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INSTITUTO DE CIÊNCIAS DA SAÚDE
FACULDADE DE ODONTOLOGIA

LEIDIANE ALENCAR DE OLIVEIRA LIMA

INTOXICAÇÃO POR METILMERCÚRIO PROMOVE RESPOSTA À METALOTIONEÍNA E DANO CELULAR EM GLÂNDULAS SALIVARES DE RATOS

BELÉM
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Trabalho de Conclusão de Curso apresentado à Faculdade de Odontologia da Universidade Federal do Pará como requisito parcial para a obtenção do grau de Cirurgião-Dentista.

Orientador: Prof. Dr. Rafael Rodrigues Lima

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Banca Examinadora:

____
Prof. Dr. Rafael Rodrigues Lima-Orientador
Instituto de Ciências Biológicas - UFPA

____
Prof. M.ª Ana Carolina Alves Oliveira
Faculdade de Medicina - UFPA Campus Altamira

____
Prof. Dra. Renata Duarte de Souza Rodrigues
Instituto de Ciências da Arte - UFPA

Julgado em: _____/_____/_____

Conceito: ___________________

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2018

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À Deus, por ter me dado saúde e força para superar as dificuldades.

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RESUMO

A exposição ambiental ou ocupacional ao mercúrio é considerada um problema de saúde pública devido aos efeitos tóxicos sobre a saúde humana. A exposição ao metilmercúrio afeta uma variedade de funções fisiológicas, mas seus efeitos sobre as glândulas salivares tem sido pouco investigado. Este estudo analisa os efeitos celulares em glândulas salivares parótida, submandibular e sublingual de ratos adultos após exposição crônica, sistêmica e em baixas doses ao metilmercúrio (MeHg). Animais adultos receberam uma dosede 0,04 mg/kg/dia, ao longo de 60 dias. Após a exposição, os animais foram eutanasiados e tiveram suas glândulas coletadas para avaliação dos níveis de mercúrio total presente nesses órgãos.Também verificou-se a expressão tecidual de metalotioneina I/II (MT I/II), actina músculo liso (α-SMA) e Citoqueratina 18 (CK-18) utilizando imunohistoquímica. Nesta investigação, verificaram-se alterações teciduais nas glândulas salivares devido ao aumento dos nívies de mercúrio, com aumento da imunomarcação de MT I/II nas três glândulas, sugerindo um mecanismo responsivo à injúria, mas não suficientemente citoprotetor para evitar dano celular, observado pela diminuição da imunomarcação de CK-18 e α-SMA, sugerindo dano estrutural ao citoesqueleto. Esses dados sugerem que a exposição crônica, sistêmica e em baixas doses ao MeHg é capaz de promover danos celulares a glândulas salivares de ratos.

Palavras-chave: Mercúrio, Metalotioneína, Glândulas salivares, Intoxicação.

ABSTRACT

Environmental and occupational mercury exposure is considered a major public health issue. Despite being well known that MeHg exposure causes adverse effects in several physiologic functions, MeHg effects on salivary glands still not completely elucidated. Here, we investigated the cellular MeHg-induced damage in the three major salivary glands (parotid, submandibular, and sublingual) of adult rats after chronic, systemic and low doses of MeHg exposure. Rats were exposed by 0.04 mg/kg/day over 60 days. After that, animals were euthanized and all three glands were collected. We evaluated total Hg accumulation, metallothionein I/II (MT I/II), α-smooth muscle actin (α-SMA), and cytokeratin 18 (CK18) immune expression. Our results have showed that MeHg is able to disrupt gland tissue and to induce a protective mechanism by MT I/II expression. We also showed that cell MT production is not enough to protect gland tissue against cellular structural damage seen by reducing marking of cytoskeletal proteins as CK18 and α-SMA. Our data suggest that chronic MeHg exposure in low-daily doses is able to induce cellular damage in rat salivary glands.

Key words: Mercury. Salivary gland. Metallothionein. Intoxication.

SUMÁRIO

1. INTRODUÇÃO.. 7

2. CONSIDERAÇÕES FINAIS... 8

REFERÊNCIAS.. 9

APÊNDICE 10

ANEXO 19

1 INTRODUÇÃO

Mercúrio é um dos metais pesados mais estudados devido à sua ampla distribuição na natureza. No meio ambiente, os seres humanos e os animais podem ser expostos a diferentes formas químicas de mercúrio, incluindo vapor de mercúrio elementar (Hg0), mercúrio inorgânico (Hg2+) como o HgC l2 e compostos orgânicos de mercúrio como etilmercúrio, metilmercúrio e dimetilmercúrio (CLARKSON, 2002; BERNHOFT, 2012).

Em 1969, um relatório escrito por uma comissão de peritos internacionais em toxicologia classifica o mercúrio e seus compostos de acordo com sua ordem decrescente de toxicidade: compostos de metil e etilmercúrio (organomercuriais), de vapor de mercúrio (mercúrio elemental), sais inorgânicos e algumas formas orgânicas adicionais, tais como sais de fenilmercúrio (BERLIM et al., 1969).

Representando um grave problema ambiental devido seu elevado risco à exposição em baixas doses por um longo período de tempo (KNOBELOCH et al., 2005). Dessa forma, a ação dos compostos mercuriais no meio ambiente já ocasionou vários episódios de intoxicação e contaminação no ecossistema (AZEVEDO, 2003).

A intoxicação por mercúrio causa vários danos à saúde humana, como por exemplo, danos endócrinos, cardiovasculares, neurológicos e renais (LI et al., 2010; TAN et al., 2009). Estudos experimentais em animais estão de acordo com os dados humanos e indicam que a exposição longa ao mercúrio pode ter consequências em um longo prazo após um determinado período de intoxicação.

No entanto, poucos estudos avaliam os efeitos do mercúrio e seus compostos durante um período longo de tempo e em baixas doses, situação que normalmente ocorre a intoxicação deste metal. Além disso, há poucos relatos sobre os efeitos desses metais pesados em tecidos glandulares (HVID et al., 2011; SCHMID et al., 2007).

O tecido epitelial glandular se mostra na literatura também suscetível a agentes tóxicos, sofrendo, dependendo da intensidade e duração da agressão, alterações volumétricas, morfológicas e comprometimento funcional (LIEBER et al, 1991; FAUSTINO &STIPP, 2003).

2 CONSIDERAÇÕES FINAIS

Nossos resultados sugerem que o MeHg também tem por alvo as glândulas salivares quando administrado por via intragastrica durante longo período, as quais conseguiram se mostrar responsivas a injuria aumentando a produção de MT I/II em uma tentativa de detoxificação, porém não sendo capaz de evitar o dano a filamentos proteicos intracelulares de células acinares, ductais e mioepiteliais, importantes células do parênquima glandular.

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APÊNDICE

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Methylmercury Intoxication Promotes Metallothionein Response and Cell Damage in Salivary Glands of Rats

Leidiane Alencar de Oliveira Lima [1] · Leonardo Oliveira Bittencourt [1] · Bruna Puty [1] · Rafael Monteiro Fernandes [1] · Priscila Cunha Nascimento [1] · Marcia Cristina Freitas Silva [1] · Sérgio Melo Alves-Junior [2] · João de Jesus Viana Pinheiro [2] · Rafael Rodrigues Lima [1]

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Abstract

Environmental and occupational mercury exposure is considered a major public health issue. Despite being well known that MeHg exposure causes adverse effects in several physiologic functions, MeHg effects on salivary glands still not completely elucidated. Here, we investigated the cellular MeHg-induced damage in the three major salivary glands (parotid, submandibular, and sublingual) of adult rats after chronic, systemic and low doses of MeHg exposure. Rats were exposed by 0.04 mg/kg/day over 60 days. After that, animals were euthanized and all three glands were collected. We evaluated total Hg accumulation, metallothionein I/II (MT I/II), α-smooth muscle actin (α-SMA), and cytokeratin 18 (CK18) immune expression. Our results have showed that MeHg is able to disrupt gland tissue and to induce a protective mechanism by MT I/II expression. We also showed that cell MT production is not enough to protect gland tissue against cellular structural damage seen by reducing marking of cytoskeletal proteins as CK18 and α-SMA. Our data suggest that chronic MeHg exposure in low-daily doses is able to induce cellular damage in rat salivary glands.

Keywords MeHg · Mercury · Salivary gland · Metallothionein · Intoxication

Introduction

Mercury (Hg) has long been considered a pollutant in environment around the world, divided into three chemical species: elemental (Hg^0), inorganic (Hg^{2+}), and an organic form, namely methylmercury (MeHg) [1, 2]. Among those, MeHg has gotten more attention due to its higher toxicity [3].

MeHg long-term exposure has been associated with several issues to human health, such as endocrine, cardiovascular, neurological, renal, and reproductive disorders [4–6]. Following long-term exposure, MeHg has the ability to accumulate in several tissues including the central nervous system, intestine, liver, and kidneys [7]. In a previous study by our group, we have demonstrated that salivary glands are also affected by MeHg exposure, with a remarkable Hg accumulation in the parenchymal tissue [8].

The main function of salivary glands are the production and secretion of saliva, performing also important functions in the maintenance of oral homeostasis—due to lubricating properties, microbiota control, buffering, and remineralization capacity [9, 10]. Damages on salivary glands could be associated with operating disorders that lead to changes in flow and quality of saliva and consequently changes in oral health [10].

In this way, we tested the hypothesis that chronic and low-daily MeHg doses are able to induce damages on acinar and myoepithelial cells of rat salivary glands. To test our hypothesis, we investigated cell-induced cytopretection events and structural changes in the cell population of the three main rat glands: parotid, submandibular, and sublingual.

✉ Rafael Rodrigues Lima
rafalima@ufpa.br; rafaelrodrigueslima@hotmail.com

[1] Laboratory of Functional and Structural Biology, Institute of Biological Sciences, Federal University of Para, Street Augusto Correa N. 01, Guamá, Belém, Para 66075-900, Brazil

[2] School of Dentistry, Federal University of Para, Belem, Para, Brazil

Material and Methods

Experimental Animals

Ninety-day-old male Wistar rats (240 g approximately; $n =$ 40) were provided by animal facility of Federal University of

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Para. All procedures were previously approved by Ethics committee on animal experimentation by Federal University of Para (BIO 225-14 - CEPAE-UFPA) following the guidelines suggested by NIH Guide to Care and Use of Laboratory Animals.

Experimental Groups and Exposure

Animals were randomly divided into two groups. Animals in the MeHg group were fed with 0.04 mg/kg/day of MeHg in oil corn ($n = 20$) by intragastric gavage over 60 days according to Kong et al. [11]. Control group ($n = 20$) were fed only with oil corn respecting the *v*/*v* ratio. Animals were weight weekly to dose adjustment.

Hg Dosage in Salivary Glands

After MeHg exposure, 10 animals of each group were anesthetized with combined solution of ketamine hydrochloride (90 mg/kg) and xylazine (9 mg/kg). After total loss of signals, animals were euthanized by cervical dislocation, and parotid, submandibular, and sublingual glands were collected. Tissues were immediately frozen in liquid nitrogen to total Hg quantification following protocol established by Akagi [12]. Total Hg content in the samples was estimated by wet digestion, reduction, and cold vapor atomic absorption spectrometry (CVAAS) (Semi-automated Mercury Analyzer, model Hg-201, Sanso Seisakusho Co. Ltd., Tokyo, Japan). The detailed methodology of this analysis is described in previously published works [13].

Tissue Preparation

Ten animals of each group were used to tissue analysis. Animals were anesthetized with combined solution of ketamine hydrochloride (90 mg/kg) and xylazine (9 mg/kg) and perfused through the left heart ventricle with saline solution 0.9% heparin, followed by 4% paraformaldehyde. The parotid, submandibular, and sublingual glands were removed, post fixed in 4% formaldehyde until processing. The glands were dehydrated in increasing solutions of ethanol (70, 80, and 90%, absolute 1 and absolute 2), diaphanized in xylol and included in paraplast. Samples were cut into the microtome to obtain sections of 5-μm thickness.

Gross Histopathology, Immunolabeling Protocol and Quantitative Analysis

Tissue sections were stained with hematoxylin and eosin (HE) for gross histopathology analysis. Sections were kept at 60 °C overnight, with subsequent clarification with xylol and rehydration with decreasing concentration of ethanol. After that, sections were stained with HE, dehydrated by increasing alcoholic and xylol solutions. For immunohistochemistry analysis, sections of the same glands were mounted on slides with 3-aminopropyltriethoxysilane (Sigma, St. Louis, MO, USA), then deparaffinized in xylol and hydrated with gradual ethanol. Antigen recovery was performed with citrate buffer (pH 6.0) in Pascal chamber (Dako, Carpinteria, CA, USA) for 30 s. Sections were immersed in 3% hydrogen peroxide (H_2O_2) in methanol for 20 min for the inhibition of endogenous peroxidase activity and then blocked with 1% bovine serum albumin (BSA, Sigma) in phosphate-buffered saline (PBS) for 1 h.

Slides were incubated with primary antibodies anti-MT-I/II (1:50 DAKO®); anti-CK 18 (1:100 DAKO®), and anti-α actin smooth muscle (α-SMA) (1:50, DAKO®). We evaluate the expression of proteins of extreme importance to defense against metal damage [14], cell-tissue architecture of epithelium [15] and myoepithelial cells of salivary glands [16].

CK18 and α-SMA are significant structural proteins of salivary glands. CK18 protein is an important component of intermediate filaments system of acinar and ductal cells in the salivary glands [15]. The α-SMA is the main protein of the microfilament system of the cytoskeleton in myoepithelial cells [16].

After the incubation with primary antibodies, sections were then incubated with horseradish peroxidase (HRP) (Spring, Pleasanton, CA, USA) for 30 min, using diaminobenzidine (Sigma) as the chromogenic and contrasting Mayer's hematoxylin (Sigma).

The evaluation of the immunostaining was performed by evaluating the fraction measure (%) of the proteins studied in both groups. Clear field images of five randomly selected areas from each sample were acquired in the Axioscope microscope (Carl Zeiss, Germany) fitted with a CCD AxiocCam HRC (Carl Zeiss) color camera with the same magnification (× 40). Areas stained with diaminobenzidine were separated and segmented using the software "deconvolution color plug-in" (Gabriel Landini, http://www.dentistry.bham.ac.uk/landinig/software/software.html) and ImageJ developed by Wayne Rasband (NIMH, NIH, Bethesda, MD, USA, http://rsbweb.nih.gov/ij/). After image segmentation, total dye fractions were measured and differences in immunoreactivity were then analyzed in the groups studied. As a negative control, the primary antibodies were replaced by non-immune serum. Adenoid cystic carcinoma samples were used as positive control for the immunohistochemical reaction.

Statistical Analysis

Data distribution of each group was tested by the Kolmogorov-Smirnov method with significance level of 0.05. To analyze body weight data, we performed one-way ANOVA for repeated measures, followed by *Tukey's* test.

Total mercury levels were analyzed by ANOVA following the pos-test *Tukey* with $p < 0.05$. The non-parametric Mann-Whitney test was used for the immunohistochemical labeling, with $p < 0.05$. GraphPad Prism 5.0 software (San Diego, CA, USA) was used for all analyzes. All data were showed as mean ± standard error.

Results

Animal Monitoring After MeHg Exposure and Tissue Metal Evaluation

Firstly, we checked the animal feeding behavior, through mass gain analysis throughout the MeHg exposure period. The temporal analysis of animal mass body gain showed a similar change over time in both experimental groups (MeHg group 258.4 ± 19.06 g vs control group 253.13 ± 23.46 g; $p = 0.2377$) (Fig. 1).

After 60 days of MeHg exposure, animals in the MeHg group showed higher Hg levels in all three glands—parotid (0.060 ± 0.0036 μg/g), submandibular (0.042 ± 0.0045 μg/g), and sublingual (0.041 ± 0.0066 μg/g)—in comparison to control group (< 0.001 μg/g, over threshold detection) ($p < 0.0001$) (Fig. 2).

MeHg Chronic Exposure Does Not Induce Histopathological Changes in Rat Salivary Glands

To analyze if MeHg exposure is able to induce changes on gland tissue morphology, we performed HE staining. We showed that MeHg chronic exposure does not induce changes in the parenchyma organization, stromal or ductal system of rat salivary glands. Therefore, salivary gland tissue showed a standard and normal morphology with no relevant histological alteration (Fig. 3).

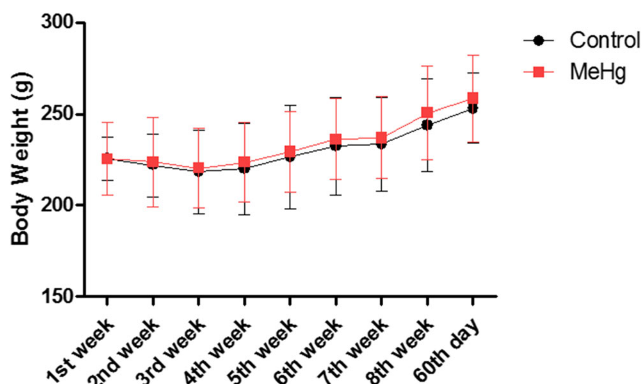

Fig. 1 Effects of MeHg chronic exposure at a dose of 0.04 mg/kg/day during 60 days on body mass gain of rats. The results are expressed as mean ± SEM with one-way ANOVA with repeated measures followed by Tukey's test

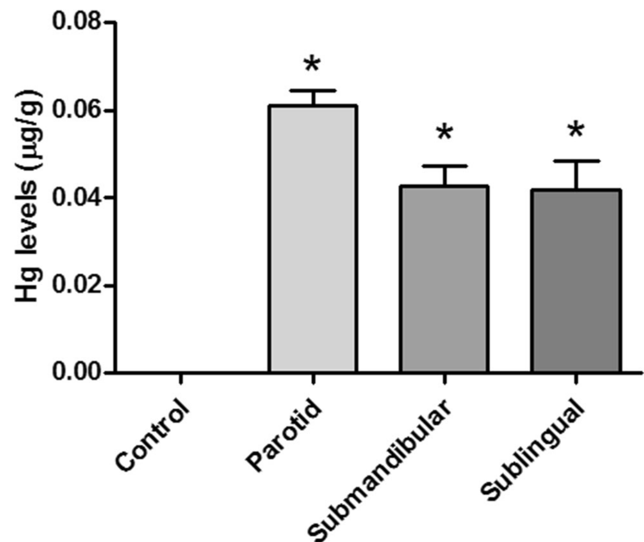

Fig. 2 Effects of MeHg chronic exposure at a dose of 0.04 mg/kg/day during 60 days on Hg levels in salivary glands of rats. The results are expressed as mean ± SEM. Salivary glands of control group showed lower levels than the threshold detected by equipment (values less than 0.001 μg/g). *$p < 0.05$ compared to control group (one-way ANOVA followed by Tukey's test)

MeHg Exposure Decrease *CK 18 and* α-*SMA Expression* in Rat Salivary Glands

Although no morphological changes were observed in rat salivary glands after MeHg exposure, we evaluate the importance to cell-tissue architecture of epithelium and myoepithelium of salivary glands. Our results showed that MeHg is able to decrease CK18 immunolabeling in parotid (MeHg 6.80 ± 0.57%; control 14.73 ± 1.04%; $p = 0.0286$), submandibular (MeHg 6.38 ± 0.70%; control 24.75 ± 1.49%; $p = 0.028$), and sublingual (MeHg 9.12 ± 1.36%; control 17.81 ± 1.78%; $p = 0.0159$) (Fig. 4).

We also showed a remarkable decrease in *α-SMA* immunolabeling in all three glands when compared to control group—parotid (MeHg 7.45 ± 0.60%; 23.50 ± 1.737%; $p = 0.028$) submandibular (MeHg 5.97 ± 0.74%; control 18.39 ± 1.27%; $p = 0.028$), and sublingual (MeHg 5.22 ± 1.35%; control 19.45 ± 3.18%; $p = 0.028$) (Fig. 5).

MeHg Chronic Exposure Increase *MT-I/II* Proteins Expression in Rat Salivary Glands

To understand the tissue/cell-like behavior against MeHg insult, we performed immunohistochemistry analysis to MT proteins, a well-known protein that acts in cellular defense against metal damage. Our result showed that MeHg chronic exposure induces a significant increase of MTI/II proteins immunolabeling in all three glands—parotid (MeHg 5.56 ± 0.74%; control 2.31 ± 0.26%; $p = 0.0050$), submandibular

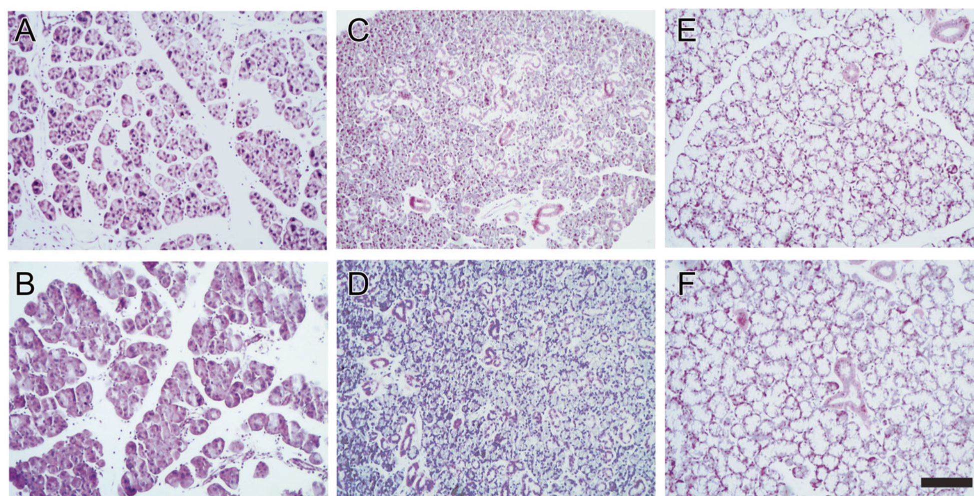

Fig. 3 Effects of MeHg chronic exposure at a dose of 0.04 mg/kg/day during 60 days on histological structure of salivary glands of rats, as revealed by hematoxylin and eosin staining. **a**, **c**, **e** From control groups and **b**, **d**, **f** from MeHg groups. **a**, **b** Parotid. **c**, **d** Submandibular. **e**, **f** Sublingual. Scale bar 100 μm

(MeHg 5.49 ± 0.43%; control 0.83 ± 0.19%; $p = 0.0023$), and sublingual (MeHg 7.87 ± 1.08%; control 0.96 ± 0.049%; $p = 0.0082$) (Fig. 6).

Discussion

In this study, we performed an evaluation of possible MeHg-induced damage in rat salivary glands after chronic MeHg exposure. Our results have showed that MeHg exposure by 0.04 mg/kg/day induced MT I/II expression in the three salivary glands, parotid, submandibular, and sublingual, suggesting an inducible protective mechanism in response to MeHg damage but not sufficiently cytoprotective to avoid structural cytoskeletal damage as showed by decrease in CK-18 e *α-SMA* expression.

In this study, we have chosen MeHg oral rout of administration by intragastric gavage since this method has been shown to be efficient in the control of variables that may cause interference in the metal consumption and absorption. In this way, the method we have chosen has low parameter commitment between animals in the same experimental group as shown in previous works [13, 17, 18]. Furthermore, intragastric gavage is an excellent choice of MeHg exposure since Hg absorption is widely absorbed by gastrointestinal tract [19]. In this way, once MeHg is absorbed by

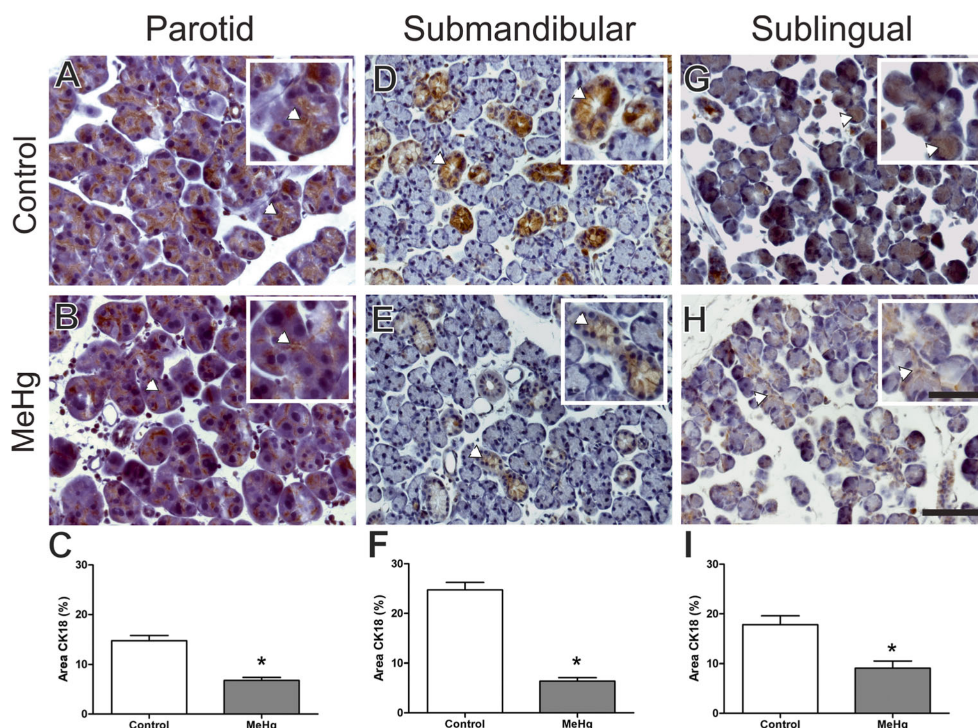

Fig. 4 Effects of MeHg chronic exposure at a dose of 0.04 mg/kg/day during 60 days on immune expression of cytokeratin 18 in rat salivary glands, as revealed by anti-cytokeratin 18 immunohistochemistry. **a**–**c** Parotid. **d**–**f** Submandibular. **g**–**i** Sublingual. Photomicrographs on **a**, **d**, **g** from control groups and **b**, **e**, **h** from MeHg groups, box shows image at higher magnification indicating the labeling region for the antibody. The results are expressed as mean ± SEM of labeled fraction area (%). *$p \leq 0.05$ compared to control group (Mann-Whitney test). Scale bar 100 μm; box 40 μm

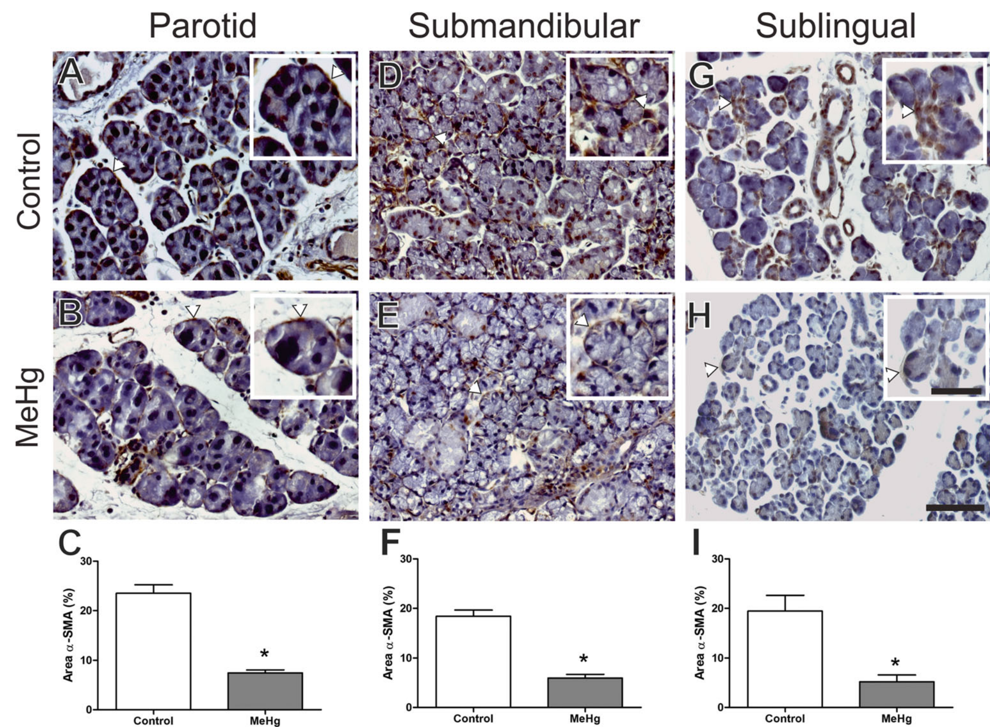

Fig. 5 Effects of MeHg chronic exposure at a dose of 0.04 mg/kg/day during 60 days on immune expression of α smooth muscle actin in rat salivary glands, as revealed by anti-α smooth muscle actin immunohistochemistry. **a**–**c** Parotid. **d**–**f** Submandibular. **g**–**i** Sublingual. Photomicrographs on **a**, **d**, **g** from control groups and **b**, **e**, **h** from MeHg groups, box shows image at higher magnification indicating the labeling region for the antibody. The results are expressed as mean ± SEM of labeled fraction area (%). *p ≤ 0.05 compared to control group (Mann-Whitney test). Scale bar 100 μm; box 40 μm

gastrointestinal tract, it could be systemically absorbed and accumulated in several organs as the kidneys [20], brain [21], and salivary glands [8].

Our results have showed that after 60 days of MeHg exposure, higher levels of Hg were found in the three main rat salivary glands (Fig. 2), suggesting that systemic absorption of MeHg is able to drive Hg to salivary glands. Kong et al. [11] showed, in a same experimental model, that over 60 days of MeHg exposure the Hg accumulation on blood was about 1.1 μg/mL while in the brain was 0.7 to 0.9 μg/mL in different areas, leading to metabolic disturbs by a low ATP and pyruvate production and the total calcium. On the other hand, higher MeHg exposure (1 mg/kg/day) over the same time could be associated to kidney damage, as renal tubular degeneration

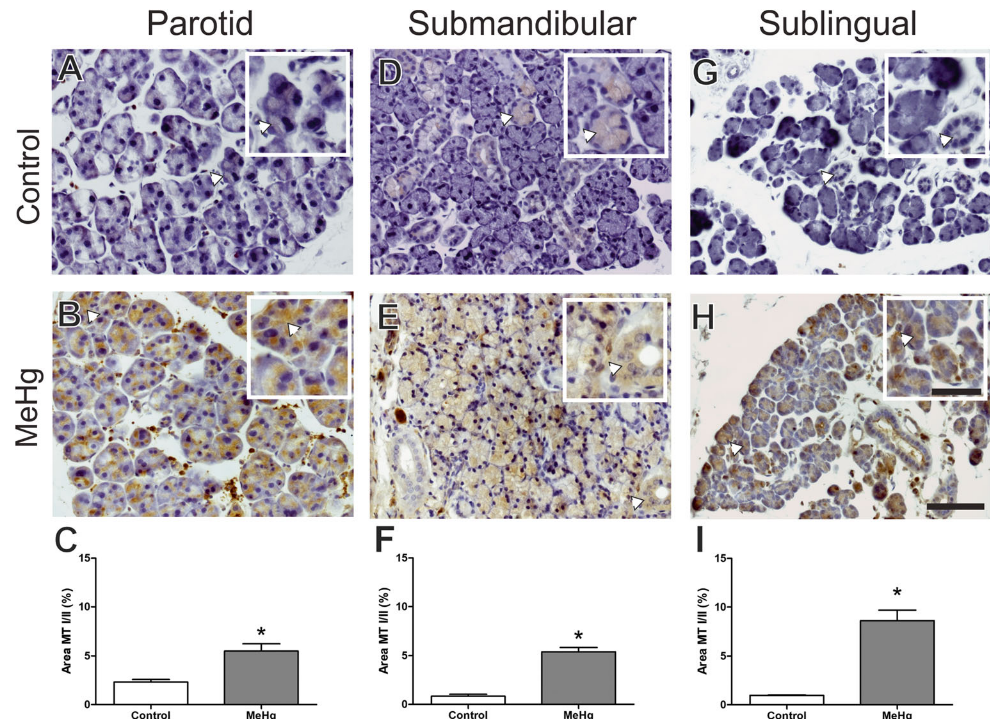

Fig. 6 Effects of MeHg chronic exposure at a dose of 0.04 mg/kg/day during 60 days on immune expression of metallothionein I/II in rat salivary glands, as revealed by anti-metallothionein I/II immunohistochemistry. **a**–**c** Parotid. **d**–**f** Submandibular. **g**–**i** Sublingual. Photomicrographs on **a**, **d**, **g** from control groups and **b**, **e**, **h** from MeHg groups, box shows image at higher magnification indicating the labeling region for the antibody. The results are expressed as mean ± SEM of labeled fraction area (%). *p ≤ 0.05 compared to control group (Mann-Whitney test). Scale bar 100 μm; box 40 μm

and necrosis, after a total Hg accumulation of 70.5 μg/g [22]. While there is no comparable data for our experimental model that has assessed damage to the liver and kidney, it is well known that kidney is the major organ for Hg accumulation from blood system. As salivary gland has a complex system to cell renewal, which is able to mediate the Hg extrusion from the cell, a higher concentration of Hg is expected to gain systemic circulation and kidney while low doses still in the salivary glands. As showed by Kong et al. [11] and Bittencourt et al. [8], our work corroborates to the knowledge that low Hg doses are able to induce cellular damage in different tissues including the three main rat salivary glands.

It is important to notice that salivary glands are also target toxic effects of MeHg by direct contact of mucosa that has a large amount of small salivary glands [23]. Previous studies have shown that rat and human salivary glands have the ability to change the phenotype of acinar, ductal, and myoepithelial cells after cell metabolism alterations [24, 25]. Histopathological analysis performed here did not show structural alterations in tissue morphology after MeHg exposure when compared to control group. But it is important to note that this does not mean that glandular function could not have been affected. We believe that in this model of long-term exposure it is possible that glandular tissue has activated protection and renewal mechanisms.

Similar Hg levels on salivary glands were found in a previous work by our group [8]. In this work, we have showed that MeHg exposure is able to induce oxidative stress that leads to changes on proteomic profile of rat salivary glands. However, the present work showed for the first time that MeHg exposure also lead to changes on mechanisms of cellular detoxification and integrity of intermediate cytoskeletal filaments in salivary gland cell population.

One of the mechanisms that are activated to protect cells against MeHg-induced cell damage is the synthesis of a class of protein known as metallothionein [26]. The MT I/II are low-weight proteins that are rich in cysteine residues and sulfhydryl groups that has the ability to bind to metals [27, 28]. Furthermore, MT proteins are important to the homeostasis maintenance of essential metals as zinc and copper as well as in the Hg detoxification. Those mechanisms could be associated with cell MeHg-induced protection [29, 30].

MT I/II syntheses have been described in several tissues as the liver, kidneys, pancreas, and intestine [31]. Hwang et al. [32] have showed that keratinocytes (HaCaT cell lineage) are able to synthesize MT after MeHg exposure [32, 33]. In the same way, our results showed that MeHg chronic exposure over 60 days increases the MTI/II in the three salivary glands. However, it is important to notice that despite the increase in MT I/II after MeHg exposure, our results also showed a remarkable change on structural proteins of salivary glands.

Several works have showed that MeHg could be associated with cellular damage by binding to important proteins as DNA repair enzymes, microtubules, and cytoskeleton proteins [34–36]. In this work, by demonstrating that MeHg could interact to cytoskeleton proteins as CK18 and α- SMA that were both decreased on acinar and ductal cells and myoepithelial cells, respectively, we showed for the first time that MeHg-induced changes on intermediate filaments of epithelial cells on rat salivary glands.

CK 18 and α-SMA are intermediate filaments of the cytoskeleton of the two main cells of the glandular parenchyma: acinar cells and myoepithelial cells, respectively. The cytoskeleton is the major mechanical structure of the cell, a dynamic biopolymer network comprising microtubules, actin, and intermediate filaments. The intermediate filaments are able to self-polymerize, forming a cytoplasmic network responsible for the mechanical resistance of the cells. In the myoepithelial cells, they also contribute, for the expulsion of the saliva of the acinar cells towards the duct system, whenever they are contracted [37, 38].

Cytokeratin filaments are important for the maintenance of mechanical integrity and stability of epithelial cells and also perform signaling function, acting in epithelial cell polarity and membrane traffic [39]. Besides that, they are also biomarkers of differentiation of epithelial cells [40]. α-SMA protein were also found to be decreases in myoepithelial cells of salivary glands. Those cells are important in the synthesis of extracellular matrix and also in the cell contraction to saliva production [41].

The decrease in CK18 and α-SMA could be associated with problems in saliva production and excretion, leading to xerostomia, a known disease to cause dry mouth sensation. Xerostomia are a regular disease with multifactorial etiology. For xerostomia, treatment is essential to understand the cause, since it could be caused by several other diseases as diabetes, sarcoidosis, Sjogren's syndrome, or even by local changes as mastication or breathing loss [42]. In this way, the proof of Hg levels on salivary glands and protein changes raises the hypothesis that MeHg models exposure could be a potential agent of xerostomia disease in population at high environmental exposure risk.

Thus, our results suggest that salivary glands are also the target of MeHg by intragastric chronic exposure. We also point that salivary glands are capable of responding to cell damage, increasing MT I/II synthesis. However, salivary glands are not able to protect cells from changes on intracellular filaments of acinar, ductal, and myoepihtelial cells, the most important cells of glandular parenchyma.

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Compliance with Ethical Standards All procedures were previously approved by Ethics committee on animal experimentation by Federal University of Para (BIO 225-14 - CEPAE-UFPA) following the guidelines suggested by NIH Guide to Care and Use of Laboratory Animals.

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ANEXO

PARECER 225-14

Projeto: CARACTERIZAÇAO DA INJURIA NO CORTEX MOTOR DE RATOS APOS ACIDENTE VASCULAR ISQUÊMICO, EM UM MODELO DE INTOXICAÇÃO CRÔNICA COM METILMERCÚRIO

Coordenador: Prof. Dr. Rafael Rodrigues Lima

Área Temática: Neurociências

Vigência: 01/2013 a 12/2015

No no CEPAE-UFPA: 225-14

O projeto acima identificado foi avaliado pelo Comitê de Ética Em Pesquisa Com Animais de Experimentação da Universidade Federal do Pará (CEPAE). O tema eleito para a investigação e de alto teor científico justificando a utilização do modelo animal proposto. Os procedimentos experimentais utilizados seguem as normas locais e internacionais para tratamento e manipulação de animais de experimentação. Portanto, o CEPAE, através de seu presidente, no uso das atribuições delegadas pela portaria No 1032/2014 do Reitor da Universidade Federal do Pará, resolve **APROVAR** a utilização de animais de experimentação (N= 80, ratos Wistar) nas atividades do projeto em questão, no período de vigência estabelecido.

As atividades experimentais fora do período de vigência devem receber nova autorização deste comitê.

Belém, 01 de janeiro de 2013