



ELSEVIER

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

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

Cadmium reduces the efficiency of Sindbis virus replication in human cells and promotes their survival by inhibiting apoptosis



Etienne Frumence, Marjolaine Roche*, Pascale Guiraud*

Université de la Réunion, UM 134 Processus Infectieux en Milieu Insulaire Tropical (PIMIT), INSERM U1187, CNRS UMR9192, IRD UMR249, Plateforme Technologique CYROI, 97490 Sainte Clotilde, France

ARTICLE INFO

Article history:

Received 27 May 2016

Received in revised form

20 August 2016

Accepted 24 August 2016

Available online 26 August 2016

Keywords:

Cadmium

Alphavirus

Sindbis virus

Viral growth

Apoptosis

ABSTRACT

Arthritogenic alphaviruses are emerging arthropod-borne viruses that occasionally cause sporadic to global outbreaks all over the world. Many environmental factors including xenobiotics have been identified as capable of influencing the spread, the susceptibility and the outcome of viral infection. Among them cadmium is a toxic non-essential heavy metal and a prevalent environmental contaminant. In the present study we evaluated the effect of cadmium exposure on alphavirus infection *in vitro*. We infected Human Embryonic Kidney (HEK) 293 cells in the presence of cadmium chloride (CdCl_2) with Sindbis virus. Cell viability, apoptosis and viral growth were then examined. Our data show that effective doses of cadmium decreased the virus mediated-cell death by inhibition of apoptosis. Moreover, virus growth in HEK 293 cells was also reduced by CdCl_2 treatment. Altogether our results demonstrate that cadmium triggers a protective response which renders HEK 293 cells resistant against Sindbis virus infection.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Arboviruses or ARthropod-BORne viruses transmitted by arthropod vectors, represent a major group of infectious viruses that infects over 100 million people each year with viruses such as Dengue virus, Chikungunya virus, West Nile virus and Zika virus. Among them, arthritogenic alphaviruses from the *Togaviridae* family occasionally cause sporadic to global outbreaks all over the world [1]. Since 2004, the Chikungunya virus has been responsible for large epidemic affecting millions in Kenya, Islands of the Indian Ocean, India, parts of the Southeast Asia, the Caribbean and South America [2,3]. Understanding the complex interactions between hosts, arthropod vectors, infectious agents and environment is becoming necessary to fight against these diseases. Many environmental factors including xenobiotics have been identified as capable of influencing the spread, the susceptibility and the outcome of viral infection [4]. Among them, heavy metals have been shown to have a potential effect on several virus infections.

Cadmium is a toxic non-essential heavy metal and a prevalent environmental contaminant. Cadmium is used in electroplating, in some industrial paints and in the manufacturing of batteries [5]. In humans, cigarette smoke is the major source of cadmium exposure

via inhalation [6]. Cadmium exposure occurs also from ingestion of contaminated food through bioaccumulation or water [7]. This metal accumulates in the kidney and the liver throughout the entire lifetime. In humans and animals, cadmium causes toxic effect in a variety of tissues and induces a number of adverse health effects and diseases, including cancer, renal dysfunction, osteoporosis and hepatotoxicity [7,8]. At the cellular level, cadmium affects cell proliferation, survival and cell death, differentiation, and many biological mechanisms [9].

Several reports showed that cadmium may have a benefic or deleterious effect on the outcome on viral diseases. Cadmium treatment *in vitro* results in an increase influenza virus replication whereas Sendai virus and Human Immunodeficiency Virus type 1 (HIV-1) are inhibited [10–12]. In mouse models, it has been shown that cadmium exposition reduces the efficiency of antiviral innate immune responses directed against cytomegalovirus [13].

In a mouse model of alphaviral encephalitis, it has been reported that a single oral dose of cadmium can enhance the neuropathogenicity of Semliki Forest virus and Venezuelan equine encephalitis virus, two members of the *alphavirus* genus [14]. Sindbis virus (SINV), which is a widely distributed zoonotic alphavirus distributed in Eurasia, Africa and Australia, has been extensively studied as a model for alphavirus replication [15]. In the present study we investigated the effects of cadmium exposure on the efficiency of SINV replication in HEK 293 cells. We showed that cadmium chloride (CdCl_2) treatment inhibits viral growth and promotes the viability of infected human cells.

* Correspondence address: GIP CYROI, Université de la Réunion, 2 Rue Maxime Rivière, 97490 Sainte-Clotilde, Réunion, France.

E-mail addresses: marjolaine.roche@univ-reunion.fr (M. Roche), pascale.guiraud@univ-reunion.fr (P. Guiraud).

2. Materials and methods

2.1. Reagents and antibodies

Cadmium chloride (CdCl_2 , Sigma Aldrich) was prepared in sterile water as 100 mM solution and stored at -20°C . The pan-caspase inhibitor, Z-VAD-fmk was purchased from Promega. Etoposide (Sigma Aldrich) was used as an inducer of apoptosis. Annexin V-FITC was purchased from BD Biosciences and propidium iodide (PI) was from Biolegend.

2.2. Virus and cell lines

Sindbis Virus (SINV, Strain AR-339) was obtained from the National Collection of Pathogenic Viruses (NCPV). In this study, SINV was amplified in Vero cells (ATCC, CCL-81) and stored at -80°C . Human Embryonic Kidney cells, (HEK 293, ATCC, CRL-1573), NIE115 cells (ATCC, CRL-2263) and Vero cells were cultured at 37°C under a 5% CO_2 atmosphere in MEM Eagle medium, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mmol L^{-1} L-Glutamine, 1 mmol L^{-1} sodium pyruvate, 100 U mL^{-1} penicillin, 0.1 mg mL^{-1} streptomycin and $0.5\text{ }\mu\text{g mL}^{-1}$ fungizone (PAN Biotech). For infection, cells were infected for 2 h at 37°C with SINV at a multiplicity of infection (MOI) of 1 PFU/cell. The inoculum was then removed and replaced with fresh medium. The cells were incubated at 37°C and 5% CO_2 for the rest of the experiment.

2.3. Cytotoxicity assays

Cell viability was assessed with the colorimetric MTT metabolic activity assay. Briefly, $20\text{ }\mu\text{L}$ of a 5 mg mL^{-1} MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, Sigma Aldrich) solution was added to HEK 293 cells cultured in a 96-well plate at a density of 10,000 cells per well. Following 1 h incubation, MTT medium was removed and the insoluble formazan salt was solubilized with $100\text{ }\mu\text{L}$ of DMSO. Absorbance of converted dye was measured at 570 nm with a background subtraction at 690 nm.

For Trypan Blue staining, cells grown in 6 well plates at a density of 6×10^5 cells per well were trypsinized and mixed with 0.4% trypan blue solution (1:10). Viable cells (unstained) and nonviable cells (stained) were then counted using a hemacytometer. The percentage of viable cells was calculated as the number of unstained cells divided by the total number of cells.

2.4. Flow cytometry assay

HEK 293 cells were grown on 100-mm Petri dishes. About 10^6 cells were harvested and resuspended in Annexin V binding buffer. Then cells were incubated with Annexin V-FITC (BD Biosciences) and propidium iodide (PI) (Biolegend) for 15 min in the dark at room temperature. Cells were washed and fixed with 3.7% formaldehyde solution for 10 min and subjected to a flow cytometric analysis using FACScan flow cytometer (Becton Dickinson). Results were analyzed with FCS Express 5 software (De Novo Software).

2.5. Caspase activity

HEK 293 cells were cultured in 96-well plates at a density of 10^4 cells per well. Caspase 3/7 activity in crude cell lysates was measured using Caspase Glo[®] 3/7 Assay Kit (Promega) according to the manufacturer's protocols. Caspase activity was quantified by luminescence using a FLUOstar Omega Microplate Reader (BMG LABTECH).

2.6. Virus titration

Virus titration assay was run using standard plaque assay. Vero

cells seeded in 24-well culture plates were incubated with tenfold dilutions of virus samples. The plates were incubated for 2 h at 37°C and unadsorbed virus was removed. Then culture medium supplemented with 0.8% carboxymethylcellulose (CMC) was added to each well, followed by an incubation at 37°C for 2 days. The CMC overlay was removed and the cells were stained with 0.5% crystal violet. Plaques were counted and expressed as plaque-forming units per mL (PFU mL^{-1}).

2.7. Statistical analysis

Results are expressed as mean \pm SEM of at least 3 independent experiments. Comparisons between different treatments have been analyzed by a one-way ANOVA test. Values with $p < 0.05$ were considered statistically significant for a post-hoc Tukey's test. All statistical tests were done using the software Graph-Pad Prism version 6.0. Degrees of significance are indicated in the figure captions as follow: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, **** $p < 0.0001$, *n.s.* = not significant.

3. Results

3.1. Cytotoxicity of CdCl_2 on HEK 293 cells

Cytotoxicity of CdCl_2 on HEK 293 cells was assessed by MTT assay (Fig. 1). The estimated CC50 value (cytotoxic concentration that reduced cell viability by 50%) was $30\text{ }\mu\text{M}$ for 24 h of treatment. The relative cell viability evaluated by Trypan Blue staining was similar to that obtained by MTT assay (data not shown). The administration of high doses of CdCl_2 ($\geq 40\text{ }\mu\text{M}$) caused cytotoxicity and reduced dramatically cells metabolic activity and viability. At concentrations lower than $10\text{ }\mu\text{M}$, there was no significant difference in terms of cell viability and cell morphology between the non-treated control cells and the CdCl_2 treated cells. Thus, concentrations of CdCl_2 up to $10\text{ }\mu\text{M}$ were considered non-cytotoxic and were used for further experiments.

3.2. Effect of CdCl_2 on SINV-mediated cell death

To evaluate the effect of CdCl_2 treatment on the infected cells viability, HEK 293 cells were infected with SINV at a multiplicity of infection (MOI) of 1 PFU/cell and cell viability was analyzed by MTT assay (Fig. 2A). We observed that 24 h post SINV infection, the

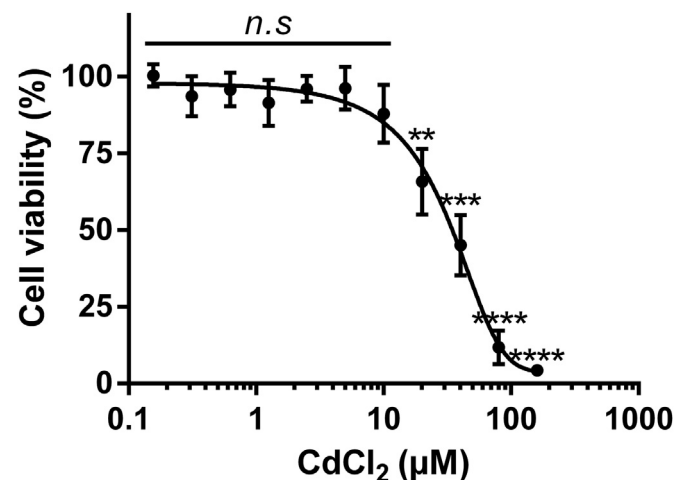


Fig. 1. Cytotoxicity of CdCl_2 on HEK 293 cells. Cells were treated with serial dilutions of CdCl_2 for 24 h. Then viability of CdCl_2 treated-cells was measured using MTT assay. Results are the mean (\pm SEM) from three independent experiments performed in triplicates. Statistical values are indicated.

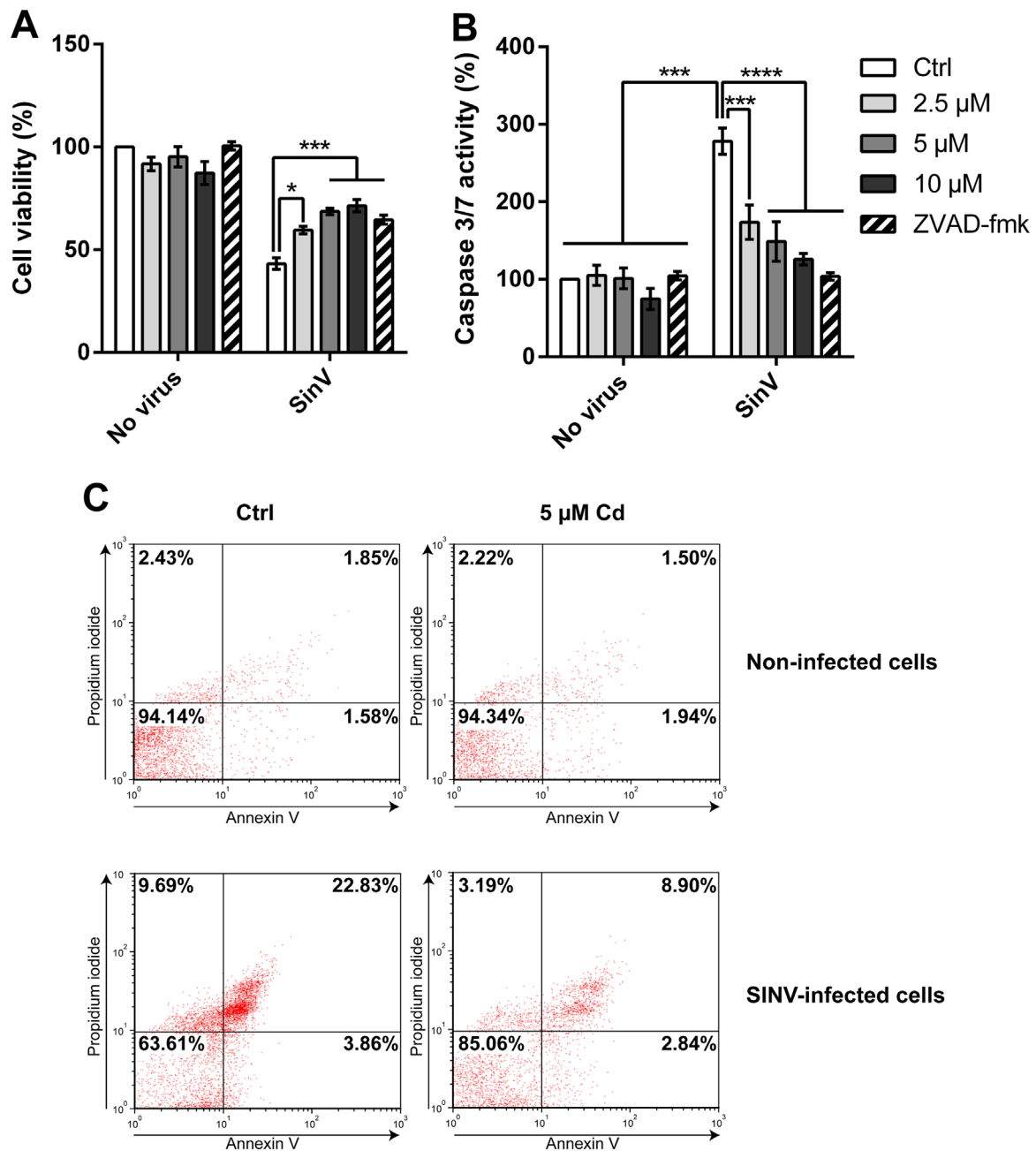


Fig. 2. Cadmium protects cells from SINV-mediated death through inhibition of apoptosis. HEK 293 cells were infected with SINV at MOI of 1 or mock infected. Then 2 h post infection, cells were treated with CdCl_2 . The *pan*-caspase inhibitor ZVAD-fmk (30 μM) served as control. (A) Viability was measured 24 h post-infection using MTT assay. (B) Caspase 3/7 activity in cell extracts was analyzed 24 h post infection using the caspase Glo 3/7 kit. Results are the mean (\pm SEM) from three independent experiments performed in triplicates. Statistical values are indicated. (C) Detection of apoptotic cells was performed 18 h post-infection by flow cytometry using annexin V/PI double staining. Data are representative of three independent experiments.

infected cell viability decreased by more than 50% compared to non-infected cells. A treatment with 2.5–10 μM of CdCl_2 applied 2 h after SINV exposure led to a significant increase of cell viability. More than 70% of SINV-infected cells treated with 10 μM of CdCl_2 were still alive 24 h post-infection. CdCl_2 concentrations below 2.5 μM , did not change the infected cells viability. Interestingly, these results showed that CdCl_2 treatment could partially rescue HEK 293 cells from SINV-mediated cell death. Thereafter, the activation of executioner caspases-3 and -7, hallmarks of apoptosis, was evaluated using a caspase-3/7 assay kit. Results revealed that SINV infection in HEK 293 cells leads to a significant increase in caspase 3/7 activity (Fig. 2B). Treatment with CdCl_2 markedly reduced caspase 3/7 activity in infected HEK 293 cells. Flow cytometric analysis performed 18 h post-infection also confirmed that SINV infection

leads to apoptosis in HEK 293 cells as cells were both stained with both Annexin V and PI, as a hallmark of apoptosis (Fig. 2C). CdCl_2 exposure decreased the proportion of infected cells in the apoptotic state. These results suggest a causal relationship between the reduction of apoptosis and the increases viability of HEK 293 cells infected with SINV and treated with CdCl_2 .

3.3. Effect of CdCl_2 on SINV growth

Given that CdCl_2 can rescue the viability of SINV-infected cells, it is important to determine whether the protective effect of the CdCl_2 might be the direct consequence of a lower efficiency of virus replication in HEK 293 cells. To investigate this issue, SINV-infected HEK 293 cells were incubated with CdCl_2 at different time points of

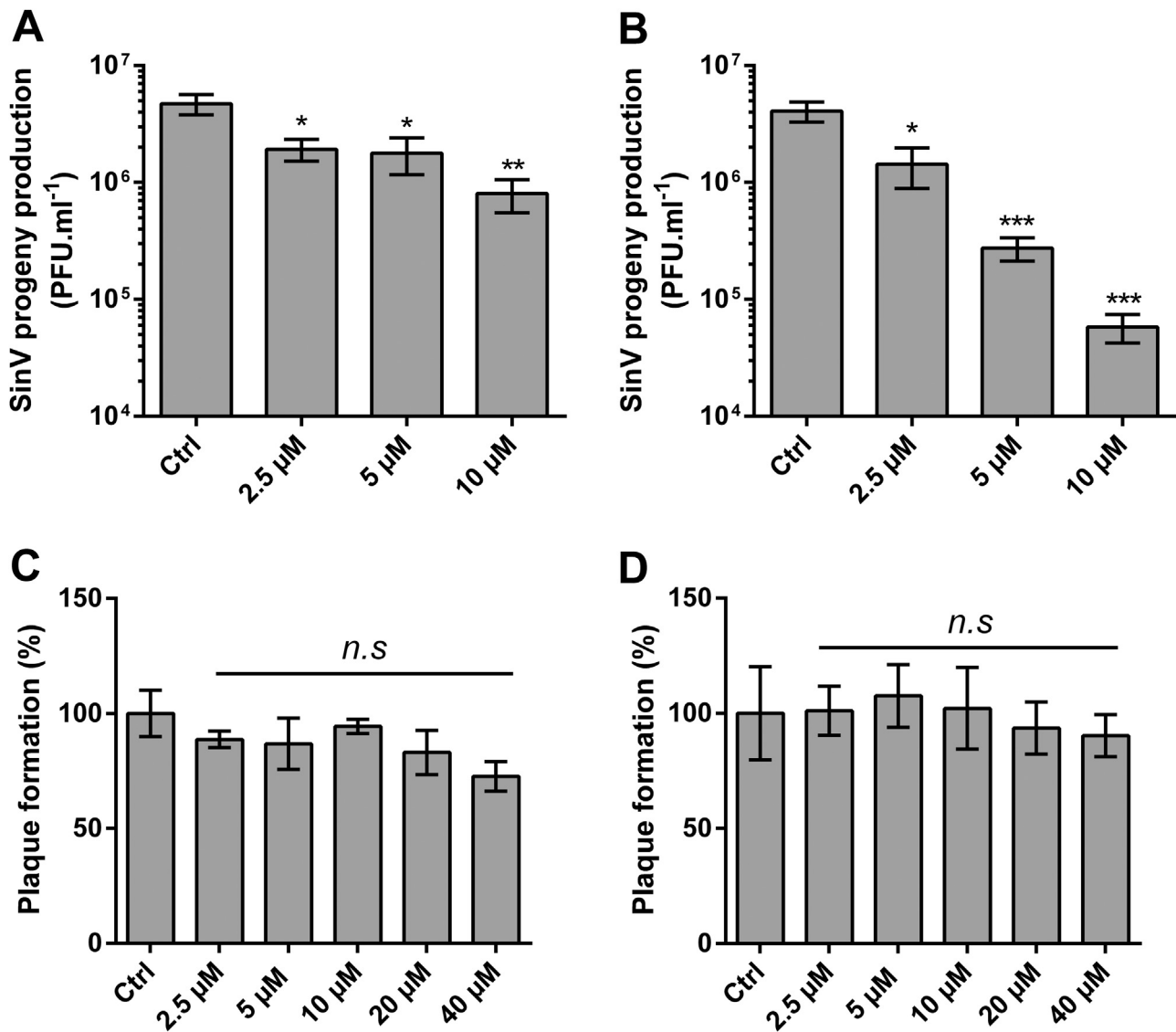


Fig. 3. Cadmium reduces SINV growth in HEK 293 cells neither by having virucidal activity nor by preventing virus entry. HEK 293 cells were treated 2 h post infection (A) or 24 h prior SINV infection (B) with CdCl₂. Viral growth was determined by plaque assay on Vero cells. (C) Virucidal activity of cadmium on SINV was performed by incubating viral inoculum with CdCl₂ at 37 °C for 2 h. Then, virus plaque formation was evaluated by plaque assay on Vero cells. (D) Effect of cadmium on SINV adsorption. Vero cells were infected for 2 h with 20 PFU of SINV in the presence of CdCl₂. Then, plaque formation was evaluated. Results are the mean (± SEM) from three independent experiments. Statistical values are indicated.

infection. Treatment with 10 μM of CdCl₂ at 2 h post-infection has a moderate effect on viral growth (~0.5 log) (Fig. 3A). In contrast, addition of CdCl₂ 24 h prior infection resulted in a marked reduction by at least 2 log of the progeny virus production (Fig. 3B). This result suggest that cadmium can impair SINV growth in HEK 293 cells. Similar results were obtained using NIE115 cells, a mouse neuroblastoma cell line (Supplemental Fig. 1).

To examine whether CdCl₂ could inactivate the virions, SINV inoculum was incubated with CdCl₂ at 37 °C 2 h before cell titration in Vero cells. No change in plaque formation was observed after incubation with CdCl₂, indicating no direct virucidal activity (Fig. 3C). Moreover, to determine if CdCl₂ could interfere with viral adsorption and entrance, Vero cells were infected with SINV in the presence of CdCl₂ for 2 h. Results showed that CdCl₂ did not significantly prevent virus adsorption (Fig. 3D).

3.4. Effect of CdCl₂ on etoposide-mediated apoptosis

To investigate if cadmium has a general inhibitory effect on apoptosis in HEK 293 cells, we exposed cells for 24 h to etoposide,

an anti-tumor agent that is commonly used *in vitro* as an apoptosis inducer. Results showed that etoposide markedly reduced the viability of HEK 293 cells (Fig. 4A). CdCl₂ co-treatment rescued cells from etoposide-mediated cell death. Moreover, using a caspase 3/7 assay kit, we also confirmed a significant increase in caspase 3/7 activity in HEK 293 cells treated with etoposide (Fig. 4B). CdCl₂ co-treatment inhibited caspase 3/7 activity in HEK 293 cells treated with etoposide. Taken together, these results suggested that cadmium reduced etoposide-mediated cell death through reduction of apoptosis.

3.5. Effect of apoptosis on SINV growth

To determine if apoptosis inhibition could contribute to the reduction of SINV growth, cells were exposed to ZVAD-fmk, a pan-caspase inhibitor. Treatment with ZVAD-fmk (30 μM) protected cells from SINV-mediated cells death (Fig. 2A) through reduction of caspase 3/7 activity (Fig. 2B). SINV progeny production in supernatant of HEK 293 cells were examined after treatment with ZVAD-fmk 2 h post infection. No significant reduction of viral

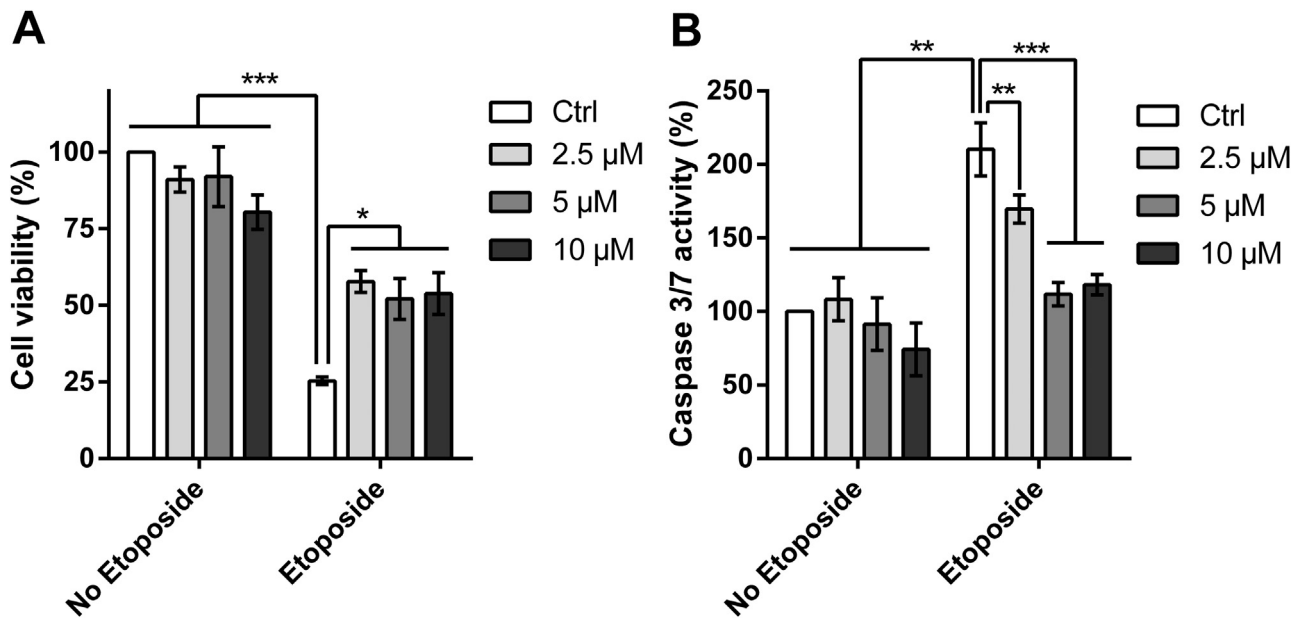


Fig. 4. Cadmium protects cells from etoposide-mediated apoptosis. HEK 293 cells were treated with or without etoposide (100 μM) for 24 h in the presence of CdCl_2 . (A) Viability was measured using MTT assay. (B) Caspase 3/7 activity in cell extracts was analyzed using the caspase Glo 3/7 kit. Results are the mean (\pm SEM) from three independent experiments performed in triplicates. Statistical values are indicated.

progeny was observed in the presence of ZVAD-fmk (30 μM) (data not shown). These results suggested that inhibition of apoptosis does not impact SINV growth in HEK 293 cells.

4. Discussion

The heavy metal cadmium is a widespread environmental contaminant that may adversely affect human health [7,16]. To date very little information is available on the effect of cadmium exposure on viral infections. It has been reported that an orally administrated dose of cadmium enhanced the growth and the neuropathogenicity of alphaviruses in a mouse model of viral encephalitis [14]. In the present study, we investigated whether cadmium can modify the replication of SINV, the prototype of alphavirus genus, in cultured human and mouse cell lines. Surprisingly, at non-cytotoxic doses, addition of 10 μM CdCl_2 on cells after SINV infection resulted in a moderate reduction in virus progeny production. In this condition cadmium had no virucidal activity and did not prevent virus adsorption and entry. Very interestingly addition of cadmium prior SINV infection suppressed viral growth in infected cells by 2 log. It has been reported that cadmium can induce oxidative stress, autophagy or heat-shock response [17–20]. Such cellular stress responses could contribute to prevent SINV replication in the host cells. Autophagy induction has been shown to protect cells against SINV *in vitro* [21]. Also, heat shock protein synthesis may be linked to the inhibition of SINV replication in Vero cells [22].

An unexpected finding was that CdCl_2 does not exert a marked antiviral effect when SINV replication has been initiated but it could rescue the viability of SINV-infected cells. In our *in vitro* infection model, we confirmed that SINV can trigger apoptosis [23]. We have investigated whether cadmium promotes cell survival through an inhibition of apoptosis. The analysis of caspase 3/7 activity, showed that apoptosis was significantly delayed in SINV-infected cells. It has been previously published that cadmium can inhibit apoptosis induced by several stressor such as chromium, hygromycin B and actinomycin D [24]. Furthermore, cadmium, like zinc, can reduce caspase 3 activity in a cell-free system [24,25]. Cadmium is a known inductor of metallothionein, a family of metal-binding proteins that has been shown to act as a negative

regulator of apoptosis in HepG2 cells [26]. In this regard, it remains to be determined their role in the reduction apoptosis induced by cadmium in the infected HEK 293 cells. Our results suggested that cadmium may have a general inhibitory effect on apoptosis in HEK 293 cells as it reduced also etoposide-mediated cell death. Although alphaviruses such as Chikungunya virus could mobilize the apoptotic machinery to spread and invades host cells through hiding in apoptotic blebs [27]. Our results showed that apoptosis inhibition using a *pan*-caspase inhibitor did not contribute to the reduction of SINV growth in HEK 293 cells.

To conclude, our results showed that cadmium exposure may have a collateral beneficial effect by mediating cell protection against SINV infection in HEK 293 cells through reduction of viral growth and apoptosis, by mechanisms that are still misunderstood necessitating the need for further investigation. It is worth cautioning that these *in vitro* results may not hold under *in vivo* conditions [14]. Many studies have described positive beneficial dose-response relationships (also known as hormesis or pre/post-conditioning) induced by toxins and stressors such as cadmium [28–30]. Low cadmium oral exposure has been shown to reduce the susceptibility of infectious colitis and to reduce arthritis disease progression in mice [31,32]. However, despite these potentially positive side effect of cadmium one should not forget the harmful long-term toxicogenic effects of this heavy metal *in vivo* (immunotoxicity, nephrotoxicity, cancer).

Acknowledgments

We thank P. Despres and W. Viranaicken for helpful discussions. E. Frumence received funding from the Conseil Régional de l'île de La Réunion, France.

Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bbrep.2016.08.023>.

Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bbrep.2016.08.023>.

References

- [1] A. Suhrbier, M.-C. Jaffar-Bandjee, P. Gasque, Arthritogenic alphaviruses – an overview, *Nat. Rev. Rheumatol.* 8 (2012) 420–429, <http://dx.doi.org/10.1038/nrrheum.2012.64>.
- [2] T.E. Morrison, Reemergence of chikungunya virus, *J. Virol.* 88 (2014) 11644–11647, <http://dx.doi.org/10.1128/JVI.01432-14>.
- [3] S.C. Weaver, N.L. Forrester, Chikungunya: evolutionary history and recent epidemic spread, *Antivir. Res.* 120 (2015) 32–39, <http://dx.doi.org/10.1016/j.antiviral.2015.04.016>.
- [4] N.-G. Ilbäck, G. Friman, Interactions among infections, nutrients and xenobiotics, *Crit. Rev. Food Sci. Nutr.* 47 (2007) 499–519, <http://dx.doi.org/10.1080/10408390600919015>.
- [5] R.A. Bernhoft, R.A. Bernhoft, Cadmium toxicity and treatment, *Sci. World J.* 2013 (2013) e394652, <http://dx.doi.org/10.1155/2013/394652>.
- [6] J. Godt, F. Scheidig, C. Grosse-Siestrup, V. Esche, P. Brandenburg, A. Reich, D. A. Groneberg, The toxicity of cadmium and resulting hazards for human health, *J. Occup. Med. Toxicol. Engl.* 1 (2006) 22, <http://dx.doi.org/10.1186/1745-6673-1-22>.
- [7] WHO, IPCS Environmental Health Criteria 134: Cadmium. (<http://www.inchem.org/documents/ehc/ehc/ehc134.htm>), 1992 (accessed 27.01.16).
- [8] G. Bertin, D. Averbeck, Cadmium: cellular effects, modifications of biomolecules, modulation of DNA repair and genotoxic consequences (a review), *Biochimie* 88 (2006) 1549–1559, <http://dx.doi.org/10.1016/j.biochi.2006.10.001>.
- [9] M. Waisberg, P. Joseph, B. Hale, D. Beyersmann, Molecular and cellular mechanisms of cadmium carcinogenesis, *Toxicology* 192 (2003) 95–117.
- [10] P. Checconi, R. Sgarbanti, I. Celestino, D. Limongi, D. Amatore, A. Iuvara, A. Alimonti, E. Garaci, A.T. Palamara, L. Nencioni, The environmental pollutant cadmium promotes influenza virus replication in MDCK cells by altering their redox state, *Int. J. Mol. Sci.* 14 (2013) 4148–4162, <http://dx.doi.org/10.3390/ijms14024148>.
- [11] Y. Haraguchi, H. Sakurai, S. Hussain, B.M. Anner, H. Hoshino, Inhibition of HIV-1 infection by zinc group metal compounds, *Antivir. Res.* 43 (1999) 123–133.
- [12] C. Amici, C. Giorgi, A. Rossi, M.G. Santoro, Selective inhibition of virus protein synthesis by prostaglandin A1: a translational block associated with HSP70 synthesis, *J. Virol.* 68 (1994) 6890–6899.
- [13] M.J. Daniels, M.G. Ménache, G.R. Burleson, J.A. Graham, M.K. Selgrade, Effects of NiCl₂ and CdCl₂ on susceptibility to murine cytomegalovirus and virus-augmented natural killer cell and interferon responses, *Fundam. Appl. Toxicol. J. Soc. Toxicol.* 8 (1987) 443–453.
- [14] P. Seth, M.M. Husain, P. Gupta, A. Schoneboom, B.F.B. Grieder, H. Mani, R. K. Maheshwari, Early onset of virus infection and up-regulation of cytokines in mice treated with cadmium and manganese, *Biomet. Int. J. Role Met. Ions Biol. Biochem. Med.* 16 (2003) 359–368.
- [15] S. Adouchief, T. Smura, J. Sane, O. Vapalahti, S. Kurkela, Sindbis virus as a human pathogen-epidemiology, clinical picture and pathogenesis, *Rev. Med. Virol.* (2016), <http://dx.doi.org/10.1002/rmv.1876>.
- [16] J. Godt, F. Scheidig, C. Grosse-Siestrup, V. Esche, P. Brandenburg, A. Reich, D. A. Groneberg, The toxicity of cadmium and resulting hazards for human health, *J. Occup. Med. Toxicol. Engl.* 1 (2006) 22, <http://dx.doi.org/10.1186/1745-6673-1-22>.
- [17] J.H. Ovelgönne, J.E. Souren, F.A. Wiegant, R. Van Wijk, Relationship between cadmium-induced expression of heatshock genes, inhibition of protein synthesis and cell death, *Toxicology* 99 (1995) 19–30.
- [18] A. Cuypers, M. Plusquin, T. Remans, M. Jozefczak, E. Keunen, H. Gielen, K. Opendakker, A.R. Nair, E. Munters, T.J. Artois, T. Nawrot, J. Vangronsveld, K. Smeets, Cadmium stress: an oxidative challenge, *BioMetals* 23 (2010) 927–940, <http://dx.doi.org/10.1007/s10534-010-9329-x>.
- [19] S.H. Wang, Y.L. Shih, W.C. Ko, Y.H. Wei, C.M. Shih, Cadmium-induced autophagy and apoptosis are mediated by a calcium signaling pathway, *Cell. Mol. Life Sci.* 65 (2008) 3640–3652, <http://dx.doi.org/10.1007/s00018-008-8383-9>.
- [20] Y. Nzengue, R. Steiman, C. Garrel, E. Lefebvre, P. Guiraud, Oxidative stress and DNA damage induced by cadmium in the human keratinocyte HaCaT cell line: role of glutathione in the resistance to cadmium, *Toxicology* 243 (2008) 193–206, <http://dx.doi.org/10.1016/j.tox.2007.10.005>.
- [21] A. Orvedahl, S. MacPherson, R. Sumpter Jr, Z. Tallóczy, Z. Zou, B. Levine, Autophagy protects against Sindbis virus infection of the central nervous system, *Cell Host Microbe* 7 (2010) 115–127, <http://dx.doi.org/10.1016/j.chom.2010.01.007>.
- [22] P. Mastromarino, C. Conti, R. Petruzzello, A. De Marco, F. Pica, M.G. Santoro, Inhibition of Sindbis virus replication by cyclopentenone prostaglandins: a cell-mediated event associated with heat-shock protein synthesis, *Antivir. Res.* 20 (1993) 209–222.
- [23] J.-T. Jan, D.E. Griffin, Induction of apoptosis by sindbis virus occurs at cell entry and does not require virus replication, *J. Virol.* 73 (1999) 10296–10302.
- [24] C. Yuan, M. Kadiiska, W.E. Achanzar, R.P. Mason, M.P. Waalkes, Possible role of caspase-3 inhibition in cadmium-induced blockage of apoptosis, *Toxicol. Appl. Pharmacol.* 164 (2000) 321–329, <http://dx.doi.org/10.1006/taap.2000.8921>.
- [25] D.K. Perry, M.J. Smyth, H.R. Stennicke, G.S. Salvesen, P. Duriez, G.G. Poirier, Y. A. Hannun, Zinc is a potent inhibitor of the apoptotic protease, caspase-3, *J. Biol. Chem.* 272 (1997) 18530–18533, <http://dx.doi.org/10.1074/jbc.272.30.18530>.
- [26] R. Shimoda, W.E. Achanzar, W. Qu, T. Nagamine, H. Takagi, M. Mori, M. P. Waalkes, Metallothionein is a potential negative regulator of apoptosis, *Toxicol. Sci. J. Soc. Toxicol.* 73 (2003) 294–300, <http://dx.doi.org/10.1093/toxsci/kfg095>.
- [27] P. Krejbich-Trotot, M. Denizot, J.-J. Hoarau, M.-C. Jaffar-Bandjee, T. Das, P. Gasque, Chikungunya virus mobilizes the apoptotic machinery to invade host cell defenses, *FASEB J. Publ. Fed. Am. Soc. Exp. Biol.* 25 (2011) 314–325, <http://dx.doi.org/10.1096/fj.10-164178>.
- [28] E.J. Calabrese, Preconditioning is hormesis (part I): documentation, dose-response features and mechanistic foundations, *Pharmacol. Res.* (2016), <http://dx.doi.org/10.1016/j.phrs.2015.12.021>.
- [29] E.J. Calabrese, Preconditioning is hormesis (part II): how the conditioning dose mediates protection: dose optimization within temporal and mechanistic frameworks, *Pharmacol. Res.* (2015), <http://dx.doi.org/10.1016/j.phrs.2015.12.020>.
- [30] A.M. Sandbichler, M. Höckner, Cadmium protection strategies – a hidden trade-off? *Int. J. Mol. Sci.* (2016), <http://dx.doi.org/10.3390/ijms17010139>.
- [31] M.M. Ansari, N. Neha, H.A. Khan, Effect of cadmium chloride exposure during the induction of collagen induced arthritis, *Chem. Biol. Interact.* 238 (2015) 55–65, <http://dx.doi.org/10.1016/j.cbi.2015.06.001>.
- [32] J. Breton, C. Daniel, C. Vignal, M. Body-Malapel, A. Garat, C. Plé, B. Foligné, Does oral exposure to cadmium and lead mediate susceptibility to colitis? The dark-and-bright sides of heavy metals in gut ecology, *Sci. Rep.* 6 (2016) 19200, <http://dx.doi.org/10.1038/srep19200>.