Vol.66: e23210014, 2023
https://doi.org/10.1590/1678-4324-2023210014
ISSN 1678-4324 Online Edition

Article - Human and Animal Health

Effect of Phenol Derivatives in the Oral Mucosa of University Laboratory Technicians

Ana Paula Nogueira Ferri[1]
https://orcid.org/0000-0001-9702-3150

Bruna Gomide[1]
https://orcid.org/0000-0002-6521-7708

Fellipe Augusto Tocchini de Figueiredo[2]
https://orcid.org/0000-0001-9924-9751

Celina Faig Lima Carta[1]
https://orcid.org/0000-0002-9305-079X

Ivan Balducci[1]
https://orcid.org/0000-0003-2569-083X

Janete Dias Almeida[1*]
https://orcid.org/0000-0003-4596-9715

[1]Universidade Estadual Paulista (Unesp), Instituto de Ciência e Tecnologia, Câmpus de São José dos Campos, São José dos Campos (SP), Departamento de Biociências e Diagnóstico Bucal, Brasil; [2]Universidade de São Paulo, Faculdade de Odontologia de Ribeirão Preto, USP, Ribeirão Preto (SP), Departamento de Materiais Dentários e Prótese, Brasil.

Editor-in-Chief: Paulo Vitor Farago
Associate Editor: Yasmine Mendes Pupo

Received: 09-Jan-2021; Accepted: 05-May-2023

*Correspondence: janete.almeida@unesp.br; Tel.: +55-12-3947-9029 | Fax: +55-12-3947-9010 (J.D.A.)

HIGHLIGHTS

- Phenolic derivatives exert a genotoxic effect on the oral mucosa.
- The use of personal protective equipment and/or exhaust laminar flow hood reduces genotoxic effects.
- Long-term exposure to genotoxic agents can be measured by oral mucosa samples.

Abstract: The objective of the present work was to quantify the effect of phenolic derivatives on in the oral mucosa of university laboratory technicians. Twenty (20) university laboratory technicians exposed to agents and 11 non-smokers without occupational exposure to phenolic derivatives for more than 10 years underwent anamnesis and extra and intra-oral clinical examination. Exfoliative cytology of the right buccal mucosa was performed. The frequency of micronuclei formation (MN) was analyzed in 1200 cells by standard microscopy. Mann-Whitney and Kruskal-Wallis test were used. The difference between the groups evaluated was observed in the frequency of more than 01 MN/cell (p=0.0397), being more present in the exposed group. The use of personal protective equipment contributed to a decrease in the frequency of 01 MN/cell (p=0.0272) for the exposed group. Genotoxic effects may be reduced by the use of PPE and/or exhaust laminar flow hood. Five years of exposure could adapt the nucleus cells to the genotoxic aggressor demonstrating that the oral mucosa could be a marker to long-term genotoxic exposure.

Keywords: micronucleus; cytodiagnosis; oral mucosa; carcinogenesis; phenol.

INTRODUCTION

The use of chemical substances in university laboratories is a routine and necessary procedure, which is performed by qualified technicians [1,2]. However, these individuals often do not receive the appropriate instructions regarding the importance of the use of personal protective equipment (PPE) such as masks, head covers, goggles, and aprons and are therefore exposed to the aspiration and direct contact with these products [1,2]. The substances most frequently used during procedures of tissue fixation and processing are formaldehyde and xylene [1-5].

Chemical agents such as formalin, hydrocarbons (soot, tar, oils) and cytotoxic drugs, among other substances, are carcinogenic to both humans and animals [1,2]. These chemically different substances are mainly transformed into active carcinogens by the cellular enzymatic system, characterizing the process of chemical carcinogenesis [1].

Laboratory technicians routinely use various chemical agents, e.g., formaldehyde as fixative, xylene as clearing agent [2], chemical preservatives and solvents, without wearing PPE [3] and without handling these substances under laminar flow hoods [1,2,3].

Exposure to these substances has harmful health effects that depend on the type of chemical agent and its concentration, frequency and duration of exposure, laboratory practices and habits, and individual susceptibility [6,7]. With respect to genotoxic agents, the inadequate use of these products can cause changes ranging from degenerative nuclear alterations to genomic alterations that can potentially be transmitted into the next generation [6,8-10].

One of the changes that occur in nucleated cells as a result of exposure to genotoxic agents and that can be easily identified is the formation of micronuclei (MN) [5-6]. Micronuclei are nuclear bodies that form whenever chromosomes are not incorporated into one of the daughter cells during cell division [6,8-10].

The progression from a normal to a neoplastic cell is a long process, with the consequent accumulation of successive exposures [5-10]. The genetic defect accumulates between the phases of pre-malignancy and malignancy and is expressed in the form of an increase in nuclear content, formation of DNA fragments in exposed cells, or expression of specific proteins [5-10]. These transformations indicate that the cells initiated the process of carcinogenesis and may serve as markers of the degree of tissue exposure to carcinogens [6,8-12].

In the oral cavity, the formation of MN in exfoliated mucosal cells has been used as an intermediate biological marker due to its simplicity, low cost and rapid response [6,8-10,12]. This marker is used to evaluate the protective effect of chemoprevention protocols and the degree of oral mucosa involvement [6,8-10,12].

The objective of the present study was to evaluate the frequency of MN on the oral mucosa of laboratory technicians occupationally exposed to mutagenic agents, and to demonstrate the possible long-term genotoxic damage to which these individuals are exposed in order to increase their awareness regarding the importance of the use of PPE.

MATERIAL AND METHODS

Thirty-one consecutive subjects matched for gender and age were selected for the present study. The present work was an observational study with a cross-sectional design. The selection of the sample was based on the convenience of locating the public universities where the laboratory technicians, recruited in the period of 2008-2010, worked. The subjects were divided into two groups. The experimental group consisted of 20 laboratory technicians from two public universities, occupationally exposed to carcinogenic substances for more than 2 years. The control group consisted of 11 non-smoking patients who had not been occupationally exposed to phenol derivatives for more than 10 years. The study was approved by the Ethics Committee (protocol number 072/2008-PH/CEP). The criteria for inclusion in the study were the absence of a history of malignant oral tumors and no visible clinical signs of anomalies in the right buccal mucosa.

All subjects were submitted to extra- and intraoral clinical examination and responded to a questionnaire administered by interview regarding the type of substances used during their working routine and frequency of use of safety equipment. The subjects were also asked about their habits of alcohol consumption and smoking and recent exposure to X-rays. The oral health condition of each patient was evaluated and classified as good (many restorations without caries and tartar), regular (presence of caries and tartar but loss of few teeth), and poor (presence of residual roots, loss of various teeth, and advanced periodontal disease).

Cytological material was collected from the right buccal mucosa with a cytobrush and the slides were processed for Feulgen staining [2]. The analysis was performed by two observers, calibrated between each other, and the results were submitted to the kappa test; kappa=1.

The slides were first evaluated qualitatively. Forty fields were analyzed at 200x magnification, counting at least 30 well-distributed, non-overlapping cells with green-stained cytoplasm and a magenta-stained nucleus per field. Only cells containing intact nuclei with a smooth and distinct nuclear perimeter and defined cytoplasm were counted. A minimum of 1200 cells/subject were analyzed [8]. The slides were examined under a light microscope at 400x magnification and at 1000x magnification for the confirmation of MN. The criteria used for MN counting were the presence of a surrounding homogeneous halo, a size less than 1/3 of the diameter of the associated nucleus, a Feulgen staining intensity similar to that of the nucleus, being on the same focal plane [13], and no association with the nucleus.

The data were analyzed statistically by the nonparametric Kruskal-Wallis and Mann-Whitney tests, with 5% significant level using the GraphPad Prisma 6 program.

RESULTS

The experimental group consisted (n=20) of twelve women and eight men with a mean age of 38.95 years (range: 22 to 53 years). Two of the subjects smoked, 12 reported alcohol consumption of 3 doses per week or more, and 95% did not use drugs. Only seven subjects had been recently submitted to radiographic examination.

Most technicians (85%) showed good oral health and 15% presented regular oral health. In this group, 60% of the subjects had been occupationally exposed to carcinogenic substances for more than 10 years, 10% for 5 to 10 years, 25% for 2 to 5 years, and 5% for less than 2 years. Of these, 85% handled xylene and formalin, 10% only formalin, 5% only xylene, and all handled other substances such as alcohol, acetone, bouin's fixative, acids, phenols, methanol, bromide, chloroform, lead citrate, propylene oxide, resin, osmium tetroxide, glutaraldehyde, tissue mount, paraffin, sodium cacodylate, and diamonobenzidine (DAB). Most technicians (65%) reported no symptoms after a day's work. However, headache was reported by four technicians, eye irritation by four, respiratory difficulties by two, cough by two, nausea by two, and skin irritation by two.

The control group consisted of 11 non-smoking patients, including six women and five men, matched for age to the experiment group (mean: 35.72 years, range: 22 to 51), who had not been occupationally exposed to phenol derivatives for more than 10 years. Six of these patients consumed alcoholic beverages once a week, one used illicit drug, and three had been recently submitted to radiographic examination. All control subjects presented good oral health.

Statistical analysis of the frequency of MN according to alcohol consumption, smoking or illicit drug use was not possible because only two subjects consumed alcohol frequently (more than once a week) and only two technicians smoked.

The median frequency of MN was higher in the experimental group (median = 5) than in the control group (median = 2). However, no significant difference was observed in the total number of MN (p = 0.16, Mann-Whitney test). There was also no significant difference in the frequency of total MN/cell (p = 0.75) or total of micronucleated cells (p = 0.22). In contrast, the frequency of more than 01 MN/cell differed significantly between the two groups (p = 0.03, Mann-Whitney test). Table 1 shows the comparison of the frequency of MN and micronucleated cells between the two groups (technicians and control) by the Mann-Whitney test.

Table 1. Frequency of micronuclei/cell and of micronucleated cells/1200 cells in the group of laboratory technicians (T) and the control group (C).
* p≤0,05

MN	1 MN		> 1 MN		Micronucleated cells		Total MN	
	T	C	T	C	T	C	T	C
Median	1	2	1	0	3	2	5	2
IQR	2.75	2	1	0	2.75	2	4.75	2
CV (%)	84.58	75.31	111.8	222.49	78.43	83.66	85.03	97.83
p value	0.75		0.0397*		0.2209		0.1671	

MN = micronuclei; IQR = interquartile range; CV = coefficient of variation.

The differences found during the time of occupational exposure to carcinogenic substances, the total number of MN was higher in the group of technicians with less than 5 years of exposure compared to those with more than 5 years of exposure, demonstrating that the chronic exposure induce lesser micronuclei

markers, which is relevant, however, there was no significant difference in the frequency of MN/cell between the two subgroups (p = 0.45, Mann-Whitney test) (Table 2).

Table 2. Frequency of micronuclei/cell and of micronucleated cells/1200 cells in the group of laboratory technicians according to time of occupational exposure.

MN	1 MN		> 1 MN		Micronucleated cells		Total MN	
	T1	T2	T1	T2	T1	T2	T1	T2
Median	1	2	0.5	1	2.5	3	3	4
IQR	2.25	2.5	1	2	3	3.5	5	4.25
CV (%)	89.87	77.46	127.94	89.44	87.73	63.25	96.81	66.88
p value	0.4457		0.4509		0.3495		0.4515	

MN = micronuclei; T1 = more than 5 years of occupational exposure; T2 = less than 5 years of occupational exposure; IQR = interquartile range; CV = coefficient of variation.

DISCUSSION

The MN test is performed for the biomonitoring of groups exposed to different genotoxic agents because of its reliability and simplicity [6,8-13].

Several studies have evaluated exposure to carcinogenic chemical agents such as phenol derivatives, especially formaldehyde, because of high reactivity upon low contact [14], by the MN test [15-18]. However, there are no studies in the literature investigating other genotoxic agents found in laboratories. Studies evaluating the effects of exposure to formaldehyde on the oral [18] and nasal mucosa [19] have demonstrated that the MN test is suitable when compared to cytotoxicity in blood samples [17]. Xylene toxicity is measured as metabolites in the urine in Brazil, the sample is used as environmental monitoring tool with an impractical and expensive method [20] demonstrating a temporary exposure, not a 5-year exposure as the present study evaluated.

In the present study, no significant differences in the total number of MN were observed between laboratory technicians and the control group. However, the median number of MN and the micronucleate cells was slightly higher in the experimental group than in the control group, a finding indicating exposure to genotoxic agents such as formaldehyde and xylene, in accordance with Martino-Roth and coauthors [21]. The present data corroborate to the main finding of our work, which a higher quantity of MN with more than one MN/Cel in the exposure groups and a lesser cell with only one MN on the exposure groups, indicates that there was an occupational exposure to genotoxic agents with a tendency to a higher number of MN/cells (more than 01 MN/cells) and a lower number of 01 MN/Cells.Significant difference was observed in the frequency of more than 01 MN/cell, indicating that the use of these substances alters the number of MN per cell but not the total number of cells with MN. One possible explanation is the fact that most technicians use PPE and laminar flow hood so that agents with a high carcinogenic potential such as formaldehyde and xylene have a low exposition effect action as an agent with a low carcinogenic potential, According to Sarto and coauthors [22], this might be explained by the higher percentage of MN formation due to spindle disturbances than to chromosome breaks. The latter results from the clastogenic action of genotoxic agents such as alcohol and smoking and exposure to agents (eg. xylene and formalin), culminating in the formation of a single MN, whereas more than one MN is formed per cell as the result of spindle disturbances during a chronic exposure.

Some qualitative variables such as alcohol consumption, smoking, illicit drug use, oral condition, and exposure to X-rays were also evaluated in the present study. However, the small size of the sample was a limitation for statistical analysis. Further studies are needed to evaluate the effect of oral condition and exposure to X-rays on the MN frequency.

No significant differences in the frequency of MN were observed while analyzing the time of exposure to carcinogenic substances. This finding might be explained by the rapid turnover of nasal and oral mucosal cells, preventing the cumulative genotoxic effects of long-term exposure [14]. Suruda and coauthors [23] demonstrated an increase of MN frequency in the nasal and oral mucosa of mortician students exposed to

small doses of formaldehyde for a prolonged period of time during the day (an average of 8 hours), during 9-day course in embalming a corpse. These findings indicate that the time of daily exposure to the genotoxic agent rather than the time of occupational exposure to these substances determines the exposure dose and is the fundamental factor for the induction of MN.

The use of PPE was associated with the frequency of MN, with the observation of a significantly higher frequency of only one MN per cell (caused by agents with a high carcinogenic potential) among technicians who used PPE less frequently. The use of PPE by the experimental group proved to be effective as a protection method, attenuating the genotoxic effect of substances to which these professionals are subjected in their work routine.

In the present study, data regarding symptoms reported by the technicians were collected by application of a questionnaire. Most technicians had no symptoms after a day's work, but some reported symptoms such as headache, eye irritation, respiratory difficulties, cough, nausea, and skin irritation.

The analysis of the frequency of MN and of the patient records permits us to conclude that, although the number of MN was higher in the group of laboratory technicians than in the control group, significant differences were only observed in terms of the frequency of more than 01 MN/cell. The use of a laminar flow hood during the handling of genotoxic substances contributed to reduce the frequency of MN. The time of 5-years compared to early exposure of less than 5-yers did not influenced the frequency of MN, but indicate that the chronic exposure to genotoxic material decrease the numbers of MN/cells, this finding may indicate that 5-years of exposure could adapt the nucleus cells to the genotoxic aggressor demonstrating that the oral mucosa could be a marker to long-term genotoxic exposure.

Conflicts of Interest: The authors declare that there is no conflict of interest in the present research.

REFERENCES

1. Brazilian Ministry of Health. Instituto Nacional do Câncer – INCA (National Cancer Institute). Formol. https://www.gov.br/inca/pt-br/assuntos/causas-e-prevencao-do-cancer/exposicao-no-trabalho-e-no-ambiente/solventes/formol; 2023.
2. Lima CF, Oliveira LU, Cabral LA, Brandão AA, Salgado MA, Almeida JD. Cytogenetic damage of oral mucosa by consumption of alcohol, tobacco and illicit drugs. J Oral Pathol Med. 2010;39(6):441-6.
3. Santos-Silva T, Ribeiro RIMdA, Alves SN, Thomé RG, Santos HB. Assessment of the Toxicological Effects of Pesticides and Detergent Mixtures on Zebrafish Gills: a Histological Study. Braz. Arch. Biol. Technol. 2022;64.
4. Macêdo AKS, Epifânio DD, Carvalho RA, Ribeiro RIMA, Santos HB, Thomé RG. Immunohistochemical analysis of P-gp, LC3-II, and cathepsin-D Associated with Histological Changes in Fish Liver: from the Impacted Environment to Clean Water. Braz. Arch. Biol. Technol. 2020;63.
5. Silva SdOV, Assis L, Campo PFGMD, Duarte KCN, Oliveira F, Castro GM, et al. Low-Level Laser Therapy and Spinal Cord Injury: Effects of 3 Different Fluences in the Intermediate Period of Repair in an Experimental Model in Rats. Braz. Arch. Biol. Technol. 2020;63.
6. Gattás GJF, de Almeida Cardoso L, de Araújo Medrado-Faria M, et al. Frequency of oral mucosa micronuclei in gas station operators after introducing methanol. Occup Med. 2001;51(2):107-113.
7. IBCC. Speech therapy and cancer. http://bvsms.saude.gov.br/bvs/publicacoes/cd06_03.pdf: Brazilian Ministry of Health. Instituto Brasileiro de Controle do Cancer.; 2000
8. Pastor S, Creus A, Parrón T, Cebulska-Wasilewska A, Siffel C, Piperakis S, et al. Biomonitoring of four European populations occupationally exposed to pesticides: Use of micronuclei as biomarkers. Mutagenesis. 2003 06/01;18:249-58.
9. Vuyyuri SB, Ishaq M, Kuppala D, Grover P, Ahuja YR. Evaluation of micronucleus frequencies and DNA damage in glass workers exposed to arsenic. Environ Mol Mutagen. 2006;47(7):562-570.
10. Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, et al. The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps. Mutation Research/Reviews in Mutation Research. 2008 05/01;659:93-108.
11. Santovito A, Cervella P, Delpero M. Evidence of genotoxicity in lymphocytes of non-smoking alcoholics. Mol Biol Rep. 2015 2015/01/01;42(1):53-59.
12. Yang H-Y, Feng R, Liu J, Wang HY, Wang YD. Increased Frequency of Micronuclei in Binucleated Lymphocytes among Occupationally Pesticide-exposed Populations: A Meta-analysis. Asian Pacific journal of cancer prevention: APJCP. 2014 08/30;15:6955-60.
13. Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: methods development. Mutat Res. 1992 Feb;271(1):69-77.
14. Speit G, Schmid O. Local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated epithelial cells. Mutat Res. 2006 Sep;613(1):1-9.
15. Burgaz S, Cakmak G, Erdem O, Yilmaz M, Karakaya AE. Micronuclei frequencies in exfoliated nasal mucosa cells from pathology and anatomy laboratory workers exposed to formaldehyde. Neoplasma. 2001;48(2):144-7.

16. Ye X, Yan W, Xie H Zhao M, Ying C. Cytogenetic analysis of nasal mucosa cells and lymphocytes from high-level long-term formaldehyde exposed workers and low-level short-term exposed waiters. Mutat Res. 2005 Dec 7;588(1):22-7.
17. Schmid O, Speit G. Genotoxic effects induced by formaldehyde in human blood and implications for the interpretation of biomonitoring studies. Mutagenesis. 2007;22(1):69-74.
18. Speit G, Schmid O, Fröhler-Keller M, Lang I, Triebig G. Assessment of local genotoxic effects of formaldehyde in humans measured by micronucleus test with exfoliated buccal mucosa cells. Mutat Res. 2007 04/01;627:129-35.
19. Edling C, Hellquist H, Odkvist L. Occupational formaldehyde exposure and the nasal mucosa. Rhinology. 1987 Sep;25(3):181-7.
20. Brazilian Ministry of Work. Regulatory Standard N15: Activities and unsanitary operations. In: Government B, editor. https://www.braziliannr.com/brazilian-regulatory-standards/nr15-unhealthy-activities-and-operations/annex-11-of-nr15-tolerance-limits-for-chemical-agents-and-inspections-in-the-workplace/ Brazilian Government Ministry of Labour; 2011.
21. Martino-Roth MG, Viégas J, Amaral M, Oliveira , L.; Ferreira, F.L.S; Erdtmann, B. Evaluation of genotoxicity through micronuclei test in workers of car and battery repair garages. Genet. Mol. Biol. 2002; 25:495–500
22. Sarto F, Finotto S, Giacomelli L, Mazzotti D, Tomanin R, Levis AG. The micronucleus assay in exfoliated cells of the human buccal mucosa. Mutagenesis. 1987 Jan;2(1):11-7
23. Suruda A, Schulte P, Boeniger M, Hayes RB, Livingston GK, Steenland K, et al. Cytogenetic effects of formaldehyde exposure in students of mortuary science. Cancer Epidemiol Biomarkers Prev. 1993;2(5):453-60.