Histol Histopathol (2015) 30: 61-67
DOI: 10.14670/HH-30.61

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

The influence of water pH on the genesis of cadmium-induced cancer in a rat model

Gisele Alborghetti Nai[1], Gisele Maria Soria Golghetto[2], Mariani Paulino Soriano Estrella[2], Larissa Di Santi Teixeira[2], Felipe do Carmo Moura[2], Hermann Bremer Neto[3] and José Luiz Santos Parizi[1]

[1]Department of Pathology, [2]Medical College and [3]Masters in Animal Science, Universidade do Oeste Paulista (UNOESTE), Presidente Prudente, SP, Brazil

Summary. Cadmium is a heavy metal that is widely used in industry and can cause tumours in multiple organs. The purpose of our study was to investigate the effect of water pH in the genesis of cadmium-induced cancer. We divided 98 male Wistar rats into 7 groups: group A - 15 rats that received cadmium chloride ($CdCl_2$ – 400 mg/L) in their drinking water at a neutral pH of 7.0; group B - 15 rats that received $CdCl_2$ (400 mg/L) in their drinking water at an acidic pH of 5.0; group C - 15 rats that received $CdCl_2$ (400 mg/L) in their drinking water at a basic pH of 8.0; group D - 15 rats that received water at an acidic pH of 5.0; group E - 15 rats that received water at a basic pH of 8.0; group F - 15 rats that received water at a neutral pH of 7.0; and group G - 8 rats that were subcutaneously injected with a single dose of cyclophosphamide (50 mg/kg). Groups A through F were euthanised 6 months after the start of the experiment and group G was euthanised 24 hours after cyclophosphamide injection. We collected the liver, kidneys, pancreas, prostate, seminal vesicles and testes for histopathological analysis and the bone marrow for micronuclei testing. In all of the groups, neither neoplastic lesions nor an increase in micronuclei ($p>0.05$) were observed in the liver, kidney, pancreas, seminal vesicles and testes. We found that animals exposed to cadmium had grade one prostatic intraepithelial neoplasia, but this was found more frequently in animals from group B ($p<0.05$). The acidic pH increased the formation of pre-neoplastic lesions in the prostate glands of cadmium-exposed animals.

Key words: Cadmium, Acidification, Neoplasms, Prostate, Micronucleus test

Offprint requests to: Gisele Alborghetti Nai, Laboratório de Anatomía Patológica e Citopatologia, Universaidade do Oeste Paulista (UNOESTE), Rua José Bongiovani, 700, 19050-680, Presidente Prudente, SP, Brasil. e-mail: gica@muranet.com.br or patologia@unoeste.br

Introduction

Cancer is widely accepted to be the result of external environmental factors acting in conjunction with individual susceptibility, with the former playing a central role in causing cancers and genetic factors having a secondary role (Terra Filho and Kitamura, 2006).

Cadmium (Cd) is a heavy metal that was discovered in approximately 1815 in ores containing carbonate and zinc. It is one of the most abundant non-essential elements found in the environment, is a component of cigarette smoke and is widely used in industrial applications (WHO, 1992). Although cadmium enters the body primarily through inhalation (Souza et al., 2010; Bernhoft, 2013), it is commonly found in most foods. Two-thirds of a person's daily cadmium intake is of plant origin (e.g., peanuts, sunflowers and rice) and

one-third is of animal origin (mainly fish, molluscs and crustaceans) (Souza et al., 2010; Bernhoft, 2013). Cadmium is obtained commercially as an industrial co-product of the mining and smelting of zinc and lead. Components containing cadmium (e.g., the batteries of mobile devices) are used as stabilisers in products, such as polymers of vinyl chloride (PVC), paint pigments, cosmetics and rechargeable nickel-cadmium batteries. Metallic cadmium is widely used as an anticorrosive agent and is a contaminant in phosphate-based fertilisers (Järup and Akesson, 2009; Bernhoft, 2013). It is also found in dental products, such as alginate and the silver alloys that are used in orthodontics (Menezes et al., 2009).

There is evidence that cadmium may increase the risk of tumour development in multiple organs, including lung, prostate, kidney, testis, liver and pancreas (Cho et al., 2013). Indeed, the International Agency for Research on Cancer (IARC) has listed cadmium as a human carcinogen (Group 1) (IARC, 2011). The cellular and molecular mechanisms for the carcinogenicity of cadmium include the activation of proto-oncogenes, the inactivation of tumour suppressor genes, the disruption of cell adhesion, the inhibition of DNA repair (Waalkes, 2003), epigenetic changes to DNA (Wang et al, 2012; Bernhoft, 2013) and oxidative stress (Bernhoft, 2013).

The micronucleus test is a statistically powerful test and is widely used as a screening tool for the safety of many substances and to classify agents as either mutagenic or non- mutagenic (Flores and Yamaguchi, 2008). The easy implementation of the micronucleus test has led to its widespread adoption as a standard genotoxicity test for monitoring the safety of agents used in human populations (Flores and Yamaguchi, 2008).

Analysis of tissue pH has shown that the microenvironment of tumours is usually more acidic than normal tissues. With the exception of brain tumours (where the microenvironment may be more alkaline than normal brain (Tannock and Rotin, 1989). Metabolic acidosis is a common feature of cancers and may affect the phenotype of tumour cells (Riemann et al., 2014). The major mechanisms that lead to tumour acidity likely include the production of lactic acid and the hydrolysis of ATP in hypoxic regions (Tannock and Rotin, 1989). Thus, both hypoxia and pH changes are two factors that can be therapeutically exploited to destroy cancer cells (Tannock and Rotin, 1989). Additionally, nutrient depletion and pH changes may contribute to cell death and necrosis of solid tumours as well (Tannock and Rotin, 1989).

Cadmium is a metal that, in addition to occupational exposure, is found in many foods, and can thus contaminate the human diet. The risk of developing cancer in patients contaminated by cadmium is high (Cheung et al., 2014); thus, there is a need for alternative and simple ways to avoid cadmium-induced tumour growth.

There are currently no studies examining the influence of drinking water pH on the toxicity of cadmium. The aim of this study was to evaluate the effect of drinking water pH on the genesis of cadmium poisoning induced cancer.

Material and methods

Animals and treatments

This study was approved by the Ethics Committee for Animal Use at the University of Oeste Paulista (CEUA - UNOESTE (Protocol no. 1167).

For our study, we used 98 male adult Wistar rats (*Rattus Norvegicus Albinus*), weighing between 200-250 g. The rats were divided into groups of four in large rectangular boxes (measuring 49x34x16 cm) suitable for the accommodation of up to five adult rats. Animals were maintained under controlled temperatures of 25±2°C, relative humidity of 50±15% and normal photoperiod (12-12 h light-dark cycles).

Exposure to cadmium was through cadmium chloride ($CdCl_2$ - Sigma Chemical Company, St. Louis, MO, USA) with a hydration of at least 98% and water content of approximately 2.5 mol/mol. For six months, cadmium chloride was given to the animals in their drinking water daily at a concentration of 400 mg/L (adapted from Motta et al., 2004). The water was acidified with hydrochloric acid and made alkaline with sodium hydroxide. Drinking water was changed three times a week to maintain the pH. Any wastewater containing cadmium was sent to the central reservoir of the Universidade do Oeste Paulista (UNOESTE) and neutralised for disposal. Left over water in the rat troughs was measured at each change of solution to estimate the average intake of each animal.

The animals were divided into seven groups: group A - 15 rats that received cadmium chloride in their drinking water at a neutral pH 7.0; group B - 15 rats that received cadmium chloride in their drinking water at an acidic pH 5.0; group C - 15 rats that received cadmium chloride in their drinking water at a basic pH 8.0; group D - 15 rats that received drinking water at an acidic pH 5.0; group E - 15 rats that received drinking water at a basic pH 8.0; group F - 15 rats that received drinking water at a neutral pH 7.0; group G - 8 rats that were subcutaneously injected with a single dose of cyclophosphamide (Genuxal, Baxter Oncology GmbH, Halle/Westfalen, Germany) (50 mg/kg) on the first day of the experiment (MacGregor, 1987). Because a previous report demonstrating that micronuclei will form in response to a 50 mg/mL dose of cyclophosphamide, group G was used as a positive control group (MacGregor, 1987). Animals in all groups received water and food *ad libitum*.

The rats in groups A through F were euthanised 6 months after the beginning of the experiment. Rats from group G were euthanised 24 hours after the administration of cyclophosphamide. Euthanasia was performed by intraperitoneal injection (Paiva et al.,

2005) of thiopental (Syntec, USA) at a dose of 100 mg/kg. Indications of death were the absence of breathing movements, the absence of a heartbeat and the loss of protective reflexes (Paiva et al., 2005). Necropsy was performed for removing the liver, kidney, pancreas, prostate, seminal vesicles and testes from each rat.

Histological study

The collected organs were fixed in 10% formalin (Cinética Indústria Química, São Paulo, Brazil) for 24 hours, embedded in paraffin (Dinâmica Reagentes Analíticos, São Paulo, Brazil), cut into 5 μm sections, stained with Hematoxylin and Eosin (H&E) (Dolles, São Paulo, Brazil), and examined under a Nikon Labophot light microscope (Nikon, Japan).

Histopathological analysis was used to determine if the liver, kidney, pancreas, prostate, seminal vesicles and testes showed abnormalities such as hyperplastic lesions, precancerous lesions, and benign and malignant neoplastic lesions. A single experienced observer (GAN) performed this analysis blinded.

Micronucleus test

Bone marrow samples were collected from the femur of each rat after euthanasia, and two sample slides were prepared per animal (MacGregor, 1987). The slides were then stained with Giemsa stain (Dolles, São Paulo, Brazil). Two thousand polychromatic erythrocytes (1,000 per slide) were counted for each animal at 400x magnification using an optical microscope to determine the number of micronuclei (MacGregor, 1987).

Micronuclei were defined as structures with probable halos surrounding the nuclear membrane and a volume less than one-third the diameter of the associated nuclei. Micronuclei staining intensity was similar to the intensity of the associated nuclei, and both structures were observed in the same focal plane (Tolbert et al., 1992). The blinded slide analysis was performed by a single person (MPSE) and reviewed by a second (GAN); results were concordant.

Statistical analysis

For the assessment of prostatic dysplasia variability, we used the Likelihood Ratio Test. We then used the Adjusted Standardised residuals test to determine where the source of the differences.

The variance in micronuclei abundance did not have a normal distribution when analysed by the Kolmogorov-Smirnov test ($p=0.0001$) and the variances were not homogenous as determined by the Levene test ($p=0.004$). We therefore chose to use the nonparametric Kruskal-Wallis test followed by multiple comparisons with the Dunn test to determine statistical significance.

All tests were considered statistically significant when $p<0.05$.

Results

Five animals died during the course of our study (one rat each from groups A, C, and D and two rats from group E). The cause of death for the animals from group A and C was acute pulmonary oedema, a complication associated with cadmium exposure (Järup and Akesson, 2009). It was not possible to establish the cause of death for the rats from groups D and E.

The average water intake per animal per day was: 55 ml for group A, 57 ml for group B, 52 ml for group C, 60 ml for group D, 70 ml for group E and 73 ml for group F. No statistically significant difference was found between the groups ($p>0.05$).

Histological study

In all of the groups (A through F), hyperplastic changes, precancerous lesions, and malignant or benign neoplasms were absent from the liver, kidneys, pancreas, testes and seminal vesicles.

Animals from all groups had nodular prostatic hyperplasia (Fig. 1A,B). However, only the animals from groups exposed to cadmium (A, B and C) had grade one prostatic intraepithelial neoplasia (PIN I) (Fig. 1C,D) ($p<0.05$). The incidence of PIN I in the animals of group B (exposure to cadmium in acidic drinking water) differed significantly from that in animals of groups A and C ($p<0.05$) (Table 1). One animal from group B had prostate adenocarcinoma (Fig. 1E,F).

Micronucleus test

The abundance of micronuclei did not increase in the cadmium-exposed groups, regardless of the water pH. Fig. 2 shows the number of micronuclei per experimental group. The number of micronuclei in the positive control group (group G, cyclophosphamide treatment) was significantly higher than was observed in all of the other experimental groups ($p<0.0001$). However, no significant difference in the number of micronuclei was observed between non-cadmium-

Table 1. Frequency of degree one prostatic intraepithelial neoplasia (PIN I) (n=85).

Group	Presence of PIN* (%)
A	3/14 (21,4%)[a]
B	4/15 (26,6%)[b]
C	3/14 (21,4%)[a]
D	0/14 (0%)[c]
E	0/13 (0%)[c]
F	0/15 (0%)[c]

*PIN: prostatic intraepithelial neoplasia. Superscript letters are a comparison between groups; a different letter superscript denotes a significant difference ($p<0.05$).

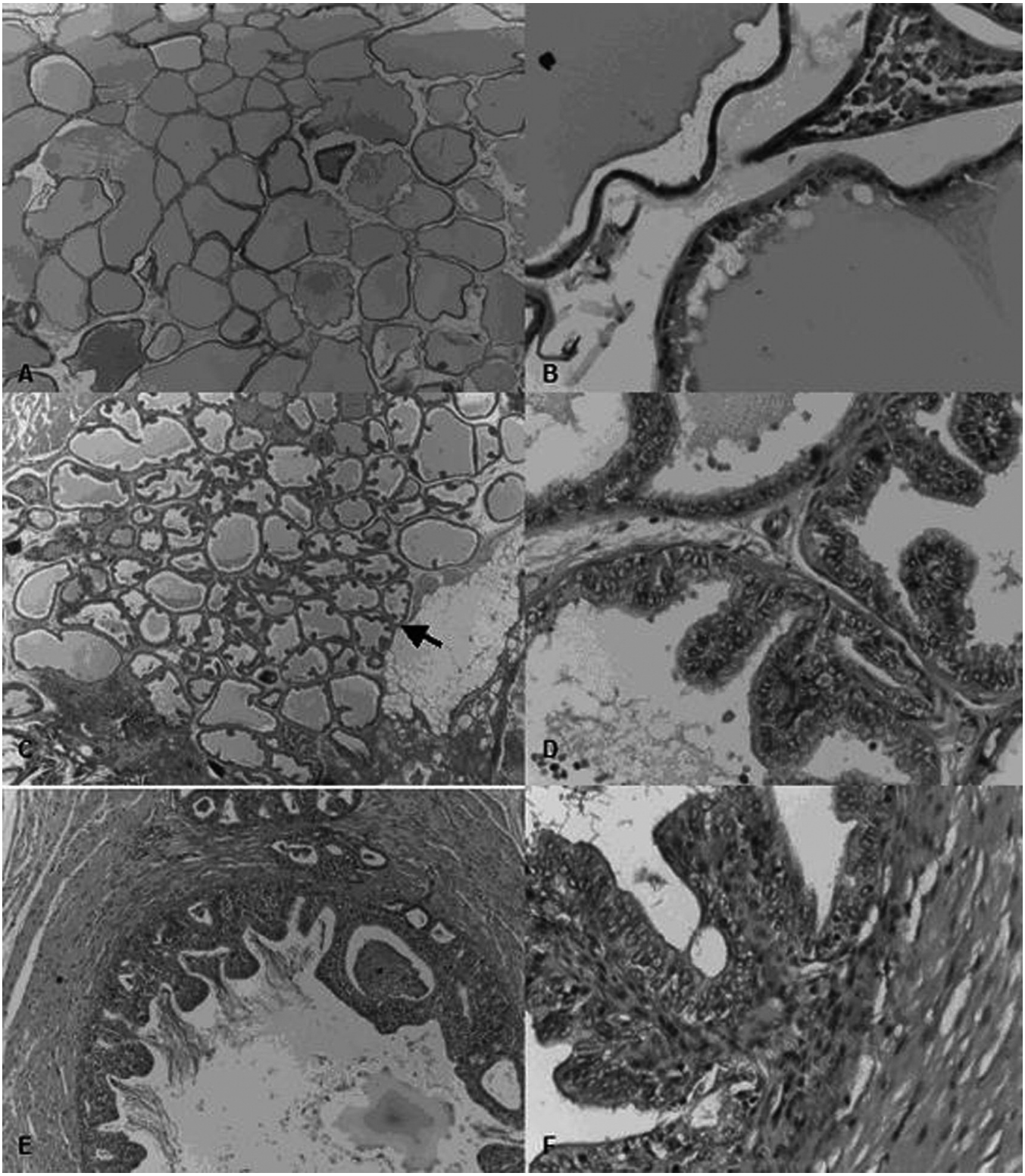

Fig. 1. Light microscopy of the prostate. **A.** Nodular hyperplasia from a group A animal (Hematoxylin-eosin). **B.** Normal prostate epithelium (Hematoxylin and eosin stain). **C.** Grade one prostatic intraepithelial neoplasia (PIN I) (arrow) from group A animals (Hematoxylin-eosin). **D.** Detail of image C showing cells with nucleoli (hematoxylin-eosin). **E.** Prostate adenocarcinoma from a group B animal (hematoxylin-eosin). **F.** Detail of image **E** showing pleomorphic cells and evident nucleoli (Hematoxylin-eosin). A, x 100; B, D, F, x 400; C, x 100; E, x 200

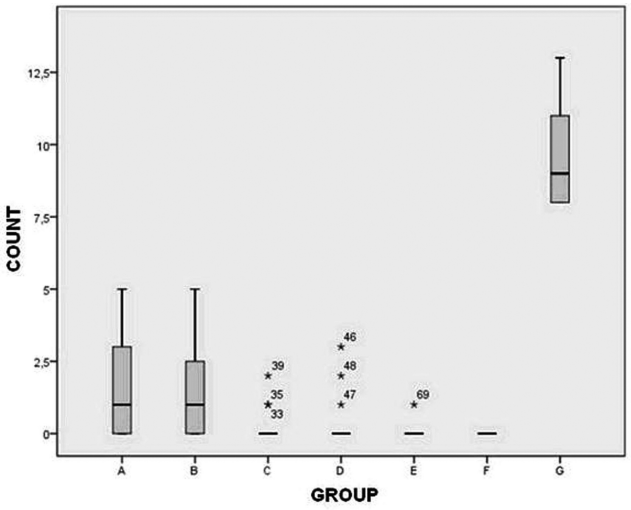

Fig. 2. Frequency of micronuclei per experimental group (median and interquartile intervals). *The number over the outlier corresponds to the number of animals.

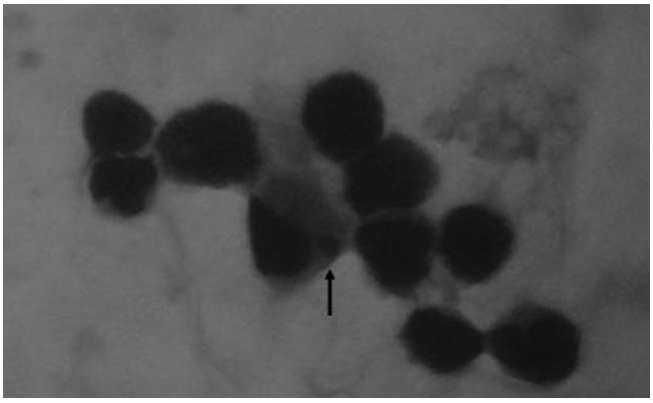

Fig. 3. Polychromatic erythrocyte with micronucleus (arrow) from an animal exposed to cadmium in neutral pH water (Giemsa stain). x 1000

exposed groups and the groups exposed to cadmium, regardless of the water pH ($p>0.05$) (Fig. 3).

Discussion

Food is a major route of exposure to cadmium for human beings and can be found in highly variable concentrations depending on the type of food (Järup and Akesson, 2009; Bernhoft, 2013). According to the World Health Organization, the maximum daily intake of cadmium should be 1μg/kg of body weight (WHO, 1992). In this study, animals were exposed to 400 mg of cadmium per litre of water, a dose well above the permissible daily intake, and therefore, a simulation of large-scale environmental contamination.

Soil pH is one of the main factors affecting the availability of heavy metals for plants and is, in general, inversely related to the availability of these elements (Cunha et al., 2008; Clemens et al., 2013). The negative effect of liming on the bioavailability of metals is primarily due to increased cation exchange capacity and the formation of hydroxides and carbonates of low solubility (Cunha et al., 2008; Clemens et al., 2013). In this study, we wanted to assess whether a difference in pH would influence the absorption and carcinogenic effects of cadmium.

The acidity of the tumour microenviroment is a major determinant of tumour progression (Barar and Omidi, 2013; Pellegrini et al, 2014) as it favours invasion and metastasis, as well as resistance to chemotherapy and immunotherapy (Barar and Omidi, 2013). Furthermore, tissue culture studies have shown that the combination of acidic pH and hypoxia is toxic to normal mammalian cells (Tannock and Rotin, 1989). It has been suggested that the acidity of tumours may allow for the development of therapeutic mechanisms that are relatively specific and can regulate pH specifically when the microenvironment is acidic (Tannock and Rotin, 1989).

Cadmium is an environmental pollutant, with relevant exposure levels in workplaces and the general population (Hartwig, 2010), and is associated with an increased risk for various cancers (Wang et al, 2012). Some studies have evaluated the mechanism by which certain substances, such as grape juice concentrate (Pires et al, 2013) and caffeine (Lacorte et al, 2013), to reduce the concentration of cadmium in animal models and to protect the male reproductive system. Additionally, there is little agreement in the literature regarding treatment for cadmium toxicity. The purpose of our study was to evaluate if the pH of the drinking water could influence the health risks associated with cadmium exposure.

Cadmium interferes with many cellular functions, particularly by the formation of complexes with external organic compound groups, such as proteins, and resulting in the inhibition of essential activities. Moreover, it can cause changes in antioxidant systems, which stimulate the accumulation of hydrogen peroxide (H_2O_2) and eventually lead to cell death (Souza et al., 2009; Bernhoft, 2013).

Cadmium can join the bases of a DNA molecule and produce a chemical modification that can damage the parent molecule. Furthermore, cadmium has a high affinity for sulfhydryl groups that can also interfere with mitotic spindle protein formation (Cho et al., 2013). The role of free radicals in the genotoxicity associated with cadmium has been confirmed. It has also been suggested that the radicals formed by metallothionein-cadmium complexes could be responsible for cadmium-induced DNA damage (Cho et al., 2013).

Prostate cancer is a common disease and is a major cause of death for men with an estimated 241.740 men being diagnosed in 2012 alone. The pathogenesis of prostate cancer is complex. Cadmium exposure has been proposed as an etiopathogenic factor for cancer

development in the prostate, as well as in other organs (Cheung et al, 2014). While smoking is not associated with an increased risk for prostate cancer, non-smoking patients have lower cadmium concentration in prostatic tissue than smokers (Neslund-Dudas et al, 2014), and therefore may be at a reduced risk. A study by Julin et al (2012) showed that cadmium ingestion plays a role in the development of prostate cancer in humans.

In our study, only the animals exposed to cadmium (groups A, B and C) had grade one prostatic intraepithelial neoplasia (PIN I) ($p<0.05$), corroborating other studies that show that cadmium causes preneoplastic and neoplastic lesions in the prostate (Cho et al., 2013). Animals of group B (exposed to cadmium in acidic drinking water) had a higher prevalence of precancerous lesions than those in group A and C ($p<0.05$). In addition, one animal from group B had an adenocarcinoma of the prostate, something not seen in any other experimental group. These data suggests that the acidity of the water may have contributed to the increased formation of PIN I and influenced the genesis of prostate preneoplastic lesions in group B animals. Therefore, prostate cancer may be associated with cadmium exposure in an acidic environment and men known to have an increased exposure to cadmium should avoid the use of acidic water. The prostatic nodular hyperplasia observed in all animals is likely due to aging.

In this study, no preneoplastic lesions or neoplasms (benign or malignant) were observed in the liver, kidneys, pancreas, seminal vesicles and testes regardless of exposure to cadmium or water pH. This may have occurred due to the poor absorption of cadmium from food and water by the gastrointestinal tract (only 5-7%) (Souza et al., 2010; Bernhoft, 2013). Although prolonged dietary exposure can lead to cadmium accumulation in the kidneys (WHO, 1992), we did not observe kidney neoplasms in our study.

Even though *in vitro* studies have demonstrated the genotoxic potential of cadmium (Waalkes, 2003), in our study, we did not observe an increase in the formation of micronuclei in exposed animals. This may be due to the route of exposure (ingestion), where there is less absorption of cadmium (Souza et al., 2010; Bernhoft, 2013). Moreover, even if a physical or chemical element does not induce an increase in micronuclei formation, that does not exclude the possibility of this element being genotoxic or mutagenic.

The micronucleus test has been extensively used to test the genotoxicity of many chemicals. Micronuclei are easily viewed in erythrocyte samples and are strongly indicative of chromosomal aberrations (Flores and Yamaguchi, 2008). These advantages prompted us to use the micronucleus test to evaluate the genotoxic effects of cadmium exposure in relation to water pH. The micronucleus test is used to evaluate the ability of a substance to break chromosomes (referred to as its clastogenicity) or affect the formation of the mitotic metaphase plate and/or spindle, both of which can lead to unequal chromosome distribution during cellular division (Flores and Yamaguchi, 2008). However, micronuclei formation is not the only means by which cadmium exposure can induce mutagenicity or genotoxicity and eventually a cancerous growth. Other mechanisms of cadmium-induced genotoxicity include direct interaction with DNA (Hartwig, 2010), interference with the DNA damage response, the dysregulation of cell growth, and resistance to apoptosis (Hartwig, 2010). Alternatively, cadmium may cause cancer via epigenetic mechanisms (Wang et al, 2012). Thus, other genotoxicity tests should be performed, as well as an examination of other forms of exposure, to better define the influence of pH on cadmium-induced cellular damage.

It must be remembered that there is a difference between the ability of an agent to produce damage and the likelihood of this agent to actually cause harm. The intrinsic potential of a toxic agent to harm health can only be achieved if there are conditions in place that allow it to reach the target organ (OPAS, 2001). This concept, associated with the data from our study, suggest that even at high doses, cadmium is poorly absorbed by the gastrointestinal tract and therefore has a low chance of causing tumours in most organs, except in the prostate.

Although currently there are no standards correlating blood or urine cadmium measurements with clinical toxicity (Bernhoft, 2013); and at low doses, the induction of tumours and hyperplastic lesions in the rat prostate is dose-dependent, whereas at high doses, there is no typical dose-response pattern because the proliferative response is lost (Waalkes, 2003), other studies that evaluate the internal exposure-dose of cadmium for each animal may provide a better understanding of the dose-response effect on the prostate epithelium.

In summary, cadmium caused precancerous lesions in the prostate, and acidic drinking water increased the prevalence of these lesions. However, even at high concentrations, cadmium exposure did not cause the formation of preneoplastic and neoplastic lesions in the liver, kidneys, pancreas, testes and seminal vesicles, or increase the number of micronuclei, regardless of the water pH.

Acknowledgments. The authors thank Edvaldo Mamede dos Santos for solution preparation. M.P. Soriano Esrella is fellow of the Institutional Program for Scientific Initiation Scholarships of the National Council for Scientific and Technological Development (PIBIC/CNPq)

References

Barar J. and Omidi Y. (2013). Dysregulated pH in tumor microenvironment checkmates cancer therapy. BioImpacts, 3, 149-162.

Bernhoft R.A. (2013). Cadmium toxicity and treatment. Scientific World J. 2013, 394652

Cheung M.R., Kang J., Ouyang D. and Yeung V. (2014). Association between urinary cadmium and all cause, all cancer and prostate cancer specific mortalities for men: an analysis of National Health and Nutrition Examination Survey (NHANES III) Data. Asian Pac. J. Cancer Prev. 15, 483-488.

Cho Y.A., Kim J., Woo H.D. and Kang M. (2013). Dietary cadmium intake and the risk of cancer: A meta-analysis. PLoS ONE 8, e75087.

Clemens S., Aarts M.G., Thomine S. and Verbruggen N. (2013). Plant science: the key to preventing slow cadmium poisoning. Trends Plant. Sci., 18, 92-99.

Cunha K.P.V., Nascimento C.W.A., Pimentel R.M.M., Accioly A.M.A. and Silva A.J. (2008). Cadmium and zinc availability, accumulation and toxicity in maize grown in a contaminated soil. R. Bras. Ci. Solo, 32, 1319-1328.

Flores M. and Yamaguchi U.M. (2008). Micronucleus test: an evaluation for genotoxic screening. Rev. Saúde Pesq. 1, 337-340.

Hartwig A. (2010). Mechanisms in cadmium-induced carcinogenicity: recent insights. Biometals. 23, 951-960.

IARC. (2011). A review of human carcinogens - part C: metals, arsenic, dusts, and fibres. Lancet Oncol. 10, 453-454.

Järup L. and Akesson A. (2009). Current status of cadmium as an environmental health problem. Toxicol. Appl. Pharmacol. 238, 201-208.

Julin B., Wolk A., Johansson J.E., Andersson S.O., Andrén O. and Akesson A. (2012). Dietary cadmium exposure and prostate cancer incidence:a population-based prospective cohort study. Br. J. Cancer 107, 895-900.

Lacorte L.M., Seiva F.R., Rinaldi J.C., Delella F.K., Moroz A., Sarobo C., Godinho A.F., Fávaro W.J., Fernandes A.A. and Felisbino S.L. (2013). Caffeine reduces cadmium accumulation in the organism and enhances the levels of antioxidant protein expression in the epididymis. Reprod. Toxicol., 35, 137-143.

MacGregor J.T., Heddle J.A., Hite M., Margolin B.H., Ramel C., Salamone M.F., Tice R.R. and Wild D. (1987). Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. Mutat. Res. 189, 103-112.

Menezes L.M., Freitas M.P.M. and Gonçalves T.S. (2009). Biocompatibility of orthodontic materials: myth or reality? Rev. Dental Press Ortodon. Ortop. Facial 14, 144-147.

Motta A.C.F., Migliari D.A., Gioso M.A., Komesu M.C., Sala M.A. and Lopes R.A. (2004). The carcinogenic potential of cadmium in the palatal and gingival epithelium of rats. A morphologic and morphometric analysis. Braz. J. Vet. Res. Anim. Sci. 41, 183-188.

Neslund-Dudas C., Kandegedara A., Kryvenko O.N., Gupta N., Rogers C., Rybicki B.A., Dou Q.P.and Mitra B. (2014). Prostate tissue metal levels and prostate cancer recurrence in smokers. Biol. Trace Elem. Res. 157, 107-112.

OPAS. (2001). Organização Pan-americana da Saúde no Brasil. Doenças relacionadas ao trabalho: manual de procedimentos para os serviços de saúde. Brasília: Ministério da Saúde. [cited 2013 Nov 05]. Available from: www.opas.org.br

Paiva F.P., Mafilli V.V. and Santos A.C.S. (2005). Curso de manipulação de animais de laboratório. Fundação Osvaldo Cruz. Centro de Pesquisas Gonçalo Muniz. [cited 2013 Nov 05]. Available from: http://www.bioteriocentral.ufc.br/arquivos/apostilha_manipulacao.pdf.

Pellegrini P., Strambi A., Zipoli C., Hägg-Olofsson M., Buoncervello M., Linder S. and De Milito A. (2014). Acidic extracellular pH neutralizes the autophagy-inhibiting activity of chloroquine: Implications for cancer therapies. Autophagy 31, 10, 562-571.

Pires V.C., Gollücke A.P., Ribeiro D.A., Lungato L., D'Almeida V. and Aguiar O. Jr (2013). Grape juice concentrate protects reproductive parameters of male rats against cadmium-induced damage: a chronic assay. Br. J. Nutr. 110, 2020-2029.

Riemann A., Schneider B., Gündel D., Stock C., Thews O. and Gekle M. (2014). Acidic priming enhances metastatic potential of cancer cells. Pflugers Arch. (in press).

Souza V.L., Silva D.C., Santana K.B., Mielke M.S., Almeida A-AF., Mangabeira P.A.O. and Rocha E.A. (2009). Effects of cadmium on the anatomy and photosynthesis of two aquatic macrophytes. Acta. Bot. Bras. 23, 343-354.

Souza M.C.B., Mancebo A.C.A., Santos H.C.N., Costa A.L.R., Taitson P.F. and Moreira M.F.R. (2010). Environmental Factors and Reproduction: metals (lead and cadmium). Rationale for research. JBRA Assist. Reprod. 14, 38-42.

Tannock I.F. and Rotin D. (1989). Acid pH in tumors and its potential for therapeutic exploitation. Cancer Res. 49, 4373-4384.

Terra Filho M. and Kitamura S. (2006). Occupational lung cancer. J. Bras. Pneumol., 32, S60-S68.

Tolbert P.E., Shy C.M. and Allen J.W. (1992). Micronuclei and other nuclear anomalies in buccal smears: methods development. Mutat. Res., 271, 69-77.

Waalkes M.P. (2003). Cadmium carcinogenesis. Mutat. Res. 533, 107-120.

Wang B., Li Y., Shao C., Tan Y. and Cai L. (2012). Cadmium and its epigenetic effects. Curr. Med. Chem. 19, 2611-2620.

WHO (1992). World Health Organization. Environmental Health Criteria, Cadmium. 1st ed. v. 134. Geneva, Switzerland: World Health Organization.

Accepted June 24, 2014