Differential Effects of Antiretroviral Nucleoside Analogs on Mitochondrial Function in HepG2 Cells

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Numerous studies have reported effects of antiviral nucleoside analogs on mitochondrial function, but they have not correlated well with the observed toxic side effects. By comparing the effects of the five Food and Drug Administration-approved anti-human immunodeficiency virus nucleoside analogs, zidovudine (3'-azido-3'-deoxythymidine) (AZT), 2',3'-dideoxycytidine (ddC), 2',3'-dideoxyinosine (ddI), 2',3'-didehydro-2',3'-dideoxycytidine (d4T), and β-L-2',3'-dideox-3'-thiacytidine (3TC), as well as the metabolite of AZT, 3'-amino-3'-deoxythymidine (AMT), on mitochondrial function in a human hepatoma cell line, this issue has been reexamined. Evidence for a number of mitochondrial defects with AZT, ddC, and ddI was found, but only AZT induced a marked rise in lactic acid levels. Only in mitochondria isolated from AZT (50 μM)-treated cells was significant inhibition of cytochrome c oxidase and citrate synthase found. Our investigations also demonstrated that AZT, d4T, and 3TC did not affect the synthesis of the 11 polypeptides encoded by mitochondrial DNA, while ddC caused 70% reduction of total polypeptide content and ddI reduced by 43% the total content of 8 polypeptides (including NADH dehydrogenase subunits 1, 2, 4, and 5, cytochrome c oxidase subunits I to III, and cytochrome b). We hypothesize that in hepatocytes the reserve capacity for mitochondrial respiration is such that inhibition of respiratory enzymes is unlikely to become critical. In contrast, the combined inhibition of the citric acid cycle and electron transport greatly enhances the dependence of the cell on glycolysis and may explain why apparent mitochondrial dysfunction is more prevalent with AZT treatment.

The use of nucleoside analogs for the treatment of human immunodeficiency virus (HIV) infection now includes a spectrum of compounds, such as zidovudine (3'-azido-3'-deoxythymidine) (AZT), 2',3'-dideoxycytidine (ddC), 2',3'-dideoxyinosine (ddI), 2',3'-didehydro-2',3'-dideoxycytidine (d4T), and β-L-2',3'-dideox-3'-thiacytidine (3TC). Some of these compounds have been associated with side effects that have been ascribed to the induction of mitochondrial dysfunction. While it is well recognized that the dose-limiting toxicity for AZT therapy is hematological, long-term treatment has also been shown to induce mitochondrial defects. This is manifested most commonly as a myopathy, with the appearance of ragged red fibers, depletion of muscle mitochondrial DNA (mtDNA), and a partial cytochrome c oxidase (COX) deficiency (10, 16, 29, 33). With other nucleosides, mitochondrial defects that are thought to contribute to peripheral neuropathy and pancreatitis are apparent. These responses are the major toxicities associated with treatment of HIV infection by ddC, d4T, and ddI and emphasize the importance of understanding the basis of mitochondrial defects and the differential sensitivities observed for these tissues (6, 20, 45).

Importantly, the emergence of hepatic failure with type B lactic acidosis is a variable response to treatment with this class of antiviral compounds, notably FIAU [1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodouracil] (21, 43). An analogous toxic side effect has been reported in response to AZT treatment, but this is an unusual syndrome with an estimated incidence of 1.3 per 1,000 person-years of follow-up in patients treated with a cohort of antiretroviral nucleoside analogs (22). This severe toxicity, while not as common as that found with FIAU, is important to understand. Liver biopsies of these patients showed massive macrovesicular steatosis and enlarged irregular mitochondria under electron microscopy (41). The association of macrovesicular steatosis and liver failure after nucleoside analog therapy is regarded as a unique clinical circumstance (41). This particular hepatotoxicity has also been reported in obese women who had received AZT for at least 6 months (23). Interestingly, these data also suggest that a mitochondrial defect underlies this toxicity with an undue reliance on glycolysis for the synthesis of ATP.

The underlying mechanism(s) responsible for nucleoside analog-induced mitochondrial abnormalities is not completely understood. Prior to exerting antiviral activities, nucleoside analogs need to be phosphorylated to their respective 5'-triphosphates. These metabolites are thought to competitively inhibit the viral reverse transcriptase or to be incorporated into the viral genome, causing termination of viral DNA chain elongation (24, 30). Although the 5'-triphosphates of these nucleoside analogs have less affinity for most of the human cellular nuclear DNA polymerases (α, β, δ, and ε), ddC, d4T, and 2',3'-dideoxyadenosine triphosphates are potent inhibitors of DNA polymerase γ. This is significant, since this is the only DNA polymerase located inside the mitochondrial matrix and it is responsible for mtDNA synthesis (28). AZT triphosphate is a 20- to 2,000-fold-less-potent inhibitor of DNA polymerase γ than ddC triphosphate. However, the accumulation of AZT monophosphate may inhibit the exonuclease and contribute to the toxicity of AZT by interfering with the repair of AZT-terminated DNA (5). Among the five nucleoside analogs, 3TC exhibits minimal cytotoxicity, which has been attributed to the lack of inhibition of DNA polymerase γ and therefore lack of disruption of mtDNA synthesis (25). Nevertheless, the interaction of nucleoside analog triphosphates with DNA polymer-
ase γ may lead to reduced mtDNA replication, resulting in further mitochondrial dysfunction.

Recent insights into mitochondrial function have revealed the fact that profound inhibition of respiratory complexes may have little or no effect on the capacity of the organelle to synthesize sufficient ATP for the cell’s requirements (18). There is then a substantial reserve metabolic capacity in the mitochondria, and each respiratory chain can be inhibited to a substantial degree before oxidative phosphorylation is affected. The point at which inhibition results in decreased synthesis of ATP may be tissue and cell specific and then the “threshold” for that respiratory complex in the control of mitochondrial function. This is particularly intriguing with respect to the effects of nucleoside analogs on mitochondrial function. For example, on the basis of histochemical studies of patients (10), AZT is thought to inhibit COX. This enzyme in the respiratory chain is not rate limiting, and loss of activity in excess of 60 to 90% would probably be required before a significant effect on mitochondrial ATP synthesis occurred (19). It thus appears likely that multiple sites of inhibition of mitochondrial function are required before significant changes in respiratory metabolism can occur. To address this issue, mitochondrial function was assessed in hepatocytes exposed to a range of nucleoside analogs commonly used in HIV therapy. Mitochondrial function was assessed in the presence of nucleosides at multiple levels to capture the complex interplay of synthesis, import, and synthesis of mitochondrial polypeptides. In addition, oxidative phosphorylation and the activity of the tricarboxylic acid cycle enzyme citrate synthase were evaluated with selected compounds.

MATERIALS AND METHODS

Materials. The HepG2 cell line was purchased from the American Type Culture Collection. Minimum essential medium with nonessential amino acids, sodium pyruvate, fetal bovine serum, and 10% trypsin-EDTA were purchased from Gibco Life Technologies, Inc. AZT, AMT (3′-amino-3′-deoxythymidine), ddC, ddI, d4T, 3TC, and AMT were purchased from Gibco Life Technologies, Inc. ddC, ddI, d4T, and cell specific analogs were then the “threshold” for that respiratory complex in the control of mitochondrial function. Isolation of mitochondria from HepG2 cells was accomplished by hypotonic lysis and separation on a Percoll-density gradient. Mitochondria were isolated from liver, brain, heart, and kidney. Mitochondria were then washed twice and resuspended in 10 mM Tris-HCl, pH 7.4, containing 5 mM MgCl2 and stored at −70°C. Protein was measured as described by Bradford (4). The concentration of microsomes was examined by determining glucose-6-phosphate activity in mitochondrial fractions compared with that in cytosol fractionates and was found to be less than 5% (7).

The activities of complexes I, II, III, and IV were measured as previously described (3). Briefly, 20 μg of freeze-dried mitochondrial protein was added to the complex I reaction mixture (25 mM potassium phosphate [pH 7.2], 5 mM MgCl2, 2.5 mg of bovine serum albumin, 5 mM KCl, 5 mM NaCl, 2 mM Na3borate, 80 mM NaCl, and 100 μg of antimycin A/ml). Complex I activity was measured through the decrease in absorbance at 340 nm of NADH oxidation with decylubiquinone as an electron acceptor in the presence or absence of rotenone (e = 6.26 × 103 M−1 cm−1). Complex II activity was assayed by measuring the absorbance at 560 nm for the rate of the reduction of succinate. The rate of succinate oxidation was measured by following the oxidation of reduced cytochrome c at 550 nm and was expressed as the first-order rate constant per milligram of protein. Complex III activity was measured by following the oxidation of reduced cytochrome c at 550 nm and was expressed as the first-order rate constant per milligram of protein. Citrate synthase activity was recorded spectrophotometrically at 412 nm (33). A background reading was obtained for freezing-thawed mitochondria (30 μg of protein) to 0.1 M 5,5′-dithiobis-2-nitrobenzoate and 0.3 mM acetyl-coenzyme A. The activity assay was performed at 30°C in a volume of 1 ml with liver mitoplasts and 600 μl of spectrophotometric standards.

Autoradiography of radiolabeled mitochrondial encoded proteins. HepG2 cells were treated with 50 μM AZT, ddC, d4T, or 3TC and 10 μM ddC for 6 days. At day 6, the medium was replaced by a medium without methionine but with 100 μg of l-phenylalanine/ml. Following 14 days of treatment, cells were treated with nucleoside analogs for 6 days. AMT had the most profound effect on cell proliferation, with a 50% inhibitory concentration of about 0.7 μM. The corresponding values for AZT and ddC were 14 and 20 μM, respectively, whereas ddT and dC, and 3TC were not toxic to HepG2 cells at concentrations up to 50 μM. Following 14 days of exposure to AZT, AMT, ddC, d4T, and 3TC, the ultrastructures of HepG2 cells were examined by electron microscopy. No discernible changes in cell structure were observed with dT and 3TC. In contrast to the studies with anti-hepatitis B compounds such as FLAV, none of the drug treatments resulted in an increase in lipid droplet formation compared with the untreated control. With respect to mitochondria, only AZT slightly enlarged the organellar size, whereas mitochondrial cristae appeared to have been disrupted in ddC- and ddd-treated cells (Fig. 1).

Effect of nucleoside analogs on the mtDNA content and extracellular lactic acid levels. Exposure of HepG2 cells for 14 days to AZT, AMT, d4T, and 3TC at concentrations up to 50 μM resulted in no deleterious effect on mtDNA levels (Fig. 2). In contrast, ddC at 1 μM and ddT at the high concentration of 200 μM decreased mtDNA levels by 85%. These data are

RESULTS

Cytotoxicity and evaluation of mitochondria by electron microscopy in HepG2 cells treated with nucleoside analogs. After treatment with various concentrations of nucleoside analogs for 6 days, AMT had the most profound effect on cell proliferation, with a 50% inhibitory concentration of about 7 μM. The corresponding values for AZT and ddC were 14 and 20 μM, respectively, whereas ddT and dC, and 3TC were not toxic to HepG2 cells at concentrations up to 50 μM. Following 14 days of exposure to AZT, AMT, ddC, d4T, and 3TC, the ultrastructures of HepG2 cells were examined by electron microscopy. No discernible changes in cell structure were observed with dT and 3TC. In contrast to the studies with anti-hepatitis B compounds such as FLAV, none of the drug treatments resulted in an increase in lipid droplet formation compared with the untreated control. With respect to mitochondria, only AZT slightly enlarged the organellar size, whereas mitochondrial cristae appeared to have been disrupted in ddC- and ddd-treated cells (Fig. 1).
FIG. 1. Electron micrograph of HepG2 cells treated with nucleosides. After 14 days of incubation of HepG2 cells with the control (A), 10 μM AZT (B), 50 μM ddI (C), and 10 μM ddC (D), electron microscopy was undertaken (magnification, ×30,000). Disrupted mitochondrial cristae were observed in ddC- and ddI-treated cells.
consistent with previous studies using ddC and ddl with other cell types (11, 13) and indicate that ddC- and ddI-dependent inhibition of mtDNA replication is probably not cell specific.

Lactic acid production can be considered a marker of impaired mitochondrial function. As shown in Fig. 3, the level of lactic acid in the medium was markedly increased, approximately 240 and 190%, respectively, by AZT (50 μM) and AMT (10 μM) treatment. In contrast the medium of cells treated with d4T, 3TC, ddC, or ddl had essentially the same lactic acid level as that of control cells. These results demonstrated a lack of direct correlation between the increase in medium lactic acid and inhibition of mtDNA replication by these nucleoside analogs. This suggests that, at this level of exposure, the inhibitory effects of ddC on mtDNA were not yet evident in terms of oxidative phosphorylation. This could occur if the turnover of mitochondrially coded proteins is slow relative to the period of the experiment (14 days) or DNA levels are not limiting for the synthesis of new protein.

Effects of nucleoside analogs on mitochondrial protein synthesis. To determine whether mitochondrial protein synthesis was affected by these nucleoside analogs, 13 mitochondrially encoded polypeptides were labeled by [35S]methionine in the presence of emetine, an inhibitor of cytosolic protein synthesis. Prior to the addition of labeled methionine, the cells were pretreated with the nucleoside analogs for 6 days. At this time point, methionine was added, and the cells were incubated for a further 3 h before lysis of the cells in denaturing buffer. After being normalized for protein content, the cell lysates were separated by urea–SDS-polyacrylamide gel electrophoresis and subjected to autoradiography (Fig. 4). In the control cells, 11 of 13 mitochondrially coded polypeptides were identified by their apparent molecular weights, and synthesis of these proteins was totally inhibited by chloramphenicol, confirming their mitochondrial origin. In the cells treated with nucleosides, no quantitative differences in polypeptide levels between the untreated control and AZT-, d4T-, or 3TC-treated cells were found (Fig. 4). In contrast, ddC greatly reduced the total mtDNA-encoded-subunit content, while ddl at this concentration (50 μM) inhibited it to a lesser extent. Quantification of the intensity of the autoradiograms indicated evidence for some selectivity in the extent of inhibition of individual polypeptides, most striking with ATP6, ATP8, and ND3 (Table 1). These data further indicate that where inhibition of mtDNA replication occurs, it has a profound effect on the rate of synthesis of mitochondrially synthesized proteins.

Effects of nucleoside analogs on the respiratory-chain complexes and citrate synthase activities. To determine whether inhibition of mitochondrial protein synthesis resulted in modification of enzyme activity, the effects of these antiretroviral drugs on the functions of isolated enzymatic components of the mitochondria were determined. Mitochondria were isolated from HepG2 cells after a 6-day exposure to 10 and 50 μM AZT, 50 μM AMT and d4T, and 10 μM ddC. The data are summarized in Table 2 and indicate that only in the case of AZT was there a significant effect on enzyme-containing subunits encoded by mtDNA. It is interesting to note that even though ddC, and to some extent ddl, inhibited mitochondrial protein synthesis, this was not reflected in the activities of critical enzymes containing these polypeptide subunits. AZT at 50 μM significantly decreased (by 30%) COX activity while having no significant effect on other mitochondrial complexes. COX activity was completely inhibited by potassium cyanide, excluding the possibility of nonspecific oxidation of cytochrome c. Therefore, the possibility of an interaction between AZT and COX was raised. However, with isolated human liver mitochondria, AZT did not inhibit COX activity at concentrations up to 1 mM. Comparable results were obtained with AMT, AZT monophosphate, and ddC (data not shown). Therefore, AZT-reduced COX activity is not the result of a direct binding of AZT to COX.
Numerous studies have shown that COX is not limiting for oxidative phosphorylation, making it unlikely that inhibition of COX by 30% would significantly affect ATP synthesis (19). However, the data showing increased lactate in the medium suggest a combination of mitochondrial defects or that inhibition of metabolism is occurring at some other point prior to electron transport. To examine the effect of AZT on a critical enzyme in the tricarboxylic acid cycle, the activity of citrate synthase was measured and found to be inhibited by 30% (Table 2).

**DISCUSSION**

Massive macrovesicular steatosis accompanied by severe lactic acidosis and hepatic failure has been reported following chronic treatment with AZT, ddC, ddI, and d4T (40). Ultrastructural examination of liver autopsy specimens from a patient revealed only enlarged mitochondria after AZT treatment (32). In HIV-infected patients who have developed myopathy after long-term AZT treatment, severe metabolic defects were observed, including accumulation of lipid droplets in myotubes (16). In addition, changes in skeletal muscle mitochondria with swelling of the organelle and disruption of cristae were observed (8, 9, 15, 37).

Since mitochondria exhibit substantial heterogeneity in differentiated cells, different organs and target cells may display different sensitivities. The mitochondrial-morphological evaluation reported here with AZT in HepG2 cells is consistent with the in vivo findings. Similarly, in vitro studies indicated that FIAU has a profound effect on lipid accumulations in both hepatocytes and myocytes (14, 36). In our study, AZT and other nucleoside analogs (ddC, ddI, d4T, and 3TC) did not lead to accumulation of lipid droplets in HepG2 cells even with extended exposure of up to 14 days, which is consistent with the reports of exposure of this duration. This may reflect the possibility that AZT and FIAU act on different target(s) in mitochondria, particularly those related to lipid metabolism.

The concentration of nucleosides that the mitochondria would be exposed to remains an area of some uncertainty; however, some realistic estimates can be made based on in vitro experiments. Interestingly, AZT was shown to be able to accumulate in the mitochondrial matrix, reaching a fourfold-higher concentration than in the surrounding medium (1). Extrapolating these data to the studies shown here would result in a concentration range of 30 to 50 μM in the mitochondria. Since accumulation of AZT in the mitochondria is also time dependent, this could represent a low estimate and is consistent with the dosing regimens used here to model acute

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**TABLE 1. mtDNA-encoded polypeptide synthesis after exposure of HepG2 cells to 50 μM AZT, d4T, and 3TC and 10 μM ddC**

<table>
<thead>
<tr>
<th>Mitochondrial polypeptide</th>
<th>AZT</th>
<th>ddC</th>
<th>ddI</th>
<th>d4T</th>
<th>3TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND5 + COI</td>
<td>96</td>
<td>14</td>
<td>52</td>
<td>91</td>
<td>103</td>
</tr>
<tr>
<td>ND4</td>
<td>115</td>
<td>45</td>
<td>87</td>
<td>106</td>
<td>100</td>
</tr>
<tr>
<td>Cyt b</td>
<td>94</td>
<td>27</td>
<td>66</td>
<td>89</td>
<td>101</td>
</tr>
<tr>
<td>ND2</td>
<td>95</td>
<td>27</td>
<td>77</td>
<td>96</td>
<td>97</td>
</tr>
<tr>
<td>ND1</td>
<td>82</td>
<td>26</td>
<td>76</td>
<td>99</td>
<td>97</td>
</tr>
<tr>
<td>COII</td>
<td>88</td>
<td>22</td>
<td>75</td>
<td>95</td>
<td>99</td>
</tr>
<tr>
<td>COIII</td>
<td>99</td>
<td>18</td>
<td>61</td>
<td>90</td>
<td>96</td>
</tr>
<tr>
<td>ATP6</td>
<td>92</td>
<td>42</td>
<td>116</td>
<td>117</td>
<td>112</td>
</tr>
<tr>
<td>ND3</td>
<td>88</td>
<td>67</td>
<td>139</td>
<td>112</td>
<td>113</td>
</tr>
<tr>
<td>ATP8</td>
<td>107</td>
<td>80</td>
<td>166</td>
<td>156</td>
<td>116</td>
</tr>
<tr>
<td>8 subunits</td>
<td>96</td>
<td>24</td>
<td>68</td>
<td>93</td>
<td>101</td>
</tr>
<tr>
<td>Total subunits</td>
<td>99</td>
<td>33</td>
<td>88</td>
<td>100</td>
<td>104</td>
</tr>
</tbody>
</table>

* ND, components of NADH dehydrogenase; CO, components of COX; cyt b, subunit of ubiquinol-COX; ATP, components of ATP synthase.

**b** The relative amount of mtDNA-encoded subunit was determined by scanning the autoradiograph using Bio-Rad imaging densitometer model GS-670. The data are normalized based on the percentage of band density in each sample compared with the respective control and represent the means of two independent experiments. In all cases, the variability was less than 5%.
and chronic exposure to the drug. Recently, similar results were obtained with cultured human muscle cells, indicating that AZT, ddC, and ddI at 1 mM significantly decreased complex II and IV activity (2). Concentrations of AZT above 5 mM were required for 50% inhibition of COX activity. These results are difficult to interpret, since the concentrations used were 100- to 500-fold higher than pharmacologically relevant AZT levels, even accounting for mitochondrial uptake.

In this study, the nucleoside found to induce lactate production to the greatest extent in HepG2 cells was AZT (Table 3). While accepting the limitations inherent in using cell cultures to investigate the cytotoxic effects of these compounds, we hypothesize that this acute response is best explained by the inhibition of both citrate synthase and COX. Citrate synthase is a key enzyme of the citric acid cycle and is an excellent marker for mitochondrial functionality. The citric acid cycle is the final common pathway for the oxidation of amino acids, fatty acids, and carbohydrates. Interference by AZT with citrate synthase would result in a low ATP/ADP ratio and favor the alternative anaerobic metabolic pathway of pyruvate, leading to an enhanced formation of lactic acid. A deficiency of COX has been associated with a number of human diseases, including Leigh syndrome, chronic progressive ophthalmoplegia, and fatal and benign infantile mitochondrial myopathy (42). COX deficiency can be caused by mutations in nuclear DNA and/or mtDNA (42). AZT has been shown to inhibit COX, while no mtDNA depletion could be detected. Incorporation of AZT into host DNA and inhibition of cellular DNA polymerases and exonucleases, as well as inhibition of hemoglobin synthesis, have been suggested as possible mechanisms for AZT-induced hematological toxicity (12, 39, 44). Therefore, the decreased COX activity may be a result of mutation of nuclear DNA induced by AZT. Previously, it has also been reported that AZT inhibited the mitochondrial ATP synthase after the exposure of cells to AZT (26). Multiple sites of inhibition by AZT appear to result in mitochondrial dysfunction, causing hepatic failure.

Compared to AZT, FIAU at 25 μM had no inhibitory effect on respiratory-chain enzyme activities and mtDNA-encoded-subunit synthesis (data not shown). The FIAU-related mitochondrial dysfunction appears to have a different mechanism than that of AZT-related dysfunction.

Three currently used anti-HIV drugs, ddC, ddI, and d4T, have been reported to induce pancreatitis, neuropathy, and liver toxicity in patients with AIDS through mitochondrial dysfunction (6, 20, 45). However, each drug displays a different potency in its effect on mitochondria. ddC and ddI increased lactic acid to a lesser extent than AZT and AMT, while no changes were observed with d4T and 3TC treatment. ddC and ddI were shown to profoundly affect mitochondrial structure, probably as a consequence of their inhibition of mtDNA replication and mtDNA-encoded-polypeptide synthesis. The mitochondrial effects of d4T appeared to be cell type specific. No mtDNA depletion was observed with d4T in PC-12 and HepG2 cells, while mtDNA synthesis of CEM cells was inhibited by d4T at concentrations less than 10 μM (11). In the present study, d4T did not increase the lactic acid level, had no effect on mtDNA-encoded-polypeptide synthesis, and did not reduce respiratory-chain enzyme activities, suggesting that other, unidentified cellular target(s) may be involved in the d4T-induced mitochondrial toxicity. Although complexes I, III, and IV contain subunits encoded by both the mitochondrial and nuclear DNAs, most of the subunits are encoded in the nucleus, synthesized in the cytosol, and imported into mitochondria. The effects of nucleoside analogs on the synthesis of nuclear-DNA-encoded polypeptides remain unknown. Nevertheless, deficiencies in mtDNA-encoded subunits caused by ddC and ddI may provide a molecular basis for the occurrence of the drug.

### Table 2. Effects of antiviral nucleoside analogs on the activities of respiratory-chain enzyme complexes

<table>
<thead>
<tr>
<th>Drug (concn [μM])</th>
<th>Activity</th>
<th>Citrate synthase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complex I</td>
<td>Complex II</td>
</tr>
<tr>
<td>AZT (10)</td>
<td>101.2 ± 14.8</td>
<td>109.8 ± 6.4</td>
</tr>
<tr>
<td>AZT (50)</td>
<td>103.3 ± 14.9</td>
<td>87.5 ± 9.2</td>
</tr>
<tr>
<td>AMT (50)</td>
<td>103.0 ± 8.5</td>
<td>96.5 ± 4.5</td>
</tr>
<tr>
<td>ddC (1)</td>
<td>110.6 ± 6.6</td>
<td>112.5 ± 1.0</td>
</tr>
<tr>
<td>ddC (10)</td>
<td>107.9 ± 7.6</td>
<td>104.4 ± 17.1</td>
</tr>
<tr>
<td>d4T (50)</td>
<td>108.2 ± 15.0</td>
<td>112.3 ± 5.6</td>
</tr>
</tbody>
</table>

* HepG2 cells were exposed to various concentrations of nucleoside analogs for 6 days. Mitochondria were then extracted as described in Materials and Methods. The mean value of complex I enzyme activity was 125.3 ± 32.4 nmol/min/mg of mitochondrial protein. The mean value of complex II enzyme activity was 157.5 ± 28.9 nmol/min/mg of mitochondrial protein. Complex III activity was 0.513 ± 0.11 first-order rate constant/mg of mitochondrial protein, and complex IV activity was 0.435 ± 0.122 first-order rate constant/mg of mitochondrial protein. Citrate synthase activity was 0.378 ± 0.05 nmol/min/mg of protein.

### Table 3. Summary of effects of anti-HIV nucleoside analogs on treated HepG2 cells

<table>
<thead>
<tr>
<th>Effect</th>
<th>AZT</th>
<th>ddC</th>
<th>d4T</th>
<th>ddI</th>
<th>3TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased lactic acid levels</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Mitochondrial morphological changes</td>
<td></td>
<td></td>
<td>+</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Inhibition of mtDNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of mitochondrial polypeptides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of COX and citrate synthase</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

* ND, not determined.
induced hepatotoxicity. During chronic exposure, it is postulated that a slow but progressive depletion of mitochondrially coded proteins will occur, resulting in emerging defects in electron transport as the period of treatment increases. This would imply that a synergistic effect on respiratory function caused by combination therapy with nucleoside analogs could occur, although no direct clinical evidence indicates that this is the case.

In summary, the results from the present study indicated that AZT selectively inhibited COX and citrate synthase activities of hepG2 while ddC, d4T, and AMT had no such effects. This AZT-related inhibition may reduce the content of intracellular ATP, resulting in the compensating enhancement of the glycolytic pathway, and finally enhancing the production of lactate. ddC and ddf significantly inhibited mtDNA content and mitochondrial protein synthesis, while none of the complex (I to IV) activities were inhibited by ddC (Table 3). This has been noted before in other models related to exposure to mitochondrial toxins (31, 38). This can be reconciled if the slow turnover of mitochondrial proteins is a critical factor leading to maintenance of enzymatic activity during inhibition of new protein synthesis. It is not clear how the myocyte is more sensitive to nucleoside-dependent mitochondrial dysfunction than hepatocytes, given the expectation that the reserve capacity of the muscle cell should be higher than that of the liver. However, little is known of the relative turnover of mitochondrial proteins in these two tissues, and in fact, inherited mitochondrial defects are more often manifested in muscle tissue than in the liver (27). This is likely to be related to differential reliance on oxidative phosphorylation of the two tissues.

The clinical relevance of these findings has yet to be established, but one may conclude that the concepts of thresholds and reserve capacity critical to our understanding of mitochondrial function should be extended to the replication and synthesis of proteins coded for by the mitochondrial genome.

REFERENCES


