Disruption of Mitochondrial Function by Suramin Measured by Rhodamine 123 Retention and Oxygen Consumption in Intact DU145 Prostate Carcinoma Cells

Randall P. Rago, Peter C. Brazy, and George Wilding

Abstract

Suramin, an antiparasitic drug, has shown antitumor activity in humans. This may occur in part through disruption of energy balance, which is believed to be part of its antiparasitic action. Suramin disrupts mitochondrial function in intact DU145 prostate carcinoma cell monolayers as seen by its causing the release of rhodamine 123 from prestained cells beginning at about 10 μM in 96-well microtiter plates measured with a fluorescent plate scanner. This effect was similar to the ionophore carbonyl cyanide m-chlorophenylhydrazone, dissolved in ethanol at 0.01 N and indicates that suramin acts as a respiratory poison or an ionophore. This effect was confirmed by studies of oxygen consumption with a Clark oxygen electrode and cellular ATP content which demonstrated uncoupling of oxidative phosphorylation by 100 μM suramin, a clinically achievable plasma drug level.

Introduction

Suramin, a polysulfonated naphthylurea, is an antitrypanosomal agent that has been used to treat African sleeping sickness and onchocerciasis since the 1920s (1). Suramin is known to inhibit respiration and glycolysis in trypanosomes, and this is believed to be part of the antiproliferative mechanism of suramin in trypanosomes (2). Suramin has been shown to have antiproliferative effects in human tumors in vivo and in vitro (3–5). Suramin is active in many in vitro assays and this has resulted in the proposal of numerous antiproliferative mechanisms. Part of the antiproliferative effect of suramin in tumors may involve inhibition of energy balance, as in trypanosomes. Inhibition of respiration in isolated mammalian mitochondria (6) and inhibition of glycolysis in colon carcinoma cells (7) have been demonstrated with suramin. We have previously shown that suramin inhibits mitochondrial activity in intact DU145 prostate carcinoma cells at concentrations which are clinically achievable and active in humans (8). This was seen by inhibition of tetrazolium conversion by mitochondrial dehydrogenases in the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, inhibition of rhodamine 123 mitochondrial uptake and retention by fluorescent microscopy, and toxic mitochondrial changes seen by electron microscopy. In this article we provide additional quantitative and confirmatory studies documenting the effect of suramin on mitochondria in intact DU145 human prostate carcinoma cells. Cell monolayers with mitochondria prestained with rhodamine 123 were measured for loss of rhodamine 123 fluorescence after suramin treatment.

Oxygen consumption and ATP were measured before and after suramin addition in cell suspensions.

Materials and Methods

DU145 human prostate carcinoma cells were grown at 37°C and 5% CO2 to approximately 75% confluency in DMEF5 and were trypsinized from plates with two rinses with 0.5% trypsin. For rhodamine 123 studies 20,000 DU145 cells/well were seeded in 100 μl of DMEF5 into 96-well microculture plates on day −1. Twenty-four h later cells were incubated with 10 μg/ml of rhodamine 123 for 10 min, and then the wells were emptied by blotting the microtiter plates on toweling. The cells were rinsed twice with phosphate-buffered saline and emptied each time. The cells were incubated with suramin in DMEF5 at final concentrations of 10, 100, and 1000 μM suramin or 1, 10, and 100 μM CCCP. At time points of interest the wells were again emptied and rinsed with phosphate-buffered saline. The retained fluorescence was measured after lysing cells by the addition of 100 μl of distilled water to the wells. The fluorescence was measured with a 96-well fluorescent plate scanner (Fluoroskan II; Flow Laboratories, McLean, VA) with filters reading fluorescence. The fluorescence in the presence of suramin or CCCP is expressed as a percentage of corresponding controls which contained no suramin or CCCP.

Oxygen consumption QO2 were determined by standard methods (9) with a Clark-type oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, OH). DU145 cells prepared as above were washed in a Krebs-Ringer bicarbonate medium that contained lactate (5 mM), alanine (1 mM), and malate (1 mM) as the metabolic substrates (no glucose). The DU145 cells were suspended in this medium at a concentration of 2–3 mg protein/1.7 ml. The suspension was oxygenated and preincubated for 20 min at 37°C and then placed in the closed, thermostated (37°C) chamber (1.7 ml) for measurement of QO2. A control QO2 was determined over the next 2 to 5 min. Then 100 μM suramin or 1 μM oligomycin followed by 100 μM suramin (final concentrations) was added to the chambers and QO2 was determined. At the end of the observation period aliquots of the cell suspension were taken for the measurement of protein (10) and ATP (11). The data are factored by the amount of protein in the suspension. Time controls for DU145 cells gave a linear rate of oxygen consumption over a 5– to 10-min observation period. Suramin was dissolved in saline and added to the suspension at a final concentration of 100 μM. Oligomycin was dissolved in 95% ethanol and was added to the suspension at a final concentration of 1 μM. The experiments were performed on five to seven preparations of DU145 cells, with duplicate samples being run for each preparation.

Results and Discussion

Rhodamine 123 is a fluorescent cation that localizes to mitochondria and its retention is dependent on the maintenance of the negative electrochemical gradient across the mitochondrial membrane. Rhodamine 123 release from prestained cells has been demonstrated with suramin addition in cell suspensions.

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been shown to be caused by compounds that are respiratory poisons or ionophores (12). The inhibitory effect of suramin on the retention of rhodamine 123 is shown in Fig. 1A. Suramin caused a dose-dependent and time course-related decrease in mitochondrial retention of rhodamine 123 in a manner similar to the ionophore CCCP (Fig. 1B). The plots show that suramin is likely a weaker respiratory poison or ionophore than the ionophore CCCP. This effect was seen at less than 1 h and beginning at approximately 10 μM suramin. This concentration is less than one-tenth the concentration of suramin routinely measured in plasma of patients in current Phase I trials. Equivalent results were obtained in four other experiments.

The data for measurements of cell oxygen consumption rates and ATP content are given on Table 1. The control rate of Q_o2 for DU145 cells was 16.8 ± 1.2 nmol/mg protein/min. The addition of suramin acutely increased this rate to 170% without an increase in ATP content. In the presence of oligomycin, an inhibitor of mitochondrial ATPase and oxygen-coupled respiration (13), Q_o2 was reduced to 5.3 ± 1.0 nmol/mg protein/min. Addition of suramin acutely increased Q_o2 to 515%. Increasing oxygen consumption in the presence of oligomycin clearly indicates that suramin behaves as an uncoupler of mitochondrial respiration in these cells.

Table 1: Acute effect of suramin on oxygen consumption by DU145 cells

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Q_o2 (nmol/mg protein/min)</th>
<th>ATP (nmol/mg protein)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>16.8 ± 1.2</td>
<td>14.0 ± 1.3</td>
</tr>
<tr>
<td>+ Suramin, 100 μM</td>
<td>28.8 ± 3.2</td>
<td>12.4 ± 1.1</td>
</tr>
<tr>
<td>Oligomycin, 1 μM</td>
<td>5.3 ± 1.0</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td>+ Suramin, 100 μM</td>
<td>27.3 ± 6.4</td>
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</tr>
</tbody>
</table>

Values are the average ± SD of n determinations. Each determination was the average of two samples run simultaneously.

The immediate effect of suramin contact with DU145 cells is to "uncouple" oxidative phosphorylation by increasing mitochondrial respiration without an increase in ATP production. This modest "uncoupling" of oxidative phosphorylation may deprive the cell of ATP. Dissipation of the mitochondrial membrane potential, seen with rhodamine 123 studies, also indicates that the cell could not generate ATP effectively. While it is not clear that these events would lead to cell death, ATP deprivation would likely be associated with antiproliferative effects.

In our previous studies (8) we showed that even a high concentration of 1000 μM suramin for 3 days had largely a static effect on DU145 cells. Greater than 50% of colonies in a colony formation assay could be counted 8 days after drug removal. There was some irreversibility toxicity seen, however, with long drug exposure. Also seen by electron microscopy were time-dependent mitochondrial changes. After a 1-h exposure of DU145 cells to 100 μM suramin, the mitochondria swelled, with progressive toxic mitochondrial changes. Later disruption of cristae with intramitochondrial precipitates, followed by progression to end-stage multivesicular bodies, was documented. These findings were similar to those seen with two sulfonilurea compounds that caused uncoupling of oxidative phosphorylation in isolated murine liver mitochondria and swelling of mitochondria at 24 h in a colon carcinoma cell line (14). In all of these studies it is unclear if mitochondrial changes or ATP deprivation accounts for cell death or is the major antiproliferative mechanism. These issues are the object of ongoing studies.

All of our mitochondrial studies have been performed using intact DU145 cells rather than cell preparations or isolated mitochondria. How suramin affects this intracellular organelle and its function is uncertain. We hypothesize that suramin affects mitochondria by interacting with the plasma membrane and perhaps by affecting ion fluxes or second messenger systems.

We have recently presented evidence that suramin can cause a mitochondrial myopathy and Fanconi's syndrome in humans, indicating a mitochondrial toxic effect in humans (15). These clinical entities of mitochondrial myopathy and Fanconi's syndrome are seen together in the De Toni-Fanconi-Debre (16, 17) syndrome which is the congenital deficiency of mitochondrial cytochrome c oxidase. This supports the concept that suramin can inhibit mitochondrial activity in humans and this mechanism may be involved in other known toxicities. Suramin has unique mechanisms which require further study to develop new therapeutic strategies and therapies and decrease toxicities.

References


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