Biochemical and Biophysical Research Communications 457 (2015) 538–541

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Cadmium exposure inhibits MMP2 and MMP9 activities in the prostate and testis

Livia M. Lacorte [a], Jaqueline C. Rinaldi [a], Luis A. Justulin Jr. [a], Flávia K. Delella [a], Andrei Moroz [b], Sérgio L. Felisbino [a, *]

[a] Univ Estadual Paulista – UNESP, Institute of Biosciences, Department of Morphology, Extracellular Matrix Laboratory, Botucatu, SP, Brazil
[b] Univ Estadual Paulista – UNESP, School of Pharmaceutical Sciences, Department of Bioprocess and Biotechnology, Cell Culture Laboratory, Araraquara, SP, Brazil

ARTICLE INFO

Article history:
Received 19 December 2014
Available online 16 January 2015

Keywords:
Prostate
Cadmium
Matrix metalloproteinases
MMP2
MMP9
Zymography

ABSTRACT

Matrix metalloproteinases (MMPs) are zinc (Zn^{2+}) and calcium (Ca^{2+}) dependant endopeptidases, capable of degradation of numerous components of the extracellular matrix. Cadmium (Cd^{2+}) is a well known environmental contaminant which could impair the activity of MMPs. In this sense, this study was conducted to evaluate if Cd^{2+} intake inhibits these endopeptidases activities at the rat prostate and testicles and if it directly inhibits the activity of MMP2 and MMP9 at gelatinolytic assays when present in the incubation buffer. To investigate this hypothesis, Wistar rats (5 weeks old), were given tap water (untreated, n = 9), or 15 ppm $CdCl_2$ diluted in drinking water, during 10 weeks (n = 9) and 20 weeks (n = 9). The animals were euthanized and their ventral prostate, dorsal prostate, and testicles were removed. These tissue samples were processed for protein extraction and subjected to gelatin zymography evaluation. Additionally, we performed an experiment of gelatin zymography in which 5 μM or 2 mM cadmium chloride ($CdCl_2$) was directly dissolved at the incubation buffer, using the prostatic tissue samples from untreated animals that exhibited the highest MMP2 and MMP9 activities in the previous experiment. We have found that $CdCl_2$ intake in the drinking water led to the inhibition of 35% and 30% of MMP2 and MMP9 ($p < 0.05$) at the ventral prostate and testis, respectively, in Cd^{2+} treated animals when compared to controls. Moreover, the activities of the referred enzymes were 80% and 100% inhibited by 5 μM and 2 mM of $CdCl_2$, respectively, even in the presence of 10 mM of $CaCl_2$ within the incubation buffer solution. These important findings demonstrate that environmental cadmium contamination may deregulate the natural balance in the extracellular matrix turnover, through MMPs downregulation, which could contribute to the toxic effects observed in prostatic and testicular tissue after its exposure.

1. Introduction

Matrix metalloproteinases (MMPs) compose a family of zinc and calcium dependant endopeptidases responsible for the remodeling and degradation of extracellular matrix (ECM) proteins, such as collagen, elastin, laminin, fibronectin, and proteoglycans [1]. These enzymes are produced as latent precursors, named zymogens or pre-MMPs, which require prior activation to be able to break the ECM components [2–4]. MMPs expression can be evaluated via different techniques; however, the gelatin zymography assay allows the determination of both active and latent forms of MMPs, mainly for MMP2 and MMP9 [5].

The expression/activity of MMP2 and MMP9 is directly correlated with ECM remodeling, in which an unbalanced activity may contribute to several diseases in different tissues such as rheumatoid arthritis, osteoarthritis, cardiovascular dysfunction, and cancer [6–8]. Therefore, any substance that has the ability to deregulate the homeostasis of the MMP activity may be potentially harmful. Interestingly, the MMPs activity is tightly regulated by divalent ions concentration, mainly Zn^{2+} and Ca^{2+}. Previous researchers reported that other metals, such as Sr^{2+}, Co^{2+}, Br^{2+}, Mn^{2+}, and Mg^{2+}, are able to interfere with the activity of these enzymes at

* Corresponding author. Univ Estadual Paulista – UNESP, Institute of Biosciences, Department of Morphology, Extracellular Matrix Lab., District of Rubião Júnior S/N, 18618-970 Botucatu, SP, Brazil.
E-mail address: felisbin@ibb.unesp.br (S.L. Felisbino).

http://dx.doi.org/10.1016/j.bbrc.2015.01.019
0006-291X/

concentrations such as 2 mM [9]. Furthermore, cadmium, lead, and zinc directly inhibited 25 kDa and 43 kDa enamel matrix proteinases in vitro activities when dissolved at the incubation buffer of gelatin zymography assays, even in low concentrations such as 110 μM [10].

Although cadmium has a well documented toxic and carcinogenic effect on the male reproductive system [11], little is known if it has also the potential to unbalance the normal activity profile of these important enzymes for prostate physiology [12,13]. Thus, this study was conducted to narrow this gap, by evaluating the effects of cadmium on the gelatinolytic activities of MMP2 and MMP9, in the rat prostate and testis.

2. Material and methods

2.1. Animals and treatments

Wistar rats were provided by the Central Stock breeder of São Paulo State University (UNESP, Botucatu, SP, Brazil), and maintained in cages (3 rats/cage) under controlled temperature (20–22 °C) and lighting conditions. The animals were fed a standard pellet diet and water *ad libitum*. Experimental protocols met the "Guidelines of Animal Experimentation" approved by the Commission of Ethics in Animal Experimentation (Protocol number 139/2009-CEEA) at the Institute of Biosciences, UNESP. After 7 days of acclimatization, the animals were randomized into three experimental groups. The control rats (untreated) received tap water (n = 9), and the Cd treated groups were exposed to 15 ppm of $CdCl_2$ (Sigma™) which was done over 10 weeks (n = 9) or 20 weeks (n = 9). This concentration was chosen after a revision of the current available data and is reported to be equivalent to non-carcinogenic exposure [14].

2.2. Tissue processing and gelatin zymography

After the exposure period, the animal's ventral and dorsal prostatic lobes were collected, along with the testis. All tissue specimens were immediately frozen in liquid nitrogen (−196 °C). Protein extraction was conducted as described [15]. Briefly, 30 mg of tissue were processed with a Polytron homogenizer in 0.1 mL of a 50 mM Tris-HCl solution, pH 7.5, supplemented with 0.25% Triton X-100, 10 mM $CaCl_2$, and protease cocktail inhibitor (Sigma™). After a 4 °C/20 min/14,000 rpm centrifugation, the supernatant was collected and subjected to protein quantification using the Bradford technique [15].

Gelatin zymography was performed as previously described [8,12] with modifications. Briefly, 30 μg of protein obtained from untreated and treated tissue extracts were loaded in 8% polyacrylamide gels co-polymerized with 0.1% gelatin (Merck™) acting as the substrate for the enzymes. Positive controls – purified human MMP2 (20 ng) and MMP9 (30 pg) (Calbiochem™) – were also

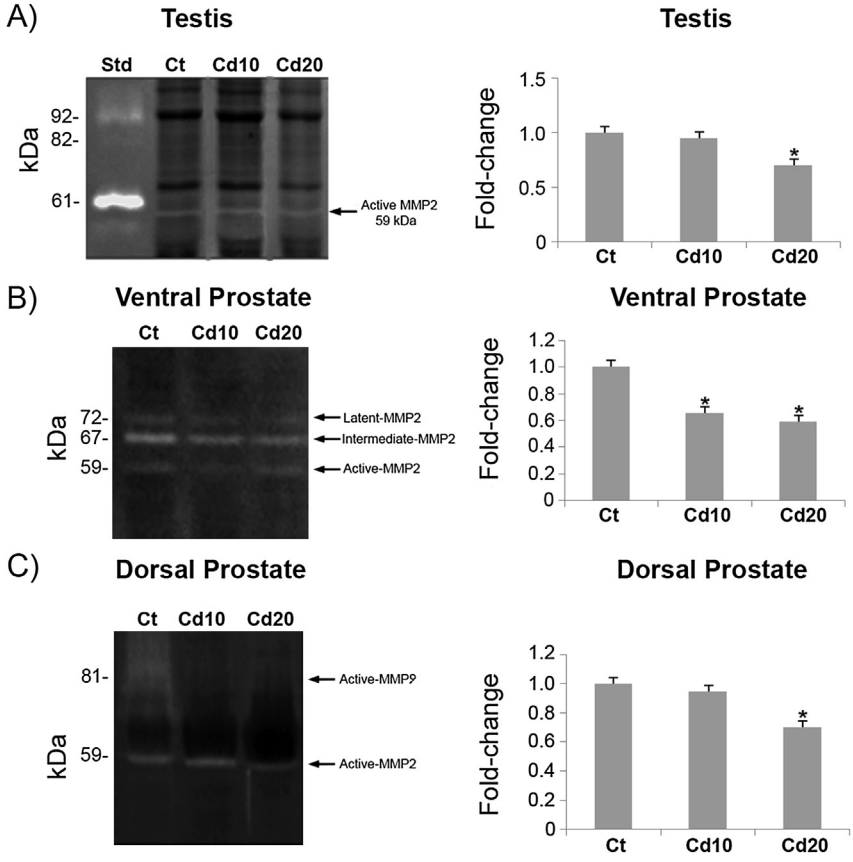

Fig. 1. Cadmium effects on the activity of MMP2 and MMP9 of animals exposed to Cd contaminated drinking water. Representative gelatin zymography of testis (A), ventral prostate (B), and dorsal prostate (C) tissue extracts of untreated animals (Ct) and animals exposed to cadmium during 10 (Cd10) and 20 (Cd20) weeks. Recombinant human MMP2 and MMP9 were loaded as positive controls (Std). Under control conditions, in the testis (A), it is possible to observe the active form of MMP2 (59 kDa). Cadmium exposure led to significant inhibition of this activity by 30% in the 20 week-treated animals (as shown in the fold-change graphic). Also, under control conditions, in the ventral prostate lobe extracts (B), it is possible to observe high activity of three bands of MMP2 (latent, intermediate and active forms; 72 kDa/67 kDa/59 kDa, respectively). Cadmium exposure also led to a significant decrease in the activity of these enzymes, for both 10 and 20 week-treated animals (35% and 40%, respectively). In the dorsal prostate (C), only the active forms of MMP9 and MMP2 were observed (81 and 59 kDa, respectively). A significant downregulation of the activity of these enzymes was also observed in the 20 week cadmium treated animals (30%). (*) Statistically significant difference from control conditions with $p < 0.01$.

loaded in the gels. After electrophoresis, the gels were washed twice in 2.5% Triton X-100 to remove sodium dodecyl sulfate and further washed in 50 mM Tris-HCl pH 8.0. Gels were incubated for the following 20 h in an activation buffer (50 mM Tris–HCl supplemented with 5 mM $CaCl_2$). Gels were stained with Coomassie brilliant blue R-250 and de-stained with 20% methanol and 10% acetic acid in distilled water until the clear bands had been visualized.

In addition, protein extracts from the ventral and dorsolateral prostates from untreated animals that exhibited the highest MMP2 and MMP9 activities were loaded in triplicate, in 3 gels, and subjected to gelatin zymography as described. One of the gels was incubated with the regular activation buffer, while the other two were incubated with the same buffer plus 5 μM $CdCl_2$ or 2 mM $CdCl_2$, to investigate the direct ability of cadmium to inhibit MMPs activities.

2.3. Image acquisition, quantification and statistical analysis

The gels were analyzed with the Image Quant350 equipment and the activities of the visualized bands were quantified using the ImageJ™ free software. The integrated optical density (IOD) was measured and the data was analyzed with INSTAT™ software using an ANOVA ($p < 0.05$) to compare the different treatments, with a post-test of Tuckey-Kramer. Values were calculated as the mean ± SD of the totality of IODs for the pro- and active forms of the MMP2 and MMP9 enzymes. Finally, a fold-change graphic was made by dividing the means of the values for the treated animals by the mean of the values for the untreated animals.

3. Results

Animals that were exposed to cadmium in their drinking water presented alterations in the activity of MMP2 and MMP9 within their tissue extracts. Testis extracts from 20 week-treated animals exhibited a significant reduction of 30% in the gelatinolytic activity of the active form of MMP2, when compared to untreated animals (Fig. 1A). Ventral prostatic lobe tissue extracts from 10 and 20 week-treated animals exhibited a significant reduction (35% and 40%, respectively) of the activity of the three different forms (latent, intermediate and active) of MMP2 (72, 67, and 59 kDa, respectively). No MMP9 activity was detected for this lobe (Fig. 1B). The dorsal prostatic lobe tissue extracts from 10 and 20 weeks-treated animals presented reduction in the activity of the active forms of MMP9 and MMP2, significant only for 20 week-treated animals (30%) (Fig. 1C).

The presence of 5 μM or 2 mM of $CdCl_2$ in the zymography incubation buffer was able to inhibit 80% and 100%, respectively, of the MMP2 and MMP9 activities in the ventral and dorsal prostates extracts from untreated animals (Fig. 2).

4. Discussion

Every day we are exposed to cadmium, an important environmental contaminant present in devices such as batteries of electronic hardware. Such exposure also results from the emission of cadmium in the atmosphere, as a result of processes such as the steel industry, waste incineration [15–17], as well as contaminants found in drinking water, food, and in tobacco smoke [15,17]. Taking into account the significant exposure to this agent, especially to the prostate, studies that enlighten the mechanisms by which this metal interferes with cellular homeostasis are required for better assessment of the real exposure risks.

Although it's well accepted that cadmium exerts a real carcinogenic stimulus to the prostate gland [14,15], little is known about its induced effects upon the expression/activity of MMPs in this gland. Given the well documented relationship between poor prognosis prostate tumors and high expression of MMPs [18,19], we aimed to investigate whether cadmium could stimulate or inhibit the activity of these important endopeptidases. Our study is the first to describe that 5 μM $CdCl_2$ inhibits the MMP2 and MMP9 gelatinolytic activity in vitro, even in the presence of 5 mM of $CaCl_2$.

It is known that the prostate is a major site for cadmium accumulation, after the liver and kidneys [20]. Animals chronically exposed to cadmium in drinking water also exhibited decreased MMP2 and MMP9 in their prostatic and testis tissues, reinforcing the relationship between cadmium consumption and accumulation in these organs. These enzymes have been reported to be potentially inhibited by Zn^{2+} and Cu^{2+}, at low concentrations of 100 μM [21] and also to higher concentrations (2 mM) of Zn^{2+}, Co^{2+}, Br^{2+}, Mn^{2+}, Mg^{2+} [9]. It is believed that these divalent ions exert their inhibitory effects by conformational changes in the catalytic domain of these enzymes [22]. Besides the potential effects on prostate carcinogenesis, we have demonstrated that cadmium also downregulated the activity of MMPs in the testicles, which could contribute to an imbalance in reproductive success, given the important role of these peptidases on the motility of spermatozoids [9].

It is well established that short-It is well established that short-term acute cadmium exposure by nebulization induces a remarkable and transient inflammation of the lung tissue, in which is followed by a transient increase of local MMPs activities due to

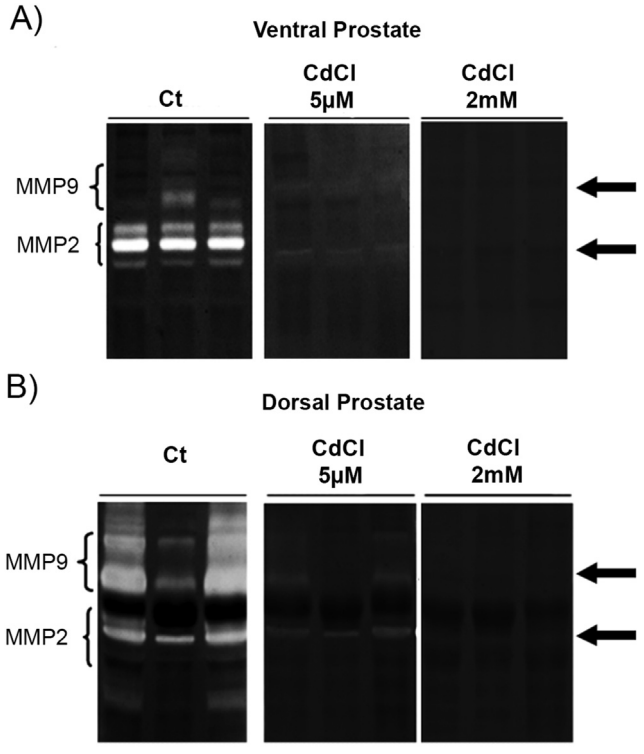

Fig. 2. Cadmium direct effects on the gelatinolytic activity of MMP2 and MMP9 when added to the incubation buffer. Gelatin zymography assay of tissue extracts of the ventral prostate (A) and dorsal prostate (B). Samples from tissue extracts (30 μg) of untreated animals (n = 3) with high gelatinolytic activities were subjected to the assay with or without cadmium (as cadmium chloride – $CdCl_2$) dissolved at the standard incubation buffer. Two concentrations were employed (5 μM or 2 mM $CdCl_2$). Under the standard incubation buffer (Ct), high MMP2 and MMP9 activities are observed. The lower concentration of 5 μM induces 80% inhibition of activity. Cadmium chloride at 2 mM totally inhibits the activities of these enzymes: no clear bands are visualized (arrow).

immune system cells infiltration and tissue remodeling [23–25]. In contrast, the dose and the route of administration of cadmium used in our study and in previous studies [14,20] does not induce significant inflammatory response in the prostate and testis, pointing to the important differences in the cadmium direct effects on MMPs activities and indirect on tissue levels of MMPs expression.

In conclusion, our results highlight that cadmium, even in a thousand times lower concentration than calcium, is able to directly inhibit the activities of both MMP2 and MMP9 when diluted on the zymography assay. Moreover, the toxic effects of this metal on the prostate and testis may be also related to an impairment of MMPs activities in these tissues, suggesting that further investigation about cadmium toxicity should take into account its impact on MMPs.

Conflict of interest

All authors report no conflicts of interest.

Acknowledgments

This article comprises part of the Ph.D. thesis of LML, supported by a FAPESP funding (processes No. 09/52747-7 and 09/50850-5), presented to the Institute of Biosciences of the São Paulo State University, UNESP, Brazil. We would also like to thank Mr. Chris Gieseke at University of Texas at San Antonio, for providing excellent assistance in the English language revision of this manuscript.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.01.019.

References

[1] R. Visse, H. Nagase, Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry, Circ. Res. 92 (2003) 827–839.
[2] L.M. Matrisian, Metalloproteinases and their inhibitors in matrix remodeling, Trends. Genet. 6 (1990) 121–125.
[3] M.D. Sternlicht, Z. Werb, How matrix metalloproteinases regulate cell behavior, Annu. Rev. Cell. Dev. Biol. 17 (2001) 463–516.
[4] I. Stamenkovic, Matrix metalloproteinases in tumor invasion and metastasis, Semin. Cancer. Biol. 10 (2000) 415–433.
[5] P.A. Snoek-van Beurden, J.W. Von den Hoff, Zymographic techniques for the analysis of matrix metalloproteinases and their inhibitors, Biotechniques 38 (2005) 73–83.
[6] G. Murphy, H. Nagase, Reappraising metalloproteinases in rheumatoid arthritis and osteoarthritis: destruction or repair? Nat. Clin. Pract. Rheumatol. 4 (2008) 128–135.
[7] N. Kaludercic, M.L. Lindsey, B. Tavazzi, G. Lazzarino, N. Paolocci, Inhibiting metalloproteases with PD 166793 in heart failure: impact on cardiac remodeling and beyond, Cardiovasc. Ther. 26 (2008) 24–37.
[8] A. Moroz, F.K. Delella, L.M. Lacorte, E. Deffune, S.L. Felisbino, Fibronectin induces MMP2 expression in human prostate cancer cells, Biochem. Biophys. Res. Commun. 430 (2013) 1319–1321.
[9] M.J. Wilson, B. Garcia, M. Woodson, A.A. Sinha, Metalloproteinase activities expressed during development and maturation of the rat prostatic complex and seminal vesicles, Biol. Reprod. 47 (1992) 683–691.
[10] R.F. Gerlach, A.P. Souza, J.A. Cury, S.R.P. Line, Effect of lead, cadmium and zinc on the activity of enamel matrix proteinases in vitro, Eur. J. Oral. Sci. 108 (2000) 327–334.
[11] M.P. Waalkes, Cadmium carcinogenesis, Mutat. Res. 533 (2003) 107–120.
[12] L.A. Justulin Jr., H.H. Della-Coleta, S.R. Taboga, S.L. Felisbino, Matrix metalloproteinase (MMP)-2 and MMP-9 activity and localization during ventral prostate atrophy and regrowth, Int. J. Androl. 33 (2010) 696–708.
[13] F.K. Delella, L.A. Justulin Jr., S.L. Felisbino, Finasteride treatment alters MMP-2 and -9 gene expression and activity in the rat ventral prostate, Int. J. Androl. 33 (2010) 114–122.
[14] S.M. Alvarez, N.N. Gómez, L. Scardapane, F. Zirulnik, D. Martínez, M.S. Giménez, Morphological changes and oxidative stress in rat prostate exposed to a non-carcinogenic dose of cadmium, Toxicol. Lett. 153 (2004) 365–376.
[15] L.M. Lacorte, F.R. Seiva, J.C. Rinaldi, F.K. Delella, A. Moroz, C. Sarobo, et al., Caffeine reduces cadmium accumulation in the organism and enhances the levels of antioxidant protein expression in the epididymis, Reprod. Toxicol. 35 (2013) 137–143.
[16] M. Hutton, Sources of cadmium in the environment, Ecotoxicol. Environ. Saf. 7 (1983) 9–24.
[17] S. Satarug, S.H. Garrett, M.A. Sens, D.A. Sens, Cadmium, environmental exposure, and health outcomes, Environ. Health. Perspect. 118 (2010) 182–190.
[18] M. Kirby, C. Hirst, E.D. Crawford, Characterizing the castration-resistant prostate cancer population: a systematic review, Int. J. Clin. Pract. 65 (2011) 1180–1192.
[19] A. Moroz, F.K. Delella, R. Almeida, L.M. Lacorte, W.J. Fávaro, E. Deffune, S.L. Felisbino, Finasteride inhibits human prostate cancer cell invasion through MMP2 and MMP9 downregulation, PLoS One 8 (2013) e84757.
[20] L.M. Lacorte, F.K. Delella, E.M. Porto Amorim, L.A. Justulin Jr., A.F. Godinho, A.A. Almeida, et al., Early changes induced by short-term low-dose cadmium exposure in rat ventral and dorsolateral prostates, Microsc. Res. Tech. 74 (2011) 988–997.
[21] A.P. Souza, R.F. Gerlach, S.R.P. Line, Inhibition of human gingival gelatinases (MMP-2 and 9) by metal salts, Dent. Mater. 16 (2000) 103–108.
[22] D.S. Auld, Removal and replacement of metal ions in metallopeptidases, Meth. Enzymol. 248 (1995) 228–2242.
[23] W. Zhang, L. Fievez, E. Cheu, F. Bureau, W. Rong, F. Zhang, Y. Zhang, C. Advenier, P. Gustin, Anti-inflammatory effects of formoterol and ipratropium bromide against acute cadmium-induced pulmonary inflammation in rats, Eur. J. Pharmacol. 628 (2010) 171–178.
[24] M. Dagouassat, S. Lanone, J. Boczkowski, Interaction of matrix metalloproteinases with pulmonary pollutants, Eur. Respir. J. 39 (2012) 1021–1032.
[25] J.L. Blum, L.K. Rosenblum, G. Grunig, M.B. Beasley, J.Q. Xiong, J.T. Zelikoff, Short-term inhalation of cadmium oxide nanoparticles alters pulmonary dynamics associated with lung injury, inflammation, and repair in a mouse model, Inhal. Toxicol. 26 (2014) 48–58.