Effect of Nucleoside Analogs on Neurite Regeneration and Mitochondrial DNA Synthesis in PC-12 Cells

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ABSTRACT

The effects of several anti-human immunodeficiency virus nucleoside analogs were examined on neurite regeneration and mitochondrial DNA (mtDNA) synthesis in nerve growth factor-primed PC-12 cells. Under pharmacologically relevant concentrations, the exposure of cells to 2',3'-dideoxyinosine (ddl), 2',3'-dideoxyxycytidine (ddC) and 2',3'-didehydro-3'-deoxythymidine (d4T) led to a marked dose-dependent inhibition of neurite regeneration with a 50% inhibitory concentration approximating 1, 5 and 15 μM, respectively. In contrast, 3'-azido-3'-deoxythymidine (AZT) and β-L-2',3'-dideoxy-3'-thiacytidine (3TC) had no effect on neurite regeneration. Inhibition of mtDNA synthesis by ddl was dose dependent, and ddC at a concentration of 10 μM strongly reduced mtDNA content by >75%. However, no inhibition of mtDNA synthesis was detected in cells exposed to 10 μM 3TC or d4T and to 25 μM AZT, suggesting a lack of definite correlation between mtDNA depletion and blockage of neurite regeneration. High performance liquid chromatographic analysis demonstrated that AZT, ddC, 3TC and d4T were anabolized to their respective monophosphate, diphosphate and triphosphate derivatives in the PC-12 cells. In addition, d4T was phosphorylated to form its monophosphate, diphosphate and triphosphate derivatives in isolated mitochondria, whereas ddC was metabolized only to its monophosphate form and no phosphorylated metabolites of 3TC were detected under the same conditions. In summary, the peripheral neuropathy induced by ddC and ddl in patients with acquired immune deficiency syndrome may be accounted for by the depletion of mtDNA content in the neurons. As for d4T, some other mechanism(s) may be involved in its clinical neurotoxicity. Both AZT and 3TC lacked any substantial toxicity in our invitro model, which is in agreement with the clinical action of these drugs.

Signs or symptoms of peripheral neuropathy have been reported in 30–60% of patients with AIDS or AIDS-related complex (So et al., 1988). The most common type of peripheral neuropathy is a distal, symmetric, primarily sensory polyneuropathy (Bailey et al., 1988; Cornblath and McArthur, 1988; Parry, 1988). Although this neuropathy is probably caused primarily by HIV infection, the precise pathogenesis remains unclear. Recently, scientific attention has expanded to include the peripheral neuropathy associated with some nucleoside analogs that are used for the treatment of HIV infection. Among the clinically approved nucleoside analogs, AZT is mainly limited by its hematological toxicity and is not known to induce any peripheral neuropathy (Mitsuya et al., 1990; Sommadossi, 1993). 3TC, which exhibits a synergistic anti-HIV effect when combined with AZT, does not cause peripheral neuropathy or hematotoxicity (Eron et al., 1995). In contrast, a reversible toxic neuropathy has been reported in phase I clinical trials of ddC (Dubinsky et al., 1989; Klecker et al., 1988; Merigan et al., 1989), and a dose-associated peripheral neuropathy has been observed in phase I studies of d4T (Browne et al., 1993). Peripheral neuropathy was also encountered with ddI, requiring drug discontinuation in 22% of patients in a phase I/II clinical trial and in 16% of patients in the expanded-access program (Cooley et al., 1990; Lambert et al., 1990; Yarchoan et al., 1990).

Early hypotheses for the mechanism(s) of ddC-induced neuropathy included potential interference by a ddC metabolite, ddC-diphosphate-choline, with production of sphingomyelin, a major constituent of myelin sheaths (Cooney et al., 1986). However, similar toxicities observed with nucleoside analogs that do not have a cytosine base, such as ddl and

ABBREVIATIONS: HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; AZT, 3'-azido-3'-deoxythymidine; ddC, 2',3'-dideoxyxycytidine; ddl, 2',3'-dideoxyinosine; d4T, 2',3'-didehydro-3'-deoxythymidine; d4TTP, 2',3'-didehydro-3'-deoxythymidine-5'-triphosphate; 3TC, β-L-2',3'-dideoxy-3'-thiacytidine; dCyd, 2'-deoxycytidine; dThd, thymidine; TMP, thymidine-5'-monophosphate; TDP, thymidine-5'-diphosphate; TTP, thymidine-5'-triphosphate; NGF, nerve growth factor; mtDNA, mitochondrial DNA; HPLC, high performance liquid chromatography; DMEM, Dulbecco’s modified Eagle’s medium.
d4T, suggest that this hypothesized mechanism is quite unlikely. The results of more recent studies have suggested that this delayed toxicity may be related to an inhibition of mtDNA synthesis (Chen and Cheng, 1989). Therefore, there is a crucial need to develop an in vitro cell culture system that mimics the clinical toxicity of some nucleoside analogs toward peripheral nerves and allows elucidation of the cellular and molecular events involved in these drug-related peripheral neuropathies. Furthermore, newly synthesized antiviral nucleosides can be tested for predictive purposes to suggest whether peripheral neuropathy may be encountered in future clinical trials.

A variety of cultured mammalian cells, including freshly isolated human peripheral neurons, have been used for the study of the interactions of drugs and/or pathogens with the peripheral nervous system (Harouse et al., 1989; Riopelle and Kennedy, 1982; Rubenstein and Price, 1983). Among these, the rat PC-12 pheochromocytoma cell line is a homogeneous model system that has been extensively characterized biochemically and physiologically (Greene and Tischler, 1982) and shown to be very useful in the study of the differentiation of peripheral sympathetic and sensory neurons (Greenberg et al., 1985; Greene et al., 1987). In addition, Stevenson et al. (1989) demonstrated the relevance of this model in determination of the mechanisms of drug-induced peripheral neuropathy. One study reported the inhibition of mtDNA steady-state levels by ddC and ddI in PC-12 cells, but experiments were performed at concentrations of ddI that were 2–3 orders of magnitude higher than the pharmacologically relevant levels, and no correlation with neural functions was investigated (Chen et al., 1991). The other study described the effects of nucleoside analogs on neurite outgrowth using GS-ras-1, a c-Ha-ras transformant of the PC-12 cells (Keilbaugh et al., 1991). The different characteristics of oncogene-induced PC-12 cells and NGF-induced original PC-12 cells (Simpson et al., 1991) and the use of nonclinically relevant concentrations (Keilbaugh et al., 1991) further emphasized the necessity for detailed investigation of the effects of these drugs on neuronal functions such as neurite regeneration and its underlying mechanism(s) in NGF-primed PC-12 cells to gain better insight into the peripheral neuropathy observed in patients treated with ddC, ddI and d4T.

**Materials and Methods**

**Materials.** The PC-12 cell line was obtained from the American Type Culture Collection (Rockville, MD). AZT, ddC, NGF and poly-l-lysine were purchased from Sigma Chemical (St. Louis, MO), and ddI and d4T were provided by Bristol-Myers Squibb Co. (Wallingford, CT). [3H]-d4T (20 Ci/mmol), [5-3H]-3TC (13.5 Ci/mmol), [5,6-methyl-3H]-AZT (14 Ci/mmol), [5,6-3H]-ddC (5 Ci/mmol), [5,6-3H]-ddI (20 Ci/mmol), [5-3H]-3TC (13.5 Ci/mmol), [6-3H]-CdCly (14 Ci/mmol) and [methyl-3H]-dThd (7 Ci/mmol) were obtained from Moravek Biochemicals (Brea, CA). DMEM, horse serum and fetal bovine serum were purchased from Gibco (Grand Island, NY). All other chemicals and reagents were of the highest analytical grade available.

**Cell culture.** PC-12 cells were grown in 75-cm² tissue culture flasks in DMEM supplemented with 7.5% heat-inactivated diazoyed horse serum, 7.5% heat-inactivated diazoyed fetal bovine serum and 1% penicillin/streptomycin. The medium was changed every 3 days, and the cells were subcultured once a week.

**Effect of nucleoside analogs on PC-12 cell proliferation in liquid suspension cultures.** PC-12 cells from stock culture were diluted and incubated in 20 ml of DMEM at a density of 2 × 10⁵ cells/ml in suspension culture flasks. Various concentrations of drugs or no drug (control) was added to each flask. After 4 days of incubation, fresh medium with drugs (at the same initial concentration) was changed every other day until termination of the experiment at day 10. Viability was assessed by trypan blue exclusion, and cells were counted with a hemocytometer under the microscope every other day.

**Effect of nucleoside analogs on the neurite regeneration of PC-12 cells.** To evaluate the effects of these nucleoside analogs on neurite regeneration, PC-12 cells were primed with 50 ng/ml NGF for 10 days in a six-well cell culture cluster with 10⁴ cells/well in 1.5 ml of medium. The cell culture cluster was precoated overnight with poly-l-lysine. The medium with NGF was replaced every other day. The cells were then mechanically deprived of their neurites by repeated aspiration of medium with a Pasteur pipette. After several washes with NGF-free medium, the cells were replated in poly-l-lysine-coated 24-well cell culture clusters with 10⁴ cells/well in the presence of 50 ng/ml NGF. Various concentrations of drugs or no drug (control) was added at the same time. Cell and neurite counts were performed in a statistically significant manner after 7 days using phase-contrast microscopy at ×200. The cell was scored positive for neurite regeneration if at least one process was observed in a length of 100 μm.

**Effect of nucleoside analogs on mtDNA content in NGF-primed PC-12 cells.** After analysis of neurite regeneration, cells (1 × 10⁵/sample) that had been treated with various concentrations of drugs and no drug (control) were then heated under alkaline conditions, and the DNA was immobilized on a Zeta-Probe membrane (BioRad, Richmond, CA) by using a slot-blot apparatus. The mtDNA was detected on the membrane with an mtDNA-specific probe (Anderson et al., 1981). To standardize the amount of total cellular DNA loaded onto the membrane, pRBA-1, a rat β-actin cDNA inserted into the Okayama-Berg vector, was used as a probe for determination of the genomic DNA as previously described (Faraj et al., 1994).

**HPLC analysis of metabolites in PC-12 cells.** To determine the intracellular 5'-phosphorylated metabolites of each drug, 2 × 10⁵ cells/ml were suspended in 75-cm² tissue culture flask in a total volume of 11 ml. After the addition of a ¹H-nucleoside analog at a specific activity of 200 dpm/pmol and a sufficient amount of nonradioactive drug to achieve a final concentration of 10 μM, cells were maintained at 37°C under an atmosphere of 5% CO₂ for 24 hr. Cells were then collected, transferred into a 15-ml conical tube and pelleted at 1200 rpm for 10 min in a Beckman GS-6R centrifuge. Cells were then washed three times with 10 ml of cold phosphate-buffered saline. Nucleotides present in the cell pellet were extracted by overnight incubation at −20°C with 1 ml of 60% methanol and then re-extracted with 500 μl of 60% methanol for 30 min in an ice bath. Combined extracts were dried under a gentle nitrogen stream at room temperature, and the samples were stored at −20°C until analysis. Separation of nucleotides was performed on a Hewlett-Packard 1050 HPLC system (Avondale, PA). The cell extracts were analyzed by anion exchange HPLC with a Partisol 10 SAX column (Whatman, Clifton, NJ). Elution was carried out at 1 ml/min with 15 mM KH₂PO₄, pH 3.5, and a 45-min linear gradient of 1 M KH₂PO₄, pH 3.5, from 0% to 100%, starting 10 min after the time of injection. The total running time was 70 min. The radioactivity associated with the fractions collected by HPLC was measured using a Beckman LS5000A scintillation counter equipped with an automatic quench correction program.

**Mitochondria isolation and phosphorylation studies.** A 1-ml pellet of PC-12 cells was washed with 20 ml of cold TD buffer containing 134 mM NaCl, 5 mM KCl, 0.7 mM Na₂HPO₄ and 2.5 mM Tris-HCl, pH 7.5, and subsequently centrifuged for 5 min at 2500 rpm and 4°C in a Beckman GS-6R centrifuge. Cells were then resuspended at 4°C in 12 ml of buffer containing 10 mM NaCl, 1.5 mM MgCl₂ and 10 mM Tris-HCl, pH 7.5, and incubated for 10 min.
Swollen cells were disrupted with a glass Dounce homogenizer, and a one-sixth volume of cold 2 M sucrose, 35 mM EDTA and 50 mM Tris-HCl solution, pH 7.5, was added immediately to stabilize mitochondria against osmotic rupture. Nuclei were then eliminated by two successive sedimentations of 4 min each at 2500 rpm in a Beckman GS-6R centrifuge. The resulting supernatant was centrifuged at 14,000 rpm for 20 min in a Beckman J2-20 centrifuge to obtain the mitochondria pellet. This pellet (600 µg of protein/sample) was then resuspended in the incubation buffer containing 10 mM succinate, 2 mM ATP, 2 mM pyruvate, 1 mM malate, 2 mM nicotinic acid, 10 mM MgCl₂, 2 mM KCl, 10 mM KH₂PO₄ and 25 mM Tris-HCl, pH 8.0. Assays were initiated with the addition of a [³H]-nucleoside analog and its nonradioactive form to achieve a final concentration of 10 µM and a specific activity of 5000 dpm/pmol. [³H]dTd and [³H]dCyd were used as experimental controls. After a 30-min incubation at 37°C, mitochondria were centrifuged at 15,000 × g for 5 min and washed three times with cold washing buffer containing 0.25 mM sucrose and 1 mM EDTA. Extraction of intramitochondrial nucleotides and HPLC analysis were performed in a similar fashion to that used for the determination of intracellular nucleotides described above.

**Incubation with alkaline phosphatase.** Approximately 1500 dpm isolated from a radioactive peak eluting at the same retention time as that of ddC-5’-monophosphate was incubated with 0.31 unit of alkaline phosphatase in 50 mM K₃PO₄ buffer containing 1 mM ZnSO₄, pH 6.5, for 4 hr at 37°C. The reaction was terminated by the addition of 30 µl of cold 50% trichloroacetic acid. After 30 min at 4°C, samples were centrifuged for 1 min at 15,000 × g in an Eppendorf model 5414 microcentrifuge. The supernatant was neutralized with 60 µl of 5 M KHCO₃ and an aliquot was analyzed by the same anion exchange HPLC system described above. Control incubations were performed with heat-inactivated enzyme.

**Results**

**Evaluation of nucleoside analogs on PC-12 cell proliferation in liquid suspension cultures.** The effects of drugs on PC-12 cell proliferation were assessed in liquid suspension cultures as described in Materials and Methods. Under these conditions, a ddC concentration of 25 µM completely inhibited cell proliferation within 8 days, whereas no substantial effects were observed at a concentration of 1 or 10 µM over the same time period. In contrast, even at a concentration of 25 µM, ddI, AZT, d4T and 3TC exhibited no inhibitory effect on the growth of these cells during a 10-day incubation period (fig. 1). These experiments were performed to determine pharmacologically relevant concentrations of drugs that are not inhibitory to cell proliferation to be used for subsequent experiments on neurite regeneration and mtDNA synthesis.

**Effect of drugs on neurite regeneration of NGF-primed PC-12 cells.** To evaluate the effect of drugs on neurite regeneration, PC-12 cells primed with 50 ng/ml NGF for 7 days were then deprived of neurites and replated in the

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**Fig. 1.** Effect of various concentrations of ddC, ddI, AZT, d4T and 3TC on PC-12 cell growth in liquid suspension culture. Each point is the average of duplicate samples. Cells were counted in the absence (□) and presence of tested compound at 1 µM (△), 10 µM (○) and 25 µM (●) except for AZT, in which cells were counted in the presence of 5 µM (△) and 25 µM (●).
A significant effect was observed on neurite regeneration in neurite regeneration starting from 1 of these compounds caused a statistically significant reduction in neurite regeneration in PC-12 cells. All three compounds showed a statistically significant reduction in neurite regeneration compared with control. The statistical analysis was further conducted using a t-test. Table 2 demonstrates that AZT and 3TC at a concentration of 25 and 10 μM, respectively, had no effect on mtDNA content in NGF-primed PC-12 cells. In contrast, ddC strongly inhibited mtDNA content by >75% at a concentration of 10 μM. A dose-dependent inhibitory effect on mtDNA content was observed after exposure to ddI of 0.5–10 μM. However, mtDNA synthesis was not affected by a ddT concentration of 10 μM, which inhibited neurite regeneration by ~40% in NGF-primed PC-12 cells (fig. 2).

**Determination of 5'-phosphorylated metabolites of nucleoside analogs in PC-12 cells.** Exposure of PC-12 cells to 10 μM 3H-nucleoside analog for 24 hr led to the detection of their 5'-monophosphate, 5'-diphosphate and 5'-triphosphate derivatives, as revealed by anion exchange HPLC. As expected, 3'-azido-3'-deoxythymidine-5'-monophosphate was the predominant intracellular metabolite, with a concentration of 14.4 pmol/10^6 cells, whereas 3'-azido-3'-deoxythymidine-5'-diphosphate and 3'-azido-3'-deoxythymidine-5'-triphosphate reached much lower levels of 0.48 and 0.10 pmol/10^6 cells, respectively. For the phosphorylation of ddC, the intracellular concentration of 2',3'-dideoxy-cytidine-5'-diphosphate was 2-fold higher than that of 2',3'-dideoxyadenosine-5'-triphosphate, with 0.14 and 0.07 pmol/10^6 cells, respectively, and a concentration of 0.10 pmol/10^6 cells was detected with 2',3'-dideoxythymidine-5'-monophosphate. For ddT, a high intracellular concentration of 0.32 pmol/10^6 cells of ddTTDP was observed, whereas 2',3'-dideoxy-3'-deoxythymidine-5'-diphosphate (fig. 3E). In addition, no phosphorylated metabolites were detected at ASPET Journals on July 9, 2017

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**TABLE 1**

Effects of nucleoside analogs on mtDNA content in NGF-primed PC-12 cells.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Ratio to Control of mtDNA Synthesis</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZT</td>
<td>μM</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>101.5 ± 14.8b</td>
<td>&gt;.05</td>
<td></td>
</tr>
<tr>
<td>3TC</td>
<td>25</td>
<td>104.7 ± 2.6</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>ddI</td>
<td>0.5</td>
<td>93.0 ± 22.6</td>
<td>&gt;.05</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>65.6 ± 12.6</td>
<td>&lt;.01</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>54.1 ± 1.8</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>ddC</td>
<td>10</td>
<td>21.5 ± 12.6</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>ddT</td>
<td>10</td>
<td>106.4 ± 11.2</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>

* Cells were continuously exposed for 7 days to drugs in poly-L-lysine-coated cell culture clusters.

* Mean ± S.D. of three experiments.

* The t-test was performed for all nucleoside analogs compared with control, and the degree of freedom was 4.
Discussion

Three of the five nucleoside analogs clinically approved for the treatment of AIDS (dDC, dDI and d4T) have been responsible for the development of a painful peripheral neuropathy that definitely limits the effectiveness of these compounds (Mitsuya et al., 1990). In the current study, by using a peripheral neuron model, we explored the cellular and molecular events involved in the neurotoxicity caused by these nucleoside analogs, focusing on their effects on mtDNA, a preferential target for toxicity of some nucleoside analogs (Cui et al., 1995; Parker and Cheng, 1994). AZT and 3TC, the two nucleoside analogs that have not been observed to cause peripheral neuropathy, were also investigated as potential negative controls.

The results of our study demonstrate that AZT and 3TC had no effect on NGF-promoted neurite regeneration, whereas dDC, dDI and d4T profoundly inhibited neurite regeneration in a dose-dependent manner. Of particular note, these experiments were performed at clinically achievable concentrations and cell growth was not affected under tested concentrations, therefore excluding that the observed toxic effects were a consequence of an inhibition of cell proliferation. These in vitro results are in agreement with the clinically observed peripheral neurotoxicity of these three nucleoside analogs and suggest that the NGF-primed PC-12 cells may be a relevant system for prediction of drug-induced peripheral neuropathy. Because mtDNA encodes subunits of enzymes involved in oxidative phosphorylation and rRNA of mitochondrial ultrastructure, a correlation of impaired mtDNA with peripheral neuropathy has been reported (Pezeshkpour et al., 1987). Furthermore, the preferential inhibition of mtDNA synthesis has been suggested as the mechanism promoting peripheral neuropathy in HIV-infected patients treated with dDC, dDI or d4T (Balzarini et al., 1989; Chen and Cheng, 1989). This hypothesis is further supported by recent studies showing that among all cellular DNA polymerases, DNA polymerase-β, the host enzyme responsible for mtDNA synthesis, was the most sensitive to some nucleoside analog 3′-triphosphates (Martin et al., 1994; Parker and Cheng, 1994). Previous in vitro studies have indicated that dDC, dDI and d4T exhibited inhibitory effects on mtDNA synthesis and interfered with cell growth in different cell lines (Chen et al., 1991; Chen and Cheng, 1989). In the current study, exposure of NGF-primed PC-12 cells to dDC and dDI at concentrations that did not affect cell growth resulted in a mtDNA depletion; however, mtDNA content of PC-12 cells exposed to d4T, at an equivalent molar concentration that led to an inhibition of neurite regeneration, was
not reduced. Therefore, the selection of the cell line in which to study effects of nucleoside analogs on mtDNA content is important as we previously demonstrated (Faraj et al., 1994), and mitochondrial abnormalities must be correlated with drug effect on cell physiological functions to provide sufficient biochemical evidence of the proposed toxicity mechanism. Overall, data from the present study suggest that depletion of mtDNA content may not be the only mechanism leading to nucleoside analog-induced neuronal damage.

The inhibition of mtDNA synthesis as a result of interaction with DNA polymerase-β by some nucleoside analogs will, in any event, require the building up of their 5’-triphosphates within the mitochondria. The formation of the 5’-triphosphate derivative within mitochondria depends on the efficiency of mitochondrial phosphorylation of the nucleoside analog or on the efficiency of its cytoplasmic anabolism to the respective nucleotides and transport of these nucleotide derivatives into mitochondria leading ultimately to the 5’-triphosphate entity (Martin et al., 1994; Parker and Cheng, 1994). Our present results demonstrated that AZT, ddC and d4T were anabolized to their respective monophosphate, diphosphate and triphosphate derivatives in intact PC-12 cells and, in particular, that d4T was phosphorylated to its 5’-triphosphate form, whereas ddC was only converted to its 5’-monophosphate derivative in isolated mitochondria. These data are in agreement with previous studies using CEM cells in which the 5’-triphosphate form of ddC was also not detected in isolated mitochondria, suggesting that 2’,3’-dideoxyctidine-5’-triphosphate probably was formed by cytoplasmic kinases and subsequently transported into mitochondria to exert its observed inhibition of mtDNA content (Chen and Cheng, 1992). The lack of inhibition of mtDNA content in PC-12 cells incubated with d4T despite the potent inhibition of DNA polymerase-β by d4TP (Martin et al., 1994) and the presence of that triphosphate derivative within mitochondria may suggest that an enzymatic repair mechanism is possibly involved. Our previous study using human bone marrow cells has shown that steady state levels of d4T incorporated into nuclear DNA were 10 to 50-fold lower than that of AZT, although the two compounds exhibited a similar affinity for DNA polymerase-α and a higher concentration of d4TP compared with 3’-azido-3’-deoxythymidine-5’-triphosphate was detected in cells. An excision of d4T from DNA was demonstrated to be in part responsible for its lack of incorporation into genomic DNA consistent with its observed limited hematological toxicity (Zhu et al., 1991). Meanwhile, recent studies have confirmed that an exonuclease activity is also highly associated with DNA polymerase-β in both human (Gray and Wong, 1992) and other species (Insdorf and Bogenhagen, 1989; Kaguni and Olson, 1989; Kunkel and Mosbaugh, 1989). Therefore, the mitochondrial exonuclease may also play an important role in the steady state levels of nucleoside analogs incorporated into mtDNA and the affinity of that enzyme toward these drugs may be responsible, in part, for the different effects of these drugs on mtDNA synthesis. This discrepancy between the potent inhibition of DNA polymerase-β and the lack of effect on mtDNA content has also been reported for 3TC (Chang et al., 1992). One recent study demonstrated the ability of DNA polymerase-β to excise 3TC 5’-monophosphate from DNA, suggesting that this DNA repair mechanism may contribute to the lack of mitochondrial toxicity by 3TC (Gray et al., 1995).

However, other researchers have proposed that the inability of transporting 3TC 5’-triphosphate into mitochondria could be responsible for its lack of effect on mtDNA (Parker and Cheng, 1994), and this hypothesis is indirectly supported by our experimental results showing that no phosphorylation of 3TC was carried out within the mitochondria.

In summary, a dose-dependent inhibition of neurite regeneration by pharmacologically relevant concentrations of ddC, ddI and d4T was demonstrated in differentiated PC-12 cells. The present study also provided evidence that ddC and ddI may cause peripheral neuropathy by depletion of mtDNA content in the neurons. As for d4T, for which inhibition of mtDNA content was not detected, which is in agreement with previous studies with other cell types that also showed a limited effect of d4T on mtDNA synthesis (Faraj et al., 1994; Martin et al., 1994), some other mechanism(s) may be responsible for its neurotoxicity. Consistent with clinical observations, no significant toxicity of AZT or 3TC was detected in this model. Although mitochondrial toxicity is probably responsible for the peripheral neuropathy observed with some nucleoside analogs, the results of this study suggest that these clinical side effects may also be due to mechanisms other than a direct inhibition of mitochondrial DNA synthesis. Based on the clinical profile, it is reasonable to consider that other neural functions, including the neuron-repair system, may also be affected by these drugs, leading to a drug-related peripheral neuropathy in patients with AIDS.

References


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