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ACCELERATED COMMUNICATION

Anti-Human Immunodeficiency Virus Type 1 Therapy and Peripheral Neuropathy: Prevention of 2′,3′-Dideoxycytidine Toxicity in PC12 Cells, a Neuronal Model, by Uridine and Pyruvate

SUE A. KEILBAUGH, GREGORY A. HOBBS, and MELVIN V. SIMPSON

Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, New York 11794-5215

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SUMMARY

A strategy for preventing or delaying the peripheral neuropathy induced by 2′,3′-dideoxycytidine (ddC) therapy in patients with acquired immunodeficiency syndrome was suggested by findings, in two laboratories, that cultured avian and mammalian cells devoid of mitochondrial DNA continue to replicate at virtually normal rates, provided that the medium is supplemented with uridine and pyruvate. Inasmuch as it is likely that a depletion of mitochondrial DNA also takes place in neuronal cells exposed to ddC, we used PC12 cells, the neuronal model we have reported on previously, in an attempt to rescue these cells from the deleterious effects of ddC. We first show, using undifferentiated PC12 cells, that DNA replication is impaired in mitochondria isolated from cells grown in the presence of ddC. Then, using growth rate as a criterion of the well-being of the cells, we show that the addition of uridine and pyruvate to uninduced cells growing in the presence of ddC results in an average rescue efficiency of 51%, based on the uridine/pyruvate-treated control. This value increases considerably at substantially higher concentrations of uridine alone. Rescue efficiencies of differentiated cells, which do not proliferate, were assessed using neurite outgrowth and neurite survival as criteria. Here the rescue efficiency is 56%, based on the uridine/pyruvate-treated control. In addition, uridine and pyruvate prolong the viability of ddC-treated cells and maintain their healthy appearance; without these compounds, the ddC-treated cells have an abnormal morphology and die off quite rapidly.

The more important toxic side effects of anti-HIV-1 therapy with ddN analogues include bone marrow suppression, myopathy, and cardiomyopathy, all induced by AZT, peripheral neuropathy induced by ddC, ddI, and d4T, and pancreatitis induced by ddI. Present evidence suggests that mitochondrial function may be causally implicated in these conditions, because various studies suggest that a cellular target of these analogues is the mitochondrion, specifically mtDNA replication. This view is based on *in vitro* studies with isolated mitochondria (1), purified DNA polymerase γ (2), intact MOLT-4F cells (3, 4), intact Friend murine erythroleukemic cells (a model for bone marrow) (2, 5), PC12 cells (a neuronal model) (6, 7), and human muscle cells (8). It is further supported by studies on mitocondria from skeletal or heart muscle derived from AIDS patients with AZT-induced myopathy or cardiomyopathy or from laboratory animals with these AZT-induced conditions (8–11).

The consequences of inhibition of mtDNA replication in AZT-treated differentiating (hemoglobin-producing) Friend cells are decreases in cell proliferation, in oxidative phosphorylation, and in cellular ATP level, with compensatory increases in glycolytic rate and in the number of mitochondria/cell (6).[1] With respect to PC12 cells, we reported previously that ddC inhibits cell proliferation in a dose-dependent manner and, in addition, has a deleterious effect on neurite-producing ability, neurite survival, and cell viability of the differentiated cells (6). A depletion of mtDNA has indeed been shown to occur when these cells are grown in the presence of ddC (7). Because this decrease could result from DNA destruction as well as inter-

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[1] G. Hobbs, S. A. Keilbaugh, P. Rief, and M. V. Simpson, unpublished observations.

ABBREVIATIONS: HIV, human immunodeficiency virus; ddN, dideoxynucleoside; AZT, 3′-azido-3′-deoxythymidine; ddC, 2′,3′-dideoxycytidine; ddI, 2′,3′-dideoxyinosine; d4T, 3′-deoxythymidine-2′-ene; mtDNA, mitochondrial DNA; AIDS, acquired immunodeficiency syndrome.

ruption of synthesis, we wished to show definitively that the latter is in fact the case.

It was demonstrated several years ago that avian (12) and mammalian (13) cells whose mtDNA had been completely depleted by cell growth in the presence of ethidium bromide, a highly potent inhibitor of DNA polymerase γ (14), continue growing. The mitochondria, which no longer carry out oxidative phosphorylation, continue to replicate along with the cell. These cells use glycolysis for ATP generation, and their continued growth depends on supplementation of the medium with uridine and pyruvate. The pyrimidine requirement arises because of the deficiency of the respiratory chain-dependent dihydroorotate dehydrogenase (15). The pyruvate requirement needs further study. Because ddN-induced inhibition of the replication of mtDNA of PC12 cells should have similar consequences for the mitochondria, we asked whether such cells could be rescued by uridine and pyruvate. We report here that, in fact, ddC inhibits mtDNA replication in PC12 cells, and we describe our attempts to rescue this cell with these substances.

Materials and Methods

Cell culture. GS-*ras*-1 and GS-*ras*-2 cells, c-Ha-*ras* transformants of PC12 cells (16) in which differentiation can be induced more conveniently and rapidly with dexamethasone than with nerve growth factor, were maintained in tissue culture dishes in Dulbecco's modified Eagle's medium (catalogue number 320–1965PJ; GIBCO), horse serum, fetal bovine serum (90:10:5), containing 1000 units/ml penicillin G sodium and 1000 μg/ml streptomycin sulfate. Cultures (maximum cell density, 2.5×10^5 to 1.0×10^6 cells/ml) were passaged every 5 days into 105 ml of fresh medium, from which 20-ml volumes were used to seed five 100-mm tissue culture plates. All cell culture incubations were at 37°, in 10% CO_2, and in 98% humidity.

Cell growth studies. For GS-*ras*-1 cells, 1.0×10^7 cells were treated with trypsin, diluted with phosphate-buffered saline (138 mM NaCl, 2.67 mM KCl, 10 mM Na_2HPO_4, 1.71 mM KH_2PO_4, pH 7.4), centrifuged, and resuspended in fresh medium as described earlier (6). The cell suspension was then diluted as noted below. For GS-*ras*-2 cells, which do not require trypsin treatment, 1.0×10^7 cells were resuspended in 105 ml of fresh medium, a fraction of which was removed for trituration and dilution as noted. Cultures used for growth studies were passaged at 0.5×10^3 cells/ml into 12- or 24-well plates and were then treated as noted previously (6).

Neurite outgrowth studies. The 2.5-ml samples of GS-*ras*-2 cells used to determine neurites/cell were passaged at 200 cells/ml into 35-mm dishes. The protocol, including dexamethasone addition and ddC addition, was as described earlier (6). Uridine and pyruvate were added with the ddC.

Incorporation of [^{3}H]thymidine into mtDNA. Approximately 1.5×10^6 cells/ml (GS-*ras*-1) were seeded into 150-mm tissue culture plates (two/sample) and incubated for 15 hr to permit attachment of the cells. The cells were exposed to varying concentrations of ddC, incubated, and harvested when there was a minimum yield of about 1.5×10^7 cells/sample. The cells from each sample were suspended in a maximum of 10 ml of medium and triturated, and then 0.5 ml of the suspension was removed to an Eppendorf tube for determination of the cell count while the remainder was centrifuged at 1000 rpm at 5° for 5 min in a Sorvall SS34 rotor. After the supernate was decanted, the cells were washed with 10 ml of phosphate-buffered saline and repelleted. The cells were resuspended in hypotonic buffer (10 mM Tris, pH 7.4, 10 mM KCl, 0.1 mM $MgCl_2$) and incubated on ice for 30 min to permit swelling. The cells were homogenized immediately after the addition of 2.3 ml of buffer A (1 M sucrose, 10 mM KCl, 0.1 mM $MgCl_2$, 10 mM Tris, pH 7.4) and the mitochondria were isolated using buffer B (0.25 M sucrose, 10 mM KCl, 0.1 mM $MgCl_2$, 10 mM Tris, pH 7.4) (1). Once isolated, the mitochondria were washed twice in buffer B and then incubated for 60 min at 37° in buffer B containing [^{3}H]thymidine (specific activity, 20 Ci/mmol), after which the mitochondrial suspension was precipitated and counted (1). Cell counts were performed as described previously (6).

Results

The effect of ddC on mtDNA replication in growing PC12 cells was measured by following the incorporation of [^{3}H]thymidine into the DNA of mitochondria isolated from cells (refer to Materials and Methods for transformant used) that had been grown in the presence of ddC for 0–48 hr (Fig. 1). An appreciable inhibitory effect (20%) occurs as early as 0.5 hr, increasing to about 55% at 47 hr, results that are in accord with those obtained with Friend cells (5).

To assess the possibility that ddC-induced peripheral neuropathy might be prevented or reversed by uridine and pyruvate, we studied the effect of these compounds first on the induced form of PC12 cells grown in the presence of ddC. When these cells are incubated in the presence of 5–50 μM ddC, concentration-dependent inhibition of cell proliferation is observed (6). We have chosen a ddC concentration (12.5 μM) that produces an accurately measurable growth inhibition but is not too far above the pharmacological range. In the experiment shown in Fig. 2, growth inhibition at this ddC concentration, with respect to the untreated control (no uridine, no pyruvate, and no ddC), is 30% at day 4.5, 29% at day 6, 53% at day 8, and 52% at day 10. In the presence of 50 μM concentrations each of uridine and pyruvate, these inhibitory values are reduced to, respectively, 11%, 13%, 26%, and 13%, relative to the untreated control, corresponding to rescue efficiencies (defined in Table 1) of 63%, 55%, 51%, and 75%. Because the control itself is stimulated somewhat when treated with uridine and pyruvate (treated control; uridine, pyruvate, and no ddC), par-

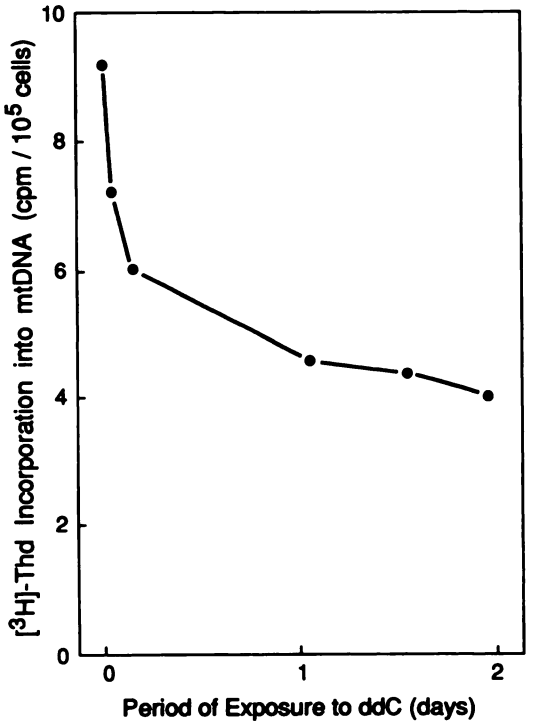

Fig. 1. DNA replication *in vitro* by mitochondria isolated from PC12 cells grown in the presence of ddC. Growth and incubation conditions are described in Materials and Methods. The ddC concentration was 10 μM.

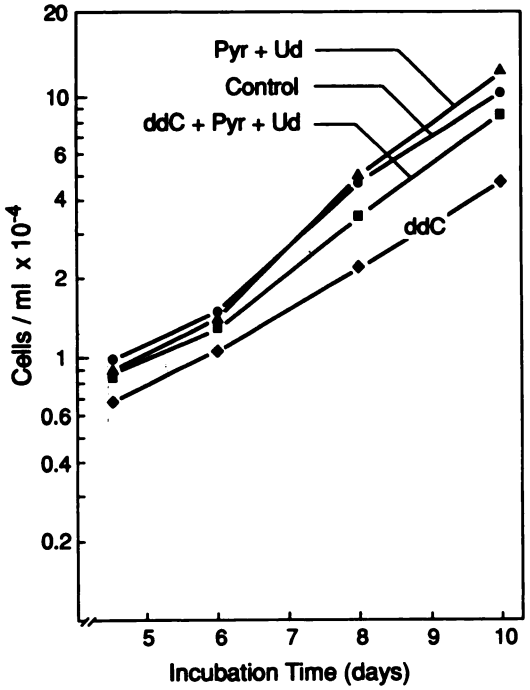

Fig. 2. Effect of uridine and pyruvate on the rescue of undifferentiated PC12 cells grown in the presence of ddC. Growth conditions are described in Materials and Methods. Concentrations of ddC, uridine (*Ud*), and pyruvate (*Pyr*) were 12.5 μM, 50 μM, and 50 μM, respectively.

TABLE 1

Effect of uridine and/or pyruvate on the rescue of uninduced PC12 cells grown in the presence of ddC

Growth conditions are described in Materials and Methods.

Conditions	Rescue efficiency[a]		
	6/7[b]	8/9	10/11
		%	
12.5 μM ddC + 50 μM uridine + 50 μM pyruvate	62	43	38
12.5 μM ddC + 100 μM uridine + 100 μM pyruvate		18	34
12.5 μM ddC + 50 μM pyruvate	60	2	−52
12.5 μM ddC + 50 μM uridine	32	−15	−60
12.5 μM ddC + 150 μM uridine		60	72
12.5 μM ddC + 200 μM uridine		71	45
12.5 μM ddC + 300 μM uridine		57	91
25 μM ddC + 50 μM uridine + 50 μM pyruvate	−23	20	46

[a] Percentage inhibition under non-rescue conditions minus percentage inhibition under rescue conditions divided by percentage inhibition under non-rescue conditions, where percentage inhibition is calculated using treated control values (i.e., control plus uridine plus pyruvate).

[b] Incubation time (days).

ticularly at the later time points, we expressed the extent of alleviation of inhibition with respect to the treated control as well. In the case of days 6, 8, and 10, this would alter the final degree of inhibition to 11%, 30%, and 32%, making the rescue efficiencies 62%, 43%, and 38%, respectively (an average of 51%). These values are in the same range as the average values from eight experiments performed under similar but slightly varying conditions.

In an attempt to improve the rescue efficiency, we varied the concentrations of uridine and pyruvate (Table 1). Increasing the uridine and pyruvate concentrations to 100 μM each provides little or no improvement over results obtained with 50 μM concentrations each of pyruvate and uridine. If either 50 μM uridine alone or 50 μM pyruvate alone is used, there is no rescue, and in fact there is further inhibition of cell growth. With higher concentrations of uridine alone (150 μM, 200 μM, or 300 μM) the overall rescue efficiency is equal to or better than that obtained with 50 μM concentrations each of uridine and pyruvate.

Because there was a reasonable rescue of ddC-treated uninduced PC12 cells by uridine and pyruvate, we were encouraged to ask how the differentiating/differentiated PC12 cells would respond to these rescue attempts. Such cells more accurately reflect the *in vivo* state. We chose to add the agents under study (ddC, uridine, and pyruvate) early in differentiation rather than at the termination of the process because at the latter point, namely about 16 days, the control cultures have aged to a point at which the neurites begin to become unstable and the cells themselves are becoming nonviable. Hence, there might not be time enough for the effects of ddC or of the rescue agents to become manifest. As shown previously, the effect of ddC on these cells is first to stimulate the production of neurites, many of which, however, can be seen to possess abnormal structure (6). Also, the life of these neurites is shortened. Finally, cell viability is appreciably decreased. Using neurite outgrowth as a measure of the well-being of the cell (Fig. 3), we tested the ability of uridine and pyruvate (7.5 μM each) to prevent the deleterious effects of ddC. This treatment over an incubation period of 12 days eliminates the stimulatory effect on neurite outgrowth. At day 12 the number of neurites/cell begins to decline in the ddC-treated cells, 4 days before this occurs in the control samples. (Microscopic observation of neurite structure shows that this decrease results both from old neurites beginning to break up and from a decline in the rate of new neurite formation.) The presence of uridine and pyruvate in these cultures (Fig. 3) permits the maintenance of a level of neurite

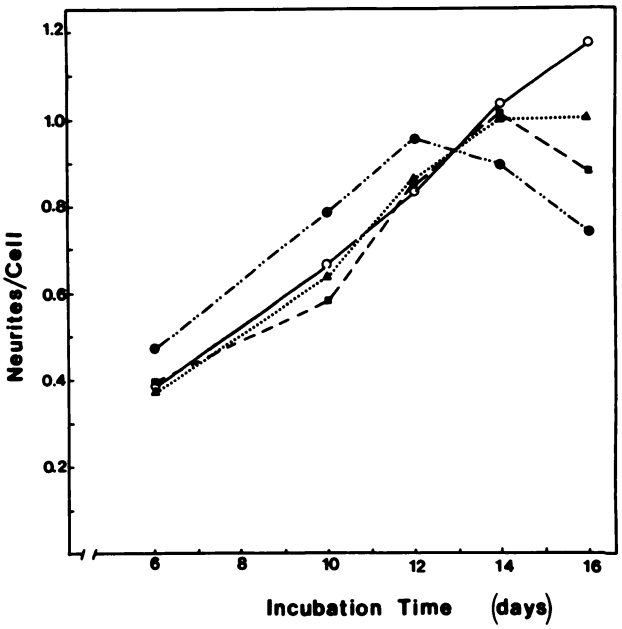

Fig. 3. Effect of uridine and pyruvate on the rescue of ddC-treated differentiated PC12 cells, as determined by the extent of neurite outgrowth. Growth conditions are described in Materials and Methods. Concentrations of ddC, uridine, and pyruvate were 12.5 μM, 7.5 μM, and 7.5 μM, respectively. Assessments of neurite condition and viability of the cells in this experiment are given in the text. ○, Control; ●, ddC; ▲, control plus uridine plus pyruvate; ■, ddC plus uridine plus pyruvate.

outgrowth similar to that of the controls for an additional 2 days, after which the control cells also exhibit a loss of neurites. Interestingly, uridine and pyruvate alone (treated control) cause a plateauing of the curve at this point, resulting in the neurites/cell value dropping below that of the untreated control. Our impression is that this results from a commencement of neurite break-up rather than a retardation of new neurite production, although this view requires further substantiation. Preliminary results indicate that this effect is dose dependent. Thus, by day 16 the rescue efficiency of uridine and pyruvate toward the cells grown in the presence of ddC is 56%, relative to the treated control.

Another way of assessing the rescue efficiency of uridine and pyruvate is by measuring the extent of prolongation of cell viability. This parameter might reflect the operation of some processes that are different from those that determine neurite outgrowth. We were able to obtain some preliminary results on this cellular state from the experiments described earlier, which were actually designed to measure the number of neurites/cell. The data were obtained by measuring the number of neurite-containing cells in several microscopic fields, rather than on the entire plate, and are therefore only semiquantitative. Cells were grown to 18 days (20 days in cases where the control cells were still healthy) and observed microscopically every 2 days. Conditions of the experiment are described in the legend to Fig. 3. It should be noted that induced cells differentiate at different rates, with virtually all cell growth ceasing at about 16 days, a point at which all the cells that are going to differentiate (i.e., grow neurites), about 75–80%, have done so. Assessing the number of neurite-containing cells at 14, 16, and 18 days, we find (by averaging four experiments) a 30–40% decrease in the ddC-treated cells, whereas the ddC/uridine/pyruvate-treated sample shows almost complete rescue. The untreated and treated controls show no change. Moreover, as pointed out in the previous experiment, the rescued cells and the controls appear healthy, whereas the untreated ddC-exposed cells are in extremely poor condition, even at 14 days.

Discussion

The peripheral neuropathy induced in humans by ddC therapy for AIDS appears to be axonal in type (17–19), as is the peripheral neuropathy induced by a mitochondrial disease studied in human patients (20). Administration of ddC to rabbits, on the other hand, leads to myelin splitting and demyelination as well as to axonal loss, and it is suggested that in rabbits the myelin pathology is the primary effect (21). However, a strong correlation of the clinical, electrophysiological, and pathological characteristics between the human and the rabbit ddC neuropathies does not exist (18), and we have sought a cell line that reflects the characteristics of the human disease to study the mechanism of the neurological toxicity induced by ddC. The PC12 cell line (22), perhaps the most frequently used system for studies on neuronal cells of the peripheral nervous system, was our choice for this purpose and we have used two *ras* transformants of this line in our study (see Materials and Methods). This cell line was derived from a tumor (pheochromocytoma) of the rat adrenal medulla, whose cells share their embryological origin with those of the peripheral nervous system and are biochemically and developmentally similar to sympathetic neurons, including their ability to synthesize and store norepinephrine and dopamine but not epinephrine. Exposure to nerve growth factor induces differentiation with the cessation of cell multiplication, accompanied by the extension of neurites (i.e., branching varicose processes similar to those produced by sympathetic neurons in primary cell culture) (23).

The rationale for using the PC12 cell line is further supported by the differential responses of this system and of Friend erythroleukemic cells to ddC, ddI, d4T, and AZT. The responses of these neuronal and erythropoietic cell models are in accord with the well known differential clinical responses to these drugs. In particular, AZT, which causes bone marrow suppression but not peripheral neuropathy, is toxic to the Friend hemopoietic model but not to the PC12 neuronal model; in contrast, ddC, which induces peripheral neuropathy but not bone marrow suppression, is toxic to the PC12 neuronal model but not to the hemopoietic model (5).

The extent of cell rescue from ddC in the experiments described in this report is high enough to be encouraging and to merit further work in an attempt to maximize the extent of rescue. Clearly, uridine and pyruvate are able to extend the time during which the uninduced and the differentiated cells remain in good condition. With respect to the differentiated cells, this assessment includes the number of neurites/cell, their physical appearance, and the viability of the cells. Translated to AIDS therapy, such supplements to ddC, ddI, d4T, etc., could extend the time during which a patient is free of peripheral neuropathy or could decrease the intensity of the neuropathy. However, considering the mechanism of action of these ddN analogues on mtDNA, it is not possible to predict whether neuronal cells could function normally for extended periods of time, although, as pointed out earlier, mitochondria devoid of DNA and the cells containing such mitochondria are capable of surviving and replicating in cell culture. Moreover, should even a limited rescue enable the neuronal cells to "gain time," the possibility exists that during this period certain cellular adaptations could take place. For example, an exonuclease capable of removing a terminal ddN (24, 25) might be induced, or the mitochondrial deoxynucleoside triphosphate pool size might increase significantly, thereby effectively competing away the dideoxy analogues.

Supplementation of ddC with uridine and pyruvate requires consideration of the possible toxic effects of these metabolites. It is known that clinical toxicity is associated with continued uridine therapy at high doses (26, 27). However, such toxicity may well be preventable, as shown in mice and dogs, by substituting for uridine the uridine phosphorylase inhibitor benzylacyclouridine. This strategy results in an effective plasma concentration of uridine but one below toxic levels (27, 28). With respect to pyruvate toxicity in humans, it is difficult to find reports in the literature. However, plasma levels in humans are high (>1 mM), and doses as high as 1 g/kg can be administered to mice with no toxicity and as high as 4 g/kg with no mortality (29).

We have not yet tested the rescue efficiency of uridine and pyruvate on the toxicity of ddI, d4T, or other ddN analogues in the PC12 cell system. However, it is of interest that, before the avian and mammalian cell mtDNA depletion studies (12, 13), Sommadossi *et al.* (30), in a study of the rescue of AZT-treated bone marrow cells by nucleotides (a study that was not based on mtDNA considerations), found that uridine could rescue these cells *in vitro*. This observation was later reflected in whole-animal (mice) experiments (28). Because there was

no requirement for pyruvate, the mechanism of rescue may differ in the different cells or the mechanisms involved in the toxicities of AZT and ddC may differ (31). Whether uridine and pyruvate are effective against AZT-induced myopathy remains untested.

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References

1. Simpson, M. V., C. D. Chin, S. A. Keilbaugh, T.-S. Lin, and W. H. Prusoff. Studies on the inhibition of mitochondrial DNA replication by 3′-azido-3′-deoxythymidine and other dideoxynucleoside analogs which inhibit HIV-1 replication. *Biochem. Pharmacol.* **38:**1033–1036 (1989).
2. Keilbaugh, S. A., J. A. Moschella, C. D. Chin, J. Mladenovic, T.-S. Lin, W. H. Prusoff, R. A. Rowehl, and M. V. Simpson. Role of mtDNA replication in the toxicity of 3′-azido-3′-deoxythymidine (AZT) in AIDS therapy and studies on other anti-HIV-1 dideoxynucleosides, in *Structure, Function and Biogenesis of Energy Transfer Systems* (E. Quagliariello, S. Papa, F. Palmieri, and C. Saccone, eds.). Elsevier Science Publishers, Amsterdam, 159–162 (1990).
3. Chen, C.-H., and Y.-C. Cheng. Delayed cytotoxicity and selective loss of mitochondrial DNA in cells treated with the anti-human immunodeficiency virus compound 2′,3′-dideoxycytidine. *J. Biol. Chem.* **264:**11934–11937 (1989).
4. Lewis, L. D., F. M. Hamzeh, and P. S. Lietman. Ultrastructural changes associated with reduced mitochondrial DNA and impaired mitochondrial function in the presence of 2′,3′-dideoxycytidine. *Antimicrob. Agents Chemother.* **36:**2061–2065 (1992).
5. Hobbs, G. A., S. A. Keilbaugh, and M. V. Simpson. The Friend erythroleukemia cell, a model system for studying the association between bone marrow toxicity induced by 3′-azido-3′-dideoxythymidine and dideoxynucleoside inhibition of mtDNA replication. *Biochem. Pharmacol.* **43:**1397–1400 (1992).
6. Keilbaugh, S. A., W. H. Prusoff, and M. V. Simpson. The PC12 cell as a model for studies on the mechanism of induction of peripheral neuropathy by anti-HIV-1 dideoxynucleoside analogs. *Biochem. Pharmacol.* **42:**R5-R8 (1991).
7. Chen, C.-H., M. Vazquez-Padua, and Y.-C. Cheng. Effect of anti-human immunodeficiency virus nucleoside analogs on mitochondrial DNA and its implication for delayed toxicity. *Mol. Pharmacol.* **39:**625–628 (1991).
8. Lamperth, L., M. Dalakas, F. Dagani, J. Anderson, and R. Ferrari. Abnormal skeletal and cardiac muscle mitochondria induced by zidovudine (AZT) in human muscle *in vitro* and in an animal model. *Lab. Invest.* **65:**742–751 (1991).
9. Dalakas, M. C., I. Illa, G. H. Pezeshkpour, J. P. Laukaitis, B. Cohen, and J. L. Griffin. Mitochondrial myopathy caused by long-term zidovudine therapy. *N. Engl. J. Med.* **322:**1098–1105 (1990).
10. Arnaudo, E., M. Dalakas, S. Shanske, C. T. Moraes, S. DiMauro, and E. A. Schon. Depletion of muscle mitochondrial DNA in AIDS patients with zidovudine-induced myopathy. *Lancet* **2:**508–510 (1991).
11. Lewis, W., T. Papoian, B. Gonzalez, H. Louie, D. P. Kelly, R. M. Payne, and W. W. Grody. Mitochondrial ultrastructural and molecular changes induced by zidovudine in rat hearts. *Lab. Invest.* **65:**228–236 (1991).
12. Morais, R., P. Desjardins, C. Turmel, and K. Zinkewich-Peotti. Development and characterization of continuous avian cell lines depleted of mitochondrial DNA. *In Vitro Cell. Dev. Biol.* **24:**649–658 (1988).
13. King, M. P., and G. Attardi. Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. *Science (Washington D. C.)* **246:**500–503 (1989).
14. Meyer, R. R., and M. V. Simpson. DNA biosynthesis in mitochondria: differential inhibition of mitochondrial and nuclear DNA polymerases by the mutagenic dyes ethidium bromide and acriflavine. *Biochem. Biophys. Res. Commun.* **34:**238–244 (1969).
15. Gregoire, M., R. Morais, M. A. Quilliam, and D. Gravel. On auxotrophy for pyrimidines of respiration-deficient chick embryo cells. *Eur. J. Biochem.* **142:**49–55 (1984).
16. Kremer, N. E., G. D'Arcangelo, M. DeMarco, J. S. Brugge, and S. Halegoua. Signal transduction by nerve growth factor and fibroblast growth factor in PC12 cells requires a sequence of *src* and *ras* actions. *J. Cell Biol.* **115:**809–819 (1991).
17. Yarchoan, R., R. V. Thomas, J.-P. Allain, N. McAtee, R. Dubinsky, H. Mitsuya, T. J. Lawley, B. Safai, C. E. Myers, C. F. Perno, R. W. Klecker, R. J. Wills, M. A. Fischl, M. C. McNeely, J. M. Pluda, M. Leuther, J. M. Collins, and S. Broder. Phase I studies of 2′,3′-dideoxycytidine in severe human immunodeficiency virus infection as a single agent and alternating with zidovudine (AZT). *Lancet* **1:**76–81 (1988).
18. Anderson, T. D., A. Davidovich, R. Arceo, C. Brosnan, V. Arezzo, and H. H. Schaumberg. Peripheral neuropathy induced by 2′,3′-dideoxycytidine. *Lab. Invest.* **66:**63–74 (1992).
19. Berger, A. R., J. C. Arezzo, H. H. Schaumberg, G. Skowron, T. Merigan, S. Bozzette, D. Richman, and W. Soo. 2′,3′-Dideoxycytidine toxic neuropathy. *Neurology* **43:**358–362 (1993).
20. Pezeshkpour, G., C. Krarup, F. Buchthal, S. DiMauro, N. Bresolin, and J. McBurney. Peripheral neuropathy in mitochondrial disease. *J. Neurol. Sci.* **77:**285–304 (1987).
21. Feldman, D., C. Brosnan, and T. D. Anderson. Ultrastructure of peripheral neuropathy induced in rabbits by 2′,3′-dideoxycytidine. *Lab. Invest.* **66:**75–85 (1992).
22. Greene, L. A., and A. S. Tischler. Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. *Proc. Natl. Acad. Sci. USA* **73:**2424–2428 (1976).
23. Greene, L. A. PC12 pheochromocytoma cultures in neurobiological research. *Adv. Cell. Neurobiol.* **3:**373–414 (1982).
24. Vazquez-Padua, M. A., M. C. Starnes, and Y.-C. Cheng. Incorporation of 3-azido-3′-deoxythymidine into cellular DNA and its removal in a human leukemic cell line. *Cancer Commun.* **2:**55–62 (1990).
25. Schinazi, R. F., A. Peck, and J.-P. Sommadossi. Substrate specificity of *Escherichia coli* thymidine phosphorylase for pyrimidine nucleosides with anti-human immunodeficiency virus activity. *Biochem. Pharmacol.* **44:**199–204 (1992).
26. Van Groeningen, C. J., A. Leyua, I. Kraal, G. P. Peters, and H. M. Pinedo. Clinical and pharmacokinetic studies of prolonged administration of high-dose uridine intended for rescue from 5-FU toxicity. *Cancer Treat. Rep.* **70:**745–750 (1986).
27. Falcone, A., J. W. Darnowski, R. M. Ruprecht, S.-H. Chu, I. Brunetti, and P. Calabresi. Differential effect of benzylacyclouridine on the toxic and theraputic effects of azidothymidine in mice. *Blood* **76:**2216–2221 (1990).
28. Calabresi, P., A. Falcone, M. H. St. Clair, M. C. Wiemann, S. H. Chu, and J. W. Darnowski. Benzylacyclouridine reverses azidothymidine-induced marrow suppression without impairment of anti-human immunodeficiency virus activity. *Blood* **76:**2210–2215 (1990).
29. Dulaney, M., Jr., and A. S. Hume. Pyruvic acid protects against the lethality of sulfide. *Res. Commun. Chem. Pathol. Pharmacol.* **59:**133–136 (1988).
30. Sommadossi, J.-P., R. Carlisle, R. F. Schinazi, and Z. Zhou. Uridine reverses the toxicity of 3′-azido-3′-deoxythymidine in normal human granulocyte-macrophage progenitor cells *in vitro* without impairment of antiretroviral activity. *Antimicrob. Agents Chemother.* **32:**997–1001 (1988).
31. Izuta, S., M. Saneyoshi, T. Sakuroi, M. Suzuki, K. Kojima, and S. Yoshida. The 5′-triphosphates of 3′-azido-3′-deoxythymidine and 2′,3′-dideoxynucleosides inhibit DNA polymerase γ by different mechanisms. *Biochem. Biophys. Res. Commun.* **179:**776–783 (1991).

Send reprint requests to: Melvin V. Simpson, Department of Biochemistry and Cell Biology, State University of New York, Stony Brook, NY 11794–5215.