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Cytostatic action of methylmercuric chloride on mammalian duodenal cells

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Summary. Adult male mice of the ICR/Swiss Webster strain received a single intragastric administration of methylmercuric chloride 1,000 ppm, at dose levels of 5,10,15,20,25 and 30 mg/kg of body weight. The animals were killed six hours later. Tissue samples from the duodenum were fixed in 10% neutral buffered formalin for light microscopy. Chromosome clumping was observed in dividing cells at all dose levels, resembling a C-mitotic effect. It would lead to reduced mitotic cell formation on account of the subsequent lysis of the arrested metaphases. The cytostatic effect was brought about by the inactivation of the microtubule spindle fiber polymerization mechanism induced by methylmercuric chloride. There was a direct positive correlation between the varying dose levels of methylmercury and the proportion of cells arrested in metaphase in the crypts of the duodenum.

Key words: Cytostatic - Methylmercuric chloride - Duodenal cells

Introduction

Methylmercury was demonstrated to cause chromosome breaks in cells of plant tissues (Ramel, 1969) and to inhibit mitosis in plant cells by causing a C-mitotic effect (Ramel, 1969, 1972). The C-mitotic effect is used to describe colchicine arrest of mitosis in metaphase. It was observed that mercury compounds produce a similar C-mitotic effect in grasshoper chromosomes at a concentration 1000 times lower than colchicine (Klasterska and Ramel, 1978). The chromosome breaking effect was attributed to a direct reaction between mercury and the chromatin, while the effect on cell division were probably ascribable to an interaction of mercury with the sulfhydryl groups of spindle proteins resulting in their deactivation (Ramel, 1972; Klaterska and Ramel, 1978; Sager et al., 1984). Several investigators concluded that even low levels of mercury exerted an important action upon aneuploidy and enhancement of C-mitoses, but the induction of chromosome aberrations appeared to occur only at higher concentrations (Gruen, 1970; Verschaeve et al., 1976; Umeda et al., 1969). The aim of the present investigation was to determine the mitosis arresting action of methylmercuric chloride at different dose levels on duodenal crypt cells which normally reveal a high degree of mitotic activity for cell renewal.

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Materials and methods

Eighteen adult male mice of the ICR Swiss Webster strain were administered a single intragastric dose of methylmercuric chloride (1,000 ppm)at dose levels of 5, 10, 15, 20, 25, and 30 mg/kg of body weight. The different dose levels were chosen so as to encompass the embryotoxic, teratogenic and embryolethal dose levels of methylmercuric chloride (MMC). Control animals were administered a corresponding volume of isotonic saline. Six hours after treatment the animals were anesthetized and killed. Tissue samples from the proximal duodenum (first centimetre) were fixed in 10% neutral buffered formalin for 48 hours. The tissues were processed, embedded in Paraplast and sectioned at 5 μm on a AO Spencer rotary microtome.

Tissue sections were stained with hematoxylin and eosin. The stained sections were examined using a light microscope. 1000 cells in the crypts of the duodenum were scored for each dose level, as well as in tissue samples from the control animals.

The cells arrested in a clumped metaphase condition were counted and their proportion determined. Photomicrographs were prepared using a Wild-Leitz microscope. The data were statistically analyzed using analysis of variance to significance involving several independent variables. The level of significance was set

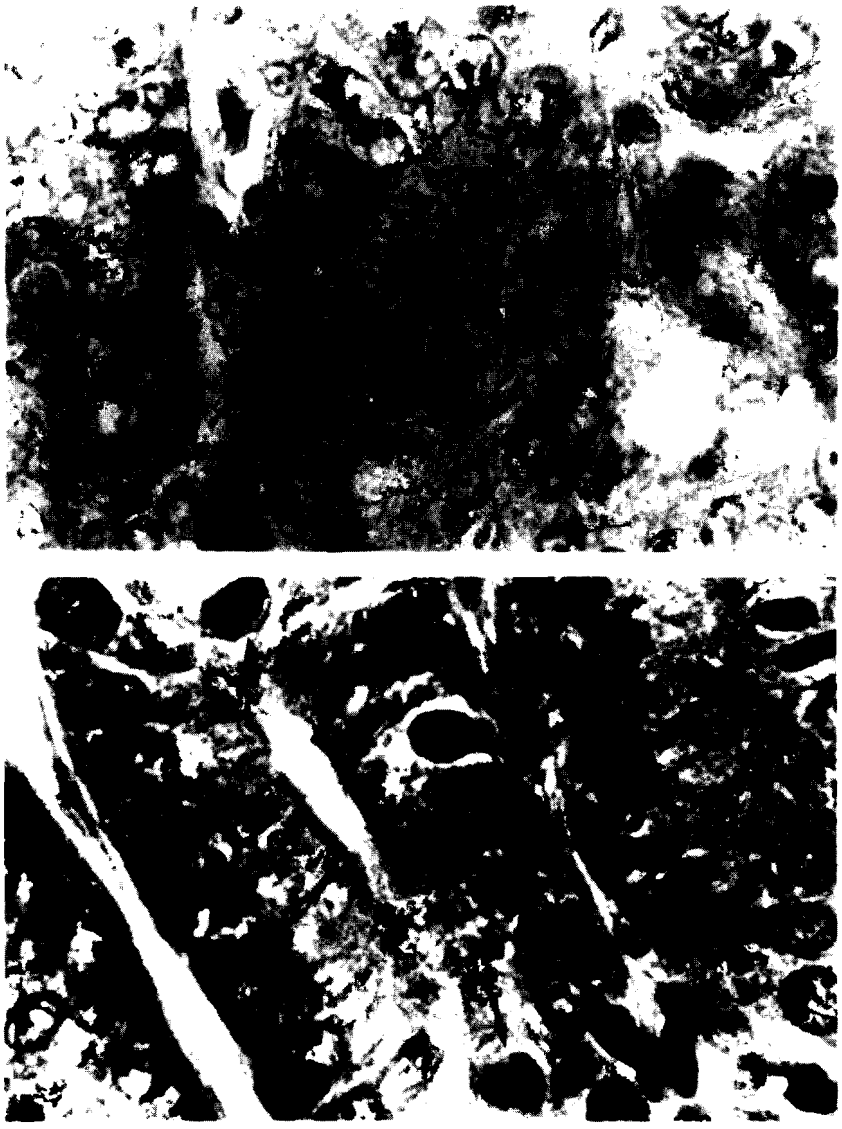
at ($p < 0.05$), meaning that observations obtained in tissue samples of treated animals were significantly different from those of controls. The coefficient of correlation (r values) was also determined to measure the linear relationship of dose-response correlation.

Results

Table 1 summarizes the results of cells arrested in metaphase by chromosomal clumping. A relatively low percentage of cells was observed in all stages in mitosis in the duodenal specimens of the control animals (9.4%). The percentage of cells arrested in metaphase increased with augmenting doses of methylmercuric chloride, that is, 11.1% in the 5 mg/kg treatment group compared to 22.6% at the 30 mg/kg dose level. Analysis of variance revealed a highly significant difference between treatment and control groups ($p < 0.001$).

Figures 1 and 2 illustrate duodenal cryptcells from both control and methylmercuric chloride (5 mg/k) treated animals, respectively. Mitotic cells in control tissues were clearly passing through metaphase into anaphase without mitotic arrest. In the tissues of all treatment groups mitotic cells became arrested in metaphase with a complete clumping of the chromosomal material (Figs. 3,4). Vacuolation, apparent as empty spaces in the duodenal cells, was first evident following treatment with 15 mg/kg methylmercury (Fig. 3) and became enhanced with increasing dose levels (Fig. 4).

Table 1 lists the proportion of mitotic cells in the duodenum of adult male mice. The control groups revealed mitotic cells in anaphase signifying that mitosis was not perturbed and halted in metaphase. In contrast, all treatment groups presented mitotic cells arrested in metaphase with none passing through to anaphase, manifesting a cytostatic effect by methylmercuric chloride treatment.



Figs. 1-4. Duodenal tissues, illustrating mitotic cells in duodenal crypts, stained with hematoxylin and eosin. Control (Fig. 1) crypt of group with two mitotic cells in anaphase (arrow); the 5 mg/kg (Fig. 2) treatment group shows several mitotic cells in a duodenal crypt arrested in metaphase (arrows); 15 mg/kg (Fig. 3) again exemplifying numerous clumped metaphase cells (arrows) and 25 mg/kg (Fig. 4) present numerous arrested metaphase cells (arrows) together with vacuoles in the epithelial lining caused by cytolysis of cells arrested in metaphase early after mercuric chloride administration (× 1,000).

Table 1. Frequency of duodenal cells suspended in metaphase following exposure to methylmercuric* chloride (MMC).

Treatment (MMC)	Total number of cells counted	Cells in metaphase	Cells in interphase	Cells suspended in metaphase (%)
Control	1000	94	916	(9.4)
5 mg/kg	1000	111	849	(11.1)
10 mg/kg	1000	157	843	(15.7)
15 mg/kg	1000	183	817	(18.3)
20 mg/kg	1000	190	810	(19.0)
25 mg/kg	1000	212	792	(21.2)
30 mg/kg	1000	226	774	(22.6)

*$p < 0.001$ (between all treatment groups & control)
$r = 0.9784$

Discussion

The degree at which mitosis was inhibited can be determined by correlating the number of arrested mitotic cells with the different doses of methylmercuric chloride. The coefficient of correlation value ($r=0.9784$) revealed a linear relationship between increasing dose levels of methylmercuric chloride and the proportion of arrested mitotic cells (C-mitosis). The occurrence of C-mitoses in the duodenal cells of methylmercuric chloride treated animals was highly significant compared to the untreated control group, where such were absent. Moreover, vacuolation of duodenal cells were observed at the higher dose levels. It was probably caused by the arrest of mitotic cells and subsequent cytolysis due to the extended duration of time the cells remained suspended in metaphase (Bertalanffy, 1960). Eventually, if methylmercuric chloride treatment were continued over extended periods, the mucosa of the duodenum would become denuded by arrest of mitosis, subsequent lysis of the arrested cells, and failure of the last cells to become replaced (Fiskesjo, 1970; Klasterska et al., 1976). For this reason, C-mitosis caused by MMC would lead to the eventual destruction of duodenal mucosa and is therefore highly significant.

Metaphase arrest (C-mitosis of animal cells is probably caused by mercury binding to spindle fiber proteins, similar to colchicine, inhibiting the polymerization of the microtubules (Thrasher and Adams 1972; Curle et al., 1983), culminating in clumping

of chromosomes and mitotic arrest (Ramel, 1972; Klasterska and Ramel, 1978; Sager et al., 1984).

In both man and laboratory animals, organic mercury has also been shown to be teratogenic. The increasing pollution of the environment with mercuric compounds is related to its widespread use in fungicides, the pulp and paper industries, and also in dental preparations. This contamination has resulted in several cases of occupational and epidemic poisoning in humans.

The adverse effects of mercury on the conceptus may be attributable to the mitosis-inhibiting activity which was seen in our studies as chromosomal clumping in duodenal tissues. The clumping phenomenon increased significantly as the dosage increased, indicating that organic mercury produces C-mitotic effects even at low concentrations. Hence, pollutant of the human environment with mercury should be a cause for concern because of its potential deleterious effects on the embryo.

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