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**Research project**

# ANTIBIOTIC-INDUCED DAMAGE TO MITOCHONDRIA

## SCIENTIFIC SUMMARY

Mitochondria are cell organelles responsible for providing energy essential in the cells of higher organisms. Mitochondria have developed about 1,3 billions of years from bacteria that migrated into cells and with them entered into a symbiosis that is stable to this day. Mitochondria are semi-autonomous; they grow and divide independently of the cell cycle. They have their own DNA and extensive equipment to synthesize their own proteins. The survival of the mitochondria and thus of the cell as a whole is imperative for intact mitochondrial DNA and protein synthesis. Studies of diseases associated with mutations in mitochondrial DNA show that a variety of degenerative processes and cell death due to defects in the mitochondrial oxidative phosphorylation(energy supply) may happen.

Antibiotics are compounds that fight against disease-causing microorganisms. They work by disrupting the cell wall structure, changing the membrane permeability, alteration of DNA, inhibition of protein synthesis or change in energy metabolism. Own as a former bacteria, mitochondria still have many properties that are otherwise only found in microorganisms.

It is therefore possible that antibiotics also in mitochondria lead to changes. Already in 1973 it was shown with yeast that the antibiotic Chloramphenicol inhibits mitochondrial protein biosynthesis. Studies at higher Eukaryotes have not yet been carried out.

We propose to study the possible mitochondrial damage by antibiotics in animal models. To do this, we will look at the livers of rats, with antibiotics administered via the feed or water and measure the number and quality of the mitochondria. We expect to be clarified by these investigations whether

mitochondria in higher organisms become damaged by antibiotics. The result will lead to a better understanding of the interaction between antibiotics and eukaryotes.

**BACKGROUND**

Mitochondrial damage and its 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 Q pi enzymatic especially superoxide dismutase, catalase, glutathione peroxidase) are weakened, ROS lipids, damage proteins and nucleic acids and lead contribute 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-dibenzo-dioxin, 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 the in a vicious circle.
Energy yield, lead to increased ROS production, 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 diseases have been identified with structurally modified liver mitochondria (Tyler, 1992). The breakthrough in understanding mitochondrial diseases provided studies of mutations in mtDNA (for a review 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 are related by individual base substitutions in mitochondrial tRNALvs and tRNALeu. It is very likely that diseases are occurring at least partly due to mitochondrial ROS (Wallace et al., 1995).

**Mitochondria and antibiotics**

Mason et al. showed in 1973 that chloramphenicol interferes with protein biosynthesis in yeast mitochondria. So far there are no studies on antibiotic induced mitochondrial damage in higher organisms. It appears an urgent need to study the effects of antibiotics on the hereditary structure and investigate the functionality of the mitochondria.
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 the 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 a1., 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 mitochondria of rats by antibiotics mixed with the feed or water given. We expect through the proposed investigations to provide evidence on drug-induced damage to mitochondria. So far it has not been of particular importance to explore the possibility that antibiotics could cause heritable mutations in mtDNA passing through the germline from the mother to the following generation. Detection of an antibiotic-induced mitochondrial damage would obviously have far-reaching political, social, and economic consequences.

**RESEARCH PLAN**

ANIMALS AND ISOLATION OF MITOCHONDRIA
treatment of animals

Adult female Wistar rats (body weight 200-250 g) are im
Drinking water and feed administered antibiotics.
(isolation of mitochondria) carried out method isolated from liver.

Mitochondria are ruptured at different times over the experimental period.Differential centrifugation according to the routine in our laboratory

DETERMINATION OF mitochondrial oxygen consumption

Oxygen consumption is measured polarographically with an oxygen electrode at 30 C in medium (final volume L2 ffiI, containing 2-3 mg protein)
Composition 150mM Sucrose, 25mM Tris-HCl, pH 7.5, 10mM Potassium phosphate and substrates of the respiratory chain were measured. phosphorylation becomes achieved by addition of ADP (250-300 nmol) and decoupling by addition of carbonyl cyanide-m-chlorophenylhydrazone. Respiratory control is through determines the ratio of respiratory rates in the presence and absence of ADP.
Determination of the mitochondrial ATP as e-activities
The mitochondrial ATPase activity is measured using a pH meter after the Procedure measured by Chance and Nishimura (1967).
Determination of the mitochondrial membrane potential
Mitochondrial ATP synthesis and Ca2+ uptake are controlled by the mitochondrial membrane potential (Ay) driven. Ary can be about the distribution of the lipophilic cation tetraphenylphosphonium (TPP+) between the mitochondrial matrix and the extramitochondrial space using a TPP+-sensitive electrode (Kamo et al., 1979) to determine which changes in Ay continuously measures.
Determination of the mitochondrial C a2+ transport.
The transport of Ca2+ across the inner mitochondrial membrane is Aminco DW2A spectrophotometer at the wavelength pair 685-675 nm in presence of 50 pM arsenazole II (Lötscher et al., 1980). ruthenium red, an inhibitor of the mitochondrial Ca2+ uptake pathway, is used to measure the cyclic uptake and release of Ca2*, a key process in mitochondrial damage. In this way we are able to measure the mitochondrial C*+ uptake capacity, the Ca2+ uptake rate and the Ca2+determine retention time.
Determination of mitochondrial pyridine nucleotide oxidation
The investigation of the redox state of the mitochondrial pyridine nucleotides provides useful information regarding damage to mitochondria (Gogvadze and Richter, 1993). The oxidation of the mitochondrial pyridine nucleotides is Measured by Aminco DW2A spectrophotometer at the wavelength pair 340-370 nm measured (Lötscher et al., 1980).

DETERMINATION OF MITOCHONDRIAL OXIDATIONAL DAMAGE

Lipid peroxidation in mitochondria
Lipid peroxidation products are classified as thiobarbituric acid (TBA) reactive Substances (TBARS) measured and as amount of malondialdehyde (MDA)/mg protein (Okhava et al., 1979). Mitochondria (0.5-1.0 mg protein) are increased a solution consisting of 5% sodium dodecyl sulfate (SDS), 50 pM of Antioxidant butylated hydroxytoluene (to prevent the formation of new radicals) and 15 pM FeCl3 (to convert lipid hydroperoxides to MDA) admitted. After shaking for 30 seconds, 1.5 ml of 2% acetic acid, pH 3.5 and 1.5 ml 0.8% TBA are added. The solution is heated for 30 min at 95 degrees Celsius

maintained, then chilled on ice and centrifuged at 3,000 x g for 10 min. The absorbance of the supernatant is measured between 600 and 450 nm. TBARS are calibrated with known amounts of MDA.

mtDNA isolation and determination of oxidation damage

Mitochondria are incubated in buffer containing 20 mM of the metal chelator diethylenetriamine contains penta acetic acid (DTPA) to suppress endonuclease activities, suspended. After that, they are lysed in neutral SDS containing 1 mM DTPA,and membranes and proteins are filled with 5 M NaCl. After centrifugation for 5 min, the mtDNA-containing excess is first mixed with phenol/chloroform (1:1) and then extracted with chloroform. DNA is then washed with 2 volumes of ethanol filled and centrifuged. The resulting mtDNA pellet is washed with TlYoiger ethanol washed, suspended and run through on a Sephacryl 5-400 mini column cleaned by centrifugation.

mtDNA oxidation is performed after isolation and enzymatic hydrolysis of the DNA to nucleosides by high pressure liquid chromatography in combination determined with electrochemical detection (Richter et al., 1988). This method analyzes the amount of 8-hydroxydeoxyguanosine, a parameter for oxidative DNA damage. In addition, the number of strand breaks in mtDNA measured, another parameter for oxidative DNA damage. For this purpose the isolated mtDNA on a O.7oÄigen agarose gel in the presence of Ethidium bromide electrophoresed and DNA exposed to UV light (365 nm) made visible.

Southern blots of the separated DNA are cleaned with digoxigenind UTP-labeled probes were performed and the hybrids using an immunoassay kit (Anti-digoxigenin/alkaline phosphatase conjugates, 5-bromo-4-chloro-3- indolyl phosphate and nitro-bluetetrazolium color reaction) visualized.

Protein peroxidation in mitochondria

The degree of oxidation of mitochondrial proteins is determined by quantifying the Reaction of tritiated sodium borohydride with oxidatively formed protein carbonyls (Levine et al., 1990; Richter et al., 1995). For this, 0.4 mg mitochondrial protein in 190 µl sucrose/mannitol, I mM EGTA, with HEPES zrLpH 7.4 buffered, suspended. To this suspension are added 10 µl I M Tris, pH 8.5 and 50 µl 20 mM tritiated sodium borohydride (specific activity approx. 100 mCi/mmol)and the mixture was incubated at 37o C for t h. After that, the proteins filled with ice-cold trichloroacetic acid and dated with Whatman GF/C glass fiber filters medium separated. The protein-bound radioactivity on the filters becomes over determined by liquid scintillation. Complies under the specified conditions 1 n mole protein carbonyl approx. 25,000 disintegrations/min.

**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**


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