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Effects of Pentamidine Isethionate on *Saccharomyces cerevisiae*

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We used *Saccharomyces cerevisiae* as a model system in which to examine the mechanism of action of the anti-*Pneumocystis* drug pentamidine. Pentamidine at low concentrations inhibited *S. cerevisiae* growth on nonfermentable carbon sources (50% inhibitory concentration [IC_{50}] of 1.25 μg/ml in glycerol). Pentamidine inhibited growth on fermentable energy sources only at much higher concentrations (IC_{50} of 250 μg/ml in glucose). Inhibition at low pentamidine concentrations in glycerol was due to cytostatic activity rather than cytotoxic or mutagenic activity. Pentamidine also rapidly inhibited respiration by intact yeast cells, although inhibitory concentrations were much higher than those inhibitory to growth (IC_{50} of 100 μg/ml for respiration). Pentamidine also induced petite mutations, although only at concentrations much higher than those required for growth inhibition. These results suggest that a function essential for respiratory growth is inhibited by pentamidine and that pentamidine affects mitochondrial processes. We propose the hypothesis that the primary cellular target of pentamidine in *S. cerevisiae* is the mitochondrion.

Pentamidine is widely used for the prophylaxis and treatment of *Pneumocystis carinii* pneumonia in AIDS patients (32). Pentamidine is also used to treat African trypanosomiasis and leishmaniasis (33). Unfortunately, pentamidine is not always effective, and it frequently causes undesirable side effects (21). Although pentamidine has been used in clinical settings for 40 years, uncertainty remains about its mechanism of action. In addition, widespread use of pentamidine as a prophylactic provokes concern over the evolution of pentamidine resistance in *P. carinii* (32, 35). Resistance to pentamidine, both natural and acquired, occurs in trypanosomes (11, 17).

Although one can investigate the effects of pentamidine on *P. carinii* by using immunocompromised animals (20) or an in vitro microculture test system (6), detailed studies are hindered by the inability to culture *P. carinii* in vitro. Therefore, we have identified a model organism, *Saccharomyces cerevisiae*, that is sensitive to pentamidine under appropriate conditions and that can be readily manipulated by sophisticated biological techniques.

In phylogenetic terms, *S. cerevisiae* is an appropriate model for *P. carinii*. Considerable molecular and morphologic evidence indicates that *P. carinii* is a fungus (29). Molecular similarities of *P. carinii* to the fungi include not only the rRNA genes (14, 30) but also many other genes, such as the dihydrofolate reductase (13) and thymidylate synthase (15) genes. An authoritative analysis of the rRNA phylogeny, including appropriate outgroups, indicates that *P. carinii* diverged from (or within) the *Ascomycetes* lineage very early in fungal evolution, such that *P. carinii* is as closely related to *S. cerevisiae* as it is to any other known fungus (5).

Pentamidine has been implicated in the inhibition of a bewildering variety of cellular processes in various systems (10, 12, 18, 23, 25, 26). Much recent work has focused upon the interactions of pentamidine with DNA (9, 12, 16). Clearly, pentamidine can affect many different cellular processes. The process most critical to pentamidine inhibition in one organism may differ from that most critical to inhibition in a second organism. We have characterized the effects of pentamidine on *S. cerevisiae* in order to identify potential inhibitory mechanisms. The taxonomic relationship of *S. cerevisiae* and *P. carinii* suggests that these mechanisms may be similar and that they should also be examined for *P. carinii*.

Our results lead us to propose the hypothesis that the primary cellular target of pentamidine is the mitochondrion. We have determined that pentamidine inhibits respiration and that it induces mutations to a petite (mitochondrially deficient) phenotype. However, we conclude that these effects are probably not the primary reason why pentamidine inhibits growth under conditions that require mitochondrial function in *S. cerevisiae*.

MATERIALS AND METHODS

Reagents. Growth media were from Difco or Gibco. Standard media were yeast nitrogen base with ammonium as nitrogen source (YNB) or yeast extract plus peptone (YP), with either dextrose (YPD) or glycerol (YPG) at 2% used as the carbon source. Other carbon sources were used at 2%. Pentamidine isethionate (Sigma) was dissolved in sterile water at 25 mg/ml and stored at −20°C. Other reagents were from standard laboratory suppliers.

Yeast strains. Our standard laboratory strain was MGD353-46D (*MATα trp1-289 ura3-52 leu2-3 leu2-112 his3-Δ1 cyh4^r*), obtained from B. Rymond, University of Kentucky. Other laboratory strains were also sensitive to pentamidine. Most laboratory strains, including MGD353-46D, derive from S288C, which carries a cryptic *gal2* (*imp1*) mutation (31). The *gal2* mutation renders strains unable to grow on galactose when mitochondrial respiration is inhibited. Strains wild type at this locus (*GAL2*) and disrupted at the locus (Δ*gal2*) were provided by J. Jaehning, Indiana University.

Assays of yeast growth and inhibition by pentamidine or other inhibitors. Quantitative assays of yeast growth were based on turbidimetry. Saturated cultures of yeast cells were diluted 1:500 into fresh medium. The cell suspension (100 μl) was added to the wells of a microtiter dish with appropriate concentrations of drug to be tested in 100 μl of medium. The microtiter plates were closed with a lid and shaken in a humidified 30°C incubator for 24 h (glucose medium) or 48 h (glycerol medium). Turbidity was measured with a Bio-Rad

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450 microtiter plate reader at 550 nm. Optical densities (above background) were plotted versus drug concentrations; we defined the 50% inhibitory concentration (IC_{50}) as the tested drug concentration that reduced the cell number to approximately 50% of that of the control.

Qualitative assays of yeast growth were based upon evaluation of growth on media solidified with agar. Petri plates containing various drug concentrations were inoculated with streaks or individual yeast colonies and incubated at 30°C. Plates containing glycerol medium were incubated 7 to 10 days before scoring for inhibition; glucose plates were typically incubated for 3 days. Qualitative assays were used to confirm results of the turbidimetry and to determine growth dependence upon the carbon source and upon the yeast strain.

Measurement of respiration. Respiration was assayed with an oxygen polarograph (G. Johnson, Baltimore, Md.) by the uptake of O_2 in a stirred oxygen electrode chamber (Yellow Springs Instruments). In general, the chamber was filled with 1.3 ml of yeast culture and the concentration of oxygen was continuously measured. Yeast cells had been grown overnight in YPG medium, centrifuged briefly, and resuspended in the original volume of water for the measurements. Small volumes (1 to 50 μl) of drugs were added to the stirred cells to determine the relationship of drug concentration to inhibition. Rates of oxygen uptake are reported relative to the rate of oxygen uptake before inhibitors were added. The IC_{50}s reported are the tested concentrations that inhibited oxygen uptake 50% relative to that of untreated control cultures. In our assays, we observed a residual rate of oxygen disappearance that did not appear to be related to oxygen uptake by the cells. This residual rate of disappearance (approximately 10% of the control rate for saturated yeast cultures) was insensitive to cyanide and other known inhibitors of yeast respiration. We believe that this apparent residual oxygen uptake is a function of the measurement system rather than of an alternate oxidase system.

RESULTS

Inhibition of yeast growth by pentamidine. Pentamidine at a low concentration (approximately 1 μg/ml) inhibited yeast growth only under conditions in which yeast cells required mitochondrial respiratory function. For example, wild-type yeast cells rely upon mitochondrial electron transport during growth on glycerol but not when fermenting glucose. As shown in Fig. 1, growth of wild-type yeast cells on glycerol medium was sensitive to low concentrations of pentamidine (IC_{50} of 1.25 μg/ml). Growth in glucose medium was sensitive only at extremely high levels of pentamidine (IC_{50} of 250 μg/ml). We investigated the pentamidine sensitivity of growth on a number of other energy sources (Table 1). Only on energy sources that required mitochondrial respiratory function was wild-type yeast growth inhibited by concentrations of pentamidine below 10 μg/ml.

Pentamidine inhibited yeast growth on all tested nitrogen sources: ammonia, urea, and glutamine (not shown). Inhibition was observed in minimal media or in complex, rich media when nonfermentable energy sources were used. (We use the term "energy source" rather than "carbon source" because carbon is available from other components, such as amino acids, in the complex medium.) Inhibition was largely independent of the KCl (0 to 500 mM), $MgCl_2$ (0 to 100 mM), or $CaCl_2$ (0 to 100 mM) concentration in the medium. Addition of polyamines (0.04 mM spermine, 1 mM spermidine, or 1 mM putrescine) to the medium did not relieve inhibition.

Independence of inhibition from cell genotype. All laboratory yeast strains that we tested were sensitive to low concentrations of pentamidine on glycerol but not on glucose. Sensitivity did vary slightly from strain to strain. Strain MGD353-46D was more sensitive than all other strains that we tested. Diploid strains were as sensitive as haploid strains. We have isolated pentamidine-resistant mutations (19) in the MGD353-46D and other cell backgrounds (22a), but we have not found such mutations in normal laboratory strain backgrounds.

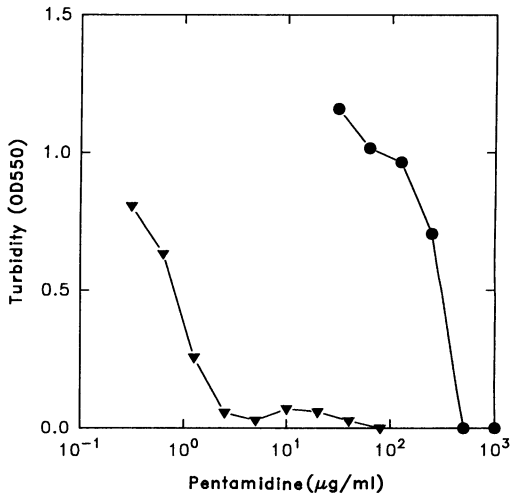

FIG. 1. Effect of pentamidine on yeast growth in glycerol or glucose medium. Turbidity (optical density at 550 nm [OD550] minus blank) is plotted versus pentamidine concentration on a logarithmic axis. ●, cultures grown in glucose medium; ▼, cultures grown in glycerol medium.

Effect of the *impl* mutation on pentamidine sensitivity. We observed that strain MGD353-46D was sensitive to pentamidine on galactose as well as on glycerol (Table 1). This initially led us to believe that pentamidine sensitivity might be due to a catabolite-repressible process. However, we became aware that many laboratory strains carry a mutation, *impl*, in the gene encoding the galactose permease *GAL2* (31). The *impl* mutation renders yeast cells unable to grow on galactose in the absence of respiring mitochondria. In fact, MGD353-46D apparently carries the *impl* mutation, because it was unable to grow on galactose anaerobically or in the presence of the respiratory inhibitor antimycin A (not shown). In contrast to MGD353-46D, strains wild type at the *GAL2* locus grew on galactose anaerobically or in the presence of antimycin A or

TABLE 1. Dependence of pentamidine inhibition of yeast growth on energy source

Energy source	Fermentation[a]	Inhibition[b]
Hexoses (glucose, fructose, mannose)	F	NI
Sorbose	NF	I
Galactose[c]	NF	I
Pentoses (arabinose, lyxose, ribose, xylose)	NF	I
Glycerol	NF	I
Lactate	NF	I
Citric acid cycle intermediates (succinate, malate, α-ketoglutarate, citrate)	NF	I

[a] NF, not fermentable; F, fermentable.
[b] I, pentamidine inhibitory at 10 μg/ml on this source; NI, not inhibitory.
[c] Galactose does not support growth in the absence of respiratory function by the MGD353-46D (*gal2*) strain tested.

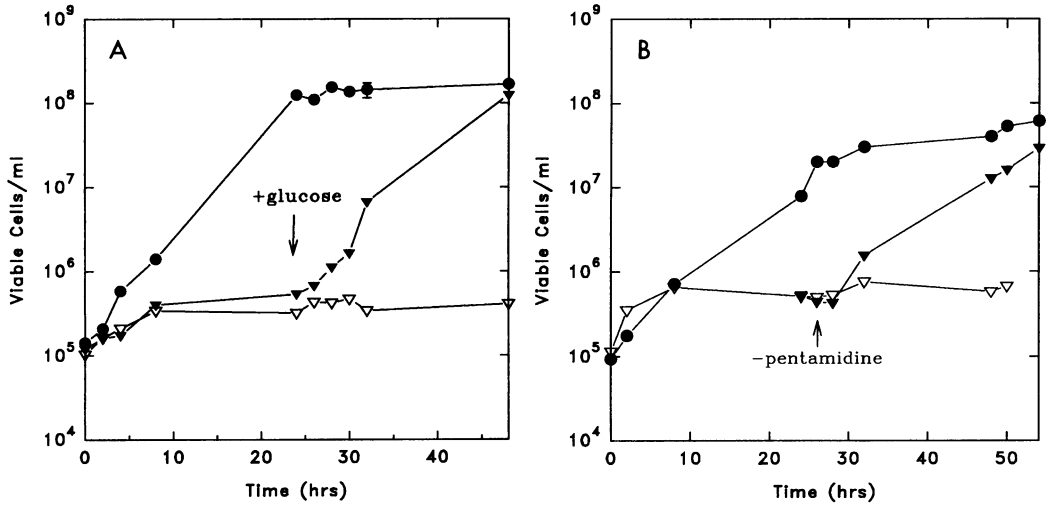

FIG. 2. Reversibility of pentamidine-induced growth inhibition by glucose. A saturated culture of yeast cells grown in glucose medium was diluted 1:1,000 into glucose (YPD [●]) or glycerol (YPG [▼ and ▽]) medium with 25 μg of pentamidine per ml. At 24 h, glucose was added to one portion of the YPG-pentamidine cultures to a final concentration of 2% (▼). The numbers of viable cells in cultures were determined at intervals by plating samples of the cultures on YPD medium at the indicated times. (B) Reversibility of pentamidine-induced growth inhibition by removal of inhibitor. A saturated culture of yeast cells grown in YPD medium was diluted 1:1,000 into YPG medium with (▽) or without (●) 25 μg of pentamidine per ml. At 24 h, the YPG-pentamidine culture was centrifuged and washed, and portions were resuspended in fresh YPG medium (▼) or fresh YPG medium with 25 μg of pentamidine per ml (▽). The numbers of viable cells in cultures were determined at intervals by plating samples of the cultures on YPD medium at the indicated times.

pentamidine. A strain disrupted at the *GAL2* locus (Δ*gal2*) was more sensitive to pentamidine than an isogenic *gal2*(*imp1*) mutant in galactose medium but was equally sensitive in glycerol medium (data not shown).

Effects of pentamidine on cell viability and induction of petite mutants. Pentamidine inhibition of yeast growth in glycerol medium was reversible (Fig. 2). Yeast cells transferred from glucose medium into YPG medium with 25 μg of pentamidine per ml doubled once, but then the culture remained static for at least 48 h. Growth inhibition was reversed either by the addition of glucose (Fig. 2A), a fermentable energy source, to the inhibited culture or by the removal of pentamidine and suspension in fresh glycerol medium (Fig. 2B). The initial doubling after suspension in glycerol medium may be due to the cell's ability to traverse the cell cycle without mitochondrial function. Growth rates in either glucose or glycerol after removal of inhibition were the same as those for cultures that had not been inhibited. This indicates that the metabolism of the viable cells was not affected by cytostasis in pentamidine. At higher levels (100 μg/ml), pentamidine did have a cytotoxic effect upon prolonged incubation (data not shown). Cytotoxicity may be due to a secondary toxic mechanism at high concentrations.

Agents like ethidium bromide, which binds to DNA, induce mutation and loss of the mitochondrial DNA. Yeast cells that lose or suffer mutations in mitochondrial DNA become unable to respire. Such mutants have a petite phenotype, forming smaller colonies on glucose medium and failing to grow on glycerol. Because this phenotype is similar to the results of pentamidine inhibition, we determined whether pentamidine induced petite mutations.

Exposure of yeast cells to pentamidine in glucose medium induced petite mutations (Table 2). Induction of petite mutations was dependent on the dose of pentamidine. However, even at the highest concentration (100 μg/ml), pentamidine induced petite mutations at a lower frequency than ethidium bromide at 10 μg/ml. Exposure to inhibitory concentrations of pentamidine in glycerol medium also induced petite mutation formation at a frequency higher than that in untreated controls (Table 2). However, the induction of petite mutations does not account for the growth inhibition observed in glycerol, since static glycerol cultures (at 10 μg/ml) contained predominantly grande (respiratory-competent) yeast cells. Consistent with this conclusion, removal of pentamidine from inhibited glycerol cultures and resuspension in fresh glycerol medium released the inhibition of respiratory growth (Fig. 2B).

Effects of pentamidine and other inhibitors on yeast growth and respiration. Pentamidine directly inhibited yeast respiration in vivo. Respiration of intact cells, measured by O_2 uptake, was rapidly inhibited by pentamidine (Fig. 3A). Known inhibitors of respiration such as antimycin A had effects on respiration similar to those of pentamidine (Fig. 3B). As expected, inhibitors of processes other than respiration (even those that affect other mitochondrial functions) did not inhibit oxygen uptake (Fig. 3C and D). Inhibition of oxygen uptake immediately after drug addition was dose dependent. Rapid inhibition

TABLE 2. Induction of petite phenotype by pentamidine[a]

Medium	Total no. of cells ml^{-1}	No. of petite cells ml^{-1}	% of petite cells
YPD	3.6×10^8	0.24×10^8	6.7
YPD + pentamidine (10 μg/ml)	2.0×10^8	0.62×10^8	31
YPD + pentamidine (100 μg/ml)	2.8×10^8	2.5×10^8	90
YPD + ethidium bromide (10 μg/ml)	1.1×10^8	1.1×10^8	100
YPG	1.5×10^7	2.5×10^5	2
YPG + pentamidine (10 μg/ml)	3.2×10^6	5.1×10^5	16
YPG + pentamidine (100 μg/ml)	1.1×10^6	4.6×10^5	41

[a] A total of 10^6 cells of an overnight yeast culture were incubated for 24 h in the indicated medium at 30°C. Aliquots were plated on YPD medium. Petite yeast cells formed small colonies on glucose medium and did not grow when transferred to glycerol.

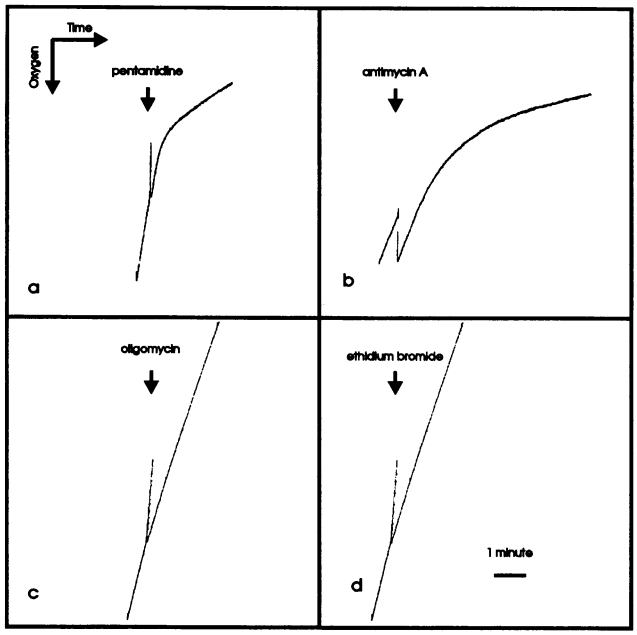

FIG. 3. Effect of pentamidine and other inhibitors on O_2 uptake by yeast cells. Polarographic traces record oxygen concentration versus time in yeast cultures treated as indicated. The slope of the trace is the rate of oxygen uptake. Discontinuities in each curve represent the addition of inhibitor. The oxygen concentration was directly proportional to the polarographic signal. With oligomycin and ethidium bromide, no inhibition of O_2 uptake was observed 20 min after inhibitor addition. The amounts of the inhibitors are as follows: a, pentamidine, 25 μg/ml; b, antimycin A, 0.04 μg/ml; c, oligomycin, 25 μg/ml; and d, ethidium bromide, 12.5 μg/ml.

of respiration required concentrations of pentamidine considerably higher (Table 3) than those that inhibited growth on glycerol over 48 h (Fig. 1). We have observed consistent inhibition of respiration by pentamidine, although the concentration required for inhibition varied considerably from experiment to experiment.

We determined the effects of other inhibitory compounds upon yeast growth in glucose and in glycerol (Table 3) and upon respiration (Fig. 3 and Table 3). Among the compounds that we tested were inhibitors of DNA topoisomerase II, mRNA translation, oxidative phosphorylation, membrane ATPases, and respiration. Nalidixic acid and hygromycin B inhibited growth on glucose as well as on glycerol, and neither inhibited respiration over a 20-min assay period. Ethidium bromide inhibited growth on glycerol at much lower concentrations than it inhibited growth on glucose but had no effect upon oxygen uptake within the 20-min assay period (Fig. 3D). The uncoupling agent oligomycin also strongly inhibited growth on glycerol but not on glucose, and it also had no effect on respiration within the 20-min assay period (Fig. 3C). Oligomycin did inhibit respiration after a longer exposure (not shown). *N,N'*-Dicyclohexylcarbodiimide (DCCD) and antimycin A inhibited growth on glycerol effectively, and at low concentrations they did not inhibit growth on glucose. However, unlike oligomycin or ethidium bromide, both compounds inhibited respiration rapidly.

DISCUSSION

Effects of pentamidine on yeast cells. At low concentrations, pentamidine inhibited yeast growth only on nonfermentable energy sources (Fig. 1 and Table 1). The IC_{50} for growth inhibition on glycerol was 1.25 μg of pentamidine isethionate per ml (equivalent to 0.7 μg of pentamidine per ml). Higher pentamidine concentrations were significantly more inhibitory to growth. The pentamidine IC_{50} for yeast growth, 2.1 μM, was also very similar to the IC_{50} observed in the *Pneumocystis* microculture assay, 7.3 μM (6). These concentrations are higher than typical reported serum pentamidine concentrations (2, 7, 34). The effective concentrations that we observed contrast with those from many other pentamidine inhibition studies in which much higher concentrations were used. For example, 50% inhibition of *Escherichia coli* RNA polymerase required 80 μg of pentamidine per ml (36). We do not infer from the inhibitory pentamidine concentrations that the mechanisms of inhibition are identical in *S. cerevisiae* and *P. carinii*. However, this comparison enriches the perspective on how our studies with *S. cerevisiae* may relate to pentamidine's effects in *P. carinii*.

Inhibition by low concentrations of pentamidine was reversible by removal of the drug or by change to a fermentable carbon source, indicating that the drug has a cytostatic rather than a cytotoxic effect in yeast cells (Fig. 2). Pentamidine is also thought to have a cytostatic effect in *P. carinii* (as stated in reference 22).

At high concentrations, pentamidine induced a petite phenotype in a dose-dependent fashion at frequencies significantly above those of the background (Table 2). The petite phenotype normally results from mutations, typically large deletions and amplifications, within the mitochondrial DNA. Such mutations occur at much higher frequency than petite phenotypes induced by nuclear mutations. Therefore, we infer that pentamidine induced the petite phenotype by inducing mutations in the yeast mitochondrial genome.

Induction of mitochondrial mutations by pentamidine is probably related to its ability to bind to DNA (9, 16). Pentam-

TABLE 3. Effects of inhibitors on yeast growth and respiration[a]

Inhibitor	Cellular target	IC_{50} (μg/ml)		
		Growth in YPD	Growth in YPG	Respiration
Pentamidine	Unknown	250	1.25	100
Antimycin A	Respiration	>4[b]	0.005	0.08
DCCD	Membrane ATPases	250	4	1
Oligomycin	Oxidative phosphorylation	>100	3	>25
Ethidium bromide	DNA binding	125	2.5	>12.5
Hygromycin B	Translation	8	4	>1,500
Nalidixic acid	Topoisomerase II	62	50	>250

[a] Growth was measured turbidimetrically. Respiration was assayed as O_2 uptake by intact yeast cells.
[b] The > sign indicates throughout that the highest tested concentration was not inhibitory.

idine binds specifically and with high affinity to AT-rich DNA sequences. The yeast mitochondrial genome is 80% AT overall, but some regions have even higher AT content, which should make yeast mitochondrial DNA a preferred target for pentamidine binding. The kinetoplast DNA of trypanosomes is also AT-rich. The nature of *P. carinii* mitochondrial DNA is less clear; the single segment of *P. carinii* mitochondrial DNA listed in the GenBank or EMBL data base (MIPCVARA) is 67% AT. These AT-rich mitochondrial DNAs contrast with human mitochondrial DNA, which is only 56% AT. Differences in AT content of the mitochondrial DNA could have a role in the selective toxicity of pentamidine to fungi and trypanosomes compared with that to humans.

Induction of petite mutations by pentamidine may involve processes in addition to DNA binding. For example, pentamidine inhibits ATP-dependent DNA topoisomerases from yeast cells and from *P. carinii* (12). Topoisomerase II is also probably involved in the cleavage of trypanosome kinetoplast DNA induced by low concentrations of pentamidine (27). Although mitochondrial topoisomerases are poorly characterized, one anticipates that they are essential to mitochondrial DNA replication and transcription. If pentamidine inhibited a mitochondrial topoisomerase, one might expect it to induce petite mutations. If the putative topoisomerase were essential for mitochondrial function as well as replication, pentamidine could also inhibit growth by this mechanism.

Pentamidine did not appear to induce nuclear mutations in yeast cells; we did not observe a higher frequency of spontaneous red Ade^- mutants after treatment. Pentamidine is nonmutagenic in Ames tests and in in vitro mammalian cell culture assays (28).

Although pentamidine had mutagenic effects on the yeast mitochondrial genome, we conclude from the reversibility of inhibition and from the pentamidine concentrations required for mutagenesis that petite mutation formation was not the primary mechanism of growth inhibition at low pentamidine concentrations in yeast cells. Activity as a mitochondrial mutagen could have a more important role in either *P. carinii* or protozoans.

An obvious mechanism by which pentamidine could inhibit mitochondrial function would be to disrupt electron transport or oxidative phosphorylation. Respiration in cells was inhibited shortly after addition of pentamidine (Fig. 3A). Such rapid inhibition could not be due to a mutagenic mechanism. However, the IC_{50} for growth (1.25 μg/ml) (Fig. 1) and the IC_{50} for respiration (100 μg/ml) (Table 3) differed by 80-fold. This difference suggests that inhibition of respiration is not the primary reason for pentamidine's cytostatic effect.

Pentamidine inhibition was not dependent on growth conditions other than the energy source. Inhibition was not affected when ammonia, urea, or glutamine was used as the nitrogen source. Inhibition was not affected by the high osmotic strength of the medium or relieved by the addition of polyamines to the medium. These results suggest that pentamidine does not enter yeast cells via the general amino acid permease, which is repressed in ammonia medium (8). Also, pentamidine must not compete with polyamines for transport. In addition, these results contradict the hypothesis that pentamidine inhibits polyamine metabolism (10, 23).

Proposed mechanism of inhibition of yeast growth by pentamidine. The dependence of inhibition on energy source is critical to our hypothesis that the mitochondrion is the primary cellular target of pentamidine. The inhibition of growth in fermentable energy sources at very high concentrations probably results from a secondary mechanism. However, the effects of pentamidine on respiration and on mitochondrial mutation, particularly the rapidity with which respiration was inhibited, suggest that pentamidine (or a rapidly produced metabolite [3]) enters the mitochondrion.

Many alternative mechanisms for pentamidine action have been proposed. To assess some of these alternatives, we compared the effects of pentamidine with the effects of other drugs upon yeast growth in glucose and glycerol (Table 3) and compared their effects upon respiration (Fig. 3 and Table 3). All of the drugs inhibited growth on glycerol media, but only those known to affect mitochondrial processes (antimycin A, oligomycin, ethidium bromide, DCCD) inhibited growth on glycerol more effectively than they inhibited growth on glucose. As expected, neither oligomycin, an uncoupler of oxidative phosphorylation, nor ethidium bromide, a very effective mutagen of mitochondrial DNA, inhibited oxygen uptake by respiring yeast cells. Only antimycin A and DCCD, like pentamidine, inhibited growth much more effectively on glycerol than on glucose and inhibited oxygen uptake. Antimycin A is a respiratory inhibitor that blocks electron transport from the cytochrome bc_1 complex. DCCD is an inhibitor of the vacuolar, mitochondrial, and plasma membrane ATPases of fungi (4), but it is also a very reactive and lipid-soluble compound that inhibits electron transport complexes (1) in addition to its effects on membrane ATPases. However, although the effects of pentamidine mimic those of respiratory inhibitors, the poor correspondence of the IC_{50}s for respiration and for growth does not support the conclusion that inhibition of respiration is the process most critical to pentamidine inhibition of mitochondrial function at low concentrations.

Pentamidine and its analogs inhibit ATP-dependent DNA topoisomerase from *P. carinii* and *S. cerevisiae* (12). In our experiments, nalidixic acid, which inhibits type II topoisomerases, inhibited yeast growth on glycerol and glucose at similar concentrations, unlike pentamidine. We infer that if pentamidine's primary target within yeast cells is a topoisomerase, either the mitochondrial topoisomerase activity is particularly sensitive to pentamidine or pentamidine is selectively concentrated in the mitochondria. Pentamidine's intracellular distribution in yeast cells has not been determined. However, its induction of petite mutations, its ability to inhibit respiration, and its chemical properties suggest that it enters the mitochondrion. These observations suggest that inhibition of a putative mitochondrial topoisomerase could be critical to pentamidine cytostasis at low concentrations in glycerol.

Our results show that *S. cerevisiae* is a valuable model for studying the inhibitory effects of pentamidine. We anticipate that pentamidine may have similar effects on other fungi that rely on mitochondrial metabolism, potentially including *P. carinii*, which has mitochondria (37) and respires (24). We propose that the primary cellular target of pentamidine in yeast cells is a process essential to respiratory growth, probably a mitochondrial function. Our data indicate that pentamidine can enter the mitochondrion but that inhibition of respiration is not likely to be the primary target of pentamidine inhibition.

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