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# Oxidative stress and antioxidant defense systems in patients after heart transplantation*

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**Summary.** Heart transplantation ranks among those surgical interventions associated with ischemia-reperfusion injury to the donor heart as well as to the recipient. These events are connected with increased production of reactive oxygen species which evoke metabolic, structural and functional disturbances. Twenty-four transplant patients were investigated for oxidative stress (plasma levels of thiobarbituric acid reactive substances, TBARS) and antioxidant capacity (plasma total antioxidant status, TAS), and for activities of erythrocyte superoxide dismutase (SOD) and glutathione peroxidase (GPx) during the first year after heart transplantation. The post-transplant period was characterized by progressive decrease of plasma TAS, indicating a significant long-term drop of antioxidant reserves in patients after successful heart transplantation. The decrease in plasma TAS is accompanied by long-lasting increase of TBARS levels, which may represent oxidative stress of the organism. We conclude that additional therapy with antioxidant substances should be an important component of the complex therapeutic programme of patients after heart transplantation.

**Key words:** Heart transplantation, oxidative stress, thiobarbituric acid reactive substances, antioxidant status, antioxidant enzymes.

## Introduction

Heart transplantation belongs to those cardiosurgical interventions that are accompanied by ischemia, both of the donor heart and during oxygenated reperfusion of the recipient's body. Despite the standard requirement of a metabolically, morphologically and functionally sufficient donor organ, iatrogenic evocation of some ischemia-reperfusion injury cannot be excluded. It is well documented that this concomitant event evokes some degree of oxidative stress of the myocardium [1], with consequent modification of heart function. The contractility of heart ventricles is decreased, with increase in the degree of cardiac failure and rising production of reactive oxygen species (ROS) that have a deleterious effect on cardiomyocyte membranes [2, 3]. Many studies have demonstrated the direct participation of ROS in various disturbances of heart function and their attenuation with antioxidants and antioxidative enzymes [4].

As several authors have demonstrated raised levels of lipoperoxides and decreased antioxidant capacity in patients with severe cardiomyopathies [5, 6] and in patients at different time periods after heart transplantation [7–10], we have investigated a homogenous group of consecutive patients just before heart transplantation and for one year afterwards.

## Patients and methods

A group of twenty-four consecutive patients undergoing orthotopic bicaval heart transplantation was investigated for a one-year period. The important characteristics of the patients in the peri- and post-transplant period are shown in Tables 1 and 2. The patients were pre-, peri- and postoperatively treated with a combination of three immunosuppressants – Sandimmun Neoral, Imuran and Urbason (replaced from the fourth day after transplatation by Prednisone) [11]. The plasma levels of cy-

**Table 1.** Perioperative characteristics of transplant patients

| | |
|---|---|
| Patients (n) | 24 (1 female) |
| Age (years) (range) | 52.7 (20–59) |
| Diagnosis | DCMP – 15, CAD – 9 |
| N.Y.H.A. classification | III – 15, IV – 9 |
| LVEF (%) before HTx (range) | 21.5 (13–25) |
| after HTX (range) | 58.2 (50–65) |
| Graft ischemia (min) (range) | 159.7 (86–241) |
| Cardiopulmonary bypass (min) (range) | 203.5 (120–286) |
| Aortic cross-clamping (min) (range) | 128.0 (80–150) |

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**Table 2.** Post-transplantation characteristics of patients

| | |
|---|---|
| Stay in I.C.U. (days) (range) | 13.3 (5–27) |
| Artificial pulmonary ventillation (hours) | 12.2 (3–96) |
| up to 6 hours | 6 pts. |
| 6–8 hours | 7 pts. |
| 8–12 hours | 7 pts. |
| 12–24 hours | 2 pts. |
| more than 24 hours | 2 pts |
| Inotropic support | Dob – 15, Dob + Adr – 3, Dob + Nor – 3, Dob + Adr+Nor – 1, Dob + Adr + Amr – 1, Dob + Adr + Nor + Amr – 1 |
| Implantation of permanent pacemaker | 5 patients |
| Acute rejection episodes | 10 patients |

*Dob* dobutamine; *Adr* adrenaline; *Nor* noradrenaline; *Amr* amrinone.

closporin A were 250–300 ng/ml during the first three months after transplantation and then 150–250 mg/ml [12].

Central venous blood samples were collected before surgery and after heart transplantation on the first, third, fifth and seventh days. Samples were collected from the cubital vein from the second week to the end of the first year after transplantation. The values of biochemical parameters in the transplant patients were compared with those in a control group of twenty-four outpatients with no clinically documented cardiovascular, renal or hepatic diseases.

The following biochemical parameters were estimated:

- plasma thiobarbituric acid reactive substances (TBARS) (on the basis of malondialdehyde concentration) as markers of lipid peroxidation [13]
- plasma total antioxidant status (TAS) (Randox Ltd, U.K.)
- erythrocyte superoxide dismutase activity (Randox Ltd, U.K.)
- erythrocyte glutathione peroxidase activity (Randox Ltd, U.K.)

The plasma levels of TBARS and TAS were calculated with respect to the standard hematocrit value of 0.40; erythrocyte enzyme activities were expressed in I.U. per gram of hemoglobin.

Data are given as arithmetic means of the values recorded and their standard errors S.E.M.). Differences among the values obtained in individual sampling periods and between the transplant patients and the control group were compared using Student´s unpaired two-tailed test (ANOVA).

## Results

During the early post-transplant period, all the patients were successfully managed in the I.C.U. (Table 2): the majority of them received inotropic support with dobutamine only and were artificially ventilated for not more than half a day. Acute episodes of cellular graft rejection (defined as mild or moderate focal and mild diffuse) occurred in ten of the patients, and all were successfully treated using more aggressive imunosuppresive therapy. All patients (12–48 months after transplantation) are under careful clinical and therapeutic management, and all have good heart function with the left ventricular ejection fraction from 50 to 65%.

Biochemical investigation of the patients revealed a gradual but significant post-transplant decrease in antioxidant status (Table 3), beginning two weeks after transplantation. In contrast, the early increase in plasma TBARS levels first peaked on the fifth day, with a second maximum twenty-eight days after transplantation. This was clearly demonstrated by a significantly increased ratio of TBARS levels to TAS from day 14 to day 28. This ratio remained higher than corresponding control values for the whole period of investigation.

Diametric differences were found in the activities of antioxidant enzymes. Activity of SOD remained significantly decreased, especially in the early days after transplantation and then markedly in the whole one-year period (Table 4), whereas the activities of GPx were increased in the early post-transplant period, although later the activity of GPx was practically the same as in the control group.

## Discussion

Oxidative stress and decreased capacity of antioxidant defense systems in patients undergoing complicated cardiosurgery with cardiopulmonary bypass are considered as general metabolic and functional consequences of this event [14, 15]. Although in long-term studies several authors have demonstrated increased production of lipid peroxides and decline of antioxidant reserves in patients after heart transplantation [16, 17], our results show that the decrease of antioxidant defense systems in transplant patients occurred very early (up to the second week). This probably reflects the immediate post-transplant rise in production of reactive oxygen species and their metabolites, which are rapidly and effectively eliminated by endogenous antioxidant systems, especially by antioxidant enzymes. The observed short-lasting significant increase

**Table 3.** Plasma total antioxidant status (TAS) (mmol/l), thiobarbituric acid reactive substances (TBARS) (mmol/l) and TBARS/TAS ratio (x $10^{-3}$) in patients after heart transplantation

| Days | TAS | TBARS | TBARS/TAS |
|---|---|---|---|
| 0 | 1.42 ± 0,07 | 2.35 ± 0.17 | 1.73 ± 0.15 |
| 1 | 1.71 ± 0.08 | 2.56 ± 0.22 | 1.66 ± 0.12 |
| 3 | 1.72 ± 0.07 | 2.70 ± 0.22 | 1.81 ± 0.14 |
| 5 | 1.60 ± 0.09 | 2.93 ± 0.16* | 1.88 ± 0.12 |
| 7 | 1.42 ± 0.09 | 2.43 ± 0.23 | 2.00 ± 0.16 |
| 14 | 1.23 ± 0.06* | 2.32 ± 0.17 | 2.22 ± 0.18* |
| 21 | 1.15 ± 0.05** | 2.50 ± 0.15 | 2.61 ± 0.24** |
| 28 | 1.17 ± 0.06** | 2.70 ± 0.19* | 2.68 ± 0.22** |
| II | 1.18 ± 0.09* | 2.57 ± 0.17 | 2.28 ± 0.20* |
| III | 1.20 ± 0.04** | 2.62 ± 0,18 | 2.21 ± 0.22 |
| IV | 1.17 ± 0.06** | 2.43 ± 0.17 | 2.20 ± 0.17* |
| Control group | 1.83 ± 0.25 | 2.05 ± 0.09 | 1.64 ± 0.09 |

Day 0 = immediately before heart transplantation; II = days 90–180; III = days 181–270; IV = days 271–360 after transplantation. Statistical significance vs. control group: * $p < 0.05$, ** $p < 0.01$.

**Table 4.** Erythrocyte activities of superoxide dismutase (SOD) (IU/g Hb) and glutathione peroxidase (GPx) (IU/g Hb) in patients after heart transplantation

| Days | SOD | GPx |
|---|---|---|
| 0 | 577.5 ± 23.40** | 47.9 ± 2.37 |
| 1 | 587.3 ± 26.16* | 49.0 ± 2.41 |
| 3 | 583.3 ± 25.44* | 52.4 ± 2.42* |
| 5 | 593.2 ± 28.11 | 49.4 ± 2.65 |
| 7 | 602.5 ± 25.77 | 48.7 ± 1.96 |
| 14 | 595.9 ± 28.23 | 48.9 ± 1.78 |
| 21 | 611.2 ± 29.38 | 44.9 ± 1.75 |
| 28 | 603.0 ± 30.26 | 46.3 ± 2.24 |
| II | 570.2 ± 28.17** | 43.2 ± 2.49 |
| III | 622.2 ± 21.88 | 44.0 ± 2.30 |
| IV | 571.9 ± 17.18** | 43.5 ±3.02 |
| Control group | 670.8 ± 20.93 | 40.3 ± 3.98 |

Notes: see Table 3.

of glutathione peroxidase activity post-transplant could be considered as a physiological compensatory reaction directed against the marked rise in production of reactive oxygen species in the early period after transplantation. The long-lasting decrease of antioxidant defense systems after transplantation, demonstrated by diminished total antioxidant status, can be explained – at least partly – by imbalance between decreased superoxide dismutase activities and stepwise normalization of glutathione peroxidase activity from the second post-transplant week.

This long-lasting effect in transplant patients is probably also maintained by the pro-oxidant effects of immunosuppressants. It has been shown that the most frequently used immunosuppressant in various kinds of transplantation, cyclosporin A, has a strong pro-oxidative effect and is one of the substances that cause increased production of reactive oxygen species [18,19]. This observation is supported by the study of Apanay et al. [20], who demonstrated that cyclosporine increased the oxidability of low-density lipoproteins in patients after renal transplantation. It is also noteworthy that a newer preparation of this immunosuppressant – Sandimmun Neoral – contains effective antioxidant, E307. The use of Sanimmun Neoral in patients after renal transplantation decreased their antioxidant capacity to less extent than in patients treated with another immunosuppressant, Tacrolimus, that did not contain any antioxidant [20]. Pro-oxidant properties are also characteristic of another immunosuppressant – the glucocorticoid derivative dexamethasone [21]. These effects of immunosuppressants may be considered important factors in the diminution of antioxidant reserves in transplant patients.

We conclude that the long-lasting increase in production of reactive oxygen species in patients after heart transplantation is associated with a long-term decreased capacity of antioxidant defense systems, probably due to the imbalance between long-term decreased superoxide dismutase activity and stepwise normalized activity of glutathione peroxidase. This imbalance could inhibit efficient protection against reactive oxygen species. It is therefore necessary to ameliorate this decreased antioxidant capacity by administration of exogenous antioxidants, particularly natural substances such as α-tocopherol, L-ascorbate, coenzyme $Q_{10}$ etc. These antioxidants should be included in the standard long-term treatment of patients after heart transplantation. The decreased plasma levels of α-tocopherol and coenzyme $Q_{10}$ in heart transplant patients [23] support the grounds for this proposed therapeutic strategy.

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