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Transplantation of donor hearts after circulatory or brain death in a rat model

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ABSTRACT

Background: Heart transplantation represents the only curative treatment for end-stage heart failure. Presently, the donor pool is restricted to brain-dead donors. Based on the lack of suitable donors and the increasing number of patients, we investigated some molecular pathomechanisms of the potential use of hearts after circulatory determination of death (DCDD) in transplantation.

Materials and methods: Rats were either maintained brain death for 5 h by inflation of a subdurally placed balloon catheter ($n = 6$) or subjected to cardiac arrest by exsanguinations ($n = 6$). Additionally, a control group was used ($n = 9$). Then the hearts were perfused with a cold preservation solution (Custodiol), explanted, stored at 4°C in Custodiol, and heterotopically transplanted.

Results: Brain death was associated with decreased left-ventricular contractility (dP/dt_{max}: 4895 ± 505 *versus* 8037 ± 565 mm Hg/s; ejection fraction: 27 ± 5 *versus* 44 ± 5%; E_{max}: 2.2 ± 0.3 *versus* 4.2 ± 0.3 mm Hg/μL; preload recruitable stroke work: 59 ± 5 *versus* 96 ± 6 mm Hg; 5 h after brain death *versus* before brain death; $P < 0.05$) and impaired cardiac relaxation (dP/dt_{min}: −4734 ± 575 *versus* −9404 ± 550 mm Hg/s and prolonged Tau, $P < 0.05$) compared with controls. After transplantation, significantly decreased systolic function and prolonged Tau were observed in brain-dead and DCDD groups compared with those in controls. Tumor necrosis factor-alpha, cyclooxygenase-2, nuclear factor-κB, inducible-NOS, and caspase-3 messenger RNA and protein-levels were significantly increased in the brain-dead compared with both control and DCDD groups. Additionally, marked myocardial inflammatory cell infiltration, edema, necrosis, and DNA-strand breaks were observed in the brain-dead group.

Conclusions: Our results show that despite the similar functional outcome in DCDD and brain-dead groups, brain-dead hearts showed marked myocardial inflammatory cell infiltration, edema, necrosis, DNA-strand breaks, and increased transcriptional and post-transcriptional expression for markers of apoptosis and inflammatory signaling pathways.

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1. Introduction

The only curative therapy in end-stage heart failure is presently heart transplantation. The main factor limiting organ donation is the availability of suitable organs. Currently, the donor pool is limited to brain-dead donors. In case of an optimal donor, the warm ischemic time is short, followed by a short cold ischemic preservation [1]. However, this ideal situation is rare in the clinical routine [2]. Although brain-dead donors provide reliable source of cardiac grafts, more than 25% of the potential donors must be excluded from transplantation because of hemodynamic instability or primary cardiac dysfunction [3]. It has been shown that brain death induces a pathophysiological cascade of early catecholamine storm (Cushing type reaction, which consists of an increase in systolic blood pressure and a reduction in heart rate in response to an increased intracranial pressure) after cerebral herniation followed by neurohumoral dysfunction including hormone depletion and the loss of the vasomotor tone. In addition, brain death induces a systemic inflammatory reaction, with generation of free radicals and expression of inflammatory cytokines [4]. The loss of sympathetic regulation of the vessels, which is responsible for the rapid vasodilation, results in a marked decrease of aortic pressure and based on that results in a reduction of coronary blood flow [3,5]. The imbalance between myocardial oxygen delivery and demand [6] may result in an ischemic injury of the donor heart. Hearts from brain-dead donors exposed to additional ischemic injury during transplantation experienced a more intense inflammatory response and an ultimately increased rate of acute graft rejection [7].

To meet the requirements of increasing waiting lists, an expansion of the donor pool is essential [8]. One potential approach to improve the number of cardiac grafts for transplantation might be the inclusion of hearts from donations after circulatory determination of death (DCDD), which is already partly established for kidney transplantations [1]. The modified Maastricht classification is widely used to categorize donation after circulatory death [9] as follows: I) dead patients (uncontrolled), II) patients who underwent unsuccessful reanimation (uncontrolled), III) patients with cardiac arrest after the ventilator was switched off (controlled), and IV) patients with cardiac arrest during brain death (controlled). Compared with brain-dead donors, DCDD have a major disadvantage; the myocardial tissue is exposed to prolonged warm ischemia. When the circulatory system is stopped, increasing warm ischemia time leads to a progressive hypoxic damage, which further affects the function of the donated organ. However, preclinical studies have revealed that donor hearts can tolerate 20 min–30 min of warm ischemia [10,11]. Paradoxically, after transplantation not only ischemia but also reperfusion itself is able to cause further cardiac injury mediated by oxidative stress, calcium overload, inflammatory responses, and apoptotic phenomena. Several research groups performed preclinical studies in porcine, canine, and primate models and have showed that functional recovery of DCDD hearts were similar to that of hearts from brain-dead donors. These works suggested the potential use of DCDD hearts for cardiac transplantation [12–14]. However, the pathomechanisms of potential heart grafts from DCDD in cardiac transplantation have not been completely elucidated and need further investigations. In the present study, we evaluated *in vivo* left-ventricular (LV) graft function after transplantation of hearts from DCDD and donations after brain death using our well-established rat model of heart transplantation [15,16]. We assessed histolopathologic changes and investigated alterations of several myocardial gene and protein expressions.

2. Material and methods

2.1. Animals

Male Lewis rats (250–350 g) were purchased from Charles River (Sulzfeld, Germany). Animals were housed in a room at a constant temperature of 22 ± 2°C with 12 h light–dark cycles, were fed a standard laboratory rat diet and water *ad libitum*. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1996) and the German animal protection code. Approval was also granted by the appropriate ethics review board (G-189/11).

2.2. Experimental groups

Rats were randomly assigned to three groups as follows: (1) control group ($n = 9$), heart transplantation was performed with intact donor hearts; (2) DCDD group ($n = 6$), heart transplantation was performed with hearts from DCDD; and (3) brain-dead group ($n = 6$), heart transplantation was performed with brain-dead donor hearts.

2.3. Model of brain death

After anesthesia with sodium pentobarbital (60 mg/kg, intraperitoneally), breathing was maintained by a respirator. For continuous measurement of arterial pressure, a 2F miniaturized pressure-volume catheter (SPR-838; Millar Instruments, Houston, TX) was inserted into the right carotid artery. For volume administration, a central venous catheter was inserted into the jugular vein. Then, the scalp was partly removed to expose the cranial bone. Using a microsurgical drill, a small 4F hole was drilled through the skull frontolateral to the bregma, and a Fogarty balloon catheter was introduced into the extradural space. After stabilization of the blood pressure and heart rate, induction of brain death was started by gradually increasing the intracranial pressure by inflating the balloon with 15 μL of saline per minute until a total volume of 750 μL was reached. After reaching the final volume, the blood pressure was stabilized. A drop of blood pressure <80 mm Hg was avoided by volume administration without inotropic or vasoactive agents. Ringer solution containing sodium chloride, potassium chloride, calcium chloride, and sodium bicarbonate (the last balanced pH) was used. A total volume of