Structural and ultrastructural study of the myocardium after 24-hour preservation in University of Wisconsin solution

E. Garcia-Poblete¹, H. Fernández¹, L. Alvarez², A. Torralba³ and C. Escudero²

¹Department of Cellular Biology, Universidad Complutense de Madrid, Services of ²Experimental Surgery and ³Hospital Pharmacy, Clínica Puerta de Hierro, Madrid, Spain

Summary. This study deals with myocardial preservation after 24 hours of continuous perfusion with University of Wisconsin (UW) solution, focusing on the morphological changes produced by preservation and reperfusion, and their possible relationship to the composition of the solution and the immediate hemodynamic findings after orthotopic heart transplantation in dogs. Following preservation, the histological images of the myocardium were normal in almost every case. After revascularization, although 50% of the hearts recovered preoperative functional levels, there were relevant histological changes in all of them. These changes consisted of interstitial edema, damage to at least 50% of the mitochondria, presence of contraction bands, loss of intercellular junction structure and vasoconstriction. According to these results, the association of continuous perfusion and UW solution appears to be effective during the ischemic period of prolonged myocardial preservation, but not during reperfusion. Perhaps the proportion of viable hearts could be increased by preventing vasoconstriction.

Key words: Prolonged myocardial preservation, University of Wisconsin solution, Continuous perfusion, Reperfusion injury

Introduction

One of the keys to successful transplantation is the preservation of the organ during the ischemic period. For this purpose, different preservation solutions have been developed which, in routine clinical practice, are used for cold storage. In the case of cardiac grafts, this technique ensures a safe ischemic time of 4 to 6 hours, although it does not completely prevent the adverse effects of ischemia-reperfusion on myocardial morphology and function (Nutt et al., 1991; Jeevanandam et al., 1992; Menasché et al., 1993; Fremes et al., 1995).

The use of University of Wisconsin (UW) solution in clinical heart transplantation is relatively recent. It appears to produce better results than other preservation solutions (Nutt et al., 1991; Menasché et al., 1993), leading to suggestions that it may play a positive role in high-risk donors or in cases of prolonged preservation (Jeevanandam et al., 1992; Fremes et al., 1995).

In most of the studies involving UW solution, the preservation consisted of cold storage technique; when continuous perfusion was employed, reperfusion was performed in vitro (Nutt et al., 1991). There are very few references to the correlation between the morphological and functional results. Thus this report deals with myocardial preservation after 24 hours of continuous perfusion with UW solution, focusing on the morphological changes produced after preservation and reperfusion, and their possible relationship to the composition of the solution and to the immediate hemodynamic findings after orthotopic transplantation.

Materials and methods

Animals

The trials were performed on 11 healthy adult dogs of unknown age and breed, weighing between 20 and 25 kg. Care of the animals complied with the rules stipulated by the Principles of Laboratory Animal Care and the Guide For The Care and Use of Laboratory Animals issue by The National Society for Medical Research and the National Academy of Sciences, respectively.

Surgical technique

Following systemic heparinization, cardiac arrest was induced in the donor by injection of a crystalloid cardioplegic solution (K⁺=30 mEq/L). The procurement of the donor heart included the aorta up to the...
commencement of the descending segment. Once the heart was harvested, the pulmonary artery was transected at the level of its bifurcation and both atria were opened wide in preparation for implantation. The heart, suspended by the aorta, was placed in a gravity-driven continuous perfusion chamber, where it remained for 24 hours at 4 °C. After this period of time, the organ was implanted orthotopically into the recipient using a technique similar to that employed in humans.

Preservation technique

Preservation involved the use of the gravity-driven continuous perfusion technique (maximum pressure 15 cm H₂O) with hypothermia (4 °C) and oxygenation (95% O₂, 5% CO₂) described by Wicomb et al. (1984). The heart was perfused with a modified form of commercially available UW solution (ViaSpan®); this process did not involve the addition of dexamethasone, insulin or penicillin.

Determinations

In the preservation solution, pH, lactate, creatine kinase (CK) and lactic dehydrogenase (LDH) were determined 0, 6, 20 and 24 hours after continuous perfusion. In the donor heart, temperature and myocardium weight were measured pre- and post-preservation. Heart rate and arterial blood pressure were also assessed in basal situation and posttransplantation.

Histological study

Samples were taken of both ventricles for light microscopy and transmission electron microscopy studies after 24 hours of continuous perfusion and 60 minutes after revascularization of the graft.

For viewing under light microscopy, the samples were fixed in 10% formaldehyde for 4 days, embedded in paraffin and cut into 7 micron-thin slices in a Minot microtome. Once freed from the paraffin, they were stained with hematoxylin-eosin and studied under a Leitz photomicroscope (Dialux model).

The samples to be used in the ultrastructural study were immersed for 2 hours in 2% glutaraldehyde in phosphate buffer and cut into 1 mm³ blocks; then they were washed in phosphate buffer and postfixed with 2% osmium tetroxide for 1 hour. After dehydration in a graded acetone series, they were embedded in Epon 812, cut into semithin slices (0.5 to 1 micron-thick) using a...
Fig. 2. Postrevascularization mitochondrial changes of variable severity. a, x 10,000; b, x 10,000.
Myocardial preservation with UW solution

Reichert Jung ultramicrotome and stained with Richardson's methylene blue for light microscopy study. Likewise, ultrathin slices (70 nm-thick) were stained with a water-based solution of 2% uranyl acetate and lead citrate for study under a Zeiss 902 electron microscope.

These studies focused mainly on the presence of cellular and/or interstitial edema, the loss of mitochondrial structure, the presence of contraction bands in the myocardial fibers, the status of the intercellular junctions and the aspect of the blood vessels.

Results

Functional findings

The pH decreased from 7.7 to 7.2 during the first 6 hours of perfusion, after which it stabilized, becoming neutral at 24 hours. The lactate levels increased progressively during the course of perfusion (0.17, 0.52, 1.06 and 1.16 mmol/L at 0, 6, 20 and 24 hours respectively. Continuous increases were also detected at these same time points in the concentrations of LDH (1.5, 17.6, 49.6 and 56.7 U/L, respectively) and CK (5.8, 39.7, 112.2 and 127.5 U/L, respectively). The weight of the myocardium did not change during this period.

With respect to recovery of the heart rate after revascularization, sinus rhythm was restored in only 3 cases; in the remainder, sinus arrest or atrioventricular dissociation was detected. Assessment of the arterial blood pressure disclosed a systolic arterial pressure over 100 mmHg in 5 of 11 cases, while it was equal to the preharvest level in 4 cases and 14% lower in 1. In the remaining 6 trials, there was a mean decrease in the systolic arterial pressure of 52% with respect to basal levels; these hearts developed an edematos appearance following reperfusion, and the left ventricle showed signs of no-reflow and the stone-heart phenomenon in 2 of them.

Morphological findings

With the exception of a considerable reduction in the intracellular glycogen content, the histological pattern after 24 hours of continuous perfusion was nearly that of

Fig. 3. Ventricular myocardium after revascularization showing contraction bands, interstitial edema and dilatation of the endoplasmic reticulum. x 3,000
Myocardial preservation with UW solution

Fig. 4. Interstitial edema, contraction band and nucleus lacking a nucleolus and presenting centrifugal arrangement of chromatin. This case shows a normal intercellular junction. x 3,000

Fig. 5. Intercalated disc presenting a wide separation of the intercellular cleft. x 8,000
normal myocardium: absence of edema; mitochondrial characteristics typical of a tissue fixed by immersion; and normal myocyte structure (Fig. 1). In this phase, contraction bands and marked changes in intracellular junctions, consisting of a widening of the intracellular space and disorganization of the anchoring filaments in the membranes of adjacent cells, were only detected in 1 case.

However, significant structural and ultrastructural changes were observed after transplantation and subsequent revascularization. There was a moderate degree of interstitial edema. Roughly 50% of the mitochondria presented changes, ranging from a reduction in the density of the mitochondrial matrix and moderate disruption of cristae to general swelling and virtual disappearance of the cristae and presence of dense amorphous structures (Fig. 2a, b). Contraction bands were observed in nearly every case, occupying broad portions of the samples (Fig. 3). The nuclei in general, but particularly in the aforementioned regions of marked changes, presented a highly irregular envelope, with centrifugal chromatin distribution arranged in irregular clumps attached to the internal aspect of the karyon and disappearance of the nucleolar structure (Fig. 4). The vast majority of intercellular junctions presented wide cleft separations and loss of structure of the filaments involved in binding (Fig. 5). Images of vasoconstriction affecting capillaries and small and medium-caliber arteries were observed, with incremented endothelial projections finally converting the vascular lumen into a virtual space, and a moderate increase in the pinocytic vesicles was also seen (Fig. 6). Another finding was the presence, in some cases, of polymorphonuclear cells partially or totally obstructing the vascular lumen (Fig. 7).

Discussion

In general, the most marked morphological changes corresponded to grafts presenting the severest ventricular dysfunction. However, there is a certain degree of disagreement since there were relevant histological lesions even when the function returned to preoperative levels. These lesions were similar to those reported by other authors (Breda et al., 1992; Kloner and Przyklenk, 1992; Mankad et al., 1992), but were less severe, perhaps because of the use of continuous perfusion during the ischemic period rather than simple cold...
Myocardial preservation with UW solution

storage (Nutt et al., 1991; Choong et al., 1992; Manciet and Copeland, 1992; Masuda et al., 1992).

The role of each component of the UW solution has not been thoroughly demonstrated, but the potassium, hydroxyethyl starch, lactobionate and raffinose concentrations are considered essential in preventing both cellular and interstitial edema (Nutt et al., 1991; Breda et al., 1992; Mankad et al., 1992; Stringham et al., 1994; Fremes et al., 1995). In our study, the UW solution was highly effective in this respect, since after 24 hours of ischemia with continuous perfusion the hearts had not increased in weight and showed no sign of edema or other relevant histological changes.

The UW solution contains no glucose, an aspect that is considered beneficial: although it can serve as a substrate for anaerobic glycolysis during hypothermic ischemia, glucose can also prove harmful as it generates lactic acid and acidosis (Breda et al., 1992). In our experience, the lactate concentrations had only a moderate impact on pH, but the absence of glucose may have been the cause of the notable reduction in intracellular glycogen detected at the end of the perfusion period, and the possibility that the lack of substrate had a negative influence on functional recovery cannot be ruled out.

The release into the blood supply of myocardial enzymes is equivalent to cell death, and the increases in LDH and CK during continuous perfusion were interpreted in that light; however, they might also indicate permeability and efficacy of the removal of cellular debris by the microcirculation. This hypothesis is supported by the normal morphology of the vascular bed during this phase.

Upon reperfusion, all the damage produced during ischemia was revealed, as was that generated by reperfusion itself; thus, it is difficult to distinguish what deleterious events are attributable to each phase and to what extent.

In addition to the absence of glucose, UW solution contains no calcium either. Experimental evidence indicates that calcium-free solutions are a potential threat to the cell membrane specially during ischemic times of over 12 hours; the reintroduction of calcium after a period of perfusion in the absence of this cation produces immediate biochemical and functional damage to the myocardium (Stringham et al., 1994). This may have been an important factor in the mechanism of the damage to the mitochondrial membranes and to the

Fig. 7. Polymorphonuclear cell partially occluding the lumen of a blood vessel. x 7,000
Acknowledgements. The authors wish to thank A. Fernández, the technician at the Luis El Bru Electron Microscopy Center of the Universidad Complutense in Madrid, for his help in processing the samples and M. Messman for her translation of the text. This project has been financed by grant no. 84/0048 from the FIS, Spain.

References


Accepted October 14, 1996

Myocardial preservation with UW solution