# Contribution of Free Oxygen Radicals to Disorders in Aerobic Metabolism Recovery in Transplanted Heart after Preservation for Different Periods

**V. I. Mil'chakov, I. I. Dement'eva, I. L. Dzemeshkevich, and M. V. Palyulina**

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 124, No. 5, pp. 528-531, May, 1998
Original article submitted February 12, 1997

Experimental transplantation of the heart after preservation for different periods in St. Thomas solution showed that recovery of aerobic metabolism during reperfusion is impaired in the transplant weakened by ischemia because of activation of free-radical oxygen-dependent processes. Functional disorders were reversible after preservation for up to 4 h and involved adaptation changes in the recipient. After longer preservation, changes in the myocardium were irreversible. They manifested by failure of recovery of heart function caused by intracellular damage. In addition, pathological changes were observed in the recipient, caused by failure of antioxidant defense. This necessitates modification of the preserving solution in order to improve the transplant stability. Moreover, antioxidant drugs should be used for protecting the recipient.

**Key Words:** *heart transplantation; free oxygen radicals*

Results of heart transplantation are determined by the duration of heart preservation which affects the recovery of aerobic metabolism during subsequent reperfusion. Postischemic reperfusion inevitably involves activation of free-radical processes (FRP) [8], and therefore it was interesting to elucidate the relationship between duration of preservation and aerobic metabolism and FRP in the myocardium during reperfusion. Time course of FRP in the recipient after transplantation of the heart preserved for different periods was of special interest.

## MATERIALS AND METHODS

The study was carried out on 17 mongrel dogs. Hearts were preserved in St. Thomas Hospital solution. The animals were divided into three groups (6, 6, and 5 animals) differing by the duration of heart preservation: 2, 4, and 8 h, respectively.

All animals were anesthesized according to the same protocol and operated using standard technique [1].

Oxygen, carbon dioxide, potassium, glucose, lactate, malonic dialdehyde (MDA) in arterial and coronary blood, and radical activity of the blood were measured before clamping of the aorta, immediately after the clamps were removed, and 5 and 15 min after reperfusion. Arteriocoronary difference was calculated from these data and expressed in percent. In addition, FRP activity and MDA level in venous blood were measured before and after artificial circulation.

The main parameters of oxygen homeostasis were measured in an ABL-3 analyzer (Radiometer). Lactate was measured by the enzyme method in a

Research Center of Surgery, Russian Academy of Medical Sciences, Moscow

0007-4888/98/0005-0467$20.00 

**Table 1.** Parameters of Myocardial Aerobic Metabolism Estimated from Aortocoronary Difference Expressed in Percent (*M±m*)

| Duration of heart preservation, stage of operation | $Po_2$ | $Pco_2$ | Potassium | Lactate | Glucose |
|---|---|---|---|---|---|
| **2 h (Group 1)** | | | | | |
| Before removal of clamp from aorta | 87.4±9.2 | -12.5±3.4 | -0.5±0.07 | -11.1±2.3 | 5.7±0.63 |
| Immediately after removal of clamp from aorta | 85.7±9.1 | -81.4±7.4 | 18.4±2.8 | -9.7±1.5 | 6.7±1.3 |
| Reperfusion: 5 min | 92.6±10.3 | -20.6±3.4 | 2.2±0.35 | -1.1±0.18 | 2.9±0.37 |
| 15 min | 82.5±10.4 | -21.3±3.5 | 1.9±0.26 | -2.4±0.33 | 5.3±0.67 |
| **4 h (Group 2)** | | | | | |
| Before removal of clamp from aorta | 85.6±11.2 | -8.9±2.1 | -4.5±0.65 | -24.0±3.7 | 4.1±0.61 |
| Immediately after removal of clamp from aorta | 83.7±10.2 | -62.8±7.9 | -20.0±3.1* | -9.1±1.8 | -8.2±1.3* |
| Reperfusion: 5 min | 82.9±11 | -63.5±8.1* | -36.2±4.0* | -8.5±1.7 | -1.92±0.21 |
| 15 min | 84.4±9.7 | -22.0±4.3 | -9.4±1.9 | -12.6±1.8* | 4.9±0.63 |
| **8 h (Group 3)** | | | | | |
| Before removal of clamp from aorta | 86.7±11.3 | 3.2±0.39 | -13.9±2.1 | -54.1±7.3** | 2.4±0.39 |
| Immediately after removal of clamp from aorta | 87.4±10.7 | -31.2±4.2** | -68.2±7.3** | -22.2±3.1** | -28.1±2.9** |
| Reperfusion: 5 min | 68.9±7.7* | -6.8±0.89** | -64.3±7.1** | -41.4±5.4** | -9.5±1.4 |
| 15 min | 74.7±9.1 | -62.2±1.9 | -21.7±3.0 | -32.2±4.7** | -4.8±0.57 |

**Note.** Here and in Tables 2 and 3: *vs. group 1, **vs. group 2.

Roche lactate analyzer. FRP activity in the plasma was measured by induced chemiluminescence (CL) [3] and MDA by modified thiobarbituric acid test [2].

Myocardial status was assessed by metabolic parameters calculated from the aortocoronary difference expressed in percent.

The results were processed by the variation statistics methods.

## RESULTS

Heart subjected to intraoperative reperfusion after 2-h preservation started to utilize glucose and lactate immediately after the clamps were removed from the aorta; it absorbed potassium and released carbon dioxide during stable consumption of oxygen (Table 1). After 4-h preservation, reperfusion was associated with glucose and potassium release by the myo-

**Table 2.** Intensity of Free-Radical Processes in the Myocardium, Estimated from Aortocoronary Difference Expressed in Percent (*M±m*)

| Duration of heart preservation, stages of operation | CL intensity | CL extinguishing rate constant | MDA |
|---|---|---|---|
| **2 h (Group 1)** | | | |
| Before removal of clamp from aorta | 2.3±0.31 | 1.7±0.21 | 0.8±0.09 |
| Immediately after removal of clamp from aorta | 8.3±0.91 | -7.7±0.81 | 4.5±0.5 |
| Reperfusion: 5 min | 11.2±1.8 | -14.8±1.9 | -2.3±0.37 |
| 15 min | 17.4±2.1 | -4.7±0.54 | -5.3±0.67 |
| **4 h (Group 2)** | | | |
| Before removal of clamp from aorta | 5.4±0.61 | -3.2±0.43 | 1.2±0.19 |
| Immediately after removal of clamp from aorta | 23.2±2.9* | -86.5±9.1* | -5.7±0.64 |
| Reperfusion: 5 min | 17.8±2.1 | -23.1±2.7 | -12.2±1.9 |
| 15 min | 16.1±2.2 | -1.7±2.4 | 11.9±1.5 |
| **8 h (Group 3)** | | | |
| Before removal of clamp from aorta | 4.8±0.59 | -2.1±0.31 | 1.4±0.17 |
| Immediately after removal of clamp from aorta | -32.2±4.1** | -183.7±27.3** | -8.4±0.97 |
| Reperfusion: 5 min | 123.2±14.1** | -217.8±19.7** | -21.3±2.9* |
| 15 min | 97.3±10.2** | -58.0±6.3** | -35.1±4.0** |

**Table 3.** Free-Radical Characteristics of Recipient Venous Blood (*M±m*)

| Duration of heart preservation, parameters | Before artificial circulation | After artificial circulation |
|---|---|---|
| **2 h (Group 1)** | | |
| CL intensity, mV/sec | 157±19 | 258±31 |
| Constant of CL extinguishing rate, (1/sec×$10^2$) | 2.54±0.37 | 3.61±0.39 |
| MDA, nmol/ml | 2.9±0.17 | 3.7±0.39 |
| **4 h (Group 2)** | | |
| CL intensity, mV/sec | 149±25 | 323±36 |
| Constant of CL extinguishing rate, (1/sec×$10^2$) | 2.33±0.29 | 1.27±0.15* |
| MDA, nmol/ml | 3.1±0.21 | 4.1±0.45 |
| **8 h (Group 3)** | | |
| CL intensity, mV/sec | 121±0.17 | 405±37* |
| Constant of CL extinguishing rate, (1/sec×$10^2$) | 2.11±0.23 | 0.67±0.16** |
| MDA, nmol/ml | 3.2±0.20 | 4.91±0.49* |

cardium during the first 5 min, and only then started glucose consumption and accumulation of carbon dioxide with stable acidosis. Prolongation of heart preservation to 8 h led to a decrease in oxygen consumption and carbon dioxide production and was associated with drastic release of potassium, lactate, and glucose into the coronary sinus (Table 1).

In group 1, there was a tendency to FRP stimulation in the myocardium, while in group 2 a short-term activation of FRP (increased intensity of CL) and release of intracellular antioxidants (decreased constant of the rate of CL extinguishing) were observed immediately after the clamp was removed from the aorta. In group 3, reperfusion caused drastic activation of FRP and significant release of intracellular contents and lipid peroxides (MDA, Table 2).

Assessment of FRP in recipient's venous blood showed increased antioxidant activity of the blood after artificial circulation in group 2 in comparison with group 1 and a decrease in this parameter in group 3 (Table 3). CL intensity and MDA level were significantly higher than in group 1.

The syndrome of reperfusion injury to the myocardium is one of the main complications of open-heart surgery. During reperfusion, disorders in aerobic metabolism recovery are observed in the myocardium weakened by ischemia, leading to dysfunctional complications [4,7]. Free oxygen radicals play an important role in the pathogenesis of these complications [8].

Crystalloid cold cardioplegia is used for protection of the myocardium [1]. In our experiments aerobic metabolism was rapidly recovered during reperfusion of the myocardium transplanted after 2-h preservation. FRP activation was negligible. This indicated an adequate hypothermic protection after 2-h preservation of the transplant. However, such operations often require longer preservation.

After 4-h preservation, recovery of aerobic metabolism in the myocardium started not immediately after the clamps were removed from the aorta, but only after 5 min of reperfusion and was associated with a short-term activation of FRP and reversible pathological changes. After 8-h preservation, aerobic metabolism was recovered only negligibly by the 15th min of reperfusion. Expressed acidosis, sharply increased activity of free oxygen radicals, and, as a result, increased lipoperoxidation leading to the reperfusion syndrome [7] were observed.

Therefore, prolongation of heart preservation in St. Thomas Hospital solution to 8 h leads to irreversible myocardial injury. Free oxygen radicals play an important role in this process; the degree of their activation helps assess the severity of heart dysfunction.

Effect of transplanted heart on the recipient organism was investigated. Transplantation of the heart after 4-h preservation involved adaptation changes aimed at improvement of antioxidant defense, while after 8-h preservation we observed failure of antioxidant defense and enhancement of FRP which led to organ and tissue injuries in the recipient.

Disorders in recovery of the transplant function and deterioration of recipient's status after a longer preservation of the heart are strongly determined by activation of free oxygen radicals. This necessitates correction of the preserving solution and improvement of antioxidant therapy of the recipient.

## REFERENCES

1. S. L. Dzemeshkevich, I. I. Dement'eva, G. F. Sheremeteva, *et al.*, *Transplantologiya*, No. 2, 11-17 (1996).

2. E. N. Korobeinikova, *Lab. Delo,* No. 7, 8-9 (1989).
3. Ya. I. Serkiz, E. E. Chebotarev, R. A. Baraboi, *et al., Blood Chemiluminescence in Experimental and Clinical Oncology* [in Russian], Kiev (1984).
4. K. Bando and Y. Senoo, *J. Surg. Res.,* **46,** No. 2, 52-56 (1989).
5. R. Bolli, *J. Am. Coll. Cardiol.,* No. 12, 239-249 (1988).
6. Y. Chancerelle, M. DeLorgeril, R. Viret, *et al., Am. J. Cardiol.,* **60,** 813-816 (1991).
7. R. Ferrari, C. Ceconi, S. Curello, *et al., Mol. Cell. Biochem.,* **111,** 61-69 (1992).
8. N. K. Ganguly, K. Nalini, S. Wahi, *et al., Ibid.,* 71-76.