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

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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 polychromatic 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 polychromatic 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 (Kew 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 1.25, 2.5, 5, 10, 20.0 or 40.0 mg/kg mercurous chloride (Sigma Chemical Co., St Louis, MO) suspended in 0.9% NaCl. The negative control was injected with physiological saline (0.9% NaCl) 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 Investigacion Biomedica del Noreste. The animal husbandry conditions were: temperature 20–25°C, water ad libitum, relative humidity 45–55%, 12 h/12 h dark/light cycle and PFI 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).

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Table 1. 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>2753.75 ± 1947.21</td>
<td>27.50 ± 4.96</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>6.00 ± 3.20</td>
<td>0.30 ± 0.09</td>
</tr>
</tbody>
</table>

*Total mercury in blood (µg/l). ANOVA test: negative control = 1.25 ± 2.5 ± 5 ± 10 = 20 = 40 mg/kg.*

b*Number 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–Keulls test 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 versus seven 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 groups (negative control and 1.25, 2.5, 5.0, 10, 20 and 40 mg/kg, respectively). The sample size for ANOVA comparison of means was estimated using MINITAB software (version 13) (α = 0.05, 1 – β = 0.80) for MPE and the PCE/RBC ratio. A value of *P* < 0.05 was considered significant for all tests.

Results

The distributions of total mercury in blood, MPE, and PCE/RBC ratio in bone marrow of hamsters treated with six different doses of calomel are shown in Table 1.

ANOVA and Newmann–Keulls tests showed that the average of total mercury in blood increased significantly (*F* = 57.521, *P* < 0.001) when the doses were higher than 2.5 mg/kg with respect to the negative control. On the other hand, the 10, 20, and 40 mg/kg doses did not show any statistical difference. The average of MPE in all the doses did not show significant differences with the negative control, but all of them were different to the positive control. There was not a significant correlation (*r* = 0.336, *n* = 30, *P* = 0.070) between MPE and the doses of calomel.

The average of the PCE/RBC ratios in bone marrow of hamsters treated with different doses of calomel. ANOVA and Newmann–Keulls tests showed a significant decrease when the doses were higher than 2.5 mg/kg with respect to the negative control. Significant correlations coefficients for the relationship between the levels of mercury in blood (*r* = 0.869, *n* = 30, *P* < 0.0001) and PCE/ RBC ratio (*r* = −0.463, *n* = 30, *P* = 0.010) versus the doses of calomel were found. With respect to the MPE no relationship was found (*r* = 0.336, *n* = 30, *P* = 0.070).

Discussion

Our findings suggest that calomel does not have a mutagenic effect on hamster bone marrow after the application of a single i.p. injection and 24 h harvest.

These results are consistent with Maiorino *et al.* (1996), who found no significant differences between MPE in buccal cells of subjects exposed to calomel as compared with the control group. On the other hand, Lazutka *et al.* (1999) reported a significant increase in chromosomal aberrations and Queiroz *et al.* (1999) reported significant differences in the percentage of MN in individuals exposed to mercury as compared with the control group.

As for the PCE/RBC ratio, we found that calomel induces an inhibitory effect on erythropoiesis at doses >2.5 mg/kg. These results are in accordance with Amorim *et al.* (2000), who found a relation between mercury concentrations in hair and the mitotic index.

In conclusion, this preliminary study shows a cytotoxic but not a mutagenic effect of calomel at one time point (24 h) *in vivo*. Given that mercurous chloride inhibited erythropoiesis, as judged by a decrease in the ratio PCE/RBC, this can be considered as an acute effect observed in a range of doses close to LD₅₀.

In order to clarify the specific toxicity of mercurous chloride, further studies are necessary: (i) injection of other mercury compounds; (ii) use of other routes of administration, e.g. dermal, oral or respiratory; (iii) use of other experimental 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.

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References


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