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3'-Azido-2'3'-dideoxythymidine (AZT) Inhibits Proliferation of Normal Bone Marrow Progenitor Cells by Reducing Mitochondrial Biogenesis. L.D. Lewis[1], S. Amin[2], T.M. Trishman[2], C.I. Civin[2], and P.S. Lietman.[1] Divisions of [1]Clinical Pharmacology and [2]Pediatric Oncology. The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.

Bone marrow suppression is a common side effect of 3'-Azido-2',3'-dideoxythymidine (AZT) therapy in HIV-1 infected individuals. We separated normal human bone marrow progenitor (CD34+) cells by immunomagnetic affinity purification. Cells were cultured in suspension in serum free medium supplemented with growth factors and exposed to AZT (0-50μM) for time periods up to twelve days. We measured growth rate, cell viability (by trypan blue dye exclusion), lactate production and mitochondrial DNA content (by dot-blot DNA-DNA hybridization). Immunofluorescent labelling of leukocyte and erythroid differentiation antigens with flow cytometry was used to enumerate different cell lineages. AZT produced time and dose dependent inhibition of myeloid cell growth with an ID_{50}=10μM after 12 days incubation, without significantly reducing viability of the cells present. Lactate production per million cells increased in a dose dependent fashion. 10μM AZT produced an increase of 86% ± 23 (mean ± SD) above control values. The mitochondrial/nuclear DNA ratio was reduced by AZT in a dose dependent manner with an ID_{50}=6μM. Erythroid cells (Glycophorin+ and CD45-) were more sensitive to AZT. 0.5μM AZT produced 78% growth inhibition of erythroid cells compared to controls. The toxic effects of AZT on bone marrow proliferation may be due to reduced mitochondrial DNA replication.

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Effects of 2'3'-Dideoxycytidine (DDC) an Anti HIV-1 Nucleoside analogue on Mitochondrial biogenesis, structure and function.

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2',3'-dideoxycytidine (DDC) is a potent inhibitor of the HIV-1 reverse transcriptase (RT) and a DNA chain terminator. In isolated mitochondrial preparations DDC has also been shown to inhibit the gamma DNA polymerase. We therefore studied Molt-4 cells grown for 4 days in the prescence of DDC and measured growth rate, cell viability by trypan blue dye exclusion, mitochondrial DNA content (using a mitochondrial DNA probe and dot-blot DNA-DNA hybridisation) and the concentration of lactate in the culture medium. DDC inhibited cell growth without significantly decreasing cell viability with an $ID_{50}(\pm SD)$= 4.70 ±.5 uM.; reduced cellular mitochondrial DNA content $ID_{50}(\pm SD)$= 0.46 ±.06 uM. and increased the lactate concentration in the culture media per 10^6 cells to 48% above control values at 4uM. Electron microscopy of the Molt-4 cells incubated in DDC 4uM for 4 days revealed no significant changes in mitochondrial structure, however after 12 days incubation in 4uM DDC the mitochondria were enlarged with severely distorted "whorled" cristae. These data demonstrate that DDC inhibits mitochondrial DNA replication producing abnormal mitochondrial ultrastructure and function. These results may have important implications for the pathogenesis of DDC induced neurological and haematological toxicity in man.