Biochemical and Ultrastructural Characterization of Human Cell Variants Resistant to the Antiproliferative Effects of Methylglyoxal Bis(guanylhydrazone)1

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ABSTRACT

Stable variants of the human cell line, VA2-B, have been developed which are 10- to 20-fold less sensitive to the antiproliferative effects of methylglyoxal bis(guanylhydrazone) (MGBG) than the parent cell line and which are not drug transport deficient. The lines were characterized biochemically giving particular attention to parameters related to the two known sites of MGBG action, mitochondria and polyamine metabolism. Dose-response studies with MGBG (0 to 30 μM for 40 to 48 hr) revealed that, of the parameters related to polyamine metabolism (i.e., polyamine pools, S-adenosylmethionine, and ornithine decarboxylase activities), only ornithine pool size reduction seemed to correlate with inhibition of cell growth by MGBG. By contrast, decreases in pyruvate oxidation (used here as a measure of mitochondrial function) closely paralleled growth inhibition in all cell lines. Similarly, MGBG-induced changes in mitochondrial ultrastructure were less conspicuous in the variants than in the parent cell line and also corresponded with growth inhibition. Respiratory inhibition of isolated mitochondria from one of the variant lines was about 2-fold more resistant to the inhibitory effects of MGBG than mitochondria from the VA2 cells. Finally, treatment with α-difluoromethylornithine, a potent inhibitor of polyamine biosynthesis having no known effect on mitochondrial function, resulted in comparable inhibition of growth in variant and parent cell lines. Overall, the data suggest that a phenotypic alteration in mitochondrial function, rather than in polyamine metabolism, may represent the basis for MGBG resistance in these variant cell lines.

INTRODUCTION

We have reported the isolation and partial characterization of several variants of the human cell line VA2-B (25) (designated hereafter as VA2) which are 10- to 20-fold more resistant to the antiproliferative effects of the anticancer agent MGBG3 than the parent line (31, 32). Although the biochemical basis for the drug resistance of these variants is uncertain, 2 possibilities are likely, based on current information regarding the mode of action of MGBG. The drug interferes profoundly with mitochondrial structure and function (17, 24) and also inhibits AdoMet decarboxylase, a key enzyme in the biosynthesis of the polyamines, spermidine, and spermine (5, 20). Since either of these drug effects can result in inhibition of cell proliferation, a phenotypic alteration at one of these sites may be responsible for the resistance of the variants to MGBG. This assumes, of course, that resistance is not based on decreased drug transport into cells, and initial studies involving MGBG or spermidine uptake and efflux indicate that it is not (31).

Our interest in determining the mechanism of resistance of the VA2 cell variants derives, first, from their potential usefulness in biological research involving mitochondrial genetics. The uniquely selective morphological effects of MGBG on mitochondria (17) could serve as a convenient marker in such studies. Second, definition of the basis for drug resistance will be particularly useful in determining which of the 2 drug actions of MGBG is responsible for its antiproliferative properties.

MATERIALS AND METHODS

VA2 and VA2/MGBG Cell Characterization. For characterization studies, the VA2/MGBG-3 and VA2/MGBG-4 lines were compared with VA2 cells. The 2 VA2/MGBG sublines were maintained continuously in the presence of 10 μM MGBG. In preparation for these experiments, the drug was removed for 48 hr before the cells were resuspended into T-150 flasks (Corning Glassworks, Corning, N. Y.) at 4 x 106 cells per 80 ml of medium per flask. VA2 cells were grown in the absence of MGBG and seeded at the same cell density. Care was taken by microscopic confirmation to ensure that all cell lines were at comparable subconfluent densities at the time of treatment with 0 to 30 μM MGBG or 0 to 10 μM DFMO (gift from Merrell-Dow Pharmaceutical Co.). After 40 to 48 hr of growth in the presence or absence of MGBG or DFMO, the cells were washed twice with warm PBS. Viability was determined to be greater than 95% by trypan blue exclusion. The detached cells were washed with cold PBS and counted electronically (Model ZF Coulter Counter). Aliquots of 105 cells were taken for the following determinations: pyruvate oxidation; ornithine and AdoMet decarboxylase activities; intracellular polyamine pools; and electron microscopy. In total, 2 experiments were performed in which the cells from triplicate flasks were pooled.

Pyruvate Oxidation. Pyruvate oxidation was measured by the amount of CO2 released from intact cells incubated for 30 min in the presence of 2-[1-14C]pyruvate (7.7 mCi/mmol; New England Nuclear, Boston, Mass.). The methodology is identical to that reported previously (24) as adapted from Braen and Scheffler (1).

Protein was determined by the method of Lowry et al. (11), and results were expressed as nmol 14CO2 released per hr per mg protein or percentage of control. All values were corrected for blanks containing PBS instead of cell suspension. The assay was linear with respect to

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6The abbreviations used are: MGBG, 1,1'-(methylethanediylidene)dinitrilo)diguanidine, also known as methylglyoxal bis(guanylhydrazone), methyl-G, or methyl-1-GAG, AdoMet, S-adenosyl-L-methionine, DFMO, α-difluoromethylornithine, PBS, phosphate-buffered saline [0.9% NaCl, 0.115% Na2HPO4, 0.02% KH2PO4, 0.05% KCl (pH 7.4)].

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time up to 60 min, and the final concentration of pyruvate (1 mM) was saturating.

Orotate and AdoMet Decarboxylase Activities. The VA2 cells and sublines were pelleted, dried with a cotton swab, and resuspended in 0.4 ml of buffer containing 25 mM Tris (pH 7.5), 0.1 mM EDTA, and 5 mM dithiothreitol at 4°. The cellular suspension was sonicated (Model LS-75; Branson Ultrasonic Corp.) twice for 4 sec at 5 amp. The broken cell suspension was centrifuged at 8000 × g for 3 min with a microcentrifuge (Brinkman Instruments, Inc., Westbury, N. Y.). The resulting supernatant was assayed for ornithine and AdoMet decarboxylase activities.

The techniques used for the determination of ornithine and AdoMet decarboxylase activities were essentially those of Pegg and Williams-Ashman (22) as described elsewhere (24). The substrates, DL-[1-14C]-ornithine (58 mCi/mmol) and [1-MC]AdoMet (51 mCi/mmol), were obtained from New England Nuclear. Data were expressed as nmol 14CO2 released per hr per mg protein.

Intracellular Polyamine Pools. Intracellular polyamine concentrations were determined on a 0.6 M perchloric acid extract using a high-pressure liquid chromatography system described in detail elsewhere (26). The system has a sensitivity range of 0.1 nmol/50-μl sample, and the percentage of error is less than 5% for standards. A chromatogram of polyamine standards has been published (26).

Electron Microscopy. For ultrastructural studies, PBS-washed cell monolayers were fixed with cold phosphate-buffered (23 ml 0.2 mM Na2HPO4-77 ml 0.1 mM EDTA) 0.2 M NaHPO4-100 ml distilled water-0.5 ml 1% NaCl-3% glutaraldehyde (pH 7.4; 490 mOsmol) while still attached to the flasks. After sitting on ice for 30 min, the cells were removed gently with a rubber scraper and pelleted. Fixed cell pellets were washed overnight in phosphate buffer, postfixed in phosphate-buffered 1% osmium tetroxide at 4° for 3 hr, dehydrated in a graded alcohol series, and embedded in Epon-Araldite plastic resin. Thick sections (500 nm) were prepared using a Porter-Blum MT-1 ultramicrotome (Ivan Sorvall, Inc., Norwalk, Conn.), stained with 1% toluidine blue in 1% aqueous sodium borate, and examined with a Leitz Ortholux microscope. Thin sections (90 nm) were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop 101 electron microscope.

Oxygen Consumption. VA2 and VA2/MGBG-3 cells, grown for 96 hr in the absence of MGBG, were homogenized as described above. Isolated mitochondria were prepared as described elsewhere (2, 3, 30). Mitochondria were suspended in 0.25 mM sucrose (British Drug House Chemical, Ltd., Poole, England) with 10 mM Tris-HCl buffer (pH 7.3) and 1 mM potassium ethyleneglycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetra-acetic acid (Sigma Chemical Co., St. Louis, Mo.). Protein concentrations were determined by the method of Lowry et al. (11), and samples were diluted appropriately to be equal. Respiration of mitochondria was measured polarographically as described elsewhere (2, 3) using a Clark oxygen electrode at 25°. The reaction mixture (final volume, 1.5 ml) contained 15 mM KCl and 50 mM Tris-HCl (pH 7.2). Oxygen consumption was recorded after addition of 50 μl of mitochondrial suspension (3.5 mg of protein) to the electrode chamber containing medium at 5 mM glutamate or 5 mM succinate (plus glutamate) as substrate. Twenty min after the addition of mitochondria, MGBG was added in a volume of 10 to 20 μl using a Hamilton microsyringe. Relative oxygen consumption was calculated by assuming that the respiration rate before the drug addition represented 100% of activity.

RESULTS

Cell Growth. The effects of varying concentrations of MGBG on the growth of VA2 cells and VA2/MGBG sublines 3 and 4 are presented in Chart 1. As reported previously (31), VA2 cells are much more sensitive to the antiproliferative effects of MGBG than the variants. At 42 hr, 3 μM MGBG decreases VA2 growth to less than 50% of control. By contrast, the growth of the variants is greater than 80% under comparable conditions, and even at 10-fold-higher drug concentrations (30 μM), growth is still above 60% of control. Overall, the 2 variants gave very similar dose-response curves.

Dose-response curves to DFMO, the inhibitor of putrescine and spermidine biosynthesis (12), indicate that the VA2 cells and sublines VA2/MGBG-3 and -4 are all equally sensitive to the cytostatic effects of the drug (Chart 2). The data tend to discount general alterations in polyamine metabolism as being responsible for the insensitivity of the variant cell lines to MGBG.

Pyruvate Oxidation. The ability of cells to liberate labeled CO2 from pyruvate labeled in position 2 is an indication of mitochondrial oxidative metabolism (Chart 3). In general, the dose-response curves for pyruvate utilization are very similar, on a relative basis, to those for growth. Pyruvate oxidation for VA2 cells is almost completely inhibited with 3 μM MGBG at 42 hr, whereas that for the 2 variant sublines is virtually unaffected at 3 μM. By 30 μM MGBG, the ability of the 2 sublines to utilize pyruvate was around 50% of control values. Although the 2 sublines are similar in their ability to oxidize pyruvate in the presence of MGBG, subline 4 appears slightly more resistant to the drug.

Polyamine Biosynthesis. In the presence of MGBG, intracellular levels of AdoMet decarboxylase are known to increase steadily (21), probably as a consequence of enzyme stabilization by the inhibitor. Chart 4 shows that, after continued maintenance of the sublines in 10 μM MGBG, the enzyme activity returns to basal levels by 24 hr after drug removal. Since dose-response studies for AdoMet decarboxylase (Chart 5) were initiated 48 hr after drug removal, it appears that the enzyme levels are indeed

![Chart 1](chart1.png)

![Chart 2](chart2.png)
Characterization of MGBG-resistant Variants

at a basal state. The basal activity of AdoMet decarboxylase was about 2-fold higher in the VA2/MGBG variants than in the parent line. It is interesting to note that, even after 96 hr in the absence of drug, the enzyme activity of the resistant sublines is about 2-fold greater than that of the VA2 cells.

In the presence of MGBG, AdoMet decarboxylase activity of the variant sublines increases steadily with drug concentration until, at 30 μM, substantial inhibition of growth occurs (Chart 3). The data confirm that the cells are permeable to MGBG. At 10 μM, the drug concentration in which the variants are maintained routinely, the enzyme activity is 5-fold greater than basal levels for VA2/MGBG-4 and about 10-fold greater for VA2/MGBG-3. AdoMet decarboxylase activity for VA2 cells rises significantly at 3 μM and then begins to decline as cytotoxicity becomes evident.

Although ornithine decarboxylase, the enzyme responsible for putrescine biosynthesis, is not affected directly by MGBG, it increases characteristically in cells treated with MGBG as a consequence of decreased spermidine biosynthesis (7, 10, 21). Thus, in the sublines, ornithine decarboxylase increases steadily, beginning at 3 μM in the dose-response curves (Chart 5), and continues to 30 μM even though inhibition of growth has been established at this concentration (Chart 1). VA2 cells, however, show no significant increase in enzyme activity at 3 μM and, as

with AdoMet decarboxylase (Chart 5), show decreased ornithine decarboxylase activity at 10 μM MGBG as a probable consequence of drug cytotoxicity.

The polyamine pool profile of the VA2 cells (Chart 6) is unusual for proliferating cells in culture. Although putrescine pools are typically very low, the ratio of spermidine to spermine (0.09) was much lower than expected for proliferating cells. For example, cultured L1210 leukemia cells in logarithmic growth typically have a ratio of spermidine to spermine of about 2.5.

Treatment with MGBG inhibits AdoMet decarboxylase, blocks conversion of putrescine to spermidine, and results typically in an accumulation of putrescine. Because of the various shifts in AdoMet and ornithine decarboxylase occurring with MGBG treatment, the most reliable indication of drug effects is the polyamine pools themselves (Chart 6). As expected with MGBG inhibition of AdoMet decarboxylase, putrescine pools increase at 3 μM of drug in both VA2 and the sublines. Putrescine levels continue to increase in the resistant sublines at 10 and 30 μM but decrease at 10 μM in the VA2 cells. Spermidine pools increase slightly in all cell lines, possibly as a consequence of MGBG-induced increases in AdoMet decarboxylase and of the increased putrescine pools. By contrast, spermine pools show an overall decrease in VA2 cells and the variants, although the latter increase slightly at 3 μM before decreasing.

7 D. L. Kramer, unpublished data.
Effects of MGBG on Cell Morphology. By light microscopy, VA2 cells and the VA2/MGBG sublines appear morphologically similar to VA2 and forms reasonably flat monolayer cultures in the absence or presence of MGBG. However, the other 3 MGBG-resistant variants differ morphologically from the parent line when grown in the presence of MGBG. Most cells attach very loosely and spread very little.

By electron microscopy, VA2 cells and the VA2/MGBG sublines appear slightly rounded in cross-section, indicating, as was apparent from light microscopy of living cultures, that cells do not assume an extremely flattened morphology in monolayer cultures. The mitochondria and elements of the endoplasmic reticulum system are the most prominent cytoplasmic organelles. Occasional primary and secondary lysosomes are also observed, especially in confluent cultures.

In untreated VA2 cells, mitochondria are uniform in size but variable in shape and internal structure (Fig. 1, A and B). When present, the cristae appear either parallel or interconnected in lacework patterns. The mitochondria contain membranous inclusions resembling small myelin figures, while others contain single round amorphous granules presumed to be deposits of calcium as described in other cell types.

Following treatment of VA2 cells with 10 μM for 41 hr, many of the mitochondria become swollen to twice their normal size (Fig. 1, C and D). Most of their cristae disappear, and the matrix becomes electron lucent. The proportion of mitochondria which are affected in this manner varies from cell to cell. Nearly all (<95%) of the cell profiles contain at least some swollen mitochondria, and in some (~20%), all of the organelles were affected. Other cellular organelles and the nuclear substructure do not appear to be affected by MGBG treatment.

Several of the swollen mitochondria contain large amorphous globular inclusions of high electron density (Fig. 1, C and D). These differ distinctly in size, quantity, and density from the granules presumed to be calcium in the mitochondria of untreated VA2 cells. Accordingly, 87% of the VA2 cells treated with MGBG contain one or more mitochondria with large amorphous densities as compared to 3% of the untreated cells (Table 1). The densities resemble closely those inclusions found in another human cell line, NALM-1 myeloid leukemia, treated with MGBG (17) and in Chinese hamster ovary cells treated with relatively high concentrations of ethidium bromide (15, 16).

The mitochondria of the untreated sublines, VA2/MGBG-3 and VA2/MGBG-4, are similar to one another and do not appear to be ultrastructurally different from those of untreated VA2 cells (Fig. 2A). Moreover, the mitochondrial morphology of the variant sublines is not altered significantly by MGBG treatment. Only 15 and 24% of the cells in VA2/MGBG-3 and VA2/MGBG-4, respectively, contain mitochondria with densities (Table 1). Among these, the average number of mitochondria with densities contained in each cell (2.3) is comparable to that of the parent line (2.1). As with the parent line, the ultrastructural effects of MGBG are restricted to mitochondria. Other cellular organelles appear identical to those of untreated VA2/MGBG cells (Fig. 2B).

All cell lines were also treated with 5 mM DFMO for 48 hr and examined by electron microscopy. Under these conditions, growth of all 3 lines was inhibited by about 30%. In no instance was ultrastructural damage to mitochondria apparent. The organelles appeared identical to those of untreated control cultures (data not shown).

Oxygen Consumption Studies. Since it became apparent from drug uptake studies that intracellular binding of MGBG might be altered in the variants, the effects of MGBG on the oxygen consumption of isolated mitochondria were examined as a possible measure of binding to that organelle. The data in present, the cristae appear either parallel or interconnected in lacework patterns. The mitochondria contain membranous inclusions resembling small myelin figures, while others contain single round amorphous granules presumed to be deposits of calcium as described in other cell types.

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Table 1
Effects of MGBG on the mitochondrial ultrastructure of VA2 cells and VA2/MGBG sublines 3 and 4

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cells with mitochondrial inclusionsa</th>
<th>MGBGb</th>
</tr>
</thead>
<tbody>
<tr>
<td>VA2</td>
<td>3</td>
<td>87</td>
</tr>
<tr>
<td>VA2/MGBG-3</td>
<td>1</td>
<td>15</td>
</tr>
<tr>
<td>VA2/MGBG-4</td>
<td>8</td>
<td>24</td>
</tr>
</tbody>
</table>

a Percentage of 200 nucleated cell profiles examined at x10,000 magnification having a minimum of one mitochondrion with an amorphous electron-density inclusion (see Fig. 12). Other mitochondrial changes were noted (see "Results") but not quantitated.

b Cells were treated for 41 hr with 10 μM MGBG.
Characterization of MGBG-resistant Variants

Table 2
Effects of MGBG on oxygen consumption by isolated mitochondria from VA2 and VA2/MGBG-3 cells

<table>
<thead>
<tr>
<th>Substrate</th>
<th>MGBG (μM)</th>
<th>VA2</th>
<th>VA2/MGBG-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutamate</td>
<td>None</td>
<td>100 (7.5)</td>
<td>100 (6.5)</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>83</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>66</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>53</td>
<td>69</td>
</tr>
<tr>
<td>Succinate</td>
<td>None</td>
<td>100 (15.9)</td>
<td>100 (10.1)</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>77</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>64</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>56</td>
<td>75</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, actual values in mg atmospheres of oxygen per min per mg of protein.

Table 2 indicate that, at equivalent protein levels, the mitochondria of the VA2/MGBG-3 cells are more resistant to the inhibitory effects of MGBG on oxygen consumption than are the mitochondria of VA2 cells. The difference in extent of inhibition for VA2 versus VA2/MGBG-3 is approximately 2-fold at all concentrations of MGBG and for either substrate.

DISCUSSION

On the basis of uptake and efflux studies described elsewhere (31), it was determined that the MGBG-resistant variants of VA2 cells are not transport defective for the drug. Rather, the data suggest that intracellular binding sites for MGBG are fewer in the variants so that, under long-term (>30 min) exposure to MGBG, they accumulate about 3- to 4-fold less drug. Because MGBG is positively charged under physiological conditions, it is conceivable that a large number and wide variety of binding sites exist intracellularly. The characterization described here deals strictly with the 2 best recognized sites of MGBG action, mitochondria and polyamine metabolism. Of these, it appears that drug effects related to mitochondrial integrity correlate best with the antiproliferative action in the various cell lines. Pyruvate oxidation was diminished significantly in VA2 cells at all concentrations of MGBG but not in the resistant variants. In fact, pyruvate oxidation proved to be the most MGBG sensitive of all parameters studied. Ultrastructural damage to mitochondria and the appearance of mitochondrial densities were also closely proportional to MGBG growth inhibition in all cell lines.

By oxygen consumption studies, mitochondria isolated from all cell lines were found to be inhibited by MGBG at concentrations reported previously (2, 3) to inhibit isolated liver mitochondria. The mitochondria isolated from the variants, however, were found to be less sensitive to inhibitory effects of MGBG than the VA2 mitochondria. The differences were about 2-fold for every drug concentration tested, but it is difficult to determine whether this difference measured over such a short time (4 min) would be sufficient to account for cellular resistance to MGBG during long-term exposure to the drug. As further indication of an altered mitochondrial phenotype in the variants, we have shown recently (32) that the variants are 2- to 5-fold more sensitive to the inhibitor of mitochondrial protein synthesis, chloramphenicol, than the VA2 cells when grown in the absence of MGBG and 5- to 10-fold more sensitive when grown in the presence of MGBG. Further, the in vivo rate of mitochondrial protein synthesis is reduced significantly in the variants when pregrown and labeled in the presence or absence of MGBG. It is possible that this decrease in the variants accounts for their increased sensitivity to chloramphenicol.

In general, drug effects on parameters related to polyamine metabolism failed to correlate with the antiproliferative action of MGBG. With increasing doses of MGBG, putrescine pools increased in all cell lines as a consequence of MGBG inhibition of AdoMet decarboxylase; however, spermidine pools did not decrease. Spermine pools were unusually high in all cell lines and decreased with increasing doses of MGBG. Although this trend correlated with growth inhibition, the spermine levels in the VA2 cells did not seem to be low enough to cause growth inhibition, since the variants reached similar levels at higher MGBG concentrations but were not growth inhibited to the same extent.

The basal activity of AdoMet decarboxylase was about 2-fold higher in the variants prior to drug treatment. While treatment with MGBG elevated AdoMet decarboxylase activity proportionally in the variants, this increase is a well-recognized phenomenon and is attributed generally to prolongation of enzyme half-life as a consequence of drug binding (7, 8, 21). Conceivably, it could afford some measure of protection to MGBG in the variants, but the difference in final AdoMet decarboxylase activities between the variant and VA2 levels was only 3- to 4-fold. This hardly seems sufficient to deal with the high intracellular levels of MGBG that are known to occur (14, 29).

Studies with the specific ornithine decarboxylase inhibitors, α-methylornithine and DFMO (12, 13), have demonstrated convincingly that polyamines are required for cell growth. Cell lines have been developed which are resistant to the effects of these inhibitors through mechanisms leading to increased ornithine decarboxylase levels (4, 13). Since MGBG is a potent inhibitor of AdoMet decarboxylase (5), it might be expected that, even if the mitochondrial effects of MGBG were minimized in the VA2 variants, subsequent depletion of polyamines would ultimately result in growth inhibition. As observed here, this allows time for drug-induced increases in AdoMet decarboxylase levels to occur (7, 8, 21) and for the accumulation of putrescine. These 2 drug effects together with increases in AdoMet might enable cells to overcome the biosynthetic block by MGBG and to maintain growth-permissive levels of spermidine and/or spermine.

Further indication that the phenotypic alteration in the resistant cell lines did not involve polyamine metabolism was provided with dose-response studies using DFMO, the highly specific inhibitor of ornithine decarboxylase (12). All cell lines including the VA2 cells were comparably sensitive to the drug (Chart 2). Although drug-resistant mutants to DFMO have been identified (see above), these particular variants are apparently not among them.

The possibility that changes in mitochondrial function rather than polyamine biosynthesis might represent the basis for MGBG resistance, and hence the critical site of drug action, is consistent with studies comparing the effects of ornithine decarboxylase inhibitors and MGBG on the growth of cultured cells. At least 3 reports (9, 18, 28) have indicated little correlation between the early antiproliferative effects of the drug and polyamine depletion by MGBG. Moreover, a number of studies have detected signifi-
icant early mitochondrial changes in cells treated with MGBG. These include significant decreases in ATP pools (23, 27), pyruvate oxidation (24), and mitochondrial DNA synthesis (25). Ultrastructurally, the mitochondria are swollen extensively and distorted, while other cellular organelles appear unaffected (17, 19, 27).

When this information is taken together with the present data, the possibility of a phenotypic change in the mitochondria of the variants seems probable. We wish to emphasize, however, that 2 intracellular sites of MGBG activity were examined. Almost certainly, other sites exist, and other alterations could lead to a decrease in intracellular MGBG and possibly to less of a drug effect on mitochondrial function. Studies comparing the subcellular distribution of MGBG binding in V2 and the resistant variants are presently under way.

ACKNOWLEDGMENTS

The authors acknowledge gratefully the skilled technical assistance of Barbara Ganis, Edwin Kelly, John Miller, and Deborah Ogden. The gift of DFMO by Merrell-Dow Pharmaceutical Co. is appreciated.

REFERENCES

Fig. 1. Electron micrographs of untreated VA2 (A and B) and VA2 treated for 41 hr with 10 μM MGBG (C and D). Note that following MGBG treatment, many of the mitochondria of the VA2 cells swell to become electron lucent, and several contain amorphous electron-dense inclusions (C and D). A, x 7,560; B, x 14,400; C, x 6,300; D, x 19,800.
Fig. 2. Untreated VA2/MGBG-3 (A) and VA2/MGBG-3 treated with 10 μM MGBG for 41 hr (B). The mitochondria of VA2/MGBG-3 cells (A) are not structurally different from those of the parent line (Fig. 1A), and most are not changed remarkably by MGBG treatment. A, × 7,200; B, × 16,200.
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