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Research Article

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Zidovudine Induces Molecular, Biochemical, and Ultrastructural Changes in Rat Skeletal Muscle Mitochondria

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Abstract

Zidovudine (AZT) inhibits HIV-1 replication in AIDS. A limiting side effect is AZT-induced toxic myopathy. Molecular changes in a rat model of AZT-induced toxic myopathy in vivo helped define pathogenetic molecular, biochemical, and ultrastructural toxic events in skeletal muscle and supported clinical and in vitro findings. After 35 d of AZT treatment, selective changes in rat striated muscle were localized ultrastructurally to mitochondria, and included swelling, cristae disruption, and myelin figures. Decreased muscle mitochondrial (mt) DNA, mtRNA, and decreased mitochondrial polypeptide synthesis in vitro were found in parallel. Mitochondrial molecular changes occurred in absence of altered abundance of cytosolic glyceraldehyde-3-phosphate dehydrogenase, or sarcomeric mitochondrial creatine kinase mRNAs. Quadriceps mitochondrial DNA polymerase gamma activity was similar in both AZT-treated and control rats. In vivo findings with rats support the hypothesis that AZT-induced inhibition of mtDNA replication has an effect of depressing the abundance of striated muscle mtDNA, mtRNA, and mitochondrial polypeptide synthesis. This experimental approach may be useful to examine mitochondrial or toxic myopathies. (*J. Clin. Invest.* 1992. 89:1354-1360.) Key words: AIDS • azidothymidine • zidovudine • DNA polymerase gamma • myopathy

Introduction

The use of zidovudine (AZT)¹ as a principal antiretroviral agent in AIDS led to awareness of a new complication of deoxynucleoside (ddN) therapy which is manifested as striated myopathy in the case of AZT (1). This myopathy poses a significant morbidity to patients who receive AZT, and limits therapy (2, 3). Early preclinical and clinical pharmacologic studies did

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1. Abbreviations used in this paper: AZT, zidovudine; G3PD, glyceraldehyde-3-phosphate dehydrogenase; mt, mitochondrial; mtDNA, mitochondrial DNA; pMM26, mouse mitochondrial genome; sMtCK, sarcomeric mitochondrial creatine kinase; SSC, standard saline citrate.

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not focus on myotoxicity of AZT, but rather on AZT's inhibition of retroviruses and the more prominent side effect of myelosuppression (4-7). Understanding of subcellular mechanisms of AZT muscle toxicity was hampered by clinical problems intrinsic to AIDS. Controversy about the pathogenesis of AZT myopathy may have stemmed from the protean manifestations of AIDS which were inseparable from the AZT myopathy per se (8). Attempts to address the myopathy clinically met with success (9), but were not definitive. An experimental model was employed here to elucidate events intrinsic to the development of AZT myopathy, to define subcellular targets of AZT, and to identify molecular and biochemical derangements induced by AZT in striated skeletal muscle. This AZT myopathy model used AZT-treated rats, which were incapable of being infected by HIV, and isolated the myopathy from related problems.

AZT doses were higher than those in current clinical use by approximately one order of magnitude (10), but were similar to doses in early preclinical studies with rodents (7). Our recent experiments focused on AZT-induced cardiac myocyte alterations in rats, and related some AZT-induced toxic ultrastructural changes to altered expression of cardiac mitochondrial mRNA (11). For striated skeletal muscle studies, fixation was optimized to identify ultrastructural alterations in an AZT myopathy model that closely resembled the human side effect (1, 9).

Methods

Animals and drug treatment. Adult Sprague-Dawley female rats (groups of 3; ~ 200-250 g each; Charles River Breeding Laboratories, Inc., Cambridge, MA) were given zidovudine 1 mg/ml (pharmaceutical grade, water soluble, white powder; lot 85/0567-177-V; Burroughs Wellcome, Research Triangle Park, NC) dissolved in drinking water ad lib. for 35 d. Control rats received drinking water ad lib. without AZT. Animals were isolated in individual cages and volumes of water consumed by individual rats were determined on alternate days when fresh drinking water was replenished with or without fresh AZT (1 mg/ml). Rats were weighed at the beginning and end of each experiment. The experiment was performed in triplicate, but polypeptide synthesis studies were performed on one pair of animals.

Preparation of striated skeletal muscle for electron microscopy. Striated skeletal muscle samples (gastrocnemius) from AZT-treated and control animals were obtained by "perfusion fixation" in 3% glutaraldehyde by vascular perfusion through the abdominal aorta, as we have done recently (11). Procedures were designed for skeletal muscle, and varied from those used in our ultrastructural studies of myocardium (11), with minor modifications (12). Rats were perfused initially with modified Locke's solution (oxygenated with bubbling 100% O₂ for 20 min) containing 0.1% NaNO₂ (9.2 g/liter NaCl, 0.42 g/liter KCl,

0.24 g/liter $\text{CaCl}_2 + 2\text{H}_2\text{O}$, 0.15 g/liter NaHCO_3 , 1.0 g/liter sucrose, and 1.0 g/liter NaNO_2 at 40°C from a reservoir height of 140 cm for 2 to 3 min. This was followed by perfusion with 150 ml of 3% glutaraldehyde in 0.1 M cacodylate (Sigma Chemical Co., St. Louis, MO), plus 2 mM CaCl_2 buffer pH 7.2 for 10 min. The gastrocnemius muscle samples were removed, rinsed briefly in cold Locke's solution, and placed in 10 ml of 3% glutaraldehyde-cacodylate at 4°C.

Electron microscopy of rat muscle samples. For electron microscopy, the perfusion-fixed muscle tissue was cut into 1 mm slices, longitudinally and transversely, fixed in 3% glutaraldehyde-cacodylate for 4 h at 4°C, rinsed in cold Locke's solution, and postfixed in 1% OsO_4 (Sigma Chemical Co.) in PBS, pH 7.4 for 2 to 3 h. After osmication and buffer rinses, the tissue was dehydrated with graded ethanols, and embedded in "Spurr" (13). Each muscle sample was sectioned (100 nm), stained with lead citrate and uranyl acetate, and examined and photographed on an electron microscope (JEOL-JEM-100CX; JEOL, Ltd., Tokyo).

DNA extraction from muscle. DNA was extracted from muscle, according to the methods of Williams and colleagues (14, 15).

Restriction enzyme digests of mitochondrial DNA (mtDNA). The mtDNA was cut, using the restriction enzyme *Hae*II (2 IU/ μg mtDNA [16]). The mixture was incubated at 37°C for 2–3 h, whereupon 10× "stop solution" was added (20% Ficoll 400, 0.1M EDTA pH 8.0, 1% SDS, 0.25% bromophenol blue) and loaded onto a 1% agarose gel in 1× TAE buffer (0.04 M Tris-acetate, 0.002 M EDTA, pH 8.5) and run overnight at 15 V. After electrophoresis, the DNA was transferred onto Hybond filter (Amersham Corp., Arlington Heights, IL), according to standard methods (17).

DNA hybridization. DNA hybridization was similar to that for Northern analysis below, with the addition of 1% SDS in the buffers.

RNA extraction from muscle samples. Parallel to DNA studies, AZT-treated and -untreated rat quadriceps femoris muscle samples were prepared for RNA extraction. RNA from individual striated muscles was extracted under RNase-free conditions, using acid guanidinium-phenol-chloroform extraction methods, as described by Chomczynski and Sacchi (18), in ways that were similar to those used by us for heart tissue (11).

Glyoxal denaturation of RNA and agarose gel electrophoresis. The methods used were those described by Maniatis et al. (19) and by Thomas (20).

Northern transfer of electrophoresed RNA. Glyoxalated RNA was transferred from agarose gels to nitrocellulose paper (Schleicher and Schuell Inc., Keene, NH), using 3 M sodium chloride + 0.3 M trisodium citrate (20× standard saline citrate [SSC], as described by Thomas (20). After overnight transfer (15–20 h), blots were dried, baked at 80°C for 2 h, and treated in 20 mM Tris pH 8.0 at 95°C for 5 to 10 min, then allowed to cool to room temperature.

Northern hybridization. The following cDNA probes were used for Northern hybridization: rat sarcomeric mitochondrial creatine kinase (sMtCK [21], courtesy of R. Mark Payne, Washington University, St. Louis, MO), glyceraldehyde-3-phosphate dehydrogenase (G3PD; American Type Culture Collection, Rockville, MD), and the BamHI fragment of mouse mitochondrial genome (pMM26 [22]), which included the 12S and 16S mitochondrial ribosomal subunits, cytochrome *b*, and four subunits of the respiratory chain NADH dehydrogenase (courtesy of R.S. Williams, UTSW, Dallas, TX). To examine mitochondrial cytochrome *b* mRNA, a PCR-generated product of the pMM26 cDNA probe which codes for 1,013 bases of the cytochrome *b* cDNA was prepared, using 21-base oligomer primers from Res. Genetics (Huntsville, AL; 5'-TGACCTACCTGCCCCATCCAA-3'; 5'-GATCGAGGCTAGTTGGCCA-3') and a thermal cycler (Cetus-Perkin Elmer, Palo Alto, CA), according to manufacturer's directions. Prehybridization and hybridization solutions used were those described by Wahl et al. (23), except that when the pMM26 cDNA probe was used, dextran sulfate was omitted. Blots were prehybridized in heat-sealable plastic bags at 42°C overnight for 18 to 20 h. The solutions were decanted and replaced with hybridization solution containing cDNA probes labeled with α - ^{32}P dCTP (specific radioactivity

3,000 Ci/mmol; New England Nuclear, Boston, MA) using the random primer method (Amersham Corp.) at 2×10^6 cpm/ml. After overnight incubation (18 to 24 h) at the same temperature used for prehybridization, blots were washed at room temperature for 10 to 15 min in two changes each of $2 \times \text{SSC} + 0.1\% \text{SDS}$, $0.5 \times \text{SSC} + 0.1\% \text{SDS}$, and if necessary, $0.1 \times \text{SSC} + 0.1\% \text{SDS}$. The radioactive bands were analyzed on a Betascope 603 blot analyzer (Betagen, Waltham, MA), and then exposed to x-ray film for varying times at -70°C , using an intensifying screen (Eastman-Kodak Co., Rochester, NY). For rehybridization with subsequent probes, blots were stripped by treatment in a solution containing $0.05 \times \text{SSC}$, 5 mM EDTA pH 8.0, and 0.1% SDS at 95°C for 5 to 10 min, then allowed to cool to room temperature.

Plasmid DNA and cDNA isolation. All plasmid DNA was isolated by following the procedure of Birnboim (24). cDNA was isolated from plasmid DNA, as described by Wieslander (25), with some minor modifications that we applied in the past (11, 26).

^{35}S -labeling of mitochondrial proteins in vitro. Mitochondrial translation products were labeled in dissociated muscle fibers with [^{35}S]-methionine for 30 min in the presence of an inhibitor of cytosolic protein synthesis, emetine (100 $\mu\text{g}/\text{ml}$). Mitochondria were isolated by differential centrifugation after disruption of the muscle fibers, as will be described elsewhere (A. Chomyn, manuscript in preparation). Protein concentration was determined, using the Bio-Rad protein assay reagent. Equal amounts of muscle mitochondrial protein were run on SDS-15 to 20% exponential polyacrylamide gradient gel in Laemmli buffer system (27). Identification of radiolabeled bands on the fluorogram (28) has been described (29). External standards for mitochondrial polypeptides were from R2 fibroblasts, as described previously (29). For densitometry of polypeptide bands, a laser densitometer of fixed wavelength (622 nm) was employed (LKB Instruments Inc., Bromma, Sweden).

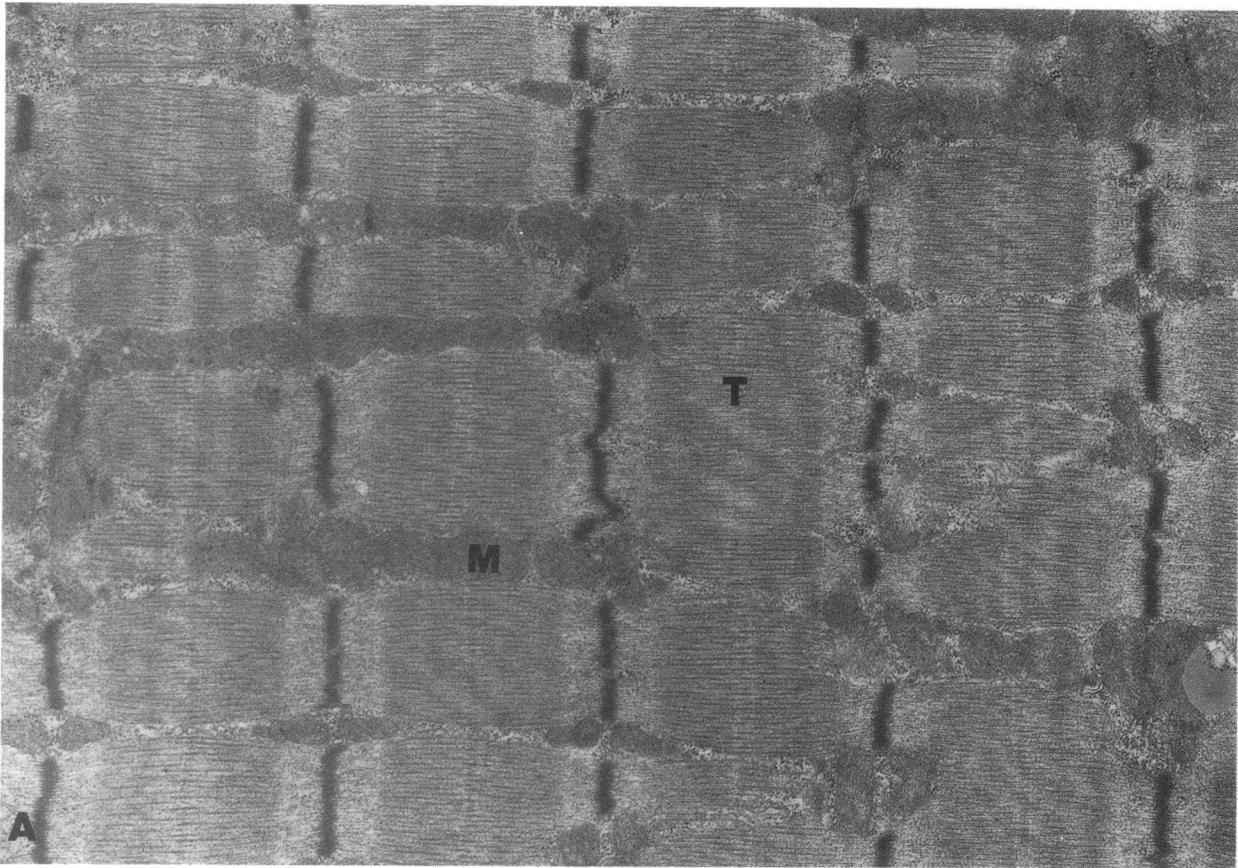
Muscle DNA polymerase gamma assays. The mitochondrial DNA polymerase, DNA polymerase gamma, was assayed from quadriceps femoris muscle extracts, according to the methods of Yamaguchi and colleagues (30). Protein concentration of the supernatant solution was determined, using the Bio-Rad protein assay and bovine albumin external standards. 5 μg of crude extract was assayed in 25 μl reaction mixture containing 50 mM Tris-HCl (pH 8.5 at 37°C), 1 mM DTT, 0.5 mM MnCl_2 , 80 $\mu\text{g}/\text{ml}$ poly (rA), 16 $\mu\text{g}/\text{ml}$ (dT) $_{12-18}$, 0.1 mM [^3H]-dTTP (80 Ci/mmol), 15% glycerol, 400 $\mu\text{g}/\text{ml}$ BSA, 110 mM KCl, 20 mM KPi , pH 8.5 at 37°C. Reactions were stopped by pipetting reaction mixture onto Whatman DE-81 filter paper (2.1 cm discs). Filters were washed 6× (4 min each) in 5% Na_2HPO_4 , then twice in water, twice in 95% ethanol, and air dried (31). Filters were suspended in 10 ml of Ecoscint and counted in a scintillation counter (model LS-7800; Beckman Instruments, Inc., Palo Alto, CA).

Results

General. The mean weights of the rats were comparable. Initially, weights were 216 ± 4 g and 199 ± 13 g for the control and test group, respectively, and weights were 249 ± 21 g and 253 ± 17 g, respectively, after 35 d. No deaths occurred during the experimental period, and the animals appeared healthy. Mean daily AZT consumption in the test group was 123 ± 15 mg/kg per d, and was comparable to the consumption of AZT by rats in past experiments (11).

Electron microscopy of rat striated skeletal muscle. Myofilaments from striated myocytes of both AZT-treated and untreated rats showed registered sarcomeres with organized Z discs. Thin filaments and thick filaments from the AZT-treated rat striated muscles revealed no disruption or splaying, compared with samples from control rat striated muscles (Fig. 1, A and B).

In both test and control striated skeletal myocytes, mitochondria were present throughout the sarcoplasm in a charac-



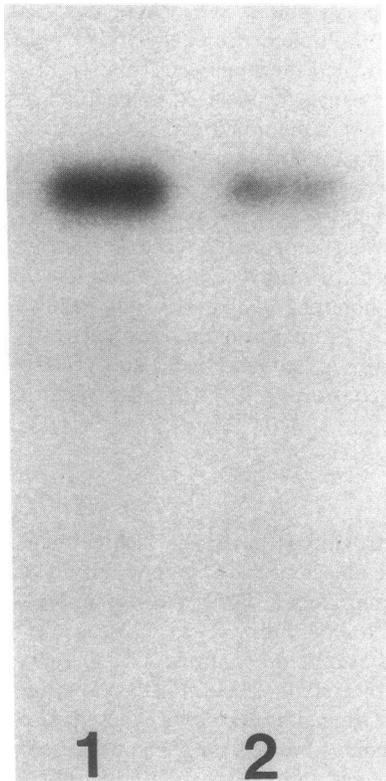


Figure 2. Southern blot analyses of extracted striated muscle mtDNA from AZT-treated rat striated muscle mitochondria hybridized with pMM26 cDNA. As above, rats received AZT (mean AZT dose: 123 mg/kg per d) or tap water. Rats were killed after 35 d of AZT therapy and striated muscle DNA rapidly extracted. Southern blot of striated muscle DNA (10 μ g/lane) from tap water controls (lane 1) and AZT-treated rats (lane 2) were probed with pMM26 cDNA (8 h exposure). Results show depressed mtDNA hybridization signal from AZT-treated rat muscles.

teristic organized pattern around the Z line. Control mitochondrial cristae were dense, and mitochondrial membranes were intact. Striking ultrastructural differences between the AZT-treated rat striated skeletal muscle mitochondrial and control rat muscle mitochondria were found. Muscle samples from AZT-treated rats revealed widespread mitochondrial swelling with disruption and fragmentation of cristae. Some mitochondrial cristae appeared focally dissolved and poorly organized within swollen mitochondrial membranes (Fig. 1 B). Other mitochondria revealed intramitochondrial myelin figures consisting of whorled lamellar bodies (which appeared continuous with mitochondrial membranes in some foci). Clumped and focally swollen mitochondrial cristae were found at higher magnification. Mitochondrial changes were present in multiple samples of striated myocytes from AZT-treated rat muscles, and absent from samples of muscles of untreated rats.

Analysis of striated skeletal muscle mtDNA. Southern analysis of the HaeII-cut mtDNA revealed decreased mtDNA radiographic signal in extracts from the quadriceps femoris of AZT-treated rats, compared with untreated controls (Fig. 2). Quantitative analysis of the Southern blot autoradiographic signals on the Betascope 603 revealed specific radioactivity of the spot to be 410 ± 77 dpm (mean \pm SD) for untreated quadriceps femoris DNA, and 194 ± 89 dpm for AZT-treated rat muscle, compared with untreated controls (53% decrease; $P < 0.05$).

Figure 1. Representative electron photomicrographs of striated muscle from control (A) and AZT-treated (B) rats. Rats drank water ad libitum with and without AZT for 35 d (1 mg/ml). (A) Myocytes from control gastrocnemius muscle sample. Ultrastructural features include registered sarcomeres with thick and thin filaments (T) and mitochondria (M). (B) Myocytes from gastrocnemius of AZT-treated rat. Severe distortion of mitochondrial internal architecture with swelling and lysis of mitochondrial cristae is seen. Myelin figures (intramitochondrial lamellar bodies) are present. Original magnification: $\times 10,000$.

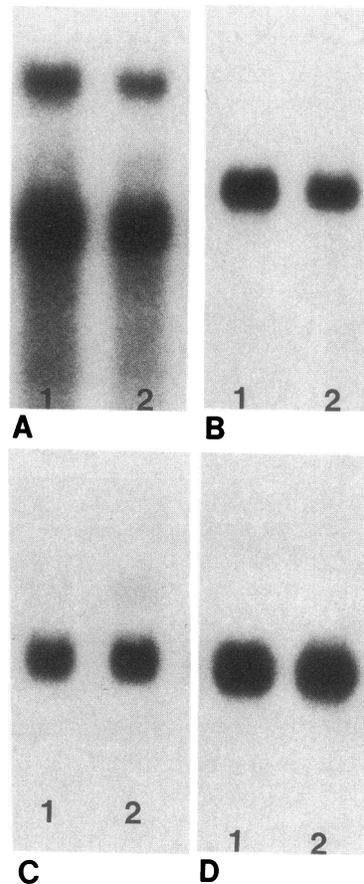


Figure 3. Northern blot analyses of extracted striated muscle RNA from control and AZT-treated rat. Extracted RNA was hybridized with specific cDNA probes. As above, rats received AZT (mean AZT dose: 123 mg/kg per d) or tap water. Rats were killed after 35 d of AZT therapy and striated muscle RNA rapidly extracted. (A) Northern blot of striated muscle RNA (10 μ g/lane) from tap water controls (lane 1) and AZT-treated rats (lane 2) were probed with pMM26 cDNA (10 h exposure); (B) (as in A) Probed with the 1,013 base PCR product of cytochrome *b* (8 h); (C) (as in A) Probed with sMtCK cDNA (8 h exposure); and (D) (as in A) probed with G3PD cDNA (5 h exposure). A relative depression in mRNA hybridization signal (particularly cytochrome *b*) from AZT-treated rat muscle is seen. sMtCK and G3PD mRNA signals (C and D) were similar in the AZT-treated group and control samples.

Analyses of striated skeletal muscle RNA. Northern analyses of abundance of selected mitochondrial (mt-) and cytoplasmic RNAs revealed discreet targeted defects in expression of selected subcellular elements of the myocyte exposed to 35 d of AZT. The Northern blot of RNA from the AZT-treated rat quadriceps showed a generalized decrement in signal of mtRNAs, including cytochrome *b* mRNA, 16S, and 12S mtRNA (Fig. 3 A). When that blot was stripped and reprobed with the cytochrome *b* probe, a 45% decrement in cytochrome *b* mRNA autoradiographic signal was found in the muscle samples of AZT-treated group. sMtCK mRNA autoradiographic signals from striated muscle extracts were unchanged between AZT-treated rats and controls (Fig. 3 C; 8 h exposure). Cytoplasmic G3PD mRNA (Fig. 3 D; 5 h exposure) also revealed no difference between AZT-treated and control muscle extracts.

Analysis of striated skeletal muscle mitochondrial polypeptide expression in vitro. Isolated muscle fibers from quadriceps of AZT-treated and control rats retained the ability to incorpo-

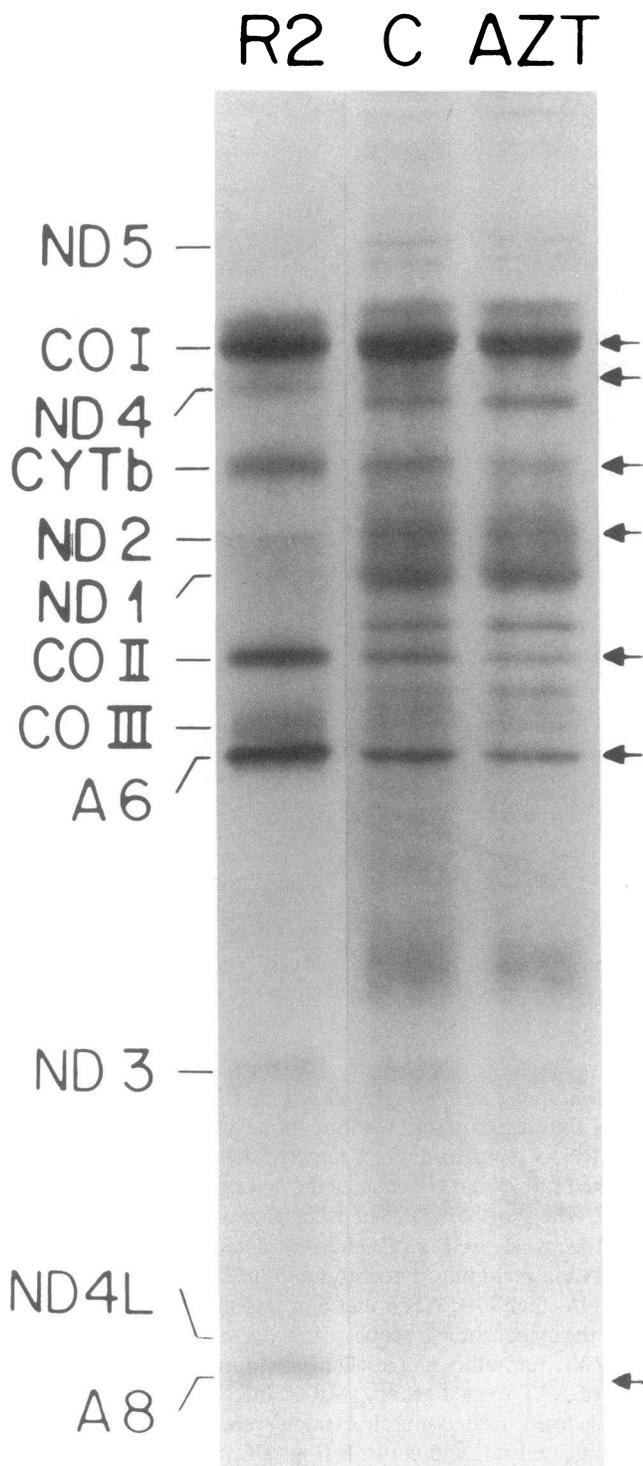


Figure 4. Patterns of mitochondrial translation products in quadriceps from control (C) and AZT-treated (AZT) rats after SDS PAGE. Isolated quadriceps femoris muscle was exposed to medium containing [³⁵S]methionine (30 min) in the presence of emetine. Mitochondrial fractions were isolated, and equal amounts of mitochondrial protein (80 μg/lane) were subjected to SDS gradient PAGE and PPO-mediated fluorography. Rat 2 fibroblast mitochondrial translation products (R2), labeled with [³⁵S]methionine and identified (as done previously), served as markers. Arrows indicate mitochondrial translation products descending from top: CO I, ND4, CYT *b*, ND 2, CO II, A 6, and A 8. Unidentified bands are visible below ND4 and between CYT *b* and CO II. CO I, II, III, subunits I, II and III of cy-

rate [³⁵S]methionine into polypeptides. SDS PAGE analysis with fluorography revealed multiple dense polypeptide bands in both control and AZT-treated rat quadriceps (Fig. 4). Decreases in autoradiographic signals for resolved mitochondrial polypeptides (Fig. 4, arrows) were noted on SDS gradient PAGE when compared with radiolabeled polypeptides synthesized by control muscle fibers. Densitometric analyses revealed reductions in band signal of 42% for cytochrome *b*, 41% for CO II, and 54% for ATPase 6 when compared to those of controls.

Analysis of striated skeletal muscle mitochondrial DNA polymerase gamma. Mitochondrial DNA polymerase gamma activity was present in extracts from frozen samples of rat quadriceps femoris muscle. Mean activity was 3.3 ± 0.4 and 3.8 ± 0.6 (nmol/min per mg [NS]) in control and AZT-treated rat quadriceps samples, respectively.

Discussion

AZT is a significant antiretroviral agent used in the treatment of AIDS (2–8). Major side effects of AZT therapy related to constitutional symptoms and nausea. Bone marrow suppression by AZT was reported to be limiting (2–5). These side effects impact on the dose, the ability to continue AZT therapy, or the ability to substitute dideoxynucleosides (ddNs) to minimize the toxic effects (32). Original protocols (7) exposed mice to orally ingested AZT in concentrations similar to those used here. Neither histopathologic nor ultrastructural muscle changes were reported. Ultrastructural examination of perfusion-fixed striated muscle, coupled with correlative biochemical and molecular studies, enabled us to evaluate pathogenetic, subcellular changes in muscle from AZT-treated rats which otherwise may not be visible.

Oral AZT in rats (in doses approximately tenfold those used currently in humans [7, 32]), had an associated skeletal myotoxicity that resembled the iatrogenic human condition in many ways (1, 2, 9, 33). The myotoxicity was manifested here ultrastructurally by mitochondrial swelling, cristae disruption, and presence of whorled lamellar bodies inside rat striated muscle mitochondrial membranes. Parallel to these changes were findings of decreased abundance of mtDNA in extracts from quadriceps femoris of AZT-treated rats, decreased mtRNA including 16S and 12S ribosomal RNA and cytochrome *b* mRNA, and decreased abundance of ³⁵S-labeled mitochondrial encoded polypeptides on SDS gradient PAGE. No comparable decrements of selected nuclear-encoded mRNAs were found.

Pathologic changes in clinical AZT-induced skeletal myopathy included histologic and ultrastructural changes (“ragged red” fibers histologically, mitochondrial crystalline inclusions ultrastructurally) in immersion-fixed muscle biopsies obtained from AZT-treated AIDS patients (1). Ultrastructural findings in our study resembled mitochondrial changes in clinical AZT myopathy (including mitochondrial lysis [1, 33]), and changes in skeletal muscle samples from AZT-treated hamsters experimentally (34). Analogies could also be made between our findings and some ultrastructural changes found in mitochondria from HeLa cells treated with chloramphenicol (including intra-

tochrome *c* oxidase; ND 2, 3, 4, 4L, and 5, subunits 2, 3, 4, 4L and 5 of NADH dehydrogenase; A 6 and 8, subunits 6 and 8 of the [H⁺]-ATPase; CYT *b*, cytochrome *b*.

mitochondrial lamellar bodies [35]), and early changes in some mitochondrial myopathies (including whorled mitochondrial lamellae [36]). This AZT myopathy model yielded localized toxic ultrastructural defects in mitochondria with molecularly related changes reflected by altered expression of products of the mitochondrial genome.

Systematic ultrastructural examination of all tissues was not undertaken. Mitochondrial changes (included swelling and disruption of mitochondrial cristae) were demonstrated in cardiac muscle from AZT-treated rats (perfusion fixed, but using a slightly different buffer system [11]). Intramitochondrial lamellar figures were not prominent in the heart. Accompanying myocardial ultrastructural alterations, mtRNA was decreased, but not nuclear-encoded mRNAs, including mRNAs which encode polypeptides that are translocated into mitochondria. Mitochondrial ultrastructural changes were absent in liver samples from AZT-treated rats (11).

Activities of quadriceps DNA polymerase gamma, the DNA polymerase of mitochondria, were similar from AZT-treated rat muscles and from controls. This supported in vitro pharmacologic and toxicologic data that suggested AZT interferes with mtDNA replication by mechanisms that involve mtDNA chain termination in vivo (37–39).

Related experimental findings included disrupted mitochondria, decreased abundance of muscle mtDNA, decreased abundance of 16S and 12S ribosomal mtRNAs and cytochrome *b* mRNA, and decreased mitochondrial polypeptide synthesis. AZT's myotoxic mechanism may relate to interference with mtDNA replication. It may be reasonable to expect that interference at this step may initiate AZT-induced toxic effects (due to decreased mitochondrial gene expression) which include changes in expression of mtRNA, mitochondrial polypeptides, and disturbed mitochondrial ultrastructure (35). All of these effects were observed experimentally here.

This effective AZT-induced myopathy model offered an opportunity to obtain data which supported clinical findings of Schon and colleagues who reported decreased mtDNA in muscle biopsies from AIDS patients with AZT-induced myopathy (9). Simpson, Prusoff, and colleagues showed that AZT (in micromolar concentrations) inhibited mtDNA replication in vitro, at the level of DNA polymerase gamma (40). At that time, analogous in vivo effects were not yet reported.

Decreased abundance of striated muscle cytochrome *b* mRNA, 16S and 12S ribosomal mtRNAs, and mitochondrial polypeptide synthesis were noted with AZT exposure. Significance of these findings was amplified in absence of changed expression of mRNAs encoding either cytosolic polypeptides (G3PD, sMtCK). Finding sMtCK mRNA unchanged was particularly important, since sMtCK is a polypeptide whose gene is nuclear derived, translated in the cytoplasm, and translocated into mitochondria. In essence, sMtCK served as a critical control for localizing the AZT toxic effect to muscle mitochondrial genome and not elements of the mitochondrial matrix that are derived from expression of nuclear genes. This was consistent with postulated mechanisms of AZT's inhibition of replication of mtDNA (37, 38, 39) and not nuclear DNA. Based on Northern analysis, cytochrome *b* does not appear to be the sole target of AZT damage to the striated muscle mitochondrial genome, but decreases in both mtRNA and polypeptide synthesis following AZT treatment were striking.

Selected cDNAs enabled us to probe distinct skeletal myocyte subcellular compartments and to determine that AZT se-

lectively leads to decreased mtDNA and mtRNA. This occurred in absence of changes of selected mitochondrial and cytoplasmic polypeptides which were nuclear encoded (sMtCK and G3PD), and in the face of correlative ultrastructural changes in skeletal muscle mitochondria. Some genetic myopathies have mutational differences in mitochondrial genomic loci and defined molecular targets. Similar phenotypic and clinical end points may be manifested in some of the associated syndromes, irrespective of the locus of the mutation in the mitochondrial genome (41, 42). It may be possible that AZT myotoxicity may serve as a model of molecular events in some genetic mitochondrial myopathies, since some phenotypic similarities exist between this model and the mitochondrial myopathies (33, 41, 42).

In summary, after 35 d of treatment, AZT caused selective changes in rat skeletal striated muscle which localized ultrastructurally to mitochondria, and which included swelling, cristae disruption, and mitochondrial myelin figures with whorled lamellae. Associated with the ultrastructural changes were decreased muscle mtDNA, mtRNA, and mitochondrial polypeptide synthesis in vitro. Mitochondrial molecular changes occurred in absence of changes in G3PD or sMtCK mRNA. Quadriceps mitochondrial DNA polymerase gamma activity was similar in the AZT-treated and control rats. Experimental observations in vivo with rats supported the concept that AZT-induced inhibition of mtDNA replication, mediated by interference with DNA polymerase gamma, has an effect of depressing the abundance of striated muscle mtDNA, mtRNA, and mitochondrial polypeptide synthesis.

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