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Effect of oral chemotherapy on the mitochondrial size of mouse intestinal cells

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Abstract Since orally given cytotoxic agents may cause intestinal disfunction, the effect of oral administration of three cytotoxics, i.e., methotrexate (MTX), cyclophosphamide (CPA), and ftoral, a derivative of 5-fluorouracil (5-FU), on the gastric, liver, and small-intestine cells of C57B1 mice was studied by transmission electron microscopy. Although no ultrastructural alterations could be detected in the cells of the first two organs, the epithelial cells of the small intestine showed a marked increase in size of their mitochondria. In the control animals the mitochondrial size was in the range of 0.04–1.8 μm (mean ± SE 0.54 ± 0.01 μm). In the treated animals the size of the mitochondria ranged between 0.15 and 4.33 μm (mean ± SE 0.73 μm) for those treated with MTX, 0.24–2.88 μm (mean ± SE 0.80 ± 0.02 μm) for those given CPA, and 0.28–5.3 μm (mean ± SE 1.18 ± 0.48 μm) for those treated with 5-FU. These findings were significantly different from those obtained in controls ($P < 0.0001$). In addition, in animals treated with MTX the mitochondria of the jejunal cells were surrounded by channels of rough endoplasmic reticulum. The cytoplasm contained long, winding channels of smooth endoplasmic reticulum, vacuoles, and myelin figures. Fluid retention in the small intestine due to administration of cytotoxic drugs is suggested as a possible mechanism for distention of the mitochondria.

Key words Cytotoxic drugs · Small intestine · Mitochondria

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Introduction

Oral chemotherapy is an important modality in the treatment of solid tumors and hematological malignancies, especially leukemias. The question arises as to how the orally given cytotoxic and/or cytostatic drugs affect the architecture of the epithelial cells of the gastrointestinal tract, mainly those of the stomach mucosa and the small intestine.

Therefore, the aim of the present work was to study the ultrastructure of the epithelial cells of these two organs in mice treated orally with methotrexate (MTX), cyclophosphamide (CPA), and 5-fluorouracil (5-FU). In addition, since these drugs are metabolized in the liver, the fine architecture of the liver cells of the treated animals was examined.

Materials and methods

A total of 20 C57B1 mice divided into 4 groups were used throughout the study. The animals were kept on a standard diet. One group of five mice served as controls. The animals of the remaining groups were treated orally with the following cytotoxic drugs dissolved in water.

The mice in the first group were given MTX (Neopharm Ltd., Petah-Tiqva, Israel) at 75 mg/kg once a day for 5 consecutive days. The animals in the second group were treated with CPA (Cytophosphan; Taro Pharmaceutical Industries, Herzlia, Israel) at daily doses of 100 mg/kg for 5 days. The mice in the third group received 25 mg/kg 5-fluorouracil (5-FU, Ftoral; kindly supplied by Mr. Philip Vennor, Abic Chemical and Pharmaceutical Industries Ltd., Netanya, Israel) once a day, 5 days a week, for a period of 3 weeks.

During treatment the animals showed a fair appearance without impairment of their vital signs. At the end of drug administration,

the animals were killed and small pieces of their gastric mucosa, the upper portion of the jejunum, and the liver were prepared for electron microscopy examination by standard methods. Fine sections cut with an LKB Ultratome III were stained with uranyl acetate and lead citrate and examined with a Philips 300 transmission electron microscope at an acceleration voltage of 60 kV.

At least 500 mitochondria were measured in cells from different blocks of animals in each experimental group. The round mitochondria were measured along their diameter, whereas in the oval ones the longest axis was determined. Statistical analysis was carried out using Student's *t*-test.

Results

Figure 1 is a representative area of a control intestinal epithelial cell containing mitochondria of round or oval shape and a moderate number of cristae. Their size was in the range of 0.04–1.8 μm (mean ± SE 0.54 ± 0.01 μm). In addition, short channels of endoplasmic reticulum and microtubules could be seen. The microvilli (not shown) were normal in size and appearance.

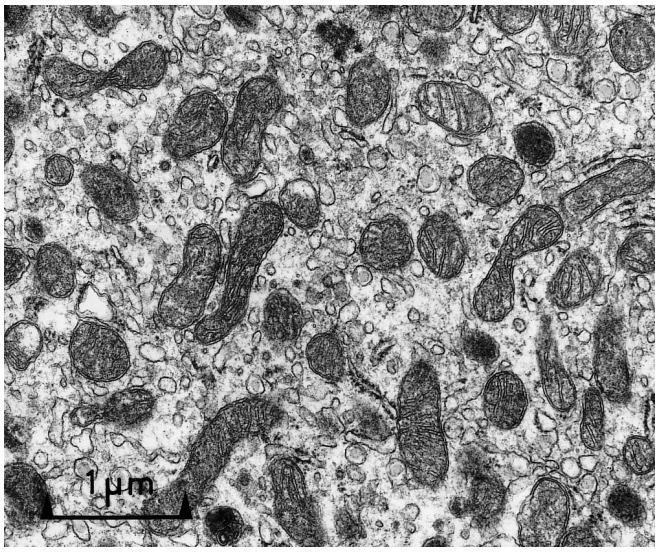

Fig. 1 Portion of a control intestinal cell containing round and oval mitochondria with well-preserved cristae

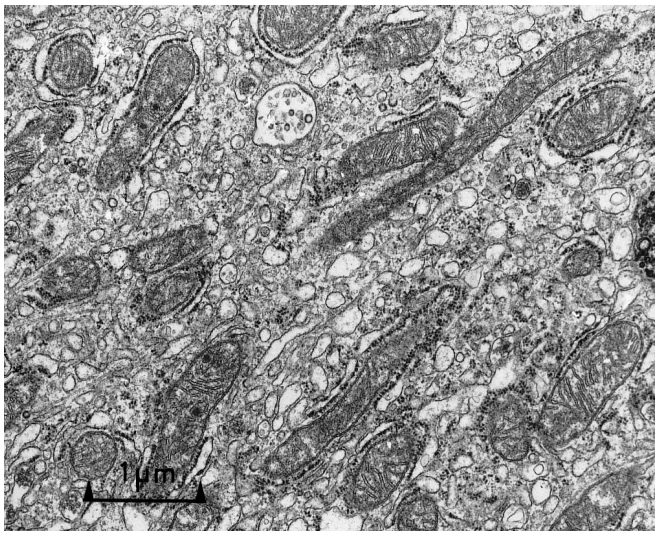

Fig. 2 Portion of an intestinal cell of an MTX-treated animal. Note the larger size of the mitochondria in comparison with those in the controls. Most of them are surrounded by rough endoplasmic reticulum

Following treatment with MTX (Fig. 2) the epithelial cells showed a pronounced enlargement of their mitochondria, their size being in the range of 0.15–4.33 μm (mean ± SE 0.73 ± 0.02 μm), the difference from the size of the control mitochondria being statistically significant ($P < 0.0001$). In addition, the majority of the mitochondria were surrounded by well-developed channels of rough endoplasmic reticulum. Myelin figures and cytoplasmic vacuoles were also observed (Fig. 3). Almost all cells examined contained numerous long and winding channels of smooth endoplasmic reticulum (Fig. 4). The microvilli were not affected.

The intestinal cells of animals treated with CPA (Fig. 5) showed an even greater enlargement of their mitochondria, whose size lay in the range of 0.24–2.88 μm (mean ± SE 0.80 ± 0.02 μm). The difference in size from that of the control was statistically

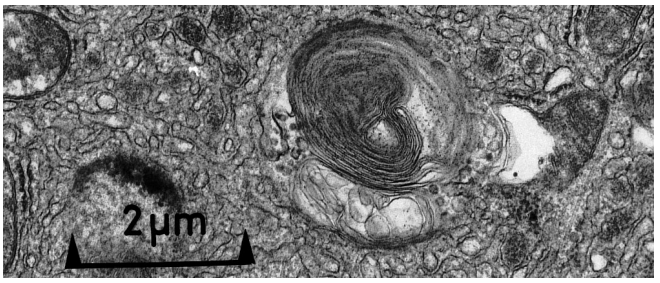

Fig. 3 Myelin figure and vacuoles in the intestinal cell of an MTX-treated mouse

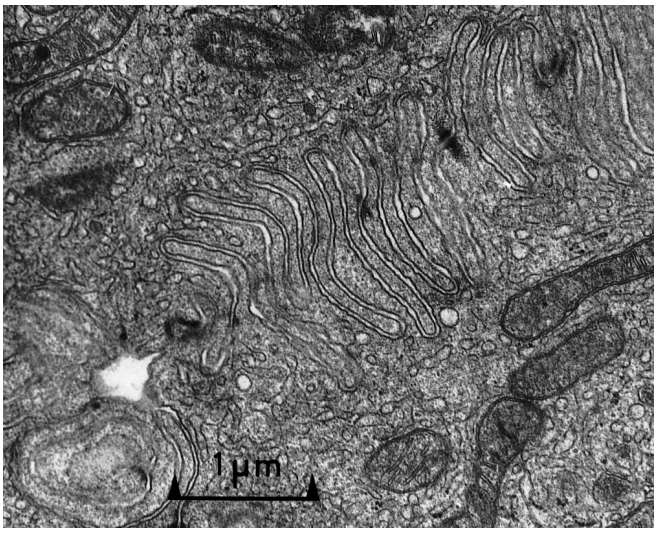

Fig. 4 Increase in the smooth endoplasmic reticulum with winding channels

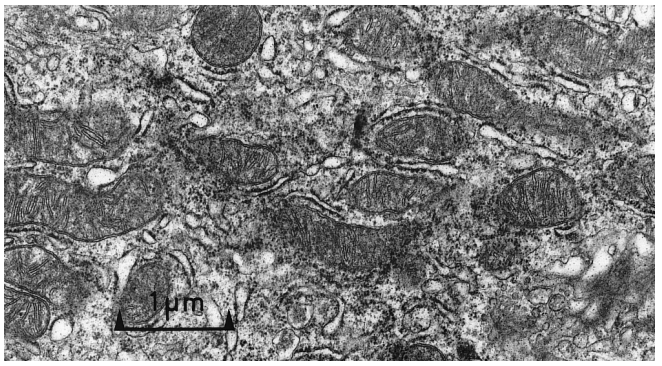

Fig. 5 Portion of an intestinal cell of a mouse treated with CPA. Also in this case the mitochondria are larger than those in the controls

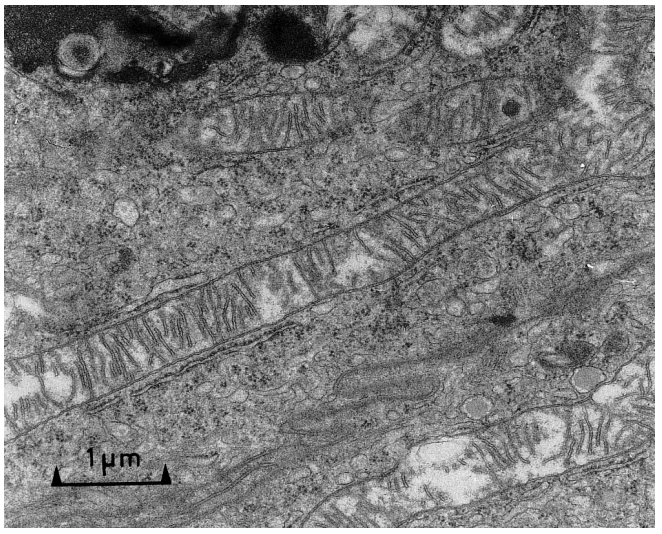

Fig. 6 Intestinal cell of a 5-FU-treated animal. Giant mitochondria with damaged cristae are visible

significant ($P < 0.0001$). The cristae were well preserved. The number of channels of endoplasmic reticulum was increased, especially around the mitochondria. Also in this case the microvilli appeared normal in structure.

Treatment with 5-FU caused a maximal increase in mitochondrial size in the intestinal cells (Fig. 6), which was in the range of 0.28–5.3 μm (mean $\pm$ SE 1.18 ± 0.48 μm; $P < 0.0001$) as compared with that of the controls. The cristae showed marked damage. The rest of the cytoplasmic organelles, including the cell membrane and the microvilli, appeared unaltered. A comparison of the mitochondrial size determined in the four groups of animals is given in Fig. 7.

The cells of the gastric mucosa as well as the liver cells did not show any difference in ultrastructure in comparison with that of untreated animals.

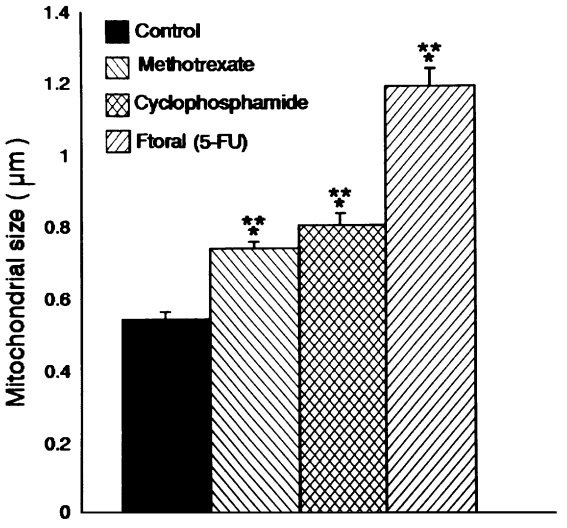

Fig. 7 Graphic presentation of the mitochondrial size determined in the intestinal cells of animals treated with cytotoxic drugs. $^{**}P < 0.0001$

Discussion

Oral administration of cytotoxic drugs is advantageous for the treatment of patients with cancer for obvious reasons. Although all three agents used in the present study may cause gastrointestinal symptoms such as vomiting, stomatitis, mucositis, and diarrhea [1, 2, 6, 12], their oral administration is better tolerated by patients. In mice injected with MTX (5 mg/kg), DNA synthesis in the small-intestine cells has been found to be suppressed by 90% at 3 h after administration of the drug [12]. Oral administration of MTX to rats has inhibited DNA synthesis in intestinal crypt cells [11]. Although in the present work the mitochondrial size was least affected by MTX in comparison with the other two cytotoxic agents, the former drug induced the most marked changes in the intestinal cells, a finding suggesting different predilection sites of the drugs on the cellular level.

CPA affects the small intestine either by immunosuppression or by a direct effect on the cells. The direct effect of CPA on the cells has been studied mainly in cardiomyocytes. In rats treated with the drug a decrease in mitochondrial respiratory function [3]; an effect on their partial volume similar to that observed in hypoxia [5]; the appearance of giant, bizarre-shaped mitochondria; and dilatation of the smooth endoplasmic reticulum [10] have been observed. Distended mitochondria, hydropic vacuoles, and dense bodies have been detected in neuroepithelial cells of rat fetuses treated with CPA [7].

Ftoral is a derivative of 5-FU that, when given orally, undergoes a gradual biotransformation to 5-FU. The drug exerts its cytotoxic effect via inhibition of DNA synthesis through blockade of the enzyme thymidylate

synthetase. It is possible that 5-FU has a direct effect on the mitochondria. Shinomiya et al. [8] have shown that treatment of EL-4 lymphoma cells with 5-FU induces an increased binding of rhodamine 123, a positively charged dye, which accumulates specifically in the mitochondria of living cells.

The results of the present work confirm the observations that cytotoxic drugs given orally induce injury in jejunal epithelial cells, a finding that might be related to the intestinal symptoms accompanying their administration. However, the damage observed in the examined organs following treatment with cytotoxics was relatively small. It is possible that epithelial cells of the jejunal mucosa are rapidly eliminated and replaced by healthy cells, which migrate up the crypt and onto the villus, as has been shown in mammalian small-intestinal mucosa by Ijiri and Potten [4] after administration of cytotoxic drugs and various types of radiation.

In the present study the mitochondria in the intestinal epithelial cells were those most affected. In all treated animals the size of the mitochondria was significantly increased in comparison with that in the controls, with 5-FU inducing the maximal effect. The reason for this phenomenon is unclear. The possibility that all three drugs affect mitochondrial DNA synthesis cannot be excluded. Another possible explanation is that treatment with cytotoxic drugs causes fluid accumulation in the small intestine with a subsequent increase in mitochondrial size. A single dose of MTX (40 mg/kg) given intraperitoneally to rats has induced fluid accumulation in their small intestine with subsequent prostaglandin E_2 (PGE_2) formation [9]. Since PGE_2 possesses an enteropooling effect, the fluid accumulation may result in diarrhea, one of the complications of cytotoxics' administration.

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