Comparing the ultrastructural effects of two different cardiac preparation- and perfusion-techniques in a porcine model of extracorporeal long-term preservation

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Abstract

Objective: In heart transplantation a well-preserved myocardial ultrastructure is an important precondition for functional regeneration. Aim of the study is to optimize the conditions in this new established model of extracorporeal cardiac perfusion. Methods: (I) In six pigs, hearts were arrested with Bretschneider Histidine-Tryptophan-Ketoglutarate cardioplegia and cold ischemia, explanted and connected to a circulating constant pressure Langendorff system (80—90 mmHg) and perfused with leukocyte depleted autologous blood. (II) Beating hearts of seven pigs were explanted and connected immediately to the Langendorff system (40—50 mmHg). Myocardial biopsies (n = 55) were taken in situ and during the following 12 h of reperfusion, and were prepared for electron microscopy. Results: Cardioplegia and hypothermia (group I) induced mitochondrial edema and myofibrillar degeneration in cardiomyocytes and severe endothelial edema. During 4 h of reperfusion, mitochondrial edema, myofibrillar, and sarcolemmal damages in cardiomyocytes increased. Moderate endothelial degeneration, interstitial edema, and bleedings appeared. In contrast, in group II after 6 h of reperfusion endothelia showed only mild alterations. Cardiomyocytes showed myofibrillar but not mitochondrial degeneration. Interstitial edema and bleedings were mild. Conclusion: Avoiding cardioplegia and hypothermia, and using lower perfusion pressure resulted in a better preservation of the ultrastructure in explanted hearts at the Langendorff system.

Keywords: Heart transplantation; Langendorff system; Ultrastructure; Pig

1. Introduction

Today, heart transplantation requires expanding donor pools and organ procurement at distant sites. Numerous factors responsible for donor heart failure [1], a common cause of early mortality and post-transplant morbidity in humans [2] have been identified. There is a cumulative effect of injuries during the whole process: brain death, neurohormonal and hemodynamic instability, cardioplegic arrest, cold ischemic storage, implantation, and reperfusion injury [3]. It has been accepted in general, that ischemia may last not longer than 4 h [1,2]. Reperfusion injury remains a limiting factor in extending ischemic storage time for heart transplantation in humans. After reperfusion the ‘no-reflow phenomenon’ [4,5], capillary microthrombosis [6], and transplant vasculopathy [7] are fatal complications. Additionally myofilament hypercontraction of the energy depleted cardiomyocytes, the so called calcium paradox, has been investigated in detail [8,9]. Furthermore, myocardial apoptosis is induced in ischemic and reperfused hearts [10,11]. Thus a well-preserved myocardial ultrastructure as a precondition for subsequent complete functional regeneration is of great clinical importance [12].

The aim of this study was to investigate the ultrastructural findings after two different methods in cardiac preparation and subsequent reperfusion at a modified Langendorff system.

2. Methods

All animals received human care in compliance with the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 85-23,
2.1 Group I

Six healthy pigs (body weight about 30 kg) were sedated with isoﬂurane (initially 2–4% followed by 1–1.5%). After standard sternotomy the heart was exposed and cardioplegia with 4 °C Bretschneider Histidine-Tryptophan-Ketoglutarate (HTK) solution (Kohler Chemie GmbH, Alsbach, Germany) and subsequently 30 min cold ischemia were performed (in ice). After explantation, the hearts were connected to a circulating constant pressure (80–90 mmHg) modiﬁed Langendorff-perfusion system. The standard Langendorff-perfusion system was modiﬁed by including a ﬁltering system, an oxygenator and a centrifugal pump (applied for a patent on 11.10.2005 at Deutsches Patentamt: Ullmann C, Dhein S, Garbade J, Gummert J. System zur nicht-kardioplegischen Konservierung von Spenderherzen für die Transplantation. AZ 10 2005 048 625).

The perfusion solution (modiﬁed Tyrode solution) consisted of 1000 ml leukocyte-depleted (autologous) blood and was supplemented by electrolytes (NaCl 120 mmol/l, NaH2PO4 0.42 mmol/l, NaHCO3 1.128 g/l, MgSO4 0.2025 mmol/l), glucose (1.1 g/l), 0.72 g/l, magnesium L-hydrogenaspartate 0.652 g/l, calcium gluconate 1.128 g/l, MgSO4 0.2025 mmol/l), glucose (1.1 g/l), insulin (Insulin Actrapid HM 20 IE/l), ketone bodies (1.26 g/l) and antibiotics (gentamycin, penicillin, and streptomycin, Sigma GmbH, Germany), anti-inﬂammatory substances (methylprednisolon-21-hydrogensuccinat 250 mg/l, dexamethaspon 80 mg/l, trasylol 1 Mio KIE/l). Left atrial pressure and aortic root pressure were analyzed and kept constant (5–6 mmHg) using processor controlled tubing clamp.

Twenty-nine myocardial needle biopsies (about 5 mm × 3 mm × 2 mm) were taken subepicardially from the apex of the left ventricle: in situ (n = 6), after 35–60 min hypothermic cardioplegia (n = 6), 2 h (n = 3), 4 h (n = 5), 6 h (n = 3), 8 h (n = 4) and 12 h (n = 2) of reperfusion.

Depending on the results of group I we changed the experimental conditions: non-ischemic beating hearts were explanted using a new developed coaxial aortic cannula (applied for a patent, s. before) and subsequently a lower perfusion pressure was used in the Langendorff system.

2.2 Group II

Seven healthy pigs (body weight about 30 kg) were sedated, and anesthetized with isoﬂurane. After sternotomy the beating hearts were explanted and immediately (within 5 min) connected to a circulating constant pressure (40–50 mmHg) modiﬁed Langendorff-perfusion system as described in experiment 1. For this procedure a special cannula was designed. Left atrial pressure and aortic root pressure were analyzed and kept constant (5–6 mmHg) using processor based closed-loop clamp.

Twenty-six myocardial needle biopsies (about 5 mm × 2 mm × 3 mm) (Biopton delicat, 1.8 mm × 510 mm, H05-04, Pillingweck GmbH, Tuttinglen, Germany) were taken subepicardially from the apex of the left ventricle: in situ (n = 7), from the explanted heart after 30 min (n = 2), 1 h (n = 5), 4 h (n = 1), 6 h (n = 3), 8 h (n = 4) and 12 h (n = 4) of reperfusion.

Monitoring of acid—base balance, hemoglobin, electrolytes, and lactate in the perfusate was performed, ATP-concentration using bioluminescent technique (Luciferase-luciferin) was measured, and contractility was quantified by pressure volume loops (dp/dt). (Data will be published elsewhere.) Contractility and myocardial color of the hearts, connected at the Langendorff system were observed at the times of biopsies and graded compared to the normal beating hearts before explantation.

All specimens were ﬁxed in 2.5% glutaraldehyde (Plano, Wetzlar, Germany) for 48 h, postﬁxed for 2 h with 1% osmiumtetroxide solution and embedded in glycicether 100. Ultrathin sections were stained with uranyl acetate/lead citrate.

Ultrastructural ﬁndings of ﬁve randomly chosen different locations in every (blinded) biopsy were examined by two investigators by the use of transmission electron microscope Zeiss EM 900 with magniﬁcations from 3000 × to 30,000 ×, following a graduation system as follows:

- **Mitochondrial degeneration**: none: normal; mild: separation of the cristae; moderate: severe swelling and disintegration of the membranes; severe: rupture of the outer membranes.
- **Nuclear degeneration**: none: normal structure; mild: marginal chromatin clumping, mild invaginations; moderate: marginal chromatin clumping and shrinkage of the nuclei, numerous deep interdigitations; severe: pyknosis and karyolysis.
- **Myofibrillar degeneration**: none: normal sarcomere structure; mild: Z-lines not straight, intramyoﬁbrillar spaces, tearing of myoﬁbrils, myoﬁbrillar contraction, or hypertension, intercalated disc organization; moderate: disruption of myoﬁbrils, severe tearing, massive hypertetration, severe contraction; severe: total loss of myoﬁbrillar structure.
- **Sarcolemmal damage**: none: continuous and normal; mild: continuous and intact but scalloped; moderate: scalloped and detached from the sarcomeres; severe: ruptured membrane.

3. Results

3.1 Ultrastructural ﬁndings in situ (both groups)

In both experimental groups the biopsies taken in situ were normal or showed slight mitochondrial edema in cardiomyocytes (Table 1). Oval nuclei were located centrally and sometimes a nucleolus was seen. Sarcomeres were relaxed, myoﬁbrils were placed longitudinally, and cross striation was clearly resolved. Sarcoplasmic reticulum was sparse, few glycogen granules and lipid droplets were present. Sarcolemma was intact and a continuous external lamina was observed. Intercalated discs (cell junctions) were arranged in a typical stepwise fashion.

Capillary endothelia showed oval heterochromatic nuclei, and contained few mitochondria, numerous pinocytotic vesicles, some primary lysosomes, ribosomes and some rough endoplasmic reticulum and Golgi apparatus. A continuous moderately electron dense basement membrane was seen.

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Fibroblasts were characterized by oval heterochromatic mildly invaginated nuclei, and small amounts of cytoplasm containing few mitochondria and some rough endoplasmic reticulum.

3.2. Ultrastructural findings after cardioplegia and 30–90 min hypothermic ischemia (group I)

The hearts from the pigs, which underwent cardioplegia and 30–90 min hypothermic ischemia before reperfusion at the Langendorff system were characterized by the following alterations, which slightly increased with the duration of ischemia.

In cardiomyocytes mitochondria were moderately swollen, cristae were distorted and ruptured, and their matrix was predominantly cleared. Nuclei showed few mild invaginations. Sarcomers were mainly intact, however, some contraction bands or thickened Z-lines occurred. Endoplasmic reticulum was mildly dilated, glycogen granules were still present. Sarcolemma was scalloped by swollen mitochondria, numerous small sarcolemmal breaks were obvious, and the external lamina appeared inhomogeneous (Fig. 1).

Capillary endothelia showed severe cloudy swelling of the cytoplasm and moderately edematous mitochondria. The capillary lumina were obturated by the swollen endothelial cells. Cellular membranes were intact or focally ruptured, and basal membranes were mildly inhomogeneous and discontinuous. Nuclei were round and euchromatic with few mild interdigitations.

Fibroblasts appeared mainly normal, only a mild mitochondrial edema was present in some cells. Bleedings or interstitial edemas were not obvious.

3.3. Findings after cardioplegia, hypothermic ischemia and subsequent 2–4 h of reperfusion (group I)

After 2–4 h of reperfusion the explanted hearts showed a moderately restricted contractility and small epicardial bleedings.

Ultrastructurally, after 2–4 h reperfusion cardiac cells were moderately damaged. Only few cells appeared normal or showed mild lesions as observed after hypothermic cardioplegia.

Nuclei of numerous cardiomyocytes were characterized by marginally clumping of chromatin, and multiple deep invaginations. Mitochondria were moderately swollen, showed cristolysis and broken membranes or contained dark matrix and cloddy electron dense material. Numerous sarcomers were hypercontracted, closely packed together with thickened Z-lines and a loss of regular structure (Fig. 2). Glycogen was predominantly absent. The lateral sarcolemma was sometimes scalloped by swollen mitochondria, separated from the underlying myofilibrils, and multifocally destructed. The cell junctions were mainly normal.

Endothelial cells showed moderate mitochondrial and intracytoplasmatic edema as well as nuclei with mild to moderate chromatin margination and nuclear invaginations.

![Fig. 1. Group I, myocardium after cardioplegia and 40 min hypothermic ischemia: mitochondria (M) of the cardiomyocyte are moderately swollen, cristae are dissolved, and matrix is cleared. The nucleus (N) shows few very mild invaginations. Sarcomers (S) has some contraction bands and thickened Z-lines. Sarcolemma is scalloped by swollen mitochondria, and numerous small sarcolemmal breaks (arrows) are obvious and the external lamina appears to be inhomogeneous (TEM, 7000x magnification, bar 1.1 μm).](image-url)
Cellular membranes and basal lamina were mildly discontinuous (Fig. 2).

Mild to severe bleedings, some debris, and few neutrophilic granulocytes appeared in the edematous interstitium. Fibrocytes showed mild mitochondrial edema and mild nuclear chromatin margination and clumping.

3.4. Findings after 6—8 h of reperfusion (group I)

The contractility of the perfused beating hearts got increasingly worse but was acceptable. Macroscopically, they showed increased bleedings and edemas.

Ultrastructurally, after 6 h reperfusion most cardiomyocytes showed severe lesions, whereas few cells were only mildly affected.

Mitochondria showed moderate swelling, cristolysis, cleared matrix, and disrupted membranes. Nuclei of the cardiomyocytes showed signs of pycnosis. In numerous cells severe myofibrillar necrosis with a loss of cross striation was obvious. Sarcomers of few cardiomyocytes were hypercontracted or hypertensed. Sarcolemma was separated from the myofibrils and partially broken, the external lamina was discontinuous and irregular. Intercalated discs were mainly intact, thus necrotic and less affected cells were attaching each other.

Capillaries showed severe lesions, characterized by pycnotic nuclei, severe intracytoplasmic edema and dissolved cellular membranes and organelles. The underlying basal lamina was thickened and irregular. Cellular debris was lying in the edematous interstitium with intensive hemorrhages, and granulocytes showed signs of phagocytosis. Fibrocytes contained pycnotic nuclei, mildly edematus mitochondria, and cellular membranes were fragmented. Furthermore in three cases bacteria were found in the interstitium.

After 8 h reperfusion the hearts showed severe signs of degeneration. Nuclei of cardiomyocytes were pycnotic, mitochondria swollen, and degenerated, sarcomers lost their regular structure, and sarcolemma was multifocally broken (Fig. 3). Endothelia were severely damaged, with pycnotic nuclei and fragmented cellular membranes and organelles (Fig. 3).

Furthermore, fibrocytes contained pycnotic nuclei and lost their organelle’s and membrane’s structures. Interstitium was severely edematous and showed intensive hemorrhages, as well as numerous neutrophil graunlocytes and some bacteria.

3.5. Findings after 12 h of reperfusion (group I)

After 10—12 h reperfusion the cardiac contractility was exhausted and edemas, bleedings and partly stoned myocardium were characteristics for the preserved hearts.

Ultrastructurally, after 10—12 h of reperfusion a total loss of the cellular structure was obvious in most cardiomyocytes. Some residuals of swollen mitochondria and occasionally mitochondrial myelin figures were observed. Single intermingled cardiomyocytes showed only mild to moderate alterations as described before.

Phagocytosis of cellular fragments was observed by neutrophilic granulocytes. Numerous erythrocytes were in the interstitium, most of them were less electron dense.

![Fig. 2. Group I, myocardium after cardioplegia and hypothermic ischemia and after subsequent 4 h reperfusion: mitochondria (M) of the cardiomyocyte are swollen, and show distortion and lysis of cristae. Sarcomers (S) lost their regular structure. The lateral sarcolemma and the external layer are discontinuous (arrows). Endothelial cells show moderate mitochondrial and intracytoplasmatic edema (e) as well as pycnotic nuclei (Ne). Cellular membranes and basal lamina are discontinuous (arrows) (TEM, 7000× magnification, bar 1.1 μm).](image1)

![Fig. 3. Group I, myocardium after cardioplegia and hypothermic ischemia and after subsequent 8 h reperfusion: cardiomyocytes show pycnotic nuclei (N), swollen mitochondria (M) with cristolysis, and sarcolemma is multifocally broken. Endothelia are severely damaged, with pycnotic nuclei (Ne) and fragmented cellular membranes (arrows) and dissolved organelles (TEM, 4400× magnification, bar 1.7 μm).](image2)
Capillary endothelia and fibrocytes were pycnotic and also severely degenerated, and in four cases bacteria were found in the interstitium.

3.6. Findings after 0.5—1 h reperfusion without previous cardioplegia and hypothermic ischemia (group II)

The hearts showed a normal contractility. No signs of bleedings or edema were detectable.

Specimens taken after 30—60 min reperfusion were ultrastructurally characterized by mild alterations. In all cardiomyocytes mitochondria were mildly swollen, their matrix was predominantly cleared, and some electron-dense granules were present. Nuclei showed mild chromatin margination and numerous mild invaginations. Myofibrils were mainly intact and small amounts of glycogen were seen. Cell junctions, sarcolemma, and external lamina appeared normal.

Capillary endothelia were normal or showed mild cloudy swelling of the cytoplasm and mildly edematous mitochondria. Nuclei showed mild chromatin margination and numerous mild invaginations. Myofibrils were mainly intact and small amounts of glycogen were seen. Cell junctions, sarcolemma, and external lamina appeared normal.

Fibroblasts appeared mainly normal, however, mitochondrial edema was present in some cells.

3.7. Findings after 4—6 h of reperfusion (group II)

Macroscopically the hearts contracted normally, only in three cases the contractility was moderately restricted. After 4—6 h of reperfusion the beating hearts showed no evidence for edemas. Nevertheless, some hearts had small epicardial bleedings.

Ultrastructurally, in group II hearts were only mildly to moderately damaged after 4—6 h reperfusion. Especially endothelial damages were mild and consequently interstitial alterations were less intensive than in group I, but cardiomyocytes showed moderate signs of degeneration.

Nuclei of numerous cardiomyocytes were characterized by marginally clumping of chromatin, and multiple invaginations. Numerous mitochondria were swollen and showed disrupted cristae, electron dense granules, granular matrix or myelin figures. In most cells sarcomeres were hypercontracted or partially lost their regular cross-striated structure. The sarcolemma was scalloped by swollen mitochondria and focally separated from the underlying myofibrils. The sarcolemma and the external lamina were multifocally discontinuous but cell junctions were normal.

Endothelial cells showed mild cloudy swelling and mitochondrial edema. Nuclei were predominantly normal. Cellular membranes were intact, but basal lamina showed areas of thickening.

Numerous erythrocytes and few neutrophilic granulocytes appeared in the edematous interstitium. Some fibrocytes were normal, others showed intensive nuclear chromatin margination and clumping and other signs of degeneration.

3.8. Findings after 8 h of reperfusion (group II)

After 8 h of reperfusion the beating hearts showed a moderate restricted globally contractility and they were relaxed. Morphologically the hearts were characterized by some small bleedings and slight edemas predominantly in the ventricular walls.

In group II after 8 h reperfusion most myocardial cells showed moderate lesions, whereas capillary endothelia were predominantly intact (Fig. 4).

Nuclei of the cardiomyocytes showed irregularly shaped nuclei with interdigitations, and chromatin clumping. Mitochondria showed mild edema or increased electron density of the matrix. In numerous cells moderate myofibrillar degeneration with a loss of cross striation was obvious. Sarcolemma and external lamina were mildly discontinuous and irregular.

Capillaries were normal or showing only mild lesions. Cellular debris, erythrocytes and granulocytes were lying in the mildly edematous interstitium. Some fibrocytes showed pycnotic nuclei, mild mitochondrial edema and fragmented cellular membranes.

3.9. Findings after 12 h of reperfusion (group II)

The global contractility of the perfused hearts got increasingly worse but was still acceptable. Macroscopically, the myocardium showed increased bleedings and edemas.

After 12 h reperfusion, the hearts of group II showed ultrastructurally severe lesions in most cardiomyocytes, but endothelia were still mildly damaged, although interstitial alterations were marked (Fig. 5).
In cardiomyocytes mitochondria showed moderate swelling and cristolysis, nuclei were pycnotic, and in numerous cells severe myofibrillary degeneration with a loss of cross striation was obvious. Sarcolemma and the external lamina were broken and discontinuous.

Capillaries showed mild lesions, characterized by margination of chromatin, invaginated nuclei of endothelia, and mild mitochondrial edema. The basal lamina was mildly thickened and irregular. In some capillaries the luminal border of the cellular membrane was dissolved and cellular debris was seen in the capillary lumina.

Cellular debris was lying in the edematous interstitium with intensive bleedings, and some granulocytes. Fibrocytes showed signs of degeneration and collagen fibers lost their cross striation. Furthermore, in two cases bacteria were found in the interstitium.

3.10. Summarized comparison of group I and group II

It can be concluded, that the cardiomyocytes in group I were damaged after 4 h reperfusion as much as cardiomyocytes in group II after 6 h. However, the most important difference was the well-preserved endothelial structure in group II compared to group I. Although alterations of the interstitium occurred after 6—8 h of reperfusion, the endothelial structure was destroyed not until 12 h of reperfusion in group II.

4. Discussion

It can be summarized that the use of the Langendorff system after cardioplegia and hypothermic ischemia (group I) was not useful in reaching a longer cardiac preservation in the porcine heart. However, the changed experimental conditions in group II led to a clearly better preservation of the ultrastructure.

Recently, reperfusion injury has been subject to intensive research. In cardiomyocytes immediate reperfusion injury is characterized by hypercontraction of myofibrils and cellular swelling. The coexistence of severe contracture, overextension of spaces between sarcomeres and sarcolummal disruption, also called ‘contraction band necrosis’, seems to be the primary cause for cardiomyocyte death during the earliest stage of reperfusion [8,13]. In group I of our study, we observed hypercontractures and cellular swelling as well as diffuse myofibrillary and contraction band necrosis shortly after reperfusion. Several authors described that functional and morphological sarcolummal alterations may lead to an increased influx of calcium resulting in hypercontraction bands [8,13]. But also the depletion of energy contributes to a calcium overload of cytosol due to a reverse mode of the sarcolummal Na/Ca exchanger. Although contraction bands may be an artifact in biopsies, they typically occur during reperfusion after myocardial ischemia [14]. In our study artifacts can be excluded by the fact, that they were not present in the in situ specimens. And, in group II we did not find contracted myofibrils until 4—6 h of reperfusion. Additionally, the evidence of other simultaneous cellular alterations and pycnosis confirmed the interpretation as true experimentally related alterations.

Cardiomyocytes in group I were damaged after 4 h reperfusion as much as cardiomyocytes in group II after 6 h. Yet, the most important difference was the well-preserved endothelial structure in group II compared to group I. Although alterations of the interstitium occurred after 6—8 h of reperfusion, the endothelial structure was not severely destroyed until 12 h reperfusion in group II. One possible reason for this may be the reduction of the perfusion pressure in group II. But even if it led to a decreased vascular damage, it could not explain the entire extent of improved preservation. Another cause may be the lack of reperfusion-associated injury, since cardiac arrest and hypothermic ischemia were excluded.

During hypothermic ischemia [group I] the endothelial alterations were more prominent than that of the cardiomyocytes reflecting their higher sensitivity to anoxia [8]. In general, our ultrastructural finding correspond well to Kuhn-Regnier et al. [15], who described that a modified HTK solution improved endothelial function and metabolic state of the porcine hearts. The degree of protection of endothelial cells during reversible deep hypothermic ischemia depends on the method used for cardiac arrest and storage [5]. Bretschneider emphasized, that cardioplegia solution is used for cardiac arrest but not for functional and morphological protection [12]. HTK cardioplegia was chosen, because this the standard cardioplegia used in the Heart Center Leipzig.

Endothelial edema probably leading to compression of capillaries is prevented by using University of Wisconsin [UW]
solution or, with slight limitations, HTK solution for arrest and storage at 5 °C [5,13,16]. Yet, in our model we observed substantial morphological alterations using HTK solution. Comparison to UW cardioplegia would be interesting, but we developed a new technique to explain the beating heart, which is the better way in any case. This technique has been applied for patent. After reperfusion the altered endothelia did not show signs of regeneration but increasing signs of degeneration resulting in a higher permeability leading to interstitial bleedings and extravasation of granulocytes. The swollen endothelial cells induced further hypoxia of the tissue by narrowing the capillary lumina corresponding to the ‘no-reflow phenomenon’ described by Marten et al. [5]. Furthermore, it is certain that the peritransplant injury of endothelia may contribute to the later development of arteriosclerosis [4]. The ultrastructural findings in our study suggest regeneration and reparation of the vascular lesions may lead to arteriosclerosis. However, depending on the pre-existing lesions of the donor heart, the alterations caused during transplantation process are probably small. In long-term observations it has been described, that marked myocardial fibrosis will be the result of the reparative mechanisms after heart transplantation, replacing the degenerated cardiomyocytes. This study also investigated the fibrocyte’s ultrastructure to describe their alterations and their potential role in further course of regeneration and reparation. We found, that fibrocytes were less sensitive to hypoxia than endothelia and cardiomyocytes and marked alterations occurred after 6 h of reperfusion in group I and after 12 h in group II. In the reimplanted heart the interstitial alterations may stimulate the fibroblast proliferation and collagen synthesis.

In group II, avoiding cardioplegia and hypothermic ischemia and using a lower perfusion pressure resulted in better functional structure of cardiomyocytes and endothelia. However, further modifications of the experimental design are necessary to reach a better preservation of cardiomyocytes. Looking at the ultrastructural alterations, it seems likely that the perfusion solution needs some adaptations concerning its content of calcium, sodium, potassium, ATP, and oxygen to preserve sarcolemmal and myofibrillar structures. Although endothelia appeared ultrastructurally quite normal, the interstitial edema and extravasation of erythrocytes increased. Therefore, colloidal and osmotic variations of the perfusion solution might be necessary.

It has to be discussed how far the findings in the pigs are comparable to humans. Ferrera et al. described that, in contrast to other species, reanimation of porcine heart failed in their experiments without any reason [17]. The animals used in our study were young, healthy and not stressed before surgery. We have chosen pigs for our experiments, because their heart’s size, function, and morphology are more comparable to the human heart than that of small animals, which have a higher heart frequency and metabolism [12].

One critical point is that donor hearts in human medicine are often mildly [age related] altered, whereas in animal models normal hearts are transplanted [12]. Furthermore, the use of live donors in animal studies excludes the considerable effect of brain death on the heart [3]. Thus, it may be suggested that pigs may serve as a very sensible animal model to optimize the system, but it has to be interpreted carefully until further indications for interspecies correlation are available.

Reperfusion with autologous blood does not effectively control variables of rejection-induced dysfunction [3]. However, Oshima et al. [18] showed, that the suppression of pro-inflammatory cytokines during 12 h perfusion improved donor heart function 3 h after transplantation in a canine model.

The finding, that erythrocytes lost their electron density may point out the necessity of refreshed blood solutions, to prevent thrombosis of the capillaries by degenerating erythrocytes.

After all it can be concluded that this model of extracorporal perfusion is very promising. Additional studies employing other methods to evaluate the efficacy of this model as well as further modifications have to be done to optimize the preservation outcomes. At least only the re-implantation of the hearts will prove the success of this preservation technique.

References


