Ultrastructural Changes in the Mitochondria of Intestinal Epithelium of Rodents Treated with Methylglyoxal-bis(guanylhydrazone)

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ABSTRACT

Ultrastructural studies of rats or mice treated for 24 hr with a toxic dose (100 mg/kg) of methylglyoxal-bis(guanylhydrazone) revealed the presence of damaged mitochondria in the crypt cells of the intestinal epithelium. Mitochondria were severely swollen and electronlucent, and appeared to be similar to those observed previously in a variety of cell types treated in vitro and in vivo with methylglyoxal-bis(guanylhydrazone). Since thymidine incorporation into the intestine was not found to be decreased until after 24 hr, it is concluded that the mitochondrial damage of methylglyoxal-bis(guanylhydrazone) could be responsible for the antiproliferative toxicities of the drug.

INTRODUCTION

Early clinical trials with MGBG revealed impressive antitumor activity that was unfortunately offset by prohibitive toxic reactions, including fetal hypoglycemia, myelosuppression, and severe ulcerations of the skin and gastrointestinal tract (4, 7, 18). Acceptable reversible toxicity was eventually achieved by administering the drug on weekly, rather than daily, infusion schedules (5). Since then, MGBG has undergone a “second look” as a clinical anticancer agent, and good activity has been reported in the treatment of certain neoplasias with the drug as a single agent (18) or in combination with the inhibitor of polyamine biosynthesis, 1,2-difluoromethylornithine (17).

Of the 2 intracellular sites of MGBG action, interference with mitochondrial function (11, 12) and depletion of certain polyamines by inhibition of S-adenosylmethionine decarboxylase (2, 19), it appears from our studies that the former is most critically involved in at least the early antitumor effects exerted by the drug (11, 12). The limited selectivity of MGBG for proliferating neoplastic tissues may be afforded by its shared uptake into cells with polyamines, spermidine in particular (3, 13).

Because of the renewed clinical interest in MGBG, it is important to determine the basis for the gastrointestinal toxicity which still appears on the weekly infusion schedule. Previously, we have determined that polyamine pools are not substantially diminished in the intestinal epithelium of mice treated with MGBG by the time that intestinal toxicity appears (14). In the present study, we have examined the ultrastructure of the gastrointestinal epithelium of rats and mice treated with MGBG to determine whether the mitochondria are affected as they are in tumor cells treated in vitro (10, 11) or in vivo (5, 12) with the drug. The results indicate that MGBG alters profoundly the ultrastructure of those mitochondria located in the crypt cells of the intestinal epithelium prior to inhibition of [3H]thymidine incorporation.

MATERIALS AND METHODS

DBA/2J mice, weighing 20 to 25 g, were given a single i.p. injection of MGBG (100 mg/kg) in sterile 0.85% NaCl solution. This dose is slightly below the 50% lethal dose for MGBG in rodents (100 to 120 mg/kg) and is known to cause diarrhea (9). Animals receiving sterile 0.85% NaCl solution alone served as controls. After 24 hr, the mice were sacrificed by cervical dislocation, and the abdominal cavity was opened. One end of the jejunum was transected, and after a section of intestine was clamped, the lumen was perfused with oxygenated phosphate-buffered (23 ml 0.2 M Na2HPO4, 77 ml 0.2 M Na2HPO4, 100 ml distilled water, and 0.5 ml 1% CaCl2) 3% glutaraldehyde (pH 7.4, 480 mOsom) at 4°. Portions of the jejunum were then excised, placed in glutaraldehyde, and cut into small blocks.

Similarly, Sprague-Dawley rats weighing 200 to 250 g were given a single i.p. injection of MGBG (100 mg/kg) suspended in sterile 0.85% NaCl solution. Control rats were given injections of sterile 0.85% NaCl solution. After 24 hr, the fasted rats were anesthetized with ether, and the tissue samples were removed as described for mice.

Tissue blocks from the intestines of mice and rats were fixed for 24 hr at 4° in phosphate-buffered 3% glutaraldehyde. The fixed tissue blocks were then washed in phosphate buffer overnight, postfixed in phosphate-buffered 1% osmium tetroxide, dehydrated in a graded alcohol series, and embedded in Epon-Araldite resin mixture. Semithin sections (~500 nm) were prepared for light microscopy with a Porter-Blum MT-1 ultramicrotome (Sorvall Corp., Norwalk, Conn.) and stained with 1% aqueous toluidine blue containing 1% sodium borate. Blocks were selected and trimmed to show longitudinal sections through the villus, including the crypt regions. Thin sections (~90 nm) were stained sequentially with uranyl acetate and lead citrate and then examined with a Siemens Elmskop 101 electron microscope at 80 kV.

As an indication of the antiproliferative effects of MGBG on the intestine, the incorporation of [3H]thymidine into various regions of the intestine was measured in mice bearing L1210 cells. Mice were first inoculated i.p. with 10⁶ L1210 cells 3 days prior to drug treatment. They were then given a single i.p. injection of MGBG (100 mg/kg) and 0 (control) 24 or 48 hr later, they were given i.p. injections of 500 μCi of [3H]thymidine (44 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) in sterile 0.85% NaCl solution. After 1 hr, the L1210 cells and segments of the intestine were removed, washed in 0.85% NaCl solution, and homogenized on ice for 2 to 4 min with a Polytron (Brinkman Instruments, Westbury, N. Y.). The homogenate was precipitated with 1% phosphotungstic acid in 0.5 N HCl and washed twice in 10% phosphotungstic acid by centrifugation. The final precipitate was completely

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dissolved at 45° in Soluene 350 (Packard Instrument Co., Inc., Downers Grove, Ill.) and placed in scintillation counting fluid. Vials were counted in a Packard Model 3320 scintillation counter. Data were expressed as dpm per mg protein per hr.

RESULTS

Treatment of mice with 100 mg/kg for 24 hr induced definite signs of drug toxicity as indicated by relative inactivity, diarrhea, piloerection, and death in ~10% of the animals. Of the 12 MGBG-treated mice which were examined morphologically, 9 mice showed drug-induced changes in their intestinal epithelium. Examination of semithin sections of jejunum at the light microscope level revealed the presence of numerous patent vacuoles in the cytoplasm of cells located in Lieberkühn’s crypts (Fig. 1). The vacuoles varied in size and appeared to be different from the secretory granules or mucin vacuoles, which were densely stained. The vacuoles were seen in the crypt cells of the jejunum but not in the cells of the villus walls (Fig. 1) or in the crypt cells of untreated mice (Fig. 2, A and B).

At the electron microscope level, the vacuoles of the crypts were observed to correlate with distended mitochondria. Its appearance (Fig. 2, C and D) was nearly identical to that described previously by this laboratory for a variety of cell types treated in vitro (10, 11) or in vivo (12, 15) with MGBG. The mitochondria were swollen, their cristae were disoriented, and the matrix appeared to be diffuse and electron lucent. Other cellular organelles and the nuclear substructure were not affected by MGBG treatment. All of the cells of the crypts seemed to be equally affected, including those undergoing mitosis and those containing mucin or secretory granules (Fig. 2D). When the intestines were examined 36 or 48 hr after the injection of MGBG, swollen mitochondria were not apparent in the crypt cells or in the villous cells, suggesting that the effect had reversed itself morphologically. However, many of the mitochondria of both crypt and villous cells contained electron-dense granules in their matrix.

In rats treated with MGBG, similar morphological effects were observed in the intestinal epithelium. The drug effect, however, was observed much more consistently, being present in all of 4 rats examined. As with the mouse, the cytoplasmic vacuolation was confined to the cells of the crypts and was not seen in the cells of the villus wall or in the crypt cells of untreated animals (Fig. 3, A and B). The mitochondria of the crypt cells, by electron microscopy, appeared swollen, as described for mice (Fig. 3, C and D), but differed in that many of the organelles contained granular electron-dense inclusions (Fig. 4). Similar inclusions have been described in cultured human cells treated with MGBG (6, 10, 11).

The ultrastructure of the liver, kidney, and spleen from MGBG-treated rodents was also examined, but neither mitochondria nor other structures appeared to be affected.

The incorporation of [3H]thymidine into intestine and L1210 leukemia cells following MGBG treatment is summarized in Table 1. Incorporation into L1210 cells was already inhibited by 24 hr, we have shown previously (12) that mitochondrial damage is apparent in 85% of these cells by 12 to 16 hr. The above findings are in keeping with what has been found in vitro systems (10, 11). Namely, mitochondrial damage precedes detectable inhibition of cell growth, and its time of onset seems to be related directly to the proliferative activity of the cells or tissues.

Subacute toxicities by MGBG have been divided into proliferative (gastrointestinal, bone marrow, and lymphoid) and nonproliferative (hepatic, renal, and cardiac) types (9). On the basis of the present findings linking mitochondrial damage to gastrointestinal toxicity and previous studies showing mitochondrial damage in subconfluent cells or lymphocytes undergoing blastogenesis (10), it seems that in general the proliferative toxicities related to MGBG are the result of drug interference with mitochondrial function. Prevention of the proliferative toxicities of MGBG by coadministration of spermidine (8) is probably due to preferred uptake of spermidine over MGBG by a shared carrier mechanism (3, 13) as opposed to prevention of drug depletion of spermidine pools by MGBG inhibition of S-adenosyl-L-methionine decarboxylase (2, 19).

The finding that the MGBG-induced mitochondrial damage to intestinal epithelium is confined to the cells of the crypts is consistent with a previous study showing that only proliferating cells are affected by the drug (10) and could be the result of increased drug uptake by dividing cells. The cells located in the base of the crypts are mitotically active and poorly differentiated. According to the report of Cheng and Leblond (1), these multipotential cells give rise to villous, columnar, mucous, enteroendocrine, and Paneth cells which, excepting the Paneth cells, experience a differentiation process as they move towards the villus tip. This could be the reason that all cells in the crypts, including those which appear to be differentiated and nondividing, contained damaged mitochondria. Apparently, the damaged mitochondria rapidly recover normal ultrastructure as circulating MGBG levels decrease, since they were not apparent in any of the epithelial cells by 36 hr after drug injection.

<table>
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<th>0 hr</th>
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<th>48 hr</th>
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<td>2068</td>
<td>4046</td>
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<td>25.6</td>
<td>27.8</td>
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<td>Colon</td>
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*On the basis of 2 experiments with 3 mice at each time point.

DISCUSSION

Selective ultrastructural damage was apparent in the crypt cells of the intestinal epithelium 24 hr after a single injection of MGBG (100 mg/kg). Since [3H]thymidine incorporation into the small intestine was not apparent until after 24 hr, the mitochondrial effect of MGBG could be responsible for its antiproliferative action. Although [3H]thymidine incorporation into L1210 cells was already inhibited by 24 hr, we have shown previously (12) that mitochondrial damage is apparent in 85% of these cells by 12 to 16 hr. The above findings are in keeping with what has been found in vitro systems (10, 11). Namely, mitochondrial damage precedes detectable inhibition of cell growth, and its time of onset seems to be related directly to the proliferative activity of the cells or tissues.

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The ultrastructural changes induced in the mitochondria of mice and rats treated with MGBG are virtually identical to those observed in L1210 cells treated in vivo with the drug (12, 15). In the latter, we have demonstrated that interference with mitochondrial function precedes by 2 hr significant reduction in spermidine or spermine pools as a consequence of MGBG inhibition of S-adenosyl-L-methionine decarboxylase (12). Mitochondrial effects by MGBG, therefore, are probably due to a direct interaction with the organelle and are not related to the inhibition of polyamine biosynthesis. Moreover, the aromatic bis(guanylhydrazone), 4,4'-diacetyldiphenyl urea bis(guanylhydrazone) (also known as DDUG), which has no effect on polyamines, does not produce mitochondrial damage to the cells of the intestinal epithelium even though, like MGBG, it causes extensive mitochondrial damage in L1210 cells. It now appears from recent studies with L1210 cells (12) and with MGBG-resistant variants of VA2 cells (16),4 that mitochondrial effects by MGBG are probably responsible for the antiproliferative action of the drug. When taken together with earlier studies (14), the present studies suggest that mitochondrial effects by MGBG are also responsible for the gastrointestinal toxicity of the drug. Previously, we found that following MGBG treatment, the polyamine pools of the intestinal epithelium are not significantly depleted by the time that intestinal toxicity appears (14). As shown here, the mitochondria are clearly damaged at this time, suggesting that interference with mitochondrial function rather than polyamine biosynthesis gives rise to at least one of the proliferative toxicities of MGBG.

ACKNOWLEDGMENTS

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REFERENCES

Changes in Mitochondria Treated with MGBG

Fig. 1. Light micrograph of a semithin section showing a villus base of intestinal epithelium taken from the jejunum of a mouse treated for 24 hr with MGBG (100 mg/kg). Note the presence of numerous patent vacuoles located in the cytoplasm of crypt cells (C). The cells of the villus (V) are not vacuolated. × 2047.
Fig. 2. Light and electron micrographs of intestinal crypt cells taken from the jejunum of untreated mice (A, C) and mice treated for 24 hr with MGBG (100 mg/kg) (B, D). The crypts of untreated mice (A) lack the vacuoles seen in MGBG-treated mice (B). A, x 800; B, x 800; C, x 6300; D, 5300.
Fig. 3. Light and electron micrographs taken from the intestinal crypts of untreated (A, C) and MGBG-treated rats (B, D). As observed in mice (Fig. 2), the cytoplasmic vacuoles in the crypts (B) correlate with distended mitochondria (D). All cells and all mitochondria appear to be uniformly affected. A, x 800; B, x 800; C, x 6300; D, x 5300.)
Fig. 4. Granular intramitochondrial inclusions observed in the intestinal crypt cells of rats treated for 24 hr with MGBG (100 mg/kg) × 20,000.
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