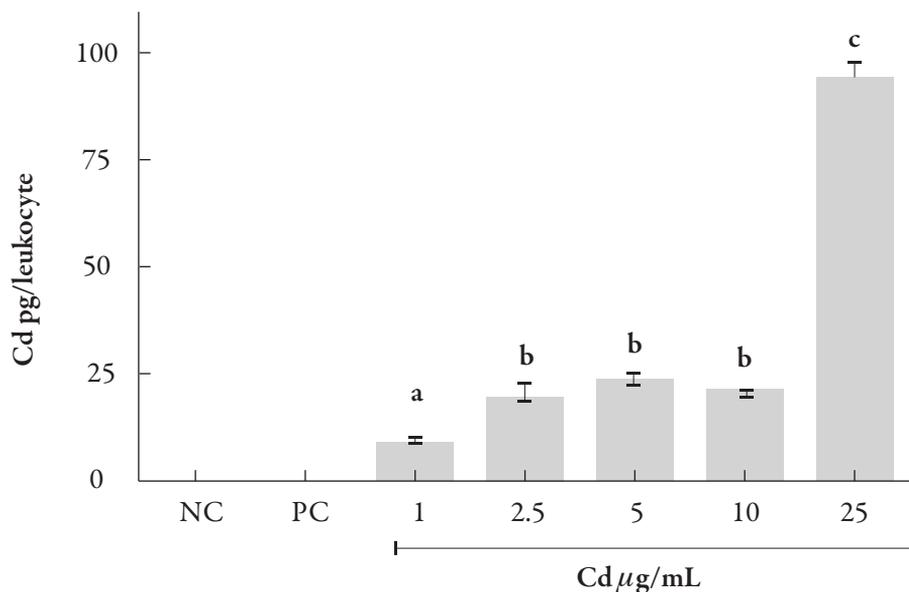
Figure 1. Intracellular Cd content in human leukocytes exposed to different concentrations of the metal. Data were analyzed by one-way ANOVA, followed by Tukey's test for multiple comparisons, and expressed as mean ± SEM, with p < 0.01. [a, b, c, d]. The superscript letters indicate statistical difference between groups; NC = negative control, PC = positive control.

The percentage of Cd cellular influx equivalent to a leukocyte related to the exposure to different $CdCl_2$ concentrations is shown in the figure 2, which allows evaluating the profile of Cd entry into a leukocyte. As shown in the figure, there is a decrease in the percentage of Cd cellular input, which occurs with a stabilization from 5 to 25 µg/mL. This phenomenon indicates a decay entry percentage but at the same time leads us to suggest that the influx kinetics is of zero-order.

Both Cd exposure and intracellular Cd concentrations were related to the percentage of cell unviability (Fig. 3) and DNA damage index (see figure 4) in human leukocytes to elucidate the Cd concentrations capable of inducing injury in these cells. Our results demonstrated that Cd exposure at concentrations ranging from 5 to 25 µg/mL significantly decreased the leukocyte cell viability compared with the negative control (p < 0.001), in particular at two higher concentrations, 10 and 25 µg/mL.

Cd input in leukocyte (%)

Cd cellular exposition (μg/mL)
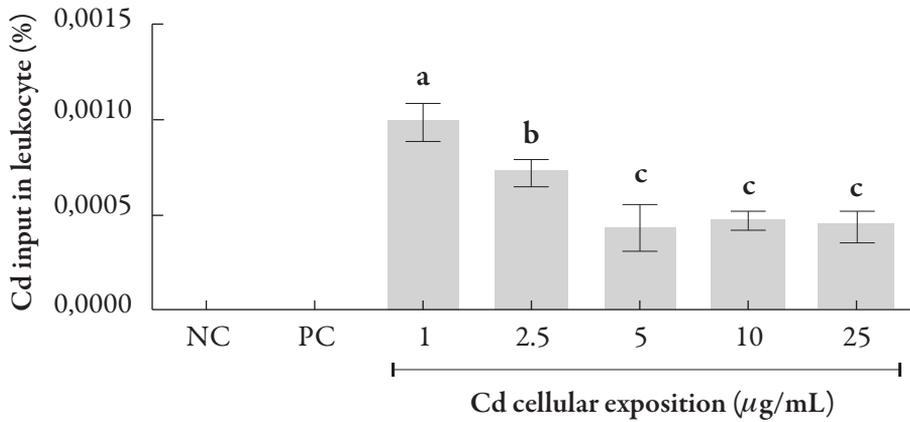
Figure 2. Percentage of cellular input of Cd in human leukocytes. Data were analyzed by one-way ANOVA, followed by Tukey's test for multiple comparisons, and expressed as mean ± SEM, with p < 0.01. [a, b, c] the superscript letters indicate statistical difference between groups; NC = negative control, PC = positive control.

Cd pg/leukocyte cell

9.85   16.22   24.22   21.21   94.38

Leukocyte viability (%)

Cd cellular exposition (μg/mL)
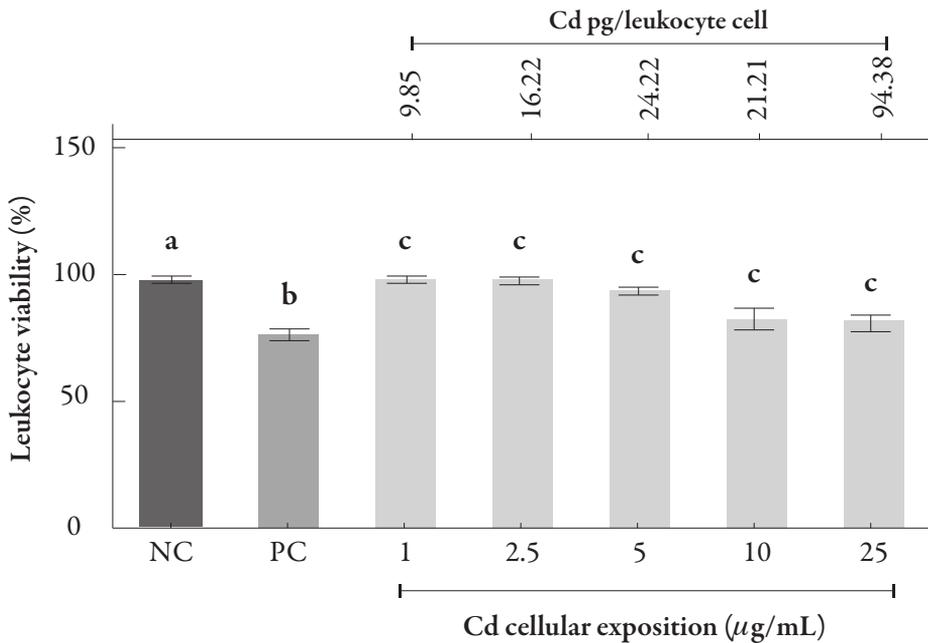
Figure 3. Percentage of cells in the cell viability assay in human leukocytes exposed to different CdCl$_2$ concentrations. Data were analyzed by one-way ANOVA, followed by Tukey's test for multiple comparisons, and expressed as mean ± SEM, with p < 0.001; [a, b, c]. The superscript letters indicate statistical difference between groups; NC = negative control, PC = positive control.

Figure 4 shows the DNA damage index of leukocytes after $CdCl_2$ exposure. The comet assay demonstrated that the exposure concentrations of 1 and 2.5 μg/mL, where the intracellular Cd levels were 9.85 and 16.22 pg/cell, respectively, did not cause significant DNA damage, since the damage levels were similar to the damage levels found in the negative control (0.001). On the other hand, at the exposure concentration range from 5 to 25 μg/mL, which is equivalent to the intracellular Cd content from 21.21 to 94.38 pg/cell, we were able to observe higher DNA damage levels, although they were lower than those observed for the positive control ($p < 0.001$).
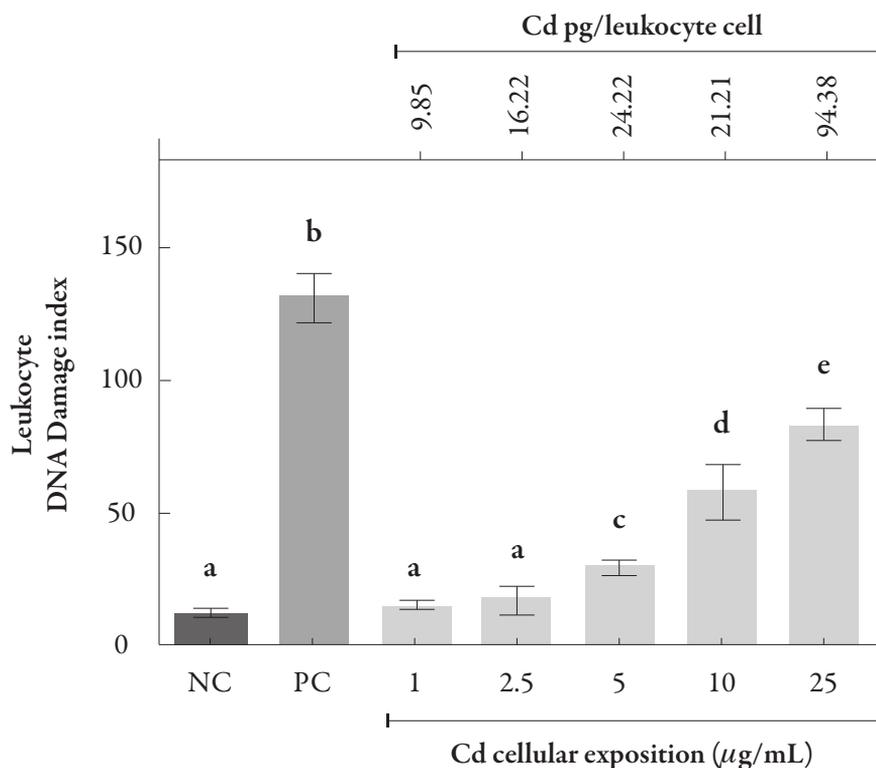
Figure 4. Rate of DNA damage in human leukocytes exposed to different $CdCl_2$ concentrations and its respective intracellular levels following exposure. Data were analyzed by one-way ANOVA, followed by Tukey's test for multiple comparisons, and expressed as mean ± SEM, with $p < 0.001$. [a, b, c, d] - the superscript letters indicate statistical difference between groups; NC = negative control, PC = positive control.

Discussion

Cd^{2+} is a nonessential heavy metal and one of the most relevant toxic pollutants associated with environmental and public health, which is due to its high water solubility and bioaccumulation capacity in living organisms [23, 24]. Furthermore, Cd^{2+} is potentially carcinogenic and teratogenic, causing several abnormalities [24, 25]. Cd^{2+} also contributes to the genotoxicity of other agents that may cause damage to the DNA structure [25, 26].

The cellular unviability and comet assays are techniques that are capable of detecting damages to cells and DNA, respectively. The literature reports different data about Cd^{2+} pertaining to cellular and DNA damages at different concentrations [27-29]. However, in this study, we demonstrated, using an unprecedented strategy, that the cellular and DNA damages begin to appear at concentration from 5 μg/mL of CdCl$_2$ considering a short exposure of 1 h.

There are several studies demonstrating Cd^{2+} toxicodynamic mechanisms. These studies address different molecular targets such as the increase in oxidative stress, evidenced by the increase in catalase and superoxide dismutase activities, besides the increase in malondialdehyde levels and the depletion of GSH concentrations [30-32]. In addition, Cd^{2+} exposure increases the cytosolic calcium levels under homeostatic conditions, i.e., at 0.1 μM [34]. This event triggers cellular destruction through the activation of phospholipases, endonucleases, proteases, and Ca^{+2}/Mg^{+2} ATPases dependent on the energy associated to the membrane [33-35].

Consistent with this, some studies corroborate with the apoptotic mechanisms deflagrated after Cd^{2+} exposure of crab hepatopancreas (30-57 mg/mL) and cortical neurons of rats (20–25 μM for 24–48 h), where it was possible to observe an increase in the activity of caspases 9 and 3, but not caspase 8, associated with a decrease in cytochrome c activity and an increase in the expression of poly-ADP-ribose-polymerase (PARP) [28, 29]. These data corroborate with the idea that cellular and DNA damages may be induced by free radicals after Cd^{2+} exposure, since cytochrome c is activated and released to activate caspase 9, especially through free radical generation. Notwithstanding, PARP is closely associated with cellular and DNA repair mechanisms, which is used as a biomarker for such.

In addition, human lungs cell strain (A549 and H441) were exposed to 20 and 40 μM of CdCl$_2$ that resulted in an increase in the activities of metallothionein (MT), HSP70, and MDR1. These data corroborate with the above-cited whether considered the mechanisms of auto defense triggered by cells under injuries against their

aggressors, as the Cd^{2+}. In this case, the cell attempts to neutralize Cd^{2+} by binding with MT; decrease the effects of stress oxidative through the HSP70, as well as to participate of protein folder; or increase the transport of metal efflux through the MDR1. However, these cytoprotective events are not effective when the metal concentrations are higher than their capacity to neutralize them [27].

The Cd^{2+} accesses the inside of the cells competing with the input pathways via ion channels, solutes carriers or ATP-requiring pumps. Once in the cell, Cd^{2+} can exert its toxic effects by binding to the thiol compounds scattered throughout the cell (cytosol and nucleus), as observed in the disruption respiratory chain function by binding Cd^{2+} to mitochondrial thiol proteins. In addition, Cd^{2+} may bind to the amino acid-cystine transporters (SLC79/SLC3A1 and SLC7A9/SLC7A13) to access the cytosol. Besides, it is accepted that Cd^{2+} has direct interaction with cellular DNA, is capable of inhibiting the nuclease of incision during DNA repair, as well as be able to exert an interference in the chromatin condensation [36].

Based on the abovementioned assumptions, the damage induced by Cd^{2+} in different types of cells is dependent on concentration and time of exposure. However, till date, no study has yet demonstrated the range of intracellular Cd^{2+} concentrations that could induce cellular damage. Although we are not aware of the fact whether intracellular Cd^{2+} concentration resulting from the exposure of 5 μg/mL has the same profile of cellular input, we can confirm that in human leukocytes, under the evaluated conditions, the Cd^{2+} content of 33 pg/leukocyte is responsible to induce cytotoxicity and genotoxicity. Furthermore, the intracellular Cd^{2+} percentages resulting from the exposure concentrations in leukocytes suggest a profile of zero-order kinetics, which may be associated with the saturation of a specific protein domain for the binding of metals in cell membranes.

Conclusion

Our results provide new insights regarding the concentration, exposure, and Cd^{2+} ($CdCl_2$) that are capable of inducing cell cytotoxicity and genotoxicity in human leukocytes. Furthermore, our study provides a better understanding about *in vitro* Cd^{2+} as a source of inducing cytotoxicity and genotoxicity at the exposure concentrations ranging from 5 to 25 μg/mL, with the intracellular levels ranging from 21.21 to 94.38 pg/cell, respectively. Using an unprecedented strategy, the intracellular Cd^{2+} level was correlated with the different exposure concentrations, and based on this, we could deduce that the Cd inflow occurs under zero-order kinetics.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

1. X. Cao, H. Wang, D. Zhuang *et al*., Roles of MSH2 and MSH6 in cadmium-induced G2/M checkpoint arrest in Arabidopsis roots, *Chemosphere*, **201**, 586-594 (2018).

2. J. Jean, V. Sirot, M. Hulin *et al*., Dietary exposure to cadmium and health risk assessment in children. Results of the French infant total diet study, *Food Chem. Toxicol*., **115**, 358-364 (2018).

3. A. Waseem, J. Arshad, A review of Human Biomonitoring studies of trace elements in Pakistan, *Chemosphere*, **163**, 153-176 (2016).

4. K. Ghosh, N. Indra, Cadmium treatment induces echinocytosis, DNA damage, inflammation, and apoptosis in cardiac tissue of albino Wistar rats, *Environ. Toxicol. Pharmacol*., **59**, 43-52 (2018).

5. M. Panjehpour, M.A. Taher, M. Bayesteh, The growth inhibitory effects of cadmium and copper on the MDA-MB468 human breast cancer cells, *J. Res. Med. Sci.,* **15**(5), 279 (2010).

6. D. Belhaj, K. Athmouni, M.B. Ahmed *et al*., Polysaccharides from Phormidium versicolor (NCC466) protecting HepG2 human hepatocellular carcinoma cells and rat liver tissues from cadmium toxicity: Evidence from in vitro and in vivo tests, *Int. J. Biol. Macromol*., **113**, 813-820 (2018).

7. D. Li, P. Wang, C. Wang, X. Fan, X. Wang, B. Hu, Combined toxicity of organophosphate flame retardants and cadmium to Corbicula fluminea in aquatic sediments, *Environ. Pollut*., **243**, 645-653 (2018).

8. G.K. Bielmyer-Fraser, B. Harper, C. Picariello, A. Albritton-Ford, The influence of salinity and water chemistry on acute toxicity of cadmium to two euryhaline fish species, *Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol*., **214**, 23-27 (2018).

9. A. Jacquet, J. Arnaud, I. Hininger-Favier *et al*., Impact of chronic and low cadmium exposure of rats: Sex specific disruption of glucose metabolism, *Chemosphere*, **207**, 764-773 (2018).

10. H. Chen, Y. Lu, Z. Cao *et al.*, Cadmium induces NLRP3 inflammasome-dependent pyroptosis in vascular endothelial cells, *Toxicol. Lett.*, **246**, 7-16 (2016).

11. R. Fan, P.C. Hu, Y. Wang *et al.*, Betulinic acid protects mice from cadmium chloride-induced toxicity by inhibiting cadmium-induced apoptosis in kidney and liver, *Toxicol. Lett.*, **299**, 56-66 (2018).

12. S. Chakraborty, A.R. Dutta, S. Sural, D. Gupta, S. Sen, Ailing bones and failing kidneys: a case of chronic cadmium toxicity. *Ann. Clin. Biochem.*, **50**(5), 492-495 (2013).

13. T. Luo, J. Lee, Z. Lu *et al.*, Effect of cadmium ion on alpha-glucosidase: an inhibition kinetics and molecular dynamics simulation integration study, *Protein J.*, **35**(3), 218-224 (2016).

14. M.J. Tamás, B. Fauvet, P. Christen, P. Goloubinoff, Misfolding and aggregation of nascent proteins: a novel mode of toxic cadmium action in vivo, *Curr. Genet.*, **64**(1), 177-181 (2018).

15. A. Sarkar, G. Ravindran, V. Krishnamurthy, A brief review on the effect of cadmium toxicity: from cellular to organ level, *Int. J. Biotechnol. Res.*, **3**(1), 17-36 (2013).

16. L.P. Pereira, F.E.B. Silva, E.M.M. Flores, H.S. Schrekker, M.M. Machado, L.F.S. Oliveira, *In vitro* ZnCl$_2$ cytotoxicity and genotoxicity in human leukocytes: Zero-order kinetic cellular zinc influx, *Acta Sci. Health Sci.*, **37**(1), 63-68 (2015).

17. T. Sliwinski, A. Czechowska, M. Kolodziejczak, J. Jajte, M. Wisniewska-Jarosinska, J. Blasiak, Zinc salts differentially modulate DNA damage in normal and cancer cells, *Cell Biol. Int.*, **33**(4), 542-547 (2009).

18. R. Guan, T. Kang, F. Lu, Z. Zhang, H. Shen, M. Liu, Cytotoxicity, oxidative stress, and genotoxicity in human hepatocyte and embryonic kidney cells exposed to ZnO nanoparticles, *Nanoscale Res. Lett.*, **7**(1), 602 (2012).

19. R. Sharif, P. Thomas, P. Zalewski, M. Fenech, Zinc deficiency or excess within the physiological range increases genome instability and cytotoxicity, respectively, in human oral keratinocyte cells, *Genes Nutr.*, **7**(2), 139-154 (2012).

20. M.E. Burow, C.B. Weldon, Y. Tang *et al.*, Differences in susceptibility to tumor necrosis factor α-induced apoptosis among MCF-7 breast cancer cell variants, *Cancer Res.*, **58**(21), 4940-4946 (1998).

21.   R.R. Tice, E. Agurell, D. Anderson *et al*., Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing, *Environ. Mol. Mutag*., **35**(3), 206-221 (2000).

22.   N.P. Singh, M.T. McCoy, R.R. Tice, E.L. Schneider, A simple technique for quantitation of low levels of DNA damage in individual cells, *Exp. Cell Res*., **175**(1), 184-191 (1988).

23.   S. Wang, Q. Dong, Z. Wang, Differential effects of citric acid on cadmium uptake and accumulation between tall fescue and Kentucky bluegrass, *Ecotoxicol. Environ. Safety*, **145**, 200-206 (2017).

24.   X. Chen, Z. Wang, G. Zhu, G.F. Nordberg, T. Jin, X. Ding, The association between cumulative cadmium intake and osteoporosis and risk of fracture in a Chinese population, *J. Exp. Sci. Environ. Epid*., doi: 10.1038/s41370-018-0057-6

25.   W.J. Zhao, Z.J. Zhang, Z.Y. Zhu *et al*., Time-dependent response of A549 cells upon exposure to cadmium, *J. App.Toxicol*., **38**(11), 1437-1446 (2018).

26.   J. Ramos-Trevino, S. Bassol-Mayagoitia, J.A. Hernández-Ibarra, P. Ruiz-Flores, M.P. Nava-Hernández, Toxic Effect of Cadmium, Lead, and Arsenic on the Sertoli Cell: Mechanisms of Damage Involved, *DNA Cell Biol*., **37**(7), 600-608 (2018).

27.   J.A. Sauvageau, C. Jumarie, Different mechanisms for metal-induced adaptation to cadmium in the human lung cell lines A549 and H441, *Cell Biol. Toxicol*., **29**(3), 159-173 (2013).

28.   H. Wang, Y. Yu, J. Li *et al*., Cadmium stimulates mouse skin fibroblast apoptosis by affecting intracellular homeostasis, *Drug Chem. Toxicol*., **40**(1), 74-84 (2017).

29.   Y. Lin, J.J. Huang, H.U. Dahms, J.J. Zhen, X.P. Ying, Cell damage and apoptosis in the hepatopancreas of Eriocheir sinensis induced by cadmium, *Aquat. Toxicol*., **190**, 190-198 (2017).

30.   S. Sarkar, P. Yadav, R. Trivedi, A.K. Bansal, D. Bhatnagar, Cadmium-induced lipid peroxidation and the status of the antioxidant system in rat tissues, *J. Trace Elem. Med. Biol*., **9**(3), 144-149 (1995).

31.   S.J. Stohs, D. Bagchi, Oxidative mechanisms in the toxicity of metal ions, *Free Rad. Biol. Med*., **18**(2), 321-336 (1995).

32.   M.W. Fariss, Cadmium toxicity: unique cytoprotective properties of alpha tocopheryl succinate in hepatocytes, *Toxicol*., **69**(1), 63-77 (1991).

33.   Y.U.A.N. Yan, J.C. BIAN, X.Z. LIU, Y. ZHANG, S.U.N. Ya, Z.P. LIU, Oxidative stress and apoptotic changes of rat cerebral cortical neurons exposed to cadmium in vitro, *Biomed. Environ. Sci.*, **25**(2), 172-181 (2012).

34.   Z. Dong, P. Saikumar, G.A. Griess, J.M. Weinberg, M.A. Venkatachalam, Intracellular Ca^{2+} thresholds that determine survival or death of energy-deprived cells. *Am. J. Pathol.*, **152**(1), 231-240 (1998).

35.   B.E. Trump, I.K. Berezesky, S.H. Chang, P.C. Phelps, The pathways of cell death: oncosis, apoptosis, and necrosis, *Toxicol. Pathol.*, **25**(1), 82-88 (1997).

36.   F. Thévenod, J. Fels, W-K. Lee, R. Zarbock, Channels, transporters and receptors for cadmium and cadmium complexes in eukaryotic cells: myths and facts, *Biometals*, **1**, 1-21 (2019).

How to cite this article

V. Fontoura dos Santos, E.A. Serpa, F.E. Barcellos da Silva, É.M. de Moraes-Flores, M. Mansur-Machado, L.F.S. Oliveira, $CdCl_2$ has zero-order kinetic cellular influx and induces cytotoxicity and genotoxicity at low concentrations in human leukocytes in vitro, *Rev. Colomb. Cienc. Quím. Farm.*, **48**(1), 145-158 (2019).