Evaluation of the mutagenic and cytotoxic effects of mercurous chloride by the micronuclei 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 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 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 polychromatized 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 polychromatized 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 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 MPE 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 Biomédica 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).
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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