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PP18.6
Persistence of chromosomal aberrations in peripheral blood lymphocytes of Hodgkin's lymphoma patients after ABVD chemotherapy

S. Ramos[1], P. Navarrete[1], B. Molina[1], D. Cervantes[1], V. Lozano[2], E. Gallardo[3], S. Frias[1,4]

[1] *Instituto Nacional de Pediatría, Mexico*
[2] *Instituto Nacional de Cancerología, Mexico*
[3] *Hospital General, Mexico*
[4] *Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Mexico*

Introduction: Hodgkin's lymphoma (HL) is a lymphoid malignancy with a worldwide incidence is 2–4/100,000 individuals/year. HL represents 5% of all childhood cancers and is 30–40% of all malignant lymphomas. Treatment for HL involves a combination of radiation and chemotherapy. One of the most common treatment schemes uses ABVD (adriamycin, bleomycin, vinblastine, dacarbazine), a cocktail of genotoxic agents. Although survival rate for HL is up to 95%, cancer therapy could produce persistent genetic damage in the survivors.

Objective: The goal of this study is to determine the time course of chromosomal damage in lymphocytes of patients with HL before, during and after ABVD/radiotherapy.

Material and methods: Five patients diagnosed with HL provided peripheral blood samples before, during and one year after ABVD chemotherapy. In addition, 5 healthy individuals provided a single blood sample. All participants in the study signed informed consent. Chromosomal aberrations were evaluated by M-FISH (SpectraVysion) in 50–100 karyotypes per sample. Spectral karyotyping for each cell was analysed with Isis software. Statistical comparisons were done with the Kruskal–Wallis and Mann–Whitney *U* tests and a $p < 0.05$ was considered significant.

Results: Average frequencies of structural chromosomal aberrations (CA) in samples from patients with HL were 6.3, 7.1 and 23.0% before treatment, during, and one year after treatment, respectively. CA frequency in healthy individuals was 2.4%. A significant difference ($p < 0.002$) was observed when comparing groups. Chromosomal damage was observed in all samples, and rejoined structural chromosomal aberrations (RCA), such as translocations and dicentrics, were the most common type. No clonal aberrations were observed.

Conclusions: Our study showed that ABVD cause genotoxic damage in peripheral blood lymphocytes of HL patients. Interestingly, the highest frequency of RCA/cell was observed in samples analyzed one year after treatment and this was significantly different from what was found before treatment (23% vs 6%). Both rejoined and non-rejoined aberrations are observed one year after treatment indicating that new aberrations are continuously produced and that there may be alterations affecting genes involved in DNA repair. These results suggest that ABVD/radiotherapy causes sublethal damage in hematopoietic stem cells and can lead to persistent genomic instability.

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PP18.7
In vivo genotoxicity evaluation and DNA intercalation potential of three acridone isosters

G. Aguilar-López Escalera, A. Gallegos-Torres, S. Merlos-De La Rosa, A. Sandoval-Lugo, E. Mauriño-Reyes, F. Moreno-Escalante, M.A. Loza-Mejía, L. Martino-Roaro

Grupo de Investigación en Alimentos y Salud, Facultad de Ciencias Químicas, Universidad La Salle, CDMX, México, Mexico

Introduction and objectives: Tricyclic compounds, like acridones, are generally regarded as genotoxic. In fact, in many drug discovery programs, they are excluded because of their capability to act as genotoxic substances. The mechanism of their genotoxic action is associated with their DNA binding through intercalation or the generation of free radicals. In our research group, we have prepared some acridone isosteres to test the effects of these compounds. The aim of this study was to evaluate the genotoxicity of these compounds in mice with the micronuclei test.

Methodology and results: Three groups were assembled with six mice each, molecules 1, 2 and 3 were assigned to a different group and were administered intraperitoneally with 300 mg/kg. Another group was administered with distilled water as a control group and the last one with ifosfamide (60 mg/kg intraperitoneally) a well-known genotoxic agent as the positive control. Blood samples were obtained from the tail of each mouse prior the administration and at 24, 48 and 72 h post-administration. Two blood smears per mouse were made, fixed in methanol for 3 min and stained for 12 min with a 5% Giemsa solution. Then, the slides were washed, dried and viewed under the microscope to evaluate the clastogenic potential as an increase in the number of micronuclei in 1000 polychromatic erythrocytes. DNA intercalation assays were carried out with the UV/Vis titration method. The results were statistically evaluated and showed that 1, 2 and 3 had no clastogenic effect. All compounds exhibited binding constants lower than ethidium bromide (EB) and were not able to displace EB in their complex with DNA. *Ab initio* calculations showed that electron density maps differed with those of classical intercalators being these a plausible explanation of their lack of DNA affinity and genotoxicity.

Conclusions: Our observations open the door for considering some acridone isosters as viable templates in drug discovery programs, as not all them are genotoxic or DNA intercalators.

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Evaluation of toxicogenetics damages of the putrescine polyamine on HepG2 cell culture

F.D. Campos-Pereira, L.B. Cagnoni, R.V. Hara, M.A. Marin-Morales

Laboratório de Mutagênese Ambiental (LMA), University Estadual Paulista (UNESP), Rio Claro, São Paulo, Brazil

Introduction: The sources of surface and groundwater have suffered constant contamination by human activities. Among these activities, the cemeteries are causing serious health and environmental problems due to production and release of necrochurume, a liquid resulting from the decomposition of the bodies, which is able to percolate into the soil and contaminate underground water sources. This liquid is composed of various organic substances, among which putrescine ($C_4H_{12}N_2$) a polyamine, which acts as an intracellular messenger and is related to the processes of differentiation and growth of the cell.

Objective: The aim of this study was to evaluate the genotoxic potential of putrescine in HepG2 cell culture, by the cytokinesis-block micronucleus assay.

Materials and methods: Approximately 5×10^6 cells were grown in culture flasks (25 cm^2), exposed for 24 h at non-cytotoxic concentrations of putrescine, corresponding to 70%, 50%, 30% and 10% of the LD_{50}, which is 463 mg/kg for rats. The assay was performed in triplicate, and 3000 cells were analyzed per treatment. Normal binucleate cells and binucleate cells bearing bridges, buds, and/or micronuclei were counted. Statistical analysis was performed by ANOVA, pos test Tukey ($p < 0.05$).

Results: According to the results of the micronucleus test, all the tested concentrations exhibited significant frequency for this marker. For the formation of bridges and nuclear buds, the results were significant only for the highest tested concentration (70%).

Conclusions: Several studies have been conducted to evaluate the necrochurume potential contamination, but little is known about its physical and chemical properties. The obtained information on the effects of putrescine present in necrochurume is extremely important, because besides of alerting for the potential as an environmental pollutant, it can lead to a better understand of the action mechanisms of this substance.

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PP18.9
Evaluation of cytotoxic and mutagenic effects of CactiNea™ nutraceutical in *A. Cepa*

F.F. Navarro [1,2], F.D.C. Pereira [2], B.S. Oliveira [2], M.A. Marin-Morales [1]

[1] *Departamento de Biologia, Instituto de Biociencias, Universidade Estadual Paulista (UNESP), Rio Claro, SP, Brazil*
[2] *Centro Universitário Hermínio Ometto – UNIARARAS, Araras, SP, Brazil*

Introduction: Natural products and functional foods have received special attention by health professionals and the general population due to the constant pursuit of welfare, as well as combating diseases. *Opuntia ficus indica* L., a species in the cactus family *Cactaceae*, it is a plant growing in dry and hot climates: northern Mexico, south-western United States, Africa, Mediterranean countries and Europe. The fruits are used in the traditional medicine. NEXIRA Health Gere Cacti-Nea™, a cactus fruit extract with natural diuretic properties, is a dehydrated water extract of the fruits of the prickly pear cactus *Opuntia ficus indica*, obtained by a process designed to preserve the nutritional and functional properties of the fruit.

Objective: This study used as test organism the *Allium cepa* to conduct the evaluation of the cytotoxic and mutagenic potential of CactiNea nutraceutical.

Materials and methods: The assay was performed in meristematic cells of *A. cepa* exposed to eight concentrations 0.12; 0.06; 0.03; 0.01; 0.008; 0.006; 0.004; 0.002 g/mL.

Results: The results showed that *A. cepa* at any concentration analyzed showed significant levels of mutagenicity when compared to the positive control after the treatment period. The concentrations 0.12 and 0.06 g/mL showed a decrease in mitotic index, which shows that within this concentration range the CactiNea solutions interfere on the cell cycle and division of cells of *A. cepa*.

Conclusions: These results demonstrate the necessity for toxicological tests in nutraceuticals, other tests will be performed to evaluate other acute and chronic toxicological parameters.

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PP18.10
Comparative study of cytotoxicity and genotoxicity of commercial Jeffamines® and polyethylenimine in CHO-K1 cells

L. Castan, C.J. Da Silva, R. Dos Santos, E. Molina

Núcleo de Pesquisas em Ciências Exatas e Tecnológicas, Universidade de Franca, Ave. Dr. Armando Salles de Oliveira, Franca, São Paulo, Brazil

Introduction: Jeffamines® are a family of polymers containing primary amine groups attached to the extremities of a polyether backbone, which can be used as biomaterials. They have been used in combination with polyethylenimine (PEI) to improve the biocompatibility in drug and gene delivery systems. Despite these facts, very few studies have been done on thecytotoxicity and genotoxicity of pure Jeffamines® or compared with PEI.

Objective: The present study aimed to evaluate and compare the cytotoxic and genotoxic effects of Jeffamines® and PEI in CHO-K1 cells. Specifically, polypropylene oxide 2000 (PPO 2000, Jeffamine® D series), polyethylene oxide 2000 (PEO 1900, Jeffamine® ED series), branched 25 kDa PEI and linear 20 kDa PEI were evaluated at different concentrations.

Materials and methods: Cell viability and proliferation were assessed by XTT and BrdU assays, respectively. Genotoxicity was evaluated using single cell gel electrophoresis and cytokinesis-blocked micronucleus assays.

Results: PPO 2000 was the most cytotoxic Jeffamine®, whereas PEO 1900 did not cause significant cell death at any tested concentration. Branched PEI was more cytotoxic than LPEI and both were more cytotoxic than Jeffamines®. Only PPO 2000 induced DNA damage when evaluated in the comet assay, probably due to its cytotoxicity. PPO 2000, PEO 1900 and PEI did not increase the frequency of micronuclei when tested at sub-cytotoxic concentrations.

Conclusions: This work provides new insights about the biocompatibility of Jeffamines® and PEI and suggests the genotoxicological safety for further investigations of PEO 1900 in drug and gene delivery systems.

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The influence of washing methods on the DNA damage levels assessed by Comet assay

A. Tirsina [1,2], C. Costa [2,3], S. Costa [2,3], J.P. Teixeira [2,3]

[1] *Department of Chemicals Surveillance, Centre of Chemical Safety and Toxicology, National Center of Public Health, Chisinau, Republic of Moldova*
[2] *Department of Environmental Health, Portuguese National Institute of Health, Porto, Portugal*
[3] *4EPIUnit-Institute of Public Health, University of Porto, Porto, Portugal*

Introduction: In the last years, the reliability and importance of the comet assay is significantly increasing. The protocol of the assay is continuously developing and although the main principles are the same, small differences in the procedure from one laboratory to the other seem to affect the sensitivity and outcomes. Therefore, among other crucial steps in performing the comet assay protocol is the washing method as this may introduce additional damage