Evaluation of the mutagenic and cytotoxic effects of mercurous chloride by the micronucleus technique in golden Syrian hamsters

Elva I. Cortés-Gutiérrez1,2, Ricardo M. Cerda-Flores1,2,3, Diego González-Ramírez4, Miguel A. Zúñiga-Charles4, Sigifredo Lazzano-Martínez4, Adriana Sampayo-Reyes4 and Carlos H. Leal-Garza1,2

1División de Genética, Centro de Investigación Biomédica del Noreste (CIBIN), Instituto Mexicano del Seguro Social (IMSS), Administración de Correos no. 4, Apdo. Postal 20, Col. Independencia, CP 64720 Monterrey, Nuevo León, Mexico, 2Facultad de Ciencias Biológicas and 3Facultad de Enfermería, Universidad Autónoma de Nuevo León, Monterrey, Mexico and 4División de Farmacología. CIBIN, IMSS, Monterrey, Mexico

The aims of this study were to evaluate the mutagenic and cytotoxic activity of mercurous chloride by the micronucleus technique in vivo on the bone marrow of golden Syrian hamsters after a single i.p. drug administration. Forty male golden Syrian hamsters were classified into eight groups: negative control, positive control and six groups treated with different doses of mercurous chloride (1.25, 2.5, 5, 10, 20 and 40 mg/kg). The negative control was injected with physiological saline i.p. and the positive control with cyclophosphamide at a dose of 80 mg/kg i.p.

With respect to mutagenic effect, the average number of micronucleated polychromatophilic erythrocytes (MPE) in hamsters treated with different doses of mercurous chloride was not significant compared with the negative control. With respect to cytotoxic effect, the average polychromatophilic erythrocyte/red blood cell ratio showed a significant decrease when the doses were higher than the 2.5 mg/kg dose compared with the negative control. In conclusion, this preliminary study shows a cytotoxic effect but not a mutagenic effect of calomel in vivo at one time point (24 h).

Introduction

Mercurous chloride (Hg₂Cl₂, calomel) is a white, odorless, tasteless, heavy powder that is very insoluble in water and poorly absorbed from the gastrointestinal tract (Clarkson et al., 1988). However, in the intestine, small amounts are converted to the more soluble mercuric salts, which are absorbed, expressing its characteristic toxic effects (Fingl, 1991).

Several diseases have been associated with calomel. For instance, pink disease has been shown to be linked to Young’s syndrome, which is found in men, who developed obstructive azoospermia resulting in reduced fertility (Hendy et al., 1993). Also, renal failure has been reported in Chinese people who have used calomel-containing medicines chronically (Kang-Yum and Oransky, 1992).

Currently, calomel-containing products manufactured in the USA are not regulated by the Food and Drug Administration and in consequence are available without prescription. In London, ethnic remedies and skin lighteners containing calomel were reported to be available for purchase in 1992 (Godlee, 1992).

The micronuclei (MN) test is a cytogenetic biomarker of occupational or environmental exposure to genotoxic agents. MN are masses of chromatin with the appearance of small nuclei, fragments of chromosomes or intact whole chromosomes lagging behind at the anaphase stage of cell division. This biomarker can be easily recognized in immature polychromatophilic erythrocytes because in mammals they extrude their nucleus at the terminal stage of maturation, leaving only MN inside the cell, which is called a micronucleated polychromatophilic erythrocyte (MPE) (Schmid, 1975). The frequency of MPE reflects the level of genetic damage induced in the erythropoietic system. The value of this test in predicting carcinogenicity has been demonstrated (Jenssen and Ramel, 1980) and it has been proposed as one of the bank of primary, short-term, in vivo mutagenicity/cytotoxicity assays (Ribeiro et al., 1993).

Given that few studies of the mutagenic/cytotoxic effects of calomel have been reported, with inconclusive results (Maiorino et al., 1996), the aims of this study were to evaluate the mutagenic and cytotoxic activities of calomel by the micronucleus technique in vivo in the bone marrow of golden Syrian hamsters after a single i.p. drug administration.

Materials and methods

Population studied

A sample of 40 males (4–5-week-old golden Syrian hamsters) were classified into eight groups, each group of five animals: negative control, positive control and six groups treated with different doses of mercurous chloride (1.25, 2.5, 5, 10, 20 or 40 mg/kg). The negative control was injected with physiological saline i.p. and the positive control received cyclophosphamide (Calbiochem, San Diego, CA) at a dose of 80 mg/kg. The positive control was used as a means of indicating that the assays worked. The six doses of mercurous chloride used were based on the LD50 reported for mercury compounds, in the range 10–40 mg/kg body wt (World Health Organisation, 1976). A preliminary study with four hamsters showed that the 40 mg/kg dose inhibited the polychromatic erythrocyte/red blood cell ratio (PCE/RBC) by ~50%. Only one time point (24 h) was used because 48 h after application of calomel at the higher dose (40 mg/kg) the frequency of PCE was very low and analysis of MPE and the PCE/RBC ratio was impossible to evaluate.

The hamsters were sourced from the bioterium of the Centro de Investigación Biomedica del Noreste. The animal husbandry conditions were: temperature 20–25°C, water ad libitum, relative humidity 45–55%, 12 h/12 h light/dark cycle and FMI Laboratory Diet 5001 ad libitum.

The institutional guide for care and use of laboratory animals was followed. The determination of total mercury levels in blood was done 24 h after mercurous chloride application as an experimental control in order to determine whether the animals absorbed mercury when the different doses were applied. No attempt was made to find a dose–time relationship.

Metal determination was performed according to the Perkin-Elmer protocols using a cold vapor generation–atomic absorption procedure (AA Perkin-Elmer model 5000 and MHS-10 mercury hydride system) (Perkin-Elmer, 1982).

To whom correspondence should be addressed. Tel: +52 81 81 90 40 35; Fax: +52 81 81 90 40 35; Email: elvairenecortes@hotmail.com
Table I. Number of MPE and the PCE/RBC ratio in bone marrow of golden Syrian hamsters treated with six different doses of calomel

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Total mercury a (mean ± SD)</th>
<th>MPE b (mean ± SD)</th>
<th>Ratio c (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>5</td>
<td>69.63 ± 65.74</td>
<td>27.50 ± 8.65</td>
<td>0.50 ± 0.04</td>
</tr>
<tr>
<td>1.25 mg/kg</td>
<td>5</td>
<td>94.00 ± 27.08</td>
<td>27.00 ± 5.20</td>
<td>0.49 ± 0.07</td>
</tr>
<tr>
<td>2.5 mg/kg</td>
<td>5</td>
<td>301.67 ± 20.61</td>
<td>15.60 ± 9.32</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>5</td>
<td>741.25 ± 169.54</td>
<td>20.00 ± 4.47</td>
<td>0.23 ± 0.06</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>5</td>
<td>2745.00 ± 92.13</td>
<td>20.66 ± 5.72</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>20 mg/kg</td>
<td>5</td>
<td>2723.75 ± 1947.21</td>
<td>27.50 ± 9.46</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>40 mg/kg</td>
<td>5</td>
<td>4110.00 ± 691.82</td>
<td>27.00 ± 8.06</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>Positive control</td>
<td>5</td>
<td>68.80 ± 32.42</td>
<td>6.80 ± 32.42</td>
<td>0.30 ± 0.09</td>
</tr>
</tbody>
</table>

aTotal mercury in blood (µg/l). ANOVA test: negative control = 1.25 ± 2.5 = positive control.

bNumber of micronucleated polychromatic erythrocytes in 2000 cells analyzed. ANOVA test: negative control = 1.25 ± 2.5 = 5 = 10 = 20 = 40 mg/kg = positive control.

Polychromatic erythrocyte/red blood cell ratio in 2000 cells analyzed. ANOVA test: negative control = 1.25 ± 2.5 = positive control = 5 = 10 = 20 = 40 mg/kg.

Micronuclei test
Each one of the 40 hamsters was killed by cervical dislocation 24 h after calomel injection and the femoral bone marrow was isolated, fixed and stained according to the Schmid criteria (Schmid, 1983).

Micronuclei count
Stained slides from each group and each animal were analyzed blind by direct observation under a light microscope at 100× magnification. For each animal there were two procedures. (i) Mutagenic effect: the number of MPE in 2000 cells was scored. (ii) Cytotoxic effect: the number of PCE was counted in 2000 cells and the PCE/RBC ratio was estimated.

Statistical analysis
The data were analyzed by one-way ANOVA with the Newmann–Keull tests for multiple comparisons at the interpopulation level to investigate any possible difference between the total mercury in blood, the numbers of MPE and the PCE/RBC ratio in hamsters treated with different doses of calomel (Lovell et al, 1989). Three Pearson’s correlation coefficients were computed for the relationship between total mercury, MPE and the PCE/RBC ratio versus seven models, such as mice or rats; (iv) use of an increased number of animals for treatment (five males and five females) according to the OECD (475) guideline for testing of chemicals.

Acknowledgement
The authors are grateful to Gerardo Lozano Garza for the management of animal facilities.

References


Received on August 28, 2003; revised on December 10, 2003; accepted on December 29, 2003