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Participation of Coenzyme Q_{10} in the Rejection Development of the Transplanted Heart: A Clinical Study

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Summary
Coenzyme Q_{10} and alpha-tocopherol concentrations were assessed in 28 endomyocardial biopsies from 22 patients and in 61 blood samples from 31 patients after heart transplantation with histologically confirmed signs of rejection. The values were compared to the group of 14 patients with cardiomyopathies of unclear etiology as candidates for heart transplantation. Blood analyses were also compared with 50 healthy persons. Myocardial and blood coenzyme Q_{10} concentrations were already significantly decreased in the incipient phase of rejection (degree 0–1) and also in rejection phase 1 and 2. In patients without rejection signs myocardial and blood coenzyme Q_{10} values were similar to those of cardiomyopathic patients. No significant differences were found in alpha-tocopherol concentrations in relation to signs of rejection. Increased plasma lipid peroxidation quantified as malondialdehyde production was detected in all groups of transplanted patients. The results contribute to the explanation of some pathobiochemical mechanisms participating in the rejection development of the transplanted heart.

Key words
Human heart transplantation – Rejection – Coenzyme Q_{10} – Alpha-tocopherol

Introduction

Pathobiochemical mechanisms participating in the rejection development of the transplanted heart have not yet been fully clarified. A significant role in this process can be played by endogenous antioxidants which are involved in some pathological events linked to increased free radicals production. Coenzyme Q_{10} is a naturally occurring cofactor in the mitochondrial respiratory chain essential for ATP synthesis and together with alpha-tocopherol (the main form of vitamin E in men) they act as antioxidants (Mitchell 1991, Beyer 1994). Decreased levels of coenzyme Q_{10} were confirmed in some types of cardiomyopathies and in the failing heart (Mortensen *et al.* 1991, Folkers 1993). Deficiency of E vitamin is a risk factor in cardiovascular diseases (Gey *et al.* 1991). Only sporadic and controversial data are available about endogenous levels of these antioxidants in patients after heart transplantation. Lower coenzyme Q_{10} values were found in the heart muscle and blood by Karlsson *et al.* (1993), while Sehested *et al.* (1993) reported that no changes occur during mild to moderate rejection in posttransplant patients. We assumed that increased oxidative stress can occur during rejection development and affect endogenous antioxidants. It is mainly coenzyme Q_{10} depletion that may deteriorate the function of the transplanted heart.

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In our study we investigated the concentrations of coenzyme Q_{10} and alpha-tocopherol in endomyocardial biopsies and in the blood of patients after heart transplantation. In these patients we also determined lipid peroxidation in the plasma quantified by malondialdehyde production. We evaluated the results in relation to histologically confirmed degree of rejection and compared these with patients with cardiomyopathies of unclear etiology as candidates for heart transplantation, and also with clinically healthy subjects.

Patients and Methods

Biochemical analyses were performed in endomyocardial biopsies and in blood or plasma samples in the following groups of patients :

CPUE-pts = patients with cardiomyopathies of unclear etiology as defined by Fabián *et al.* (1996) – 13 endomyocardial biopsies and 14 blood samples. Mean age of patients was 47 years, range 30–55 years, 2 females.

HTx-pts = patients 1–9 years after heart transplantation monitored in the Slovak Institute of Cardiovascular Diseases. Mean age of patients was 45 years, range 19–63 years. Twenty-eight endomyocardial bioptic samples from 22 patients (3 females) were divided according to a histologically confirmed degree of rejection: without rejection = rejection 0 (7 samples), incipient rejection = rejection 0–1 (10 samples), mild rejection = rejection 1 (7 samples) and moderate rejection = rejection 2 (4 samples). Sixty-one blood or plasma samples from 31 patients (4 females) were divided as follows: rejection 0 (23 samples), rejection 0–1 (19 samples), rejection 1 (13 samples) and rejection 2 (6 samples).

Healthy persons: mean age 48 years, range 28–64 years (23 females), 50 blood or plasma samples.

Coenzyme Q_{10} and alpha-tocopherol were determined simultaneously by the isocratic high-performance liquid chromatography method (LKB) according to Takada *et al.* (1982) and Lang *et al.* (1986) with some modifications. One ml of heparinized blood, plasma or 1–3 mg tissue from endomyocardial biopsy were twice vortexed for 5 min with hexane/ethanol (5/2 v/v), the organic phases were collected, evaporated under nitrogen, the residue dissolved in ethanol and 20 μl of extract were injected into SGX C18, 7 μm column (Tessek). Elution was performed with methanol/acetonitril/ethanol (6/2/2 v/v/v, Merck); flow rate 0.85 ml/min; detection spectrophotometrically at 275 nm. External standards of coenzyme Q_{10} and alpha-tocopherol (Sigma) were used. All steps of sample preparation were carried out in the dark, samples were measured within 2 h. Concentrations of coenzyme Q_{10} were determined in whole heparinized blood, alpha-tocopherol in the plasma. Both value were expressed in μg/ml. Concentrations of coenzyme Q_{10} in bioptic tissues were expressed in μg/g wet weight and alpha-tocopherol in mg/wet weight. Lipid peroxidation in the plasma from healthy subjects and Htx-patients were determined by malondialdehyde formation spectrophotometrically at 532 nm according to Janero and Burghardt (1989). Malondialdehyde concentrations in CPUE-patients were not measured in this study. Concentrations of malondialdehyde in the plasma were expressed in μmol/l.

The results are mean values ± S.E.M. Student's t-test for unpaired data was used for statistical analysis, $P<0.05$ were considered statistically significant. The investigation conforms with the principles outlined in the Declaration of Helsinki.

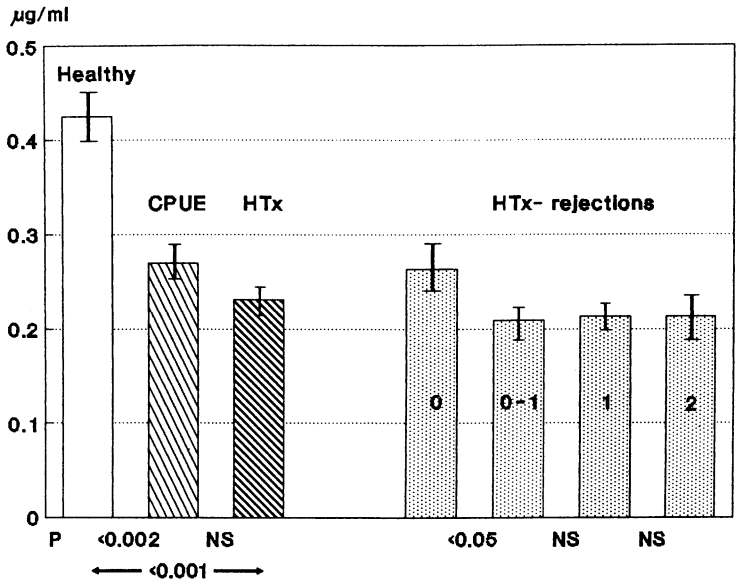

Fig. 1. *Blood coenzyme Q_{10} concentrations: Healthy persons (n = 50), CPUE = patients with cardiomyopathies of unclear etiology (n = 14), Htx = all blood samples from transplanted patients (n = 61), rejection 0 = Htx-pts without rejection (n = 23), 0–1 = incipient rejection (n = 19), 1 = mild rejection (n = 13), 2 = moderate rejection (n = 6). Values are means ± S.E.M.*

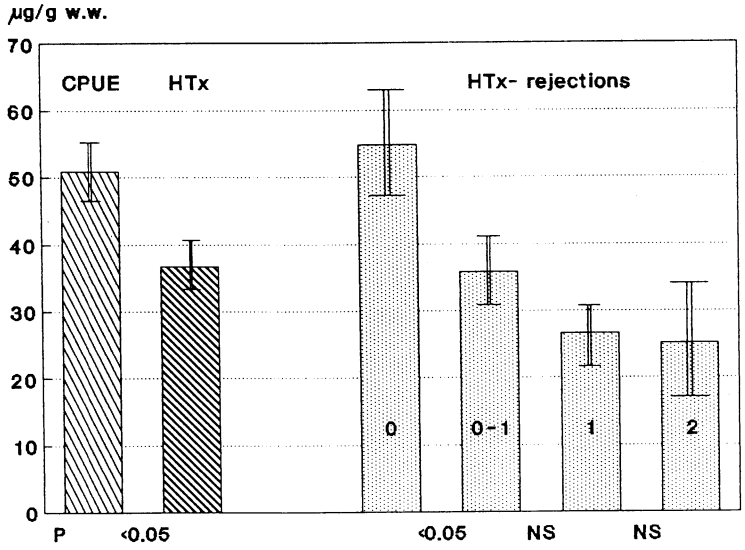

Fig. 2. *Coenzyme Q_{10} concentrations in endomyocardial biopsies (EMB) : CPUE = patients with cardiomyopathies of unclear etiology (n = 13), Htx = all EMB from transplanted patients (n = 28), rejection 0 = EMB without rejection (n = 7), 0–1 = EMB with incipient rejection (n = 10), 1 = EMB with mild rejection (n=7), 2 = EMB with moderate rejection (n = 4).*

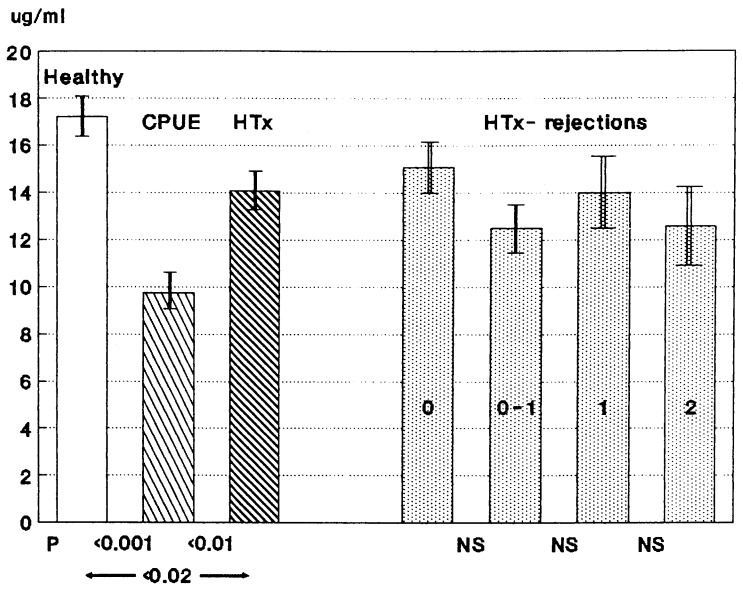

Fig. 3. *Plasma alpha-tocopherol concentrations: groups as in Figure 1. Healthy persons (n = 50), CPUE-pts (n = 14), Htx-pts (n = 61), rejection 0 (n = 23), rejection 0–1 (n = 19), rejection 1 (n = 13), rejection 2 (n = 6).*

Results

The mean blood coenzyme Q_{10} concentration (Fig. 1) in healthy persons was 0.425±0.026 µg/ml, but it was significantly lower in CPUE-pts and HTx-pts were (0.270±0.018, P<0.002 and 0.231±0.015 µg/ml, P<0.001, respectively). Significant differences were found in HTx-pts without rejection (0.263±0.025 µg/ml) or with signs of rejection. In the group with rejection 0–1, the concentration of coenzyme Q_{10} was 0.209±0.017 µg/ml (P<0.05 vs rejection 0), in rejection groups 1 and 2 the concentrations were similar (0.213±0.014 and 0.213±0.023 µg/ml). Mean myocardial coenzyme Q_{10} concentration (Fig. 2) was significantly lower in HTx-pts (all HTx-pts with or without rejections) in comparison with CPUE-pts (36.7±3.72 and 50.9±4.45 µg/g w.w., respectively, P<0.05). Significant differences were found between HTx-pts without rejection (54.9±7.97 µg/g w.w.) and those with rejection symptoms. In rejection group 0–1, myocardial coenzyme Q_{10} was 35.9±5.19 µg/g w.w. (P<0.05 vs rejection 0), in the rejection group 1 – 26.6±4.65 and in the rejection group 2 – 25.2±8.74 µg/g w.w. Plasma alpha-tocopherol in healthy persons was 17.2±0.88 µg/ml, significantly lower were the concentrations in CPUE-pts and HTx-pts (9.75±0.80 µg/ml, P<0.001 and 14.1±0.86 µg/ml, P<0.02, respectively) (Fig. 3). Alpha-tocopherol concentrations

did not differ significantly in relation to the degree of rejection. Mean myocardial alpha-tocopherol was higher in HTx-pts in comparison with CPUE-pts (0.574±0.12 mg/g w.w. and 0.353±0.095 mg/g w.w.) but because of the great variability the differences were not statistically significant (Fig. 4). We found no significant differences in concentrations in relation to the degree of rejection. The mean concentration of malondialdehyde was significantly higher in transplanted patients in comparison with healthy persons (5.94±0.20 and 4.73±0.12 μmol/l, P<0.001) (Fig. 5), but no significant differences were found in relation to the degree of rejection.

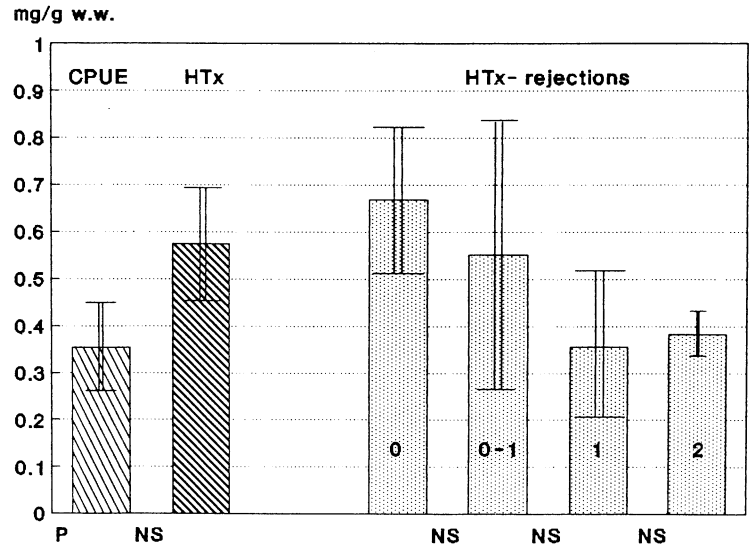

Fig. 4. *Alpha-tocopherol concentrations in endomyocardial biopsies (EMB): groups as in Figure 2. CPUE (n = 13), Htx (n = 28), rejection 0 (n = 7), rejection 0–1 (n = 10), rejection 1 (n = 7), rejection 2 (n = 4).*

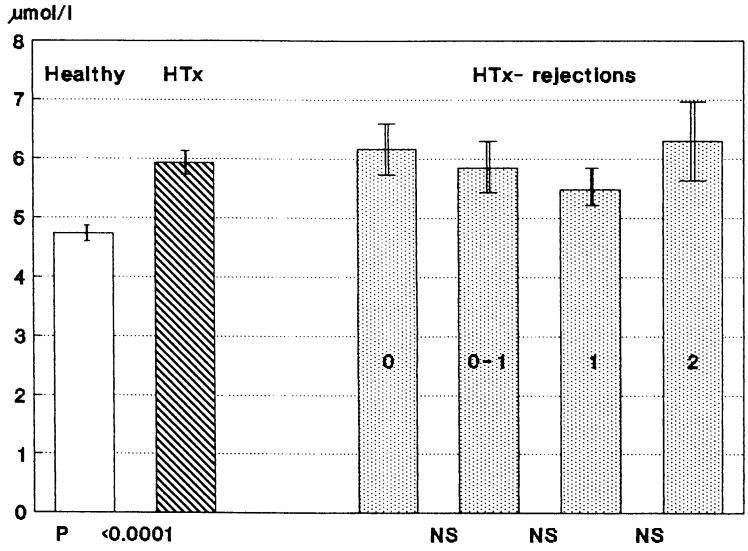

Fig. 5. *Plasma malondialdehyde concentrations: groups as in Figure 1. Healthy persons (n = 50), Htx-pts (n = 61), rejection 0 (n = 23), rejection 0–1 (n = 19), rejection 1 (n = 13), rejection 2 (n = 6).*

Discussion

In our previous studies, we detected early metabolic changes in mitochondria and the level of endogenous antioxidants in patients after heart transplantation (Gvozdjáková *et al.* 1996, Kucharská *et al.* 1996). We found decreased levels of myocardial and blood coenzyme Q_{10} in these patients in comparison with pretransplant patients. In the present study we tried to find out whether these changes are related to histologically confirmed signs of rejection. Mean coenzyme Q_{10} concentration in all Htx-pts was significantly decreased in comparison with healthy subjects in the blood and with CPUE-pts in the heart (Figs 1 and 2). Significant differences were found in patients without rejection (rejection 0) and in patients with some signs of rejection – incipient (0–1), mild (1) and moderate (2) rejection. Coenzyme Q_{10}

decreased with incipient signs of rejection and myocardial coenzyme Q_{10} decreased with the severity of rejection. Concentrations of coenzyme Q_{10} in patients without rejection were similar to CPUE-patients (Figs 1 and 2). Our results are in agreement with Karlsson *et al.* (1993) who found reduced plasma and myocardial coenzyme Q_{10} levels in patients with symptoms of rejection but in contrast to Sehested *et al.* (1993) who did not find such deficiency in patients with rejections. We also compared mean blood coenzyme Q_{10} levels with a group of 50 healthy persons (mean age 48 years), in which mean levels were 0.427 μg/ml. This value is lower than reported in healthy subjects by other authors – about 0.7 μg/ml determined by similar methods (Johansen *et al.* 1991, Karlsson *et al.* 1992, 1993, Weber *et al.* 1994). No information is available about the levels of coenzyme Q_{10} in the Slovak population, but nutritional deficiencies of antioxidants in Slovakia and other postcommunist countries have been reported (Ginter 1997). We suppose that it can also be applied to a lower intake of coenzyme Q_{10}. Physical and psychical stress can also influence endogenous biosynthesis and degradation of coenzyme Q_{10}. Myocardial concentrations of coenzyme Q_{10} in our patients can be compared with the data of Kalén *et al.* (1989). These authors reported age-related concentrations of coenzyme Q_{10} in healthy human hearts. They found values 75.0 μg/g w.w. in the age group of 39–43 years and 47.2 μg/g w.w. in the age group of 77–81 years. Our patients with cardiomyopathies had mean myocardial concentrations of coenzyme Q_{10} 50.9 μg/g w.w. (mean age 47 years) and transplanted patients without rejection 54.9 μg/g w.w. (mean age 41 years). In our study we found significantly lower plasma alpha-tocopherol values in patients with cardiomyopathies and also in patients after heart transplantation in comparison with healthy persons. However, all values were in the range of reference values 5–20 μg/ml (Fig 3). No significant changes were ascertained in plasma and myocardial alpha-tocopherol concentrations in patients after heart transplantation in relation to the degree of rejection. Moreover, a considerable variability was found in myocardial alpha-tocopherol concentrations (Fig. 4). Decreased levels of antioxidants together with increased lipid peroxidation found by us in the plasma from patients after heart transplantation (Fig. 5) can contribute to the increased oxidative stress in these patients. A dysfunction of cellular bioenergetics and lack of coenzyme Q_{10} are regarded as molecular causes of heart failure (Folkers *et al.* 1992, Folkers 1993). In our study, we found depletion of coenzyme Q_{10} in relation to rejection episodes in transplanted patients together with disturbances of mitochondrial oxidative phosphorylation (Gvozdjáková *et al.* 1997). In recent years, the clinical benefits of coenzyme Q_{10} treatment in various forms of cardiovascular diseases has been proved (Folkers *et al.* 1992, Langsjoen *et al.* 1994) and we suppose that such supplementary therapy could also be beneficial in patients after heart transplantation by enhancing cellular bioenergetics and preventing cardiac damage.

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