Disruption of Cellular Energy Balance by Suramin in Intact Human Prostatic Carcinoma Cells, a Likely Antiproliferative Mechanism

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ABSTRACT

The antiparasitic drug, suramin, has antiproliferative effects in human carcinoma cells. It has been suggested that this occurs through blockade of growth factor-receptor interactions. Three types of evidence that suramin rapidly inhibits cellular respiration or disrupts cellular energy balance in intact cells of the human prostate carcinoma cell line, DU145, are presented. Beginning at approximately 10^{-8} M, suramin rapidly causes dose-dependent inhibition of tetrazolium conversion by mitochondria, demonstrating an inhibition of respiration. This effect is reversed by exchange with suramin-free media but not by pretreatment with serum, epidermal growth factor, insulin-like growth factor I, acidic and basic fibroblast growth factors, or calcium. Rhodamine 123 (10 µg/ml) uptake by mitochondria in intact DU145 cells is inhibited in the presence of 10^{-3} M suramin. Treatment with 10^{-3}–10^{-5} M suramin causes the loss of rhodamine 123 from cells with mitochondria prestained with rhodamine 123, indicating that suramin is acting as an ionophore or respiratory poison. Also shown by electron microscopy are progressive toxic changes in mitochondria of DU145 cells within 1 h after treatment with 10^{-3} M suramin.

These data indicate that in intact DU145 cells 10^{-4} M suramin rapidly disrupts cellular energy balance or respiration as seen by three studies of mitochondrial state. Disruption of energy balance or respiration represents a likely antiproliferative mechanism, as is thought to be a primary mechanism for the action of suramin in parasitic diseases. This proposed mechanism of action for suramin can explain the most prominent observed clinical toxicities of nephrotoxicity, adrenal toxicity, coagulopathy, and demyelinating neuropathy.

INTRODUCTION

Suramin is a polysulfonated naphthylurea traditionally used to treat human trypanosomiasis (African sleeping sickness) caused by Trypanosoma brucei and human onchocerciasis caused by Onchocerca volvulus (1). Suramin has recently been shown to have antitumor effects in human tumors (2, 3). Antiproliferative effects are seen with numerous human cancer cell lines, including human prostate carcinoma cells (4). A favorable response was seen in patients with prostate cancer (5) which led to our interest in the drug and further examination of its activity in prostate carcinoma cell culture.

The antiproliferative mechanism(s) of suramin is not known. Suramin, at low concentrations, has been shown to interfere with numerous assays, including those of of isolated enzymes (1, 6) and growth factor-receptor binding (7–9). It is likely that this compact polyion binds nonspecifically on the basis of charge at regulatory sites or active sites of enzymes and macromolecules, thereby interfering with function. For example, suramin competes for the ATP-binding site of the trypanosomal protein kinase I (10). Nuclear magnetic resonance analysis of the yeast phosphoglycerate kinase indicates that suramin binds to both the ATP- and triose-substrate-binding sites (11). These experiments indicate that suramin may, in particular, inhibit enzymes that are energy dependent (ATP) or that utilize charged substrates.

The ability to inhibit many isolated assays nonspecifically makes it difficult to determine a specific antiproliferative mechanism(s) for suramin. For this reason, in this paper, the proposed mechanism of action of suramin in parasites will be reviewed briefly, and similar effects in intact cells of the human prostate cell line DU145 (12) will be discussed.

In 1904, Ehrlich demonstrated antitrypanosomal activity of trypan red (1). Suramin was developed as a colorless derivative of trypan red by Bayer in 1916 and bears a close structural relationship to trypan blue and trypan red (13). Trypan blue has been used in low concentrations in mammalian cell viability studies, but at higher concentrations it is toxic and effects respiration (14–17). Trypan blue can inhibit cellular hydrolytic activity within 1 h after injection into mice, thus demonstrating a rapid in vivo enzymatic inhibition by a polyanionic substance structurally similar to suramin (18).

A major antitrypanosomal mechanism of suramin is inhibition of glycolysis and respiration. It has been known since 1928 and 1933 that suramin inhibits glycolysis and respiration, respectively, in trypanosomes. This is believed to occur primarily by the inhibition of two key glycolytic enzymes in T. brucei, glycerol-3-phosphate oxidase and glycerol-3-phosphate dehydrogenase (19). Interestingly, both T. brucei (bloodstream forms) and O. volvulus are unusual in that they depend exclusively on glycolysis to obtain energy (20, 21). In a bovine trypanosomiasis treated with suramin, Trypanosoma evansi, an altered malic dehydrogenase confers high level resistance to suramin (22). Suramin resistance via mutation in malic dehydrogenase substantiates interference with respiration or energy balance as a prime site of action for suramin in T. evansi.

Studies of isolated mammalian cell preparations and cells also have shown that suramin effects respiration and energy balance. Suramin inhibits oxygen consumption and ATP synthesis in intact rat liver mitochondrial preparations by inhibition of succinate dehydrogenase, the ATPase complex, and/or adenine nucleotide translocase (23). The H+-ATPase in organelle preparations of bovine adrenal medulla and rat liver lysosomes and mitochondria is inhibited by suramin (24). Suramin has also been shown to inhibit glycolysis in intact human colon adenocarcinoma cells (25).

Knowing that suramin can affect respiration or energy balance in parasites, and in mammalian organelle preparations and cells, we further examined its effect on respiration or energy balance in intact cells by three different assays: (a) conversion of tetrazolium by mitochondrial dehydrogenases, (b) uptake and retention of rhodamine 123 by functional mitochondria, and (c) ultrastructural examination of mitochondria by electron microscopy.
were again frozen and thawed. Next, 100 μM of 10 μg/ml bisbenzimide (bisbenzimidazole; Calbiochem, La Jolla, CA) was added to each well. After 1 h incubation at 37.5°C with 5% CO2, the plates were emptied, and 100 μl of distilled water was added to each well and incubated for 1 h. The plates were mixed, they were measured for absorbance at 515 nm on a 96-well plate reader (Biotek Microplate, Winooski, VT). The wells used as blanks contained no cells.

For the measurement of inhibition of tetrizolium conversion by suramin, the MTT assay was performed as above, immediately after the addition of suramin to microculture plates. Also, various serum-free media; F5, 5% fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; EGF, epidermal growth factor; FGF, fibroblast growth factor; IG F, insulin-like growth factor I; ECGF, endothelial cell growth factor.

RESULTS

Antiproliferative Effect of Suramin. The antiproliferative effect of suramin was demonstrated using the MTT assay (Fig. 1A), DNA assay (Fig. 1B), and anchorage-dependent colony formation assay (Fig. 2). These three assays show that there was no proliferation of DU145 cells after 8 days at a concentration of 10^{-3} M suramin. The antiproliferative effect was dose dependent, and at 10^{-3} M suramin there was no inhibition of growth by the MTT and DNA assays. There was good correlation between the MTT and DNA assays for cell proliferation.

Effect of Suramin on Tetrizolium Conversion. The MTT assay measures a tetrizolium salt conversion to a colored formazan product by mitochondrial enzyme activity and is commonly used to estimate cell number during a period of days (27). However, Fig. 3 demonstrates that suramin rapidly reduced the conversion of tetrizolium dye during 4 h in a dose-dependent fashion. After 4 h, the reduced production of formazan crystals in the mitochondria of DU145 cells can be seen by light microscopy. Fig. 4A shows formazan crystals deposited in micrographs. Du 145 cells were photographed by an electron microscopist who was unaware of the treatments. Electron micrographs were then reviewed by a second electron microscopist who noted and recorded significant findings.

DNA Assay. At time points of interest, 96-well microculture plates were assayed by a fluorometric DNA assay (29) using the fluorochrome rhodamine 123. Rhodamine 123 (Eastman Kodak, Rochester, NY) was dissolved in DME and added to microculture wells for final added concentrations of 0-200 ng/ml. Likewise, acidic FGF (0.5, 5, and 50 ng/ml), basic FGF (1, 10, and 50 ng/ml), and IGF (0.5, 5, and 100 ng/ml) (all from Collaborative Research Inc., Bedford, MA) were added as final concentrations to microculture wells.

Anchorage-dependent Colony-forming Assay. DU145 cells (500/well) were seeded in 1 ml DMEF5 on day -1 into 6-well plates (Corning, Corning, NY). On day 0, suramin was added in 1 ml DMEF5 to achieve the final concentration of 10^{-3} M. Untreated control wells received no suramin. Suramin was removed by exchange with 2 ml suramin-free media at 1 h and 1 and 3 days. Colonies were counted after 8 days in the cultures in which the suramin was not removed and in the untreated control cultures. In the cultures in which the suramin was removed, the colonies were counted 8 days after the removal of the suramin. At time points of interest, wells were stained with crystal violet, and colonies estimated to contain >40 cells were counted with an inverted lens microscope.

Rhodamine 123. Rhodamine 123 (Eastman Kodak, Rochester, NY) was made fresh and used (30) at a final concentration of 10 μg/ml in DMEF5. Cells were grown to 75% confluency on glass coverslips in DMEF5. The coverslips were incubated (incubation media) for 10 min in DMEF5 with rhodamine 123 (10 μg/ml) with or without 10^{-3} M suramin. Coverslips were rinsed several times with DMEF5. Next, coverslips were inverted over slides with depressions wells containing DMEF5 (observation media) with no additives or 1, 0.5, 0.25, and 0.1 x 10^{-3} M suramin. These slides were observed for initial fluorescence, as well as subsequent changes in fluorescence while incubated at 37°C with 5% CO2. Observations were made with a fluorescent microscope at λ485 nm. Suramin and rhodamine 123 did not form a precipitate over the concentration ranges used in these experiments.

Electron Microscopy after Suramin Treatment. DU145 cells were grown in DMEF5 to approximately 50% confluency in 10-cm tissue culture plates. Media was exchanged with media that contained 10^{-4} M suramin in 10 ml DMEF5. At time points of interest, cells were trypsinized from plates by 2 rinses with 0.5% trypsin and prepared for electron microscopy as described previously (31). Briefly, horizontal ultrathin sections were cut, double stained with lead citrate and uranyl acetate, and then viewed in a Phillips 301 electron microscope. Representative cells were photographed by an electron microscopist who was unaware of the treatments. Electron micrographs were then reviewed by a second electron microscopist who noted and recorded significant findings.

The abbreviations used are: DME, Dulbecco's modified Eagle's; F5, 5% fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor I; ECGF, endothelial cell growth factor.

MATERIALS AND METHODS

Cell Culture. The human prostate carcinoma cell line, DU145, was used in experiments. Cells were incubated at 4°C with 5% CO2 in DME media with F5 (both from Gibco BRL, Grand Island, NY). Cells were serially passed into 10-cm tissue culture dishes after trypsinization with two rinses of 0.5% trypsin (Gibco BRL) and then diluted in DMEF5. Cells were counted using a standard hemacytometer. For assays, cells were seeded into 6-well or 96-well plates in 1 ml or 100 μl DMEF5, respectively, 24 h prior to the addition of test substances.

Assays in the Presence of Suramin. A stock solution of 10^{-2} M suramin from FBA, Federal Republic of Germany, was made fresh for each experiment by dissolving in DME. Serial dilutions of this stock in DME or DMEF5 were added to wells containing DME or DMEF5 and to yield the desired final concentrations. Cell proliferation was measured at points of interest by MTT, DNA, and anchorage-dependent colony formation assays.

MTT Assay. At time points of interest, 96-well microculture plates were assayed by the MTT assay (27, 28). MTT (30 μl of 5 mg/ml; Sigma Chemical Co., St. Louis, MO) was added in phosphate-buffered saline to microculture wells containing cells and media. After 4 h incubation at 37.5°C with 5% CO2, the plates were emptied, and 100 μl of dimethyl sulfoxide (Sigma) was added to each well. After the plates were mixed, they were measured for absorbance at 515 nm on a 96-well plate reader (Biotek Microplate, Winooski, VT). The wells used as blanks contained no cells.

For the measurement of inhibition of tetrizolium conversion by suramin, the MTT assay was performed as above, immediately after the addition of suramin to microculture plates. Also, various serum-free media; F5, 5% fetal bovine serum; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor I; ECGF, endothelial cell growth factor.
Effect of Suramin on Rhodamine 123 Uptake and Retention. We further examined the ability of suramin to inhibit mitochondrial function by demonstrating altered rhodamine 123 uptake and retention by the mitochondria of DU145 cells. Suramin interfered with rhodamine 123 (10 μg/ml) uptake and retention (Table 2). There was little or no fluorescence developed by rhodamine 123 in the presence of 10^{-3} M suramin, indicating interference with the ability of mitochondria to accumulate rhodamine 123. If suramin (1, 0.5, 0.25, and 0.1 × 10^{-3} M) was added after cells had accumulated rhodamine 123, the fluor was released into the surrounding media, demonstrating disruption of the ability of the mitochondria to retain rhodamine 123.

Effect of Suramin on Mitochondrial Ultrastructure. Finally, 10^{-4} M suramin caused toxic mitochondrial changes within 1 h as seen by electron microscopy in Fig. 5. These changes became progressively more pronounced with time. There was advanced cell damage by 3 days, as evidenced by the presence of multivesicular bodies that were not observed in control cells.

Reversal of Effects of Suramin. The inhibition by suramin of tetrazolium conversion was reversible by exchange with medium that did not contain the drug as seen by the MTT assay. Inhibition of tetrazolium conversion could not be reversed by the addition of serum, multiple growth factors, or calcium. The reversibility of the antiproliferative effect of suramin after removal of suramin was also demonstrated by anchorage-dependent colony formation at the concentration 10^{-3} M in Fig. 2. If suramin was removed after 1 h or 1 or 3 days by exchange with fresh medium, there was a return of colony formation. However, total colony count decreased with increased time of suramin exposure, indicating some irreversible toxicity at the concentration 10^{-3} M. The reversibility of the antiproliferative effect of suramin by medium exchange and some irreversible toxicity at high suramin concentrations were also seen with the DNA and MTT assays (results not shown).

DISCUSSION

In addition to measuring cell proliferation, the MTT assay was used in this study to measure the immediate inhibition of tetrazolium conversion after the addition of suramin. The conversion of tetrazolium to the formazan product occurs by reduction with NADH or NADPH. At physiological pH and temperature, this is a specific enzymatic reaction that directly
DISRUPTION OF CELLULAR ENERGY BALANCE BY SURAMIN

Fig. 4. Light micrographs of suramin-treated cells and MTT assay. Light micrographs of DU145 cells after 4 h incubation for MTT assay without (A) and with 10^{-3} M (B) suramin.

Table 1 Reduction of tetrazolium conversion during 4 h by 10^{-3} M suramin in the presence of serum, epidermal growth factor, or calcium

DU145 cells (5 x 10^4) were plated for 1 h with 10^{-2} M suramin (as described for Fig. 3). After 1 h, fetal calf serum, EGF, or Ca^{2+} were added in 100 µl DMEF5 to achieve final concentrations. At this time, an MTT assay was performed, and tetrazolium conversion was measured after 4 h. Tetrazolium conversion by A is expressed as a percentage of a control that did not contain suramin. Each entry represents the average of eight determinations ± SD.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% A vs. control</th>
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<tbody>
<tr>
<td>Fetal calf serum (%)</td>
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</tr>
<tr>
<td>5</td>
<td>59.7 ± 6.7</td>
</tr>
<tr>
<td>7</td>
<td>51.3 ± 7.6</td>
</tr>
<tr>
<td>10</td>
<td>47.9 ± 7.6</td>
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<tr>
<td>12</td>
<td>48.7 ± 9.2</td>
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<tr>
<td>14</td>
<td>47.9 ± 10.1</td>
</tr>
<tr>
<td>16</td>
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</tr>
<tr>
<td>EGF (ng/ml)</td>
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</tr>
<tr>
<td>0</td>
<td>58.8 ± 5.9</td>
</tr>
<tr>
<td>1</td>
<td>55.5 ± 4.2</td>
</tr>
<tr>
<td>10</td>
<td>52.1 ± 5.0</td>
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<td>15</td>
<td>50.4 ± 5.0</td>
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<tr>
<td>20</td>
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</tr>
<tr>
<td>Ca^{2+} (mM)*</td>
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</tr>
<tr>
<td>1</td>
<td>51.3 ± 3.6</td>
</tr>
<tr>
<td>2</td>
<td>51.6 ± 2.5</td>
</tr>
<tr>
<td>3</td>
<td>52.7 ± 3.3</td>
</tr>
<tr>
<td>11</td>
<td>50.3 ± 2.5</td>
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* A total of 2 x 10^4 cells were plated.

Table 2 Rhodamine 123 fluorescence in the presence of suramin

DU145 cells were plated on glass coverslips and allowed to proliferate to about 75% confluency in DMEF5. Coverslips were incubated for 10 min in DMEF5 (incubation media) containing rhodamine 123 (10 µg/ml, suramin (10^{-3} M), or both. Coverslips were then washed with several rinses of DMEF5. Next, coverslips were inverted over slides with depression wells containing DMEF5 (observation media) with no additives or suramin (1, 0.5, 0.25, or 0.1 x 10^{-3} M).

<table>
<thead>
<tr>
<th>Additives to incubation media</th>
<th>Additives to observation media</th>
<th>Fluorescence*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodamine</td>
<td>None</td>
<td>+ (+)</td>
</tr>
<tr>
<td>Rhodamine + Suramin</td>
<td>None</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Rhodamine + Suramin</td>
<td>Suramin</td>
<td>+ (becomes 0)</td>
</tr>
</tbody>
</table>

* +, positive fluorescence; 0, negative fluorescence. Fluorescence was observed by fluorescent microscopy at the excitation wavelength 485 nm. The initial presence or absence of fluorescence is indicated, and subsequent loss or development of fluorescence at 4 h is indicated in parentheses.

reflects activity of cellular dehydrogenases and is not affected by glutathione or other strong reducing substances in the cell (32, 33). The MTT assay measures dehydrogenase activity in the respiratory chain in at least two sites (34). The NADH dehydrogenases are involved primarily in the energy-producing reactions of the respiratory chain: glycolysis, TCA cycle, and oxidative phosphorylation. The NADPH dehydrogenases are primarily involved in biosynthetic reductive reactions such as the hepatic P-450 system and the production of adrenal steroid hormones (35).

These dehydrogenases are located primarily in the mitochondria and, in some cells, to a small degree in the endoplasmic reticulum. Mosmann (27) demonstrated that up to 200,000 RBCs, metabolically active cells that contain no mitochondria or endoplasmic reticulum, convert no or little tetrazolium. This correlates with the deposition of the insoluble formazan crystals in the mitochondria (32) and can be seen in DU145 cells in Fig. 4A. Dehydrogenase activity directly reflects cellular respiration and has been used in the past to study the effect of drugs on respiration (36, 37).

To our knowledge this is the first study to use the MTT assay to evaluate the effect of a drug on respiration or energy balance. The rapid and reversible inhibition of tetrazolium conversion by suramin in intact DU145 cells demonstrates inhibition of dehydrogenase activity, a direct reflection of diminished cellular respiration and mitochondrial activity. This inhibition of tetrazolium conversion by suramin begins at approximately 10^{-3} M (143 µg/ml), which is below clinically desired plasma levels (>200 µg/ml) at which antitumor effects are seen (3).

The inhibition of tetrazolium conversion by suramin is a rapid event, occurring during <4 h. Various substances known to stimulate DU145 cell growth or substances known to be antagonized by suramin were tested for their ability to reverse the rapid inhibition of tetrazolium conversion by suramin. In our laboratory, serum (1-10%) stimulates DU145 cell growth. EGF and EGF receptors are present in DU145 cells and EGF stimulates proliferation in DU145 cells (38-40). IGF and heparin-binding growth factor-stimulated proliferation are antagonized by suramin in other cell systems (41, 42), and calcium has been shown to reverse suramin inhibition of growth (43). None of these substances reversed the rapid inhibition of tetrazolium conversion by suramin in DU145 cells when they were tested over a broad range of concentrations. In addition, we have examined >12 primary prostate epithelial cultures and have not been able to reverse the effects of suramin with the addition of EGF, ECGF, basic FGF, calcium, bovine pituitary extract, or transforming growth factor β (44). Thus, inhibition of tetrazolium conversion was not competitively reversed by the addition of serum, multiple growth factors, or calcium. The rapid inhibition by suramin of tetrazolium conversion in intact DU145 cells indicates disruption of cellular energy balance or respiration at suramin concentrations that are routinely achieved in human plasma.

The ability of mitochondria to accumulate and retain the fluorescent compound, rhodamine 123, is dependent on maintenance of the energy-dependent transmembrane potential of the mitochondria (30). Demonstrated in this report is rapid inhibition by suramin of the uptake and retention of rhodamine 123 by mitochondria of DU145 cells. This indicates the loss of the energy-dependent transmembrane potential of the mito-
Fig. 5. Electron micrographs of suramin-treated DU145 cells. a, untreated control cells at 0 h. Mitochondria (M) have normal cristae (× 18,000). b, 10⁻⁴ M suramin at 1 h. Loss of cristae is already present (*) (× 18,000). c, 10⁻⁴ M suramin at 2 h. Mitochondria show extreme loss of cristae (arrow) (× 18,000). d, 10⁻⁴ M suramin at 24 h. Swollen mitochondria are present (arrowhead) (× 23,700). e, 10⁻⁴ M suramin at 3 days. Multivesicular bodies are now evident in the cytoplasm (arrow) (× 18,000). f, control at 3 days. Neither multivesicular bodies nor swollen mitochondria (M) are present (× 13,000).

Mitochondria. Interference with rhodamine 123 staining of this type has been described as characteristic of inhibitors of electron transport in the respiratory chain or ionophores (45). Again, these effects can be demonstrated at 143 mg/ml (10⁻⁴ M) suramin, a concentration that can be achieved in human plasma. The rapid disruption by suramin of rhodamine 123 uptake and retention by mitochondria in intact DU145 cells indicates that suramin acts as a respiratory poison or ionophore in intact cells at suramin concentrations that are achievable in human plasma.

Finally, our findings document the rapid toxic mitochondrial changes by electron microscopy, further demonstrating an effect by 10⁻⁴ M suramin on cellular energy balance. These effects occur rapidly, within 1 h, again, at suramin concentrations that are achievable in human plasma.

Kopp and Pfeiffer (46) demonstrated that suramin had a noncompetitive interaction with EGF receptors in HT-29 cells and showed its effect on phospholipid metabolism. The studies we performed with tetrAZolium conversion also show noncompetition with respect to suramin and growth factors. We believe that suramin may inhibit growth factor-stimulated proliferation but at a site beyond the binding of the growth factor to its receptor.

The effects seen with suramin at low concentrations on respiration and energy balance of intact cells occurs so rapidly that we think it may involve interference with surface membrane structures. Our data from the rhodamine 123 studies show that suramin may act as an ionophore. Further studies are under way in our laboratory to answer this question. In addition, Dunn and Blakely (47) showed that suramin functionally is a reversible antagonist of cell surface P₂-purinoreceptors. Inhibition of intracellular calcium release and growth factor-induced increases in cytoplasmic free calcium (48) and phosphoinositide
synthesis (46) illustrates how suramin could inhibit growth factor-stimulated second messenger signaling. Extramitochondrial calcium concentrations have been shown to regulate activity of calcium-dependent mitochondrial dehydrogenases (49). Interference by suramin at any of these sites could explain how suramin might inhibit growth factor stimulation of respiration and, hence, proliferation.

The inhibition of glycolysis and respiration by suramin in trypanosomiasis and onchocerciasis provides a model to suggest a mechanism of action of suramin on intact organisms. It is probable that the antiproliferative mechanism of suramin on intact DU145 cells involves the inhibition of respiration or disruption of cellular energy balance as seen by three different studies in this report. The inhibition by suramin of glycolysis in a human colon adenocarcinoma cell line also suggests that suramin inhibits respiration or energy balance in intact mammalian cells (25).

The disruption of cellular energy balance or respiration may explain the most prominently observed clinical toxicities of nephropathy, adrenal toxicity, coagulopathy, and demyelinating neuropathy. The function of mitochondrial dehydrogenases are crucial to the function of the kidney and adrenal gland. The adrenal mitochondrial P-450 enzymes are responsible for steroid biosynthesis and have been shown to be inhibited by suramin (50). Coagulation factors must undergo micosomal enzymatic carboxylation in order to have functional activity (51). Demyelinating neuropathy may reflect the effect of suramin on the oligodendroglia cells which are especially sensitive to cytotoxic anoxic damage (52).

In summary, it is apparent that suramin may act as an antiproliferative agent, at least in part, via the disruption of respiration or cellular energy balance. Energy balance and cellular respiration will need to be considered with regard to drug interactions, toxicities, and therapy.

REFERENCES

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