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Research project

AZT (AZIDOTHYMIDINE, ZIDOVUDIN)-INDUCED DAMAGE TO BLOOD CELLS

SCIENTIFIC SUMMARY

Reactive oxygen species (ROS) are produced in large quantities in mitochondria and cause mutations in mitochondrial DNA mtDNA). Studies of diseases associated with mutations in mtDNA show that a variety of degenerative processes and cell death due to defects in the mitochondrial oxidative phosphorylation (OXPHOS).

AZT (azidothymidine, zidovudine) is one of the most commonly used Drugs to fight AIDS the (acquired Immunodeficiency syndrome) designated disease which may be caused by HIV (human immunodeficiency virus) infections.
Previous studies have shown that the use of AZT is restricted by the occurrence of myopathies. These express themselves for the patient in muscle weakness, at the cellular level in "Ragged Red Fibers" (RRF), swollen, dysfunctional mitochondria and loss of mtDNA. In an animal model simulating these side effects of AZT treatment massive ROS-induced oxidation of mtDNA was demonstrated.

The etiology of "AIDS" is still uncertain. There is the urgent suspicion that the disease is not caused by HIV, but also by AZT and related nucleotide analogues, since it has not been ruled out so far that AZT not only in muscle, but also in

lymphocytes causes damage to mitochondria leading to cell loss. Evidence of such Drug-induced damage to the immune system could have far-reaching political, social and economic consequences.

We propose to investigate damage to mitochondria of blood cells, in particular of lymphocytes, in an animal model, which has already been developed for AZT-induced myopathies. For this we will count the number of blood cells and measure the number and quality of their mitochondria. We expect through these investigations that we can clarify whether blood cells, in particular Lymphocytes can be damaged by AZT and related nucleotide analogues. The result will lead to a better understanding of the etiology of "AIDS".

BACKGROUND

Mitochondrial myopathies and their causes: Reactive oxygen species (ROS), such as the superoxide radical, Hydrogen peroxide, or the hydroxyl radical, are physiological reactants (for an overview see Chance et al., 1979). Produced in excess, or when the antioxidant defense systems (non-enzymatic) in particular Vitamin C and E, glutathione, coenzyme Q10, (enzymatic) especially superoxide dismutase, catalase, glutathione peroxidase) are weakened. ROS lipids, damaged proteins and nucleic acids lead to the development of pathological conditions Examples include acute and chronic inflammation, arteriosclerosis, infarct damage and some forms of cancer (Halliwell and Gutteridge,1992).

Mitochondria are the most productive cellular source of ROS. Different Parts of the mitochondrial respiratory chain already produce ROS in the normal state.

The "redox cylers" (substances that are cyclically reduced and transfer electrons to molecular oxygen) alloxan, rotenone, Methylphenylpyridinium or tetrachloro-p-dylbenzodioxin, increased Ca2+ levels, Hypoxia/reperfusion, or tumor necrosis factor-cr stimulate ROS production of the mitochondria (for an overview see Richter, 1992).ROS damage directly mitochondrial lipids, proteins and nucleic acids (Richter et al., 1995; Yakes and Van Houten, 1997). ROS also cause the formation of the mutagenic 8-Hydroxyguanine and strand breaks in mtDNA (Richter et al., 1988), whereby ROS indirectly alter the properties of polypeptides attached to OXPHOS involved. OXPHOS defects in turn degrade in a vicious circle the energy yield, leading by increased ROS production to further damage and increasing OXPHOS defects.

The possibility that defective mitochondria can cause disease,

was first discussed by Luft et al. (1962). Since then, a number of myopathies have been identified associated with structurally altered muscle mitochondria (Tyler, 1992). The breakthrough in understanding mitochondrial myopathies provided studies of mutations in mtDNA (for an overview see Wallace, 1992a,b). It is now known that deletions in mtDNA lead to chronic external ophthalmoplegia (CEOP) and Kearns-Sayre (KS)syndrome while other syndromes by individual Base substitutions in mitochondrial are caused tRNALvs and tRNA. It is very likely that myopathies are caused at least partly due to mitochondrial ROS (Wallace et al., 1995).

Azidothymidine (AZT) and mitochondrial myopathies

AZT is one of the drugs most commonly used in "AIDS". A serious side effect of AZT treatment is the occurrence of myopathies (Groopman, 1990; Lewis and Dalakas, 1995), which, like "classical" Myopathies at the cellular level as RRF with swollen mitochondria (Peters et al., 1993). AZT inhibits retroviral reverse transcriptase of HIV-I, but also mtDNA polymerase (Lewis et al., 1994). That's why it's in "AlDS" patients presenting with AZT-induced myopathy, the content of mtDNA in muscle are decreased (Amaudo et al., I99I) and mitochondrial functions are impaired (Lewis et al., 1992).

In an animal model of AZT toxicity Ozawa and co-workers (Hayakawa et al., 199I) found that AZT in a Dosage one tenth of the amount used in "AlDS" patients corresponds to a massive ROS-related oxidation of the mtDNA. In cultured human muscle cells, AZT decreases the ability to proliferate and differentiate (Benbrik et al., 1997).

Mitochondrial damage and cell death

As the cell's "power plants", mitochondria are of paramount importance for their survival. In recent years it has been shown that damage to the mitochondria leads to apoptotic and necrotic cell death (Richter, 1996; Richter et al., 1996; Zanzami et al., 1996; Judges, 1997; Kroemer, 1997).

An intact mtDNA is essential for the maintenance of the mitochondria. In contrast to the DNA in the cell nucleus, damage to the mtDNA is not or only incompletely repaired according to the current state of knowledge (Wallace et aL,1995; Yakes and Van Houten, 1997).

GOALS OF THE PROJECT AND ITS IMPORTANCE

The aim of the investigations proposed in the project is the analysis of possible damage to blood cells by AZT-mediated mitochondrial changes are induced in an animal model. We

expect through the proposed studies providing evidence of a drug-induced damage to the immune system. Obviously this would have far-reaching consequences political, social and economic consequences.

RESEARCH PLAN

treatment of animals
Adult female Wistar rats (body weight 200-250 g) are divided into two Divided into groups: A group receives AZT dissolved in the drinking water at a Concentration of 1 mg/ml ad libitum for 30 days (Lewis et al., 1992). The other group (control animals) receives drinking water without AZT.

Analyses
The animals are bled and the blood cell count becomes measured differentially. The amount of mitochondria and the amount of mitochondrial membrane potential is measured using our established methods (Hennet et al.,1993).

DNA is isolated from the blood cells and mtDNA using the polymerase Chain reaction and quantitatively examined for deletions (Filser et al., 1997).
The basic structure of the Biochemistry Laboratory, ETH Zürich. All planned Experiments can be done here.

FINANCIAL NEEDS
It is planned to employ a postdoc for an initial period of two years. The costs (salary,
Social security contributions, materials) amount to Sfr. 150'000 p.a..
Total costs: Sfr. 300'000.

Literature:

Arnaudo, E., Dalakas, M., Shanske, S., Moraes, C.T., DiMauro, S. und Schon, E.A'

(1991) Lancet 337, 508-510.

Benbrik, E., Chariot, P., Bonavaud, S., Ammi-Said, M., Frisdal,E., Gherardi, R. und

Barlovatz-Meimon, G. (1997) J. Neurol. Sci. 149, 19-25.

Chance, B., Sies, H. und Boveris, A. (1979) Physiol. Rev. 59, 527-605.

Filser, N.S., Margue, C. und Richter, C. (1997) Biochem. Biophys. Res. Commun.

233, r02-r07.

Groopman, J.E. (1990) Rev. Infect. Dis. 12, 5500-5506.

Halliwell, B. und Gutteridge, J.M.C. (1992) Free Radicals in Biology und Medicine,

Clarendon, Oxford.

Hennet, T., Peterhans, E., Richter, C., und Bertoni, G. (1993) Cancer Res. J3, 1456-

1460,

6

Hayakawa, M., Torii, K., Sugiyama, S., Tanaka, M. und Ozawa, T. (1991) Biochem.

Biophys. Res. Commun. 179, 1023-1029.

Kroemer, G. (1997) Nature Medicine 3,614-620.

Lewis, W. und Dalakas, M.C. (1995) Nature Med. 1, 417-422.

Lewis, W., Chomytr, A., Gonzales, B. und Papoian, T. (1992) J. Clin. Invest. 89,

13s4-r360.

Lewis, W., Simpson, J.F. und Meyer, R.R. (1994) Circ. Res. 74,344-348.

Luft, R., Ikkos, D., Palmieri, G., Ernster, L. und Afzelius, B. (1962) J. Clin. Invest.

4r,1776-1804.

Peters, R.S., Winer, J., Lundon, D.N., Stotter, A. und Pinching, A.J. (1993) Quart. J'

Med. 86,5-15.

Richter, C. (1992) Mutation Res. 275, 249-255.

Richter, C. (1996) Basic Appl.Myology 6,215-219.

Richter, C. (1997) Biosci. Rep. 17, 53-66.

Richter, C., Park, J.-W. und Ames, B.N. (1988) Proc. Natl. Acad. Sci. USA. 85,

6465-6467.

Richter, C., Gogvadze,Y., Laffranchi, R., Schlapbach, R., Schweizer, M., Suter, M.,

Walter, M. und Yaffee, M. (1995) Biochim. Biophys. Acta 127I,67-74.

Richter, C., Schweizer,M., Cossarizza. A., und Franceschi, C. (1996) FEBS Lett'

378,107-110.

Tyler, D. (1992) The Mitochondrion in Health und Disease, VCH Publishers, New

York.

Wallace, D.C. (1992a) Science 256,628-632.

Wallace, D.C. (I992b) Annu. Rev. Biochem. 61, 1175-1212.

Wallace, D.C., Richter, C., Bohr, V.A., Cortopassi, G., Kadenbach, B., Linn, S',

Linnane, A.W. und Shay, J.W. (1995) In: Dahlem Konferenz 74 "Molecular

aspects of aging", John Wiley & Sons, Chichester,pp,199-225.

Yakes, F.M. und Van Houten,B. (1997) Proc. Natl. Acad. Sci. 94, 514-519.

Zamzami, N, Marchetti, P, Castedo, M, Zanin, C, Vayssiere, JL, Petit, PX, und

Kroemer, G. (1996) J. Exp. Med. 181,1661-1672.