Capillary endothelia and cardiomyocytes differ in vulnerability to ischemia/reperfusion during clinical heart transplantation

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Abstract

Objective: The development of accelerated graft arteriosclerosis is a major cause of late death after orthotopic heart transplantation. The influence and the extent of peritransplant injury, especially of cardiomyocyte or capillary endothelial cell edema is discussed.

Methods: A morphometric ultrastructural analysis of myocardial biopsies from 29 donor hearts (21 male, age 34 ± 11 years) was performed. Right ventricular biopsies were obtained before cardioplegia (A), immediately following cardioplegia (B) (Custodiol®, Dr. F. Köhler Chemie GmbH, Alsbach-Hähnlein, Germany), before implantation (C), after 30 (D) or 60 (E) min of reperfusion and 1 week after transplantation (F). Mean ischemic time was 185 ± 68 min. Quantitative electron microscopy was carried out in five samples per heart and time point and in 30 test fields per sample by 'random systematic sampling' and 'point and intersection counting'. As parameters for cell edema the volume density of myofibrils in cardiomyocytes and the mean barrier thickness of capillary endothelia were analyzed. P-values of less than 0.05 were regarded as significant. Significant differences in contrast to the previous values are marked by *.

Results: The volume density of myofibrils (vol.%) was as follows: (B) 63.6 ± 3.2, (C) 61.8 ± 3.2, (D) 62.9 ± 3.2, (E) 63.6 ± 4.5. The mean barrier thickness (nm) was as follows: (A) 353 ± 21, (B) 376 ± 59, (C) 416 ± 71, (D) 473 ± 45*, (E) 453 ± 50*, (F) 379 ± 39.

Conclusions: Apart from a generally accepted edema of cardiomyocytes a relevant capillary endothelial cell edema develops during clinical heart transplantation. In contrast to cardiomyocytes the cell edema of endothelia shows a more pronounced and significant progression during cold ischemia and early reperfusion. After 60 min of reperfusion it is still significantly more pronounced than at the onset of ischemia. After 1 week there are no statistical differences compared to the initial values. Thus, an edema of capillary endothelia probably will trigger inhomogeneities in capillary perfusion. Peritransplant injury of endothelia may contribute to the later development of accelerated allograft arteriosclerosis. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Heart transplantation; Capillary endothelia; Cell edema; Ischemia

1. Introduction

Today orthotopic heart transplantation has become one of the most effective therapeutic efforts in the treatment of congestive heart failure [1]. But in the last years there has been a growing incongruity between patients waiting for heart transplantation and the amount of potential organ donors [2]. With growing experience the criteria for the acceptance of donor organs were adjusted [1]. The most important reason for an early postoperative lethality is initial graft failure, especially of the right ventricle [3]. A cold ischemia of more than 4 h is statistically highly significant contributing to an early death after heart transplantation [1,3,4].

Thus, a well preserved myocardial ultrastructure as a precondition for subsequent complete functional recovery after global ischemia is of great clinical importance. The knowledge about the fundamental processes that are initiated during cardiac arrest with different cardioplegic solutions and during ischemia and reperfusion will contribute to better processes of myocardial protection [5,6]. Operation times of 2–5 h are achieved by the lowering of temperature to reduce the energy consumption in combination with coronary perfusion with cardioplegic solutions. For heart transplantations the incubation of the explanted heart in protection solution in deep hypothermia allows a prolongation of the ischemic period without deterioration of function after a sufficient resuscitation period [7–9]. After the end of ischemia an altered myocardial reperfusion may
lead to the ‘no-reflow phenomenon’ [10]. Blood flow during reperfusion is inhibited because of clumped erythrocytes and capillary microthrombosis. However, intraschemic endothelial membrane damage also contributes to the ‘no-reflow phenomenon’. Thus, an adequate endothelial protection during ischemia and reperfusion will lead to a complete restitution of tissue perfusion [6].

The degree of alterations of different cells, their specific vulnerability and their reversibility can only be estimated by electron microscopy and has to be confirmed by morphometry [11–13]. A precondition for such stereological examinations is the fixation of tissue immediately after harvesting in a specific fixative and the further embedding in resin [6].

Even if the mid-term results are encouraging, meaning that more than 75% of the patients survive the first year after transplantation, the long-term follow-up is limited by the development of severe complications such as accelerated graft arteriopathy, a special type of arteriosclerosis [2,24]. In combination with these vascular alterations an interstitial myocardial fibrosis and myocyte hypertrophy contribute to the development of a chronic graft failure [14,24,25]. The influence and extent of peritransplant injury, especially of cardiomyocyte or capillary cell edema, are discussed herein.

2. Materials and methods

During 29 clinical heart transplantations right ventricular biopsies were obtained before cardioplegia (A), immediately following cardioplegia (B), before implantation (C), after 30 (D) and 60 (E) min of reperfusion, and in the course of 1 week after transplantation (F). Cardioplegia for conservation of the donor-hearts was performed with the crystalloid cardioplegic solution Custodiol® according to Bretschneider (HTK) (Dr. F. Köhler Chemie GmbH, Alsbach-Hähnlein, Germany). The composition of the solution was (in mmol/l): 15 NaCl, 9 KCl, 4 MgCl₂, 180 histidine, 18 histidine–HCl, 30 mannitol, 2 tryptophan and 1 K-α-glutamate. The hearts were perfused with, 2000–4000 ml of solution in 10 ± 3 min [9,15] at a temperature of 4°C. Biopsies (A), (B), (D) were taken with Tru-Cut® biopsy needles (Travenol Laboratories Inc., Deerfield, IL, USA). Endomyocardial biopsies (E) were harvested with Konno biotomes [16]. The right ventricular trabecules for (C) were cut with scissors and were taken from the branches into the fixative. Fixation for electron microscopy was carried out by immersion of the samples in a fixative containing 1.5% paraformaldehyde and 1.5% glutaraldehyde in 0.1 M phosphate buffer at room temperature for several hours. After rinsing the samples in phosphate buffer with 7.5% glucose added, postfixation was carried out in osmium tetroxide. Dehydration was performed automatically (Histomat, Bio-med, Theres, Germany) in a graded series of ethanol continuously followed by embedding in Araldite. Polymerization lasted 24 h at 70°C. Ultrathin sections (Ultracut, Reichert, Vienna, Austria) (70–80 nm) were contrasted with lead citrate and uranyl acetate. Electron microscopy was performed with an EM 10 equipped with TV camera and monitor (Zeiss, Oberkochen, Germany). Because of ‘random embedding’ and ‘random sectioning’, cardiomyocytes and capillary endothelia showed no preferred orientation in the samples. Per heart and time point, usually ultrathin sections of five samples from different tissue blocks, and in each sample 30 test fields were evaluated according to the ‘systematic random sampling’ method [17]. All ultrathin sections were examined on-line by means of the TV equipment at a magnification of ×25869. As parameters for cell edema the volume densities of myofibrils in cardiomyocytes and the mean barrier thickness of capillary endothelia were analyzed by ‘point and intersection counting’. The volume density of the myofibrils served as parameter for cell edema under the condition that there was no myofibrilolysis or myofibrilogenesis at the time of transplantation [13]. Myofibrillar volume density was related to the cardiomyocytes as reference space [11,12]. The cell edema of capillary endothelia was evaluated as the mean barrier thickness (τ), represented as a surface-to-volume-ratio [17]. A point-lattice test system of test points and test lines was projected on-line on a cross-section of an endothelial cell. Then the number of test points projected on the cytoplasm was related to the intersections with the outer surface membrane of the endothelial cell. The mean barrier thickness (τ) was calculated according to the following formula [17]:

\[ \tau = \frac{V_b}{S_b} = \frac{L_t}{2P_vI_b} \]

where \( V_b \) = volume density; \( S_b \) = surface density of endothelial surface; \( L_t \) = length of test lines; \( P_v \) = number of points in projection on the barrier; \( I_b \) = number of intersections with the barrier’s surface.

All results were given as mean values ± standard deviation. Significant differences were noted for \( P \)-values of 0.05 or less using the Friedman test. In the results the significant differences versus previous values at the different time points (e.g. A versus B, C versus D, etc.) are marked by an asterisk (*).

3. Results

We enrolled 29 heart transplant recipients in the study. Twenty-four were male and five were female. Mean age at transplantation was 51 ± 8 years. All patients were diagnosed to have dilatative cardiomyopathy. Functional class of all patients (according to the New York Heart Association grading system) was either III or IV. Average total ischemic time of the graft was 185 ± 68 min.

The donor population consisted of 21 male and 8 female organ donors. The mean age at brain death was 34 ± 11 years. Except for smoking there were no cardiac risk factors in the donors’ medical history. Main cause of brain death
was head injury in 18 patients followed by intracranial hemorrhage in nine patients. One patient died in status asthmaticus and one of carbon monoxide intoxication. The mean amount of catecholaminergic support was dopamine $4.5 \pm 3.0 \mu g/kg$ per min in 26 patients, dobutamine $6 \pm 6 \mu g/kg$ per min in seven patients. Mean central venous pressure was $8 \pm 3$ mmHg. Systolic pressure was $120 \pm 19$ mmHg.

Electron microscopic morphometric investigations revealed only moderate changes of the myocardial ultrastructure during the time course of ischemia. Immediately after cardioplegia (B) the volume density of myofibrils was $63.6 \pm 3.2$ vol.\% (Fig. 1). Before implantation (C) there was a small decrease to $61.8 \pm 3.2$ vol.\% (not significant (n.s.) compared with B). After 30 min of reperfusion (D) the volume density of myofibrils increased to $62.9 \pm 3.2$ vol.\% (n.s. compared with C) (Fig. 2), to show a further increase to $63.6 \pm 4.5$ vol.\% (n.s. compared with D) after 60 min of reperfusion (E). There were no statistically significant differences at any time point. As there were no statistically significant differences at any of these time points, no morphometric measurements were performed at (A) and (F) (Fig. 3).

In capillary endothelia the cell swelling (measured as the mean barrier thickness) showed a statistically significant increase from explantation (B) $376 \pm 59$ to $416 \pm 71$ nm* ($P < 0.05$) at implantation (C). Values between before cardioplegia (A) of $353 \pm 21$ nm and immediately following cardioplegia (B) were on a comparable level. During the first 30 min of reperfusion (D) the value of $473 \pm 45$ nm* increased significantly compared with the situation before implantation (C) ($P < 0.05$). After 60 min of reperfusion (E) there was a slight decrease to $453 \pm 50$ nm*, but these values (D, $P < 0.05$) and (E, $P < 0.05$) were still significantly elevated compared to those immediately after cardioplegia (B). After 1 week (F) mean barrier thickness $379 \pm 39$ nm was equivalent to the values before transplantation (n.s.). (Fig. 4).

4. Discussion

During clinical heart transplantation the acceptance of donor hearts is limited by the extent of ischemia [1]. Extensive knowledge about ischemic alterations on a cellular level, interstitial and cellular edema can only be achieved by electron microscopy and must be confirmed by morphometry [18,19]. Stereology as a special aspect of morphometry describes three-dimensional quantities, as for example a cell swelling from measurements on two-dimensional sections [17,20]. The mean barrier thickness of the capillary endothelium is a suitable parameter for estimating endothelial cell swelling [17,21]. The definition of a barrier is a mass of tissue or cytoplasm lying between two surfaces. Thus, barriers form closed compartments. With increasing volume, the mean barrier thickness between the surfaces increases. An endothelial cell swelling results in an increase of barrier thickness. This can narrow or even obliterate the capillary lumina and disturb further perfusion [5,21].

There are some principally different methods for myocardial protection successfully used in clinical heart transplan-
A direct comparison is not possible because of the different extent of alterations from the previous donor’s history, for example catecholamine-release during brain death and intensive care treatment [4,6,22]. The different methods of myocardial protection have been examined and compared experimentally [11,19,20]. The combination from induction of cardiac arrest with the use of cardioplegic solutions and deep hypothermia during the following ischemic period successfully prevents ischemic damage and achieves an excellent functional recovery during reperfusion [8,12,15,23]. The reduction of metabolism rates supports the reduced energy consumption during hypothermia [8,21]. Best functional results are reported for storage at deep hypothermic temperatures between 2 and 6°C [21,23]. But during isolated hypothermia different ion constellations may lead to cellular edema and impaired electrical activity to pronounced heart fibrillation. Before the onset of cardiac arrest, the energy consumption is increased [16,21,23]. Thus hypothermia should be combined with a cardioplegic heart arrest.

During clinical heart transplantation donor hearts were perfused at a temperature of 4°C. Temperature at explantation (biopsies B) is between 8 and 10°C. The lowest achievable temperature during cold storage is about 4°C (biopsies...
C). During implantation temperature slowly rises to hypothermic temperature (24–28°C) of the recipient on cardiopulmonary bypass (biopsies D and E). After removal of the aortic cross-clamp during reperfusion temperature reaches normothermia again.

The cardioplegic principle of the HTK solution is to induce cardiac arrest by an intracellular ionic composition [15]. HTK lowers the extracellular concentration of sodium and calcium to approximately cytoplasmic values [15]. A low concentration of calcium impairs electromechanical coupling, and a low concentration of sodium leads to an immediate stop of the electric activity. The sodium–calcium exchange is converted because the low sodium concentration is followed by a washout of calcium ions [20]. Thus, accumulating calcium ions stabilize the cell membranes and the intracellular calcium concentration rises to 10–20 μmol/l. An important ingredient of the HTK solution is the histidine/histidine HCl buffer system. This buffer is hardly able to diffuse into the cells. The high buffer capacity and the additional antiosmotic effect of mannitol seem mainly responsible for the good structural protection [16,21].

In different species a different vulnerability of the cardiac tissues to ischemia has been shown in response to the cardioplegic solution applied [11,12,19,20]. Indeed the capillary endothelia after application of various protective processes showed similar ultrastructural alterations to those during clinical heart transplantation [12–14,19].

There was a wide range of ischemic times (minimum 74 min, maximum 292 min) in different patients. In our results we were not able to show a statistical correlation between the duration of ischemia and the extent of ultrastructural alterations under the condition that ischemic time does not exceed 300 min. Severe ultrastructural alterations under preservation with HTK solution appear after prolongation of ischemia over 6–8 h. A comparison of patients divided into subgroups according to an ischemic time about and shorter than 160 min did not show any statistical difference. Therefore this wide range of ischemic times as occurring in clinical practice seems to be acceptable.

Apart from a generally accepted edema of cardiomyocytes, capillary endothelia also develop a marked and significant cellular edema during clinical heart transplantation, as shown in this study. In contrast to cardiomyocytes the cell edema of endothelia shows a more pronounced and significant progression during cold ischemia and early reperfusion. Even after 30 and 60 min of reperfusion, cell edema still is significantly more pronounced than at the onset of ischemia.

So there is a specific vulnerability of capillary endothelia to ischemia/reperfusion. But neither cardiomyocyte nor capillary endothelial cell swelling reaches an extent that limits postischemic reperfusion described as the ‘no-reflow phenomenon’ [10,12]. In this study biopsies taken before onset of cardioplegia served as intraindividual controls. At this time there should be no alterations regarding surgical procedure. But in comparison, with a healthy reference population the fact that an organ donor had undergone brain death has to be emphasized. There might be structural alterations due to catecholamine-storm at this time point.

A description of normal myocardial biopsies from a healthy reference population may be desirable but these biopsies are too rare to be examined systematically. Further studies might focus on this point.

All alterations observed in this study are potentially reversible. The ‘no-reflow phenomenon’ is mainly caused by the simultaneous development of endothelial cell swelling and myocyte cell edema and/or overcontraction during ischemia. This results in a mechanical compression of the capillaries [10]. Thus, an edema of capillary endothelia probably triggers inhomogeneities in capillary perfusion [12]. Furthermore, peritransplant injury of endothelia as initial damage during brain death and intensive care may initiate immunological processes and may contribute to the later development of accelerated graft arteriopathy [5,24]. On the other hand similar alterations of myocardial ultrastructure may also occur during prolonged cardiac arrest in other forms of cardiac surgery. In conclusion, this may also lead to alterations of myocardial ultrastructure and of myocardial function during postischemic reperfusion. But in contrast to heart transplantation there will be a differing extent of immunological processes. So protection of the myocardial and especially of the endothelial ultrastructure is of great importance in achieving good long-term results in all forms of cardiac surgery.

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