Mitochondrial toxicity of antiviral drugs

Long-term treatment with antiviral nucleoside analogue drugs, such as AZT, can give rise to delayed and at times severe mitochondrial toxicity. Although these toxic effects are manifest in many tissues, a common disease mechanism can explain the diverse clinical events. A better understanding of these disorders will shed light on genetic mitochondrial diseases and lead to the design of safer and more effective antiviral drugs.

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Mitochondrial diseases are multisystem disorders with unique genetics. Changes in mitochondrial structure, function and molecular biology occur in a very diverse group of disorders including viral infections, diabetes mellitus, heart disease, Parkinson's disease and even the biologic process of ageing[1,2]. Recently, clinical and experimental events in mitochondrial toxicity caused by antiviral nucleoside analogues (ANAs) were found to resemble those of genetic mitochondrial diseases.

The ANA acyclovir was the archetypal nucleoside analogue for the treatment of herpes virus infections with minimal toxicity[3]. Other ANAs, some of which are used to treat human immunodeficiency virus type 1 (HIV-1) and hepatitis B virus (HBV) infection, include zidovudine (AZT), zalcitabine (ddC), didanosine (ddI), stavudine (D4T), and 2′-deoxy-3′-thiacytidine (3TC)[4-6].

In structure, ANAs resemble natural nucleotide bases that serve as the building blocks in DNA. The pharmacologic effectiveness of ANAs depends upon their relatively selective interference with viral DNA replication in the absence of significant toxicity to cellular DNA replication in the patient. Toxicity occurs when cellular DNA polymerases are inhibited and the subcellular toxic target is frequently the mitochondrial DNA polymerase (DNA pol-γ) in selected tissues.

Although short-term ANA therapy appears to be relatively safe, long-term therapy has revealed unique toxic effects on oxidative phosphorylation in various tissues. Features of this toxicity suggest that defective mitochondrial DNA (mtDNA) replication may be the common event. These features resemble those that occur in genetic mitochondrial diseases and include mitochondrial myopathy, cardiomyopathy, neuropathy, lactic acidosis, exocrine pancreas failure, liver failure and bone marrow failure[2-6]. The clearest example is AZT mitochondrial myopathy[7] with a prevalence of up to 20% in long-term AZT-treated AIDS patients. Manifestations of the disorder relate to defective mitochondrial gene expression and to ANA triphosphates' (ANATP's) inhibition of mitochondrial DNA pol-γ in the target tissues[7,8]. Recent experience with the fluorinated ANA fialuridine [FIAU, 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodouridine] have further dampened expectations for the long-term safety of ANA treatments. FIAU yielded serious toxicity and was linked to several deaths in an aborted clinical trial for chronic hepatitis B virus (HBV). Toxic manifestations of the FIAU treatment included profound lactic acidosis, hepatic failure, fat accumulation in the liver, coma, skeletal and cardiac myopathy, pancreatitis and peripheral neuropathy[9-12].

In spite of the established effectiveness of ANAs in AIDS, enthusiasm has diminished because long-term therapy causes multi-organ side-effects to striated muscle[7], peripheral nerves[13], pancreas[14], liver[15] and heart[16]. In some cases, reversal of symptoms corresponds to cessation of therapy; in others, toxicity persists. In this review we present evidence that ANA-induced inhibition of mitochondrial DNA pol-γ partially explains the pathophysiologic changes to mtDNA replication.

The potential impact of ANA mitochondrial toxicity in viral infections other than HIV may be formidable given their widespread use. For example, chronic hepatitis due to HBV (which afflicts approximately 250 million people worldwide) is currently treated with the ANA 3TC in clinical studies (ref. 17). ANA-induced mitochondrial toxicity offers insights into the mechanisms of mitochondrial and genetic diseases. Studies using ANAs in animal models explain normal and abnormal mitochondrial function.

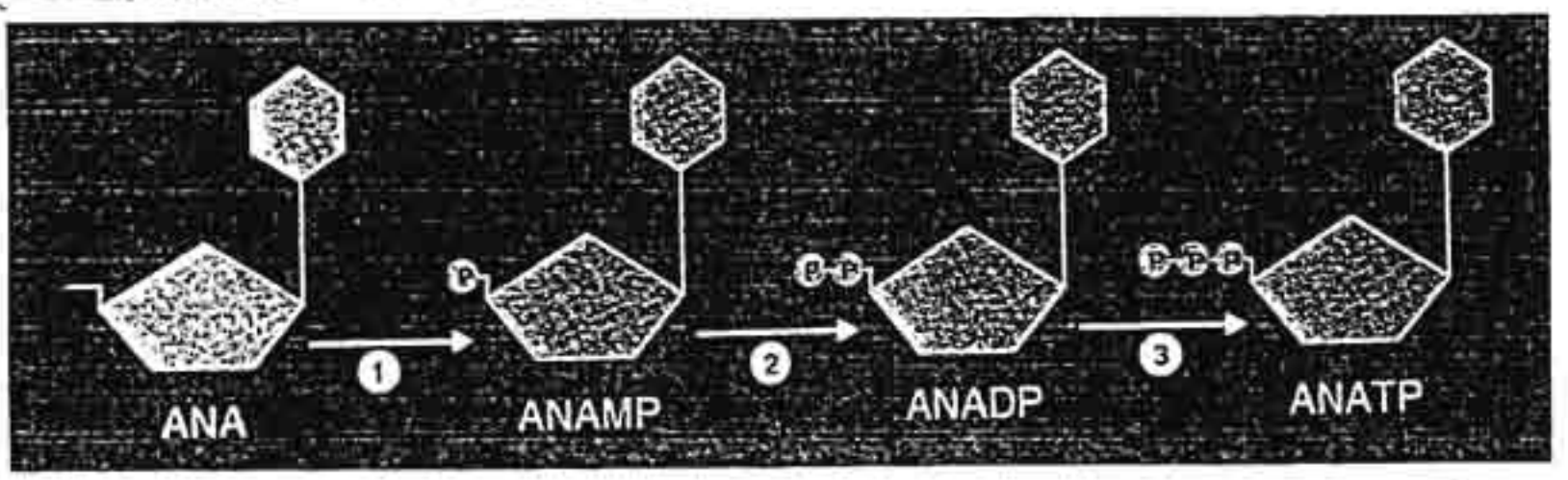

Fig. 1 Phosphorylation of thymidine analogue ANAs. ANA phosphorylation occurs intracellularly and thymidine kinase (1) is the enzyme responsible for the first anabolic phosphorylation to ANAMPs. ANAMPs are phosphorylated by thymidylate kinase (2) to ANADPs and these in turn are phosphorylated to ANATPs by nucleoside diphosphate kinase (3) or other enzymes.

Cellular metabolism of ANAs

Nucleoside kinases are enzymes responsible for the intracellular activation of ANAs. Activation is accomplished using the same enzymes that phosphorylate the natural ribonucleosides and deoxynbonucleosides of RNA and DNA[18]. AZT, ddC and FIAU are phosphorylated by cellular

thymidine kinase (TK); (Fig. 1, Step (1)). In mammals, two tissue-specific forms of TK exist: TK1 is cytosolic, with low activity in skeletal muscle, and TK2 is mitochondrial, with higher activity in muscle[19]. The tissue distribution of these two forms of TK may relate to the selective toxicity of some ANAs. After monophosphorylation, ANAs are phosphorylated by other cellular enzymes, to ANATPs (Fig. 1, Steps (2) and (3)) where they function as inhibitors of viral DNA polymerases by competing with the natural substrates or by leading to chain termination of viral DNA.

Effect Of ANAs on mitochondria

Interactions with DNA polymerases. On a structural basis, ANATPs could inhibit mammalian nuclear DNA polymerases (DNA pol-α, DNA pol-β, DNA pol-δ, and DNA pol-ε) and DNA pol-γ used for mtDNA replication. Both the mechanisms for DNA polymerization and the choice of nucleotide substrates are similar among eukaryotic DNA polymerases, and some ANATPs have been shown to inhibit nuclear DNA pols *in vitro*[20]. However, neither clinical nor laboratory evidence support inhibition of nuclear DNA polymerases by ANATPs, whereas it strongly suggests that ANATPs do inhibit the mitochondrial DNA pol-γ. This relatively selective polymerase inhibition may in part explain ANA toxicity to mtDNA replication.

Role of DNA pol-γ. One important clinical and experimental observation in some ANA toxicities is a decreased steady-state mtDNA abundance. This may relate to the function of DNA pol-γ, a nuclear-encoded DNA polymerase for mtDNA replication[21]. The *polymerase* function of DNA pol-γ is fundamental, but an associated 3′→5′ *exonuclease* is also present. DNA pol-γ is *processive*. Processivity allows each DNA pol-γ molecule to replicate each mtDNA molecule to completion after one initiation event.

Processivity of DNA pol-γ may also relate to *heteroplasmy* (an intracellular mix of normal and mutant mtDNA molecules)[22]. Since DNA pol-γ is processive, mtDNA deletion mutants (shortened mtDNA) may be replicated more quickly than native mtDNA. As a result, the proportion of mtDNA mutants may increase and yield functional changes beyond a certain threshold. In some ways, analogies exist between this mechanism of ANA toxicity (as with AZT therapy) and accumulated mtDNA deletions seen in heritable mitochondrial illnesses[23].

ANA toxicity and its relationship to ANA structure. ANA toxicity can be classified based on ANA chemical structure and fundamental chemical properties. In one case, phosphorylated ANAs could be internalized into nascent mtDNA by their substitution for the natural base. In another, mtDNA chain termination could be pivotal[24] (Fig. 2).

The first form of inhibition (represented by ANAs like FIAU), involves ANA incorporation at internucleotide linkages, with, for example, fialuridine substituting for the natural base, thymidine (Fig. 2*a*). ANATPs (like FIAUTP) that contain 3′-hydroxyl groups (3′-OHs) serve as competitive, alternate substrates for DNA pol-γ in mtDNA synthesis (by substituting for thymidine triphosphate). The 3′-OH on members of this class of ANAs enables DNA pol-γ to extend the mtDNA chain after the ANA is incorporated into mtDNA. No mitochondrial postreplicational repair mechanism exists to remove the internally incorporated ANA, and as a result, mtDNA damage is essentially permanent. Moreover, changes in mtDNA caused by fialuridine incorporation could lead to alterations in mitochondrial transcription and ultimately to defective mitochondrial polypeptide synthesis.

In the second case, ANATPs (like AZTTP) also compete with natural thymidine triphosphate at the nucleotide binding site of DNA pol-γ (as above). However, their incorporation into mtDNA terminates nascent mtDNA non-competitively, because they lack the 3′-OH group necessary for nascent mtDNA chain extension with a new base (Fig. 2*b*). This correlates mechanistically with mixed inhibition (competitive and non-competitive inhibition[25]) of DNA pol-γ by AZTTP *in vitro*. It follows that reversibility of ANA toxicity may be possible if the toxic ANA can be removed from the mtDNA terminus (possibly by the exonuclease associated with DNA pol-γ).

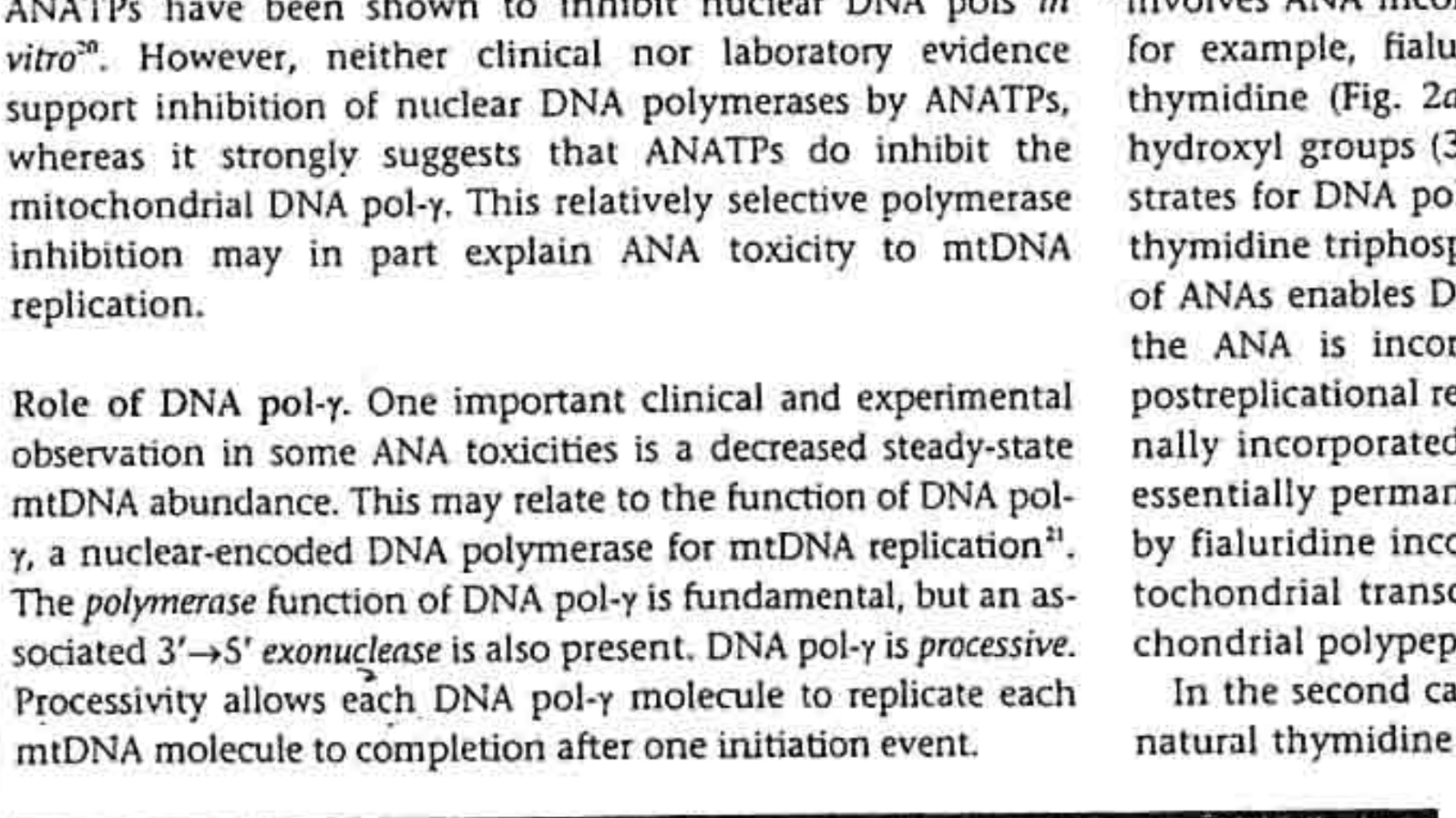

Fig. 2 Proposed mechanism of ANA toxicity to mtDNA (small double circle), with attached DNA pol-γ (in block) in which ANATPs serve as inhibitors of mtDNA replication. *a*, Enlarged view of interaction of mtDNA with DNA pol-γ. Incorporation of ANAs into mtDNA as alternate substrates for DNA pol-γ. FIAUTP serves as an alternate substrate for thymidine triphosphate with DNA pol-γ and is incorporated (inserted into mtDNA as F) into the nascent chain because it possesses a 3′-OH group. The nascent mtDNA can be extended beyond the inserted FIAU. *b*, In contrast, ANATPs (like AZTTP) that lack a 3′-OH also compete with thymidine triphosphate as substrates of DNA pol-γ. The latter ANAs terminate mtDNA synthesis (inserted into DNA as Z) because bases cannot be added to the nascent chain after AZT is inserted.

Clinical manifestations of ANA toxicity

It is self-evident that ANAs, like all drugs, have side-effects. However, the prevalent and at times serious ANA mitochondrial toxic side-effects are particularly broad ranging with respect to their tissue target and mechanisms of toxicity.

Haematologic toxicity. The most common side effect of AZT is haematologic toxicity, occurring in up to 21% of patients[26]. The clinical importance of this effect relates to the fact that it limits AZT ther-

apy. Clinical manifestations of AZT haematologic toxicity include anaemia, leukopenia, thrombocytopenia and bone marrow suppression. AZT produces significant loss of haematopoietic precursors in the peripheral blood before affecting bone marrow itself. This toxicity is attributed to inhibition of cellular DNA polymerases[28], (possibly DNA pol-γ[8]) or to depletion of thymidine. Effects of AZT on bone marrow cultures include time- and dose-dependent inhibition of marrow precursors[29].

Clinical trials used recombinant human erythropoietin (r-HuEPO) to treat the anaemia associated with HIV infection and AZT therapy[30]. In immunosuppressed mice, AZT-induced erythroid toxicity was ameliorated by hemin and stem factor[31] and 2',3'-dideoxythymidine reversed AZT-induced bone marrow toxicity[32]. AZT-induced perturbation of deoxyribonucleotide pools suggests the use of benzylacyclouridine to reverse AZT haematotoxicity[33].

Analogies exist between AZT myelotoxicity and one heritable mtDNA illness. Mitochondrial deletions and bone marrow failure are main components of Pearson's marrow-pancreas syndrome[34]. It should be noted also that an important manifestation of didanosine (ddI) toxicity is acute pancreatitis; exocrine pancreas dysfunction is part of Pearson's syndrome.

Myopathy. Clinically, AZT is the aetiologic agent in a skeletal myopathy in AIDS. The myopathy presents with fatigue, myalgia, muscle weakness, wasting and elevated serum creatine kinase[7,35]. We reported that zidovudine induces a mitochondrial myopathy with "ragged red fibres"[7,35] seen microscopically (Fig. 3). Ragged red fibres are characteristic histopathologic changes in the skeletal muscle of patients with mitochondrial myopathies and result from subsarcolemmal accumulation of mitochondria. By transmission electron microscopy the mitochondria are enlarged and swollen, contain disrupted cristae, and paracrystalline inclusions (Fig. 4)[36–39].

Biochemical assays performed on muscle homogenates reveal abnormal mitochondrial respiratory function. Enzyme histochemical analysis of patients' muscle biopsies shows partial deficiency of cytochrome *c* oxidase activity[40,41] (complex IV) and a high lactate/pyruvate ratio (consistent with abnormal mitochondrial function) is seen in the blood of patients with AZT-myopathy[42]. Assessment of muscle metabolism *in vivo* using magnetic resonance spectroscopy shows marked phosphocreatine depletion with slow recovery only in AZT-treated, HIV-positive patients[43]. This suggests impaired oxidative metabolism as a result of AZT-induced abnormalities in mitochondria.

Because of mitochondrial dysfunction in AZT-induced myopathy, long-chain fatty acids may be mobilized ineffectively for β-oxidation. Consequently, fat accumulates intracellularly and can be seen histopathologically[44] (Fig. 5). Muscle biopsies from AZT-myopathy patients contain decreased mtDNA[35], and this correlates with decreased mtDNA, mtRNA, polypeptide synthesis and altered histochemistry and ultrastructure in animal systems[36–39]. These morphological, functional and molecular changes are, however, absent in AZT-naive, myopathic AIDS patients' muscle samples[7,35,36,42,45]. The pathological changes in AZT-induced myopathy are reversed when AZT treatment is discontinued and clinical improvement accompanies these histological improvements and reversal[7,35]. Pathologic and clinical reversibility of AZT myopathy is consistent with AZT terminating nascent mtDNA (Fig. 2*b*)[8].

AZT myopathy develops slowly after at least 6 months of therapy and occurs in up to 17% of AZT-treated patients[46]. It occurs not only with the high-dose therapy but with current low-dose regimens. In paediatric populations with AIDS, AZT myopathy is less frequently recognized and may be masked by coexistent encephalopathy[47].

Experimental evidence in non-HIV-infected tissues supports the myotoxicity of AZT. In various human cell lines, AZT exposure causes a reduction in mtDNA abundance and gives rise to abnormal mitochondria with extensive lipid accumulation in human myotubes[39,48,49]. Rats treated with AZT develop ultrastructural abnormalities in their skeletal and cardiac muscle mitochondria associated with depression of muscle mtDNA and mitochondrial polypeptide synthesis, impaired cytochrome *c* reductase and an uncoupling effect[37–39,48,49].

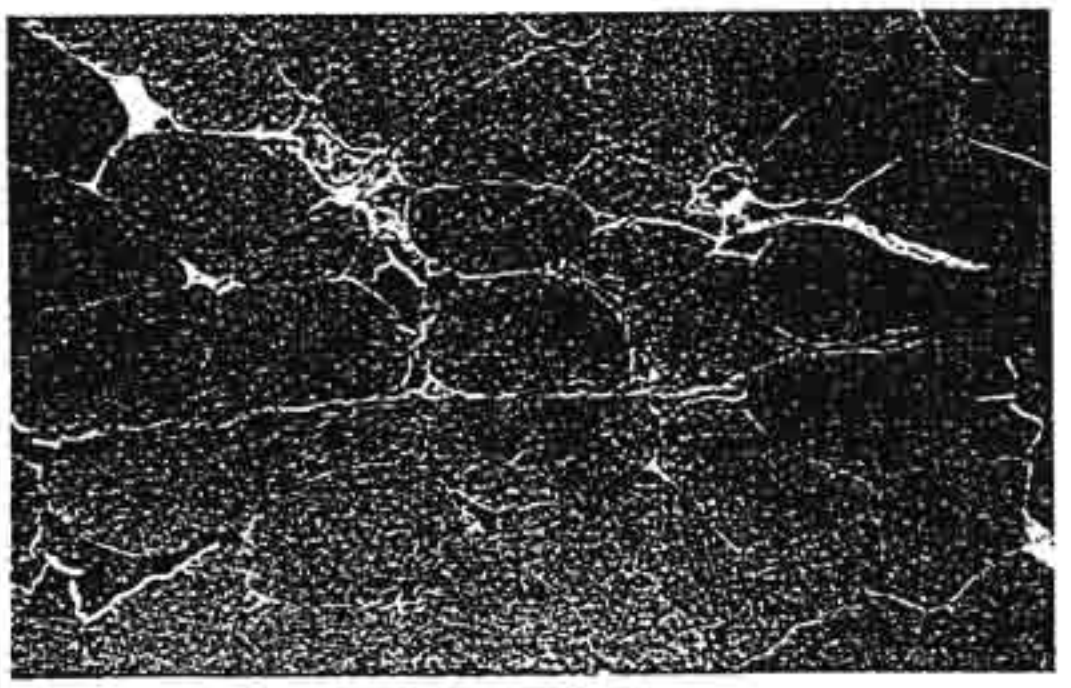

Fig. 3 Cross section of a snap-frozen muscle biopsy from a patient with muscle weakness, myalgia and fatigue after 8 months of AZT-therapy. Note several "ragged-red" fibres with subsarcolemmal cracks shown to be due to mitochondrial myopathy and mtDNA depletion. A few degenerated fibres are noted. The tiny vacuoles in a few fibres represent accumulated fat. Trichrome stain (x 320).

Cardiotoxicity. Dilated cardiomyopathy related to AZT or other antiretroviral therapy was reported in AIDS patients. Cessation of the presumed toxic ANA therapy resulted in improved left ventricular function on echocardiographic examinations[50]. AZT cardiomyopathy occurs after prolonged treatment and includes congestive heart failure, left ventricle dilation and reduced ejection fractions. Endomyocardial biopsies show intramyocytic vacuoles, myofibrillar loss, dilated sarcoplasmic reticulum and mitochondrial cristae disruption[16]. In AZT-treated paediatric AIDS patients, it has been reported that impaired cardiac function was not attributed to AZT therapy[51]. AZT-skeletal myopathy is also uncommon in children with AIDS[47].

Hepatic toxicity. Hepatic toxicity of AZT, ddI and ddC was reported recently[16,52,53] and may relate to ANA toxic effects on liver mitochondria. Fatal hepatomegaly with intracellular fat accumulation[13], lactic acidosis[52] and adult Reye's syndrome[53] in AZT-treated HIV-seropositive patients is linked to AZT-induced hepatotoxicity. Clinical features resemble those seen in FIAU toxicity (see below), and some uncommon metabolic illnesses in which neutral fat accumulates in liver cells.

Peripheral neuropathy. An axonal peripheral neuropathy is caused by zalcitabine (ddC) and stavudine (D4T) (2',3'-dideoxy-

dro-2',3'-dideoxythyinidine)[27,34]. It is characterized by painful tingling sensations (dysesthesias) in the feet and toes, loss of tendon reflexes (areflexia), distal sensory loss and mild muscle weakness. Electrophysiologic studies confirm axonal involvement. Histological findings in nerve biopsies include axonal degeneration. We observed mitochondria with disrupted cristae in some nerve axons. These findings resemble the mitochondrial "Schwannopathy" induced in the peripheral nerves of rabbits fed ddC[35]. The dideoxynucleoside-related neuropathy reverses when ddC or ddI is discontinued. However, clinical evidence suggests that these agents can aggravate pre-existing neuropathy related to HIV.

The role of tissue selectivity in ANA toxicity is highlighted with the contrast between AZT myopathy and ddI or ddC neuropathy. Interestingly, AZT does not cause neuropathy and neither ddI nor ddC exacerbate AZT myopathy. When AZT is discontinued, myopathy improves even in the face of continued ddI or ddC therapy[36]. ANA tissue selectivity may be related to differential phosphorylation of ANAs or specificity of cellular kinases for ANA phosphorylation in different tissues.

ANA neurotoxicity of ddC (and ddI) has been confirmed in tissue cultures. AZT decreases mtDNA in myoblasts and lymphoblasts[19,37]. ddC and ddI decrease mtDNA abundance, cause destruction of mitochondria and increase lactate production in a neuronal cell line (PC 12) (ref. 58). Rabbits treated with ddC develop an axonopathy with abnormal Schwann cell mitochondria and decreased myelin mRNA[35] *in vivo*. Rats treated with ddI develop an axonal neuropathy with characteristic electrophysiological abnormalities, and ultrastructural changes in the axonal mitochondria.

Neuropathy also occurs with FIAU treatment. FIAU-induced neuropathy has a later onset and persists longer than that of ddC or ddI. Toxic mechanisms may relate to the triphosphate of FIAU inhibiting DNA pol-γ and fialuridine incorporating into mtDNA (Fig. 1*a*), which may explain the persistence of FIAU neurotoxicity after ANA therapy is stopped.

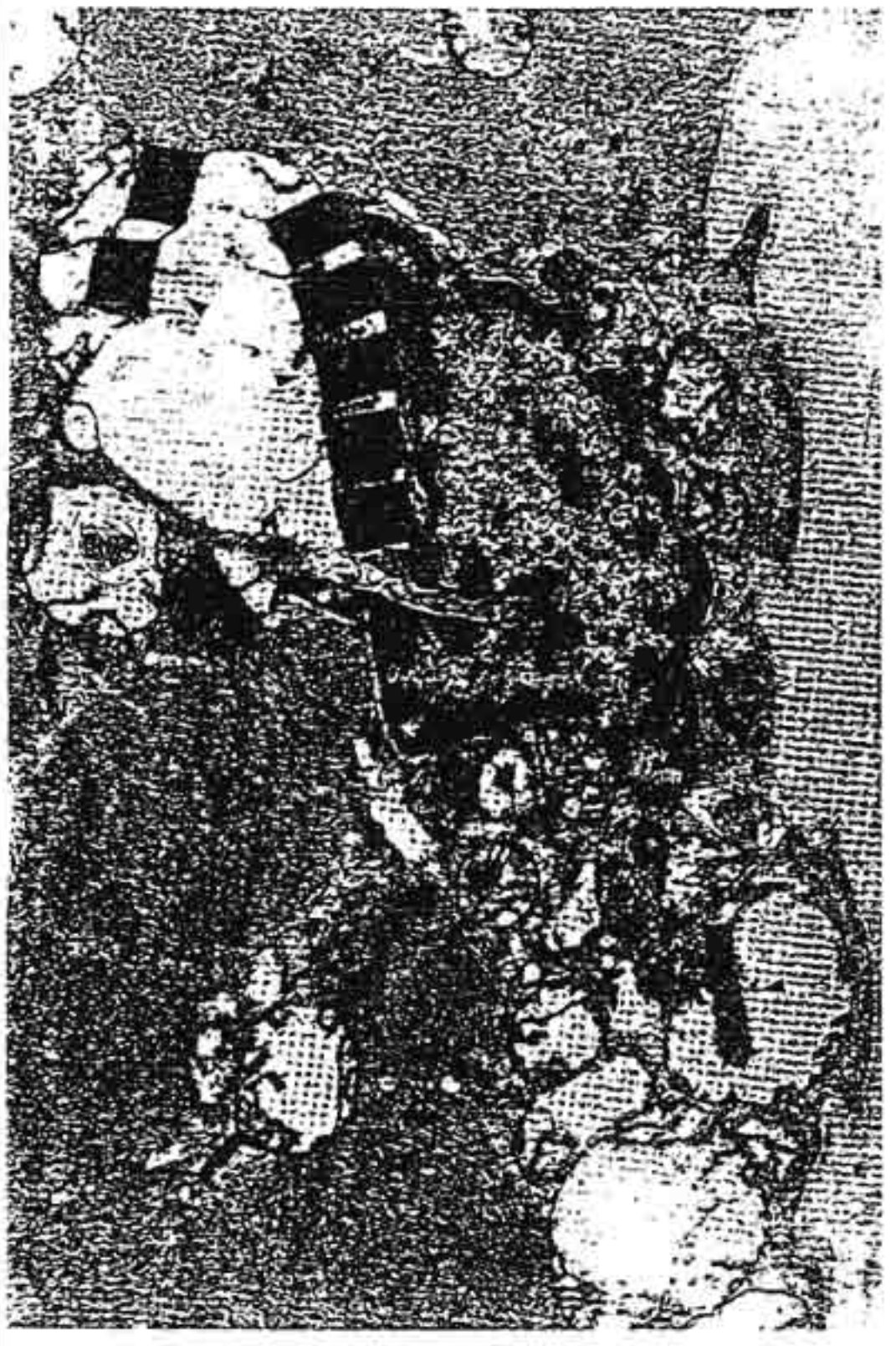

Fig. 4. Transmission electron micrograph of a muscle biopsy from a patient with AZT-myopathy. Note mitochondrial enlargement and destruction of cristae with vacuolization. Intramitochondrial paracrystalline inclusions (arrowheads) are prominent (original magnification x 14,000).

Toxicity of FIAU. The documented anti-HBV activity of FIAU[39] was the basis for a clinical trial in patients with chronic HBV. However, serious FIAU toxicity occurred in clinical trials (including liver failure requiring liver transplantation and the death of some patients)[10-12]. Toxic manifestations included profound lactic acidosis, hepatic failure, skeletal and cardiac myopathy, pancreatitis and neuropathy. Pathologic findings from autopsies and liver explants showed marked micro- and macrovesicular steatosis (D. Kleiner, pers. commun.).

In vitro, FIAU increases lactate abundance in myotube and hepatoblast culture medium, alters mitochondrial ultrastructure and causes accumulation of intracellular neutral fat. In contrast to AZT treatment, FIAU-induced changes appear to be irreversible and are consistent with FIAU's incorporation into mtDNA. FIAUTP competitively inhibits hepatic DNA pol-γ[40]. Woodchucks infected with hepatitis and treated with FIAU develop hepatic steatosis (B. Tennant, pers. commun.) and we found neutral lipid droplets in their myocardium. Rodents, canines and primates incorporate FIAU into DNA[41].

A hypothesis common to ANA toxicities

At first glance, ANA toxicities to diverse target tissues (including cardiac and skeletal muscle, peripheral nerve, liver and pancreas) do not show an obvious pattern linking features of the illnesses. A unifying hypothesis (that we will call the 'DNA pol-γ hypothesis') addresses this. It was suggested in part by work of Cheng[37], Wallace[22], and Wright and Brown[24]. The hypothesis states that manifestations of ANA toxicity in selected tissues reflect the combined effects of four principal factors: the subcellular availability and abundance of the ANA in the target tissue; the ability of cellular thymidine kinase to use the ANA as a competitive alternate substrate and for the ANA to become monophosphorylated intracellularly; the ability of the ANA triphosphate to inhibit DNA pol-γ either by serving as a competitive alternate substrate and incorporating into mtDNA or by terminating the nascent mtDNA chain non-competitively; and the metabolic requirements in the target tissues for oxidative phosphorylation.

In essence, the DNA pol-γ hypothesis suggests that DNA pol-γ function is pivotal to mitochondrial DNA homeostasis. Alterations in the abundance of substrates for DNA pol-γ may result in altered mtDNA genomes and lead to altered mitochondrial polypeptide expression and ultrastructural changes like those in AZT myopathy. In some ways, parts of the hypothesis serve as corollaries to the oxidative phosphorylation (OXPHOS) paradigm of Wallace[22]. As such, ANA toxicity may serve as a model to study genetic illnesses of mitochondria in which disturbed mtDNA replication is crucial. This was exemplified

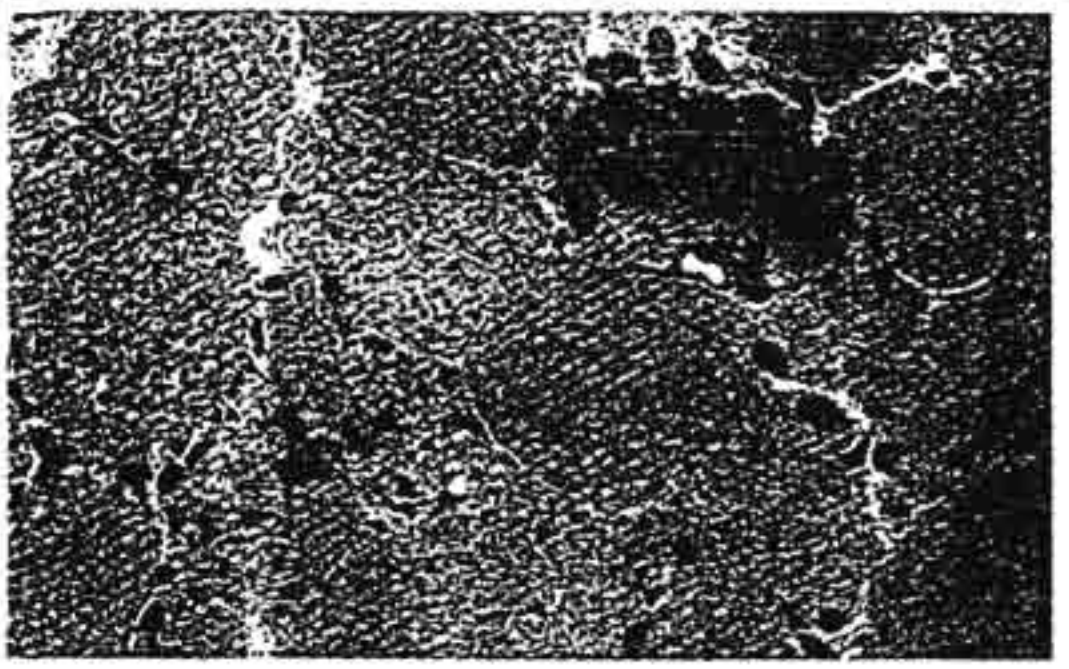

Fig. 5 Cross-section of snap-frozen muscle biopsy from a patient with AZT-myopathy shows abundant orange-staining lipid droplets. (Oil red O stain, original magnification ×560).

with AZT-induced mitochondrial myopathy's resemblance to the genetic mitochondrial DNA-depleting syndromes[62,63].

Analogies to mitochondrial diseases and clinical implications

Genetic mitochondrial illnesses include those with point mutations, mtDNA deletions or duplications, and mtDNA depletion[62-64]. These syndromes include Kearns-Sayre syndrome; myoclonus epilepsy and ragged red fibres syndrome; Leber hereditary optic neuropathy; mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes; mitochondrial DNA deletion syndromes and cytochrome *c* oxidase deficiency; Leigh disease; chronic progressive external ophthalmoplegia; Alper syndrome; and mtDNA depletion syndrome)[62-64].

OXPHOS is the main source of mitochondrial energy in many target tissues of ANA toxicity[65]. OXPHOS subunits are encoded by mtDNA. Experience with ANAs suggests that defective OXPHOS may relate to defective mitochondrial gene expression in selected target tissues. Consequently, defining molecular events in acquired OXPHOS defects, such as AZT myopathy, can help to elucidate mechanisms of some hereditary OXPHOS defects.

Pathologically defining ANA-induced changes is necessary to pinpoint subcellular targets of ANA mitochondrial toxicity. Such an approach was employed successfully in elucidating molecular mechanisms of some mitochondrial illnesses[66]. By understanding processes involved in mtDNA replication, more effective ANAs may be designed for antiviral therapy. Conversely, mitochondrial toxic ANAs serve as experimental tools to clarify mtDNA replication mechanisms, and thus based on clinical and experimental studies of ANA toxicity, mitochondrial pharmacology has become an integral part of mitochondrial medicine.

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1. Luft, R. The development of mitochondrial medicine. *Proc. natn. Acad. Sci. U.S.A.* 91, 8731–8738 (1994).
2. Wallace, D.C. Mitochondrial genetics: a paradigm for aging and degenerative diseases? *Science* 256, 628–632 (1994).
3. Elion, G.B. Acyclovir: Discovery, mechanism of action, and selectivity. *J med. Virol.* (*supp 1*) 1, 2–6 (1993).
4. McGowan, J.J. *et al.* Overview of the preclinical development of an antiretroviral drug, 2',3'-dideoxyinosine. *Rev. infect. Dis.*12, S513–S521 (1990).
5. Prusoff, W., Lin, T-S, Pivazyan, A., Sun, A.S. & Birks, E. Empirical and rational approaches for development of inhibitors of the human immunodeficiency virus - HIV-1. *Pharmac. Ther.* 60, 315–329 (1993).
6. Mitsuya, H., Yarchoan, R. & Broder, S. Molecular targets for AIDS therapy. *Science* 249, 1533–1544 (1990).
7. Dalakas, M.C., Illa, I., Pezeshkpour, G., Laukaitis, J., Cohen, B. & Griffin, J. Mitochondrial myopathy caused by long term zidovudine therapy. *New Engl. J. Med.* 322, 1098–1105 (1990).
8. Lewis, W., Simpson, J.F. & Meyer, R.R. Cardiac mitochondrial DNA polymerase-γ is inhibited competitively and noncompetitively by phosphorylated zidovudine. *Circulation Res* 74, 344–348 (1994).
9. Cui, L., Toon, S., Schinazi, R.F. & Sommadossi, J-P. Cellular and molecular events leading to mitochondrial toxicity of 1-[2'-deoxy-2'-fluoro-β-D-arabinofuranosyl]-5-iodouracil in human liver cells. *J. clin. Invest.* 95, 555–563 (1995).
10. Brahams, D. Deaths in US fialuridine trial. *Lancet* 343, 1494–1495 (1994).
11. Marshall, E.. Drug trial deaths deemed unavoidable. *Science* 264, 1530 (1994)
12. Macilwain, C. US health agencies at odds over trial deaths. *Nature* 369, 433 (1994).
13. Rozencweig, M. *et al.* Overview of phase 1 trials of 2',3'-dideoxyinosine (ddI) conducted on adult patients. *Rev. infect. Dis.* 12, S570–S575 (1990).
14. Butler, K.M. *et al.* Dideoxyinosine in children with symptomatic human immunodeficiency virus infection. *New Engl. J. Med.* 324, 137–144 (1993).
15. Freiman, J.P., Helfert, K.E., Hamrell, M.R. & Stein, D.S. Hepatomegaly with severe steatosis in HIV-seropositive patients. *AIDS* 7, 379–385 (1993).
16. d'Amati, G., Kwan, W. & Lewis, W. Dilated cardiomyopathy in a zidovudine treated AIDS patient. *Cardiovasc. Pathol.* 1, 317–320 (1992).
17. Chang, C-N, Skalski, V., Zhou, J.H. & Cheng, Y-C. Biochemical pharmacology of (+)-and (−)-2',3'-dideoxy-3'-thiacytidine as anti-hepatitis B virus agents. *J. biol. Chem.* 267, 22414–22420 (1992).
18. Reichard, P. Interactions between deoxyribonucleotide and DNA. *Annu. Rev. Biochem.* 57, 349–374 (1988).
19. Munch-Petersen, B., Cloos, L., Tyrsted, G. & Eriksson, S. Diverging substrate specificity of pure human thymidine kinase 1 and 2 against antiviral dideoxynucleosides. *J. biol. Chem.* 266, 9032–9038 (1991).
20. Fry, M. & Loeb, L.A. *Animal Cell DNA Polymerases* 221(CRC Press, Boca Raton, Florida, 1986).
21. Wang, T.S.F. Eukaryotic DNA polymerases. *Annu. Rev. Biochem.* 60, 513–552 (1991).
22. Wallace, D.C. Diseases of the mitochondrial DNA. *Annu. Rev. Biochem.* 61, 1175–1212 (1992).
23. Wallace, D.C. Mitochondrial DNA sequence variation in human evolution and disease. *Proc. natn. Acad. Sci. U.S.A.* 91, 8739–8746 (1994).
24. Wright, G.E. & Brown, N.C. Deoxyribonucleotide analogs as inhibitors and substrates of DNA polymerases. *Pharmac. Ther.* 47, 447–497 (1990).
25. Dixon, M. & Webb, E.C. *Enzymes* 3rd edn. (Academic, New York, 1979).
26. Richmann, D.D. *et al.* The toxicity of AZT in the treatment of patients with AIDS and AIDS-related complex. *New Engl. J. Med.* 317, 192–197 (1987).
27. Yarchoan, R., Mitsuya, H., Myers, C.E. & Broder, S. Clinical pharmacology of 3'-azido-2',3'-dideoxythymidine (zidovudine) and related dideoxynucleosides. *New Engl. J. Med.* 321, 726–738 (1989).
28. Nickel, W., Austermann, S., Bialek, G. & Grosse, F. Interactions of azidothymidine triphosphate with the cellular DNA polymerases α, δ, and ε and with DNA Primase. *J. biol. Chem.* 267, 848–854 (1992).
29. Dainiak, N., Worthington, M., Riordan, M.A., Kreczko, S. & Goldman, L. 3'-Azido-3'-deoxythymidine (AZT) inhibits proliferation in vitro of human haematopoietic progenitor cells. *Br. J. Haematol.* 69, 299–304 (1988).
30. Scadden, D.T., Zon, L.I. & Groopman, J.E. Pathophysiology and management of HIV-associated hematologic disorders. *Blood* 74, 1455–1463 (1989).
31. Hamburger, A.W. & Chen, R-B. Amelioration of azidothymidine-induced erythroid toxicity by hemin and stem cell factor in immune-suppressed mice. *Exp. Hematol.* 22, 348–352 (1994).
32. Yoshida, Y. & Yoshida, C. Reversal of azidothymidine-induced bone marrow suppression by 2',3'-dideoxythymidine as studied by hemopoietic clonal culture. *AIDS Res. hum. Retrovir.* 6, 929–932 (1990).
33. Falcone, A., Darnowski, J.W., Ruprecht, R.M., Chu, S.H., Brunetti, I. & Calabresi, P. Different effect of benzylacyclouridine on the toxic and therapeu-

tic effects of azidothymidine in mice. *Blood* 76, 2216–2221 (1990).
34. Rötig, A. *et al.* Pearson's marrow-pancreas syndrome. *J. clin. Invest.* 86, 1601–1608 (1990).
35. Arnaudo, E., Dalakas, M.C., Shanske, S., Moraes, C.T., DiMauro, S. & Schoen, E.A. Depletion of muscle mitochondrial DNA in AIDS patients with zidovudine-induced myopathy. *Lancet* 337, 508–510 (1991).
36. Pezeshkpour, G., Illa, I. & Dalakas, M.C. Ultrastructural characteristics and DNA immunocytochemistry in human immunodeficiency virus and zidovudine-associated myopathies. *Hum. Pathol.* 22, 1281–1288 (1991).
37. Lewis, W., Chomyn, A., Gonzalez, B. & Papoian, T. Zidovudine induces molecular, biochemical and ultrastructural changes in rat skeletal muscle mitochondria. *J. clin. Invest.* 89, 1354–1360 (1992).
38. Lewis, W. *et al.* Mitochondrial ultrastructural and molecular changes induced by zidovudine in rat hearts. *Lab Invest.* 65, 228–236 (1991).
39. Lamperth, L., Dalakas, M.C., Dagani, F., Anderson, J. & Ferrari, R. Abnormal skeletal and cardiac mitochondria induced by zidovudine (AZT) in human muscle in vitro and in an animal model. *Lab Invest.* 65, 742–751 (1991).
40. Dalakas, M.C. Carnitine loss and AZT-myopathy. *Ann. Neurol.* 36, 680–681 (1994).
41. Chariot, P. & Gherardi, R. Partial cytochrome *c* oxidase deficiency and cytoplasmic bodies in patients with zidovudine myopathy. *Neuromusc. Dis.* 5, 357–363 (1991).
42. Chariot, P. *et al.* Blood lactate/pyruvate ratio is a non invasive test for the diagnosis of zidovudine myopathy. *Arthritis Rheumat.* 37, 583–586 (1994).
43. Weissman, J.D., Constantinides, I., Hudgins, P. & Wallace, D.C. ^{31}P magnetic resonance spectroscopy suggests impaired mitochondrial functions in AZT-eated HIV-infected patients. *Neurology* 42, 619–623 (1992).
Dalakas, M.C., Monzon, M.E., Bernardini, I., Gahl, W.A. & Jay, C.A. Zidovune-induced mitochondrial myopathy is associated with muscle carnitine deficiency and lipid storage. *Ann. Neurol.* 35, 483–487 (1994).
45. Mhiri, C. *et al.* Zidovudine myopathy: a distinctive disorder associated with mitochondrial dysfunction. *Ann. Neurol.* 29, 606–614 (1991).
46. Peters, R.S., Winer, J., Landon, D.N., Stotter, A. & Pinching, A.J. Mitochondrial myopathy associated with chronic zidovudine therapy in AIDS. *Q. J. Med.* 86, 5–15 (1993).
47. Jay, C. & Dalakas, M.C. Myopathies and neuropathies in HIV-infected adults and children. in *Pediatric Aids* (Williams & Wilkins, Baltimore, 1994).
48. Semino-Mora, M.C., Leon-Monzon, M.E. & Dalakas, M.C. The effect of L-carnitine on the AZT-induced destruction of human myotubes. Part 1: L-Carnitine prevents the myotoxicity of AZT in vitro. *Lab. Invest.* 70, 102–112 (1994).
49. d'Amati, G. & Lewis, W. Zidovudine azidothymidine causes early increases in mitochondrial ribonucleic acid abundance and induces ultrastructural changes in cultured mouse muscle cells. *Lab. Invest.* 71, 879–884 (1994).
50. Herskowitz, A., Willoughby, S.B., Baughman, K.L., Schulman, S.P. & Bartlett, J.D. Cardiomyopathy associated with antiretroviral therapy in patients with HIV infection: a report of 6 cases. *Ann. intern. Med.* 116, 311–313 (1992).
51. Lipschultz, S.E., Oray, J., Sanders, S.P., Hale, A.R., McIntosh, K. & Colan, S.D. Cardiac structure and function in children with human immunodeficiency virus infection treated with zidovudine. *New Engl. J. Med.* 327, 1260–1265 (1992).
52. Chatta, G., Atieff, A.I., Cummings, C. & Tierney, L.M. Lactic acidosis complicating the acquired immunodeficiency syndrome. *Ann. intern. Med.* 118, 37–39 (1993).
53. Jolliet, P. & Widman, J.J. Reye's syndrome in adult with AIDS. *Lancet* 335, 1457 (1990).
54. Dubinsky, R.M., Yarchoan, R., Dalakas, M.C. & Broder, S. Reversible axonal neuropathy from the treatment of AIDS and related disorders with 2',3'-dideoxycytidine (ddC). *Muscle Nerve* 12, 856–860 (1989).
55. Anderson, T.D. *et al.* Mitochondrial schwannopathy and peripheral myelinopathy in a rabbit model of dideoxycytidine neurotoxicity. *Lab Invest.* 70, 724–739 (1994).
56. Jay, C. Ropka, M. & Dalakas, M.C. The drugs 2',3'-dideoxyinosine (ddI) and 2',3'-dideoxycytidine (ddC) are safe alternatives in people with AIDS with zidovudine-induced myopathy. *J. AIDS* 7, 630–631 (1994).
57. Chen, C-H. & Cheng, Y-C. Delayed cytotoxicity and selective loss of mitochondrial DNA in cells treated with the anti-human immunodeficiency virus compound 2',3'-deoxythymidine. *J. biol. Chem.* 264, 11934–11937 (1989).
58. Keilbaugh, S.A., Prusoff, W.H. & Simpson, M.V. The PC 12 cell as a model for studies of mechanism of induction of peripheral neuropathy by anti-HIV-1 dideoxynucleoside analogs. *Biochem. Pharmacol.* 42, R5–R8 (1991).
59. Fourel, I., Li, J., Hantz, O., Jacquet, C., Fox, J.J. & Trepo, C. Effects of 2'-fluorinated arabinosyl-pyrimidine nucleosides on duck hepatitis B virus DNA level in serum and liver of chronically infected ducks. *J. med. Virol.* 37, 122–126 (1992).
60. Lewis, W., Simpson, J.F., Meyer, R.R., Colacino, J.M. & Perrino, F.W. Mammalian DNA polymerases α, β, γ, δ, and ε incorporate fialuridine (FIAU) monophosphate into DNA and are inhibited competitively by FIAU triphosphate. *Biochemistry* 33, 14620–14624 (1994).
61. Richardson, F.C., Engelhardt, J.A. & Bowsher, R.R. Fialuridine accumulates in DNA of dogs, monkeys, and rats following long-term oral administration. *Proc. natn. Acad. Sci. U.S.A.* 91, 12003–12007(1994).
62. Moraes, C.T. *et al.* mtDNA depletion with variable tissue expression: an novel genetic abnormality in mitochondrial diseases. *Am. J. hum. Genet.* 48, 492–501 (1991).
63. *Progress in Neuropathy* Vol 7 (eds. Sato, T. & DiMauro, S.) (Raven Press, New York, 1991).
64. Morgan-Hughes, J.A. Mitochondrial diseases. in *Myology* (eds. Engel, A.G. & Franzini-Armstrong, C.) 1610–1660 (McGraw Hill, New York, 1994).
65. Shoffner, J.M.V.I. & Wallace, D.C. Oxidative phosphorylation diseases. Disorder of two genomes. *Adv. hum. Genet.* 19, 267–330 (1990).
66. Moraes, C.T. *et al. Mitochondrial DNA in human pathology.* (eds. DiMauro, S. & Wallace, D.C.) 97–108 (Raven, New York, 1993).