The Impact of Donor Brain Death on Graft Integrity after Transplantation: Insights from Chronic Rat Cardiac Allograft Rejection

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In the present study, the effect of donor brain death on long-term intragraft changes was investigated in a model of chronic rat cardiac allograft rejection. Hearts of brain-dead (BD) Lewis (LEW) rats (BD) were transplanted heterotopically in F344 rats treated with cyclosporine; hearts from normal anesthetized LEW rats served as controls (CON). At the time of explantation, BD and CON hearts exhibited normal morphology. Fifteen days after transplantation, infiltration by CD4+ (P < 0.005) and CD8+ (P < 0.001) T cells, as well as by macrophages (P < 0.01) was significantly more intense in hearts from BD than in hearts from CON. The expression of IL-2R+ cells; the production of IL-2, IFN-γ, and TNF-α; and the expression of profibrotic cytokines (TGF-β, PDGF, FGF) was more pronounced in hearts from BD than in those from CON (each P < 0.01). By 90 days following engraftment, hearts from CON exhibited some fibrosis, predominately in the subendocardium and subepicardium. The myocardium, in general, was well preserved. In contrast, in hearts from BD, fibrosis was widespread throughout the graft (P < 0.001). The insult to the donor heart induced by brain death may trigger an increased host alloresponsiveness, which in the long term, ultimately results in fibrotic remodeling and loss of integrity.

Introduction
Donor-associated risk factors, such as ischemic time and age, markedly influence outcome after organ transplantation. It becomes increasingly evident that brain death may also play a role as a donor-associated risk factor. In a model of acute rejection of rat cardiac allografts, we recently demonstrated that hearts from brain-dead (BD) donors were rejected significantly earlier than hearts from control (CON) donors (9.3 ± 0.6 vs 11.6 ± 0.7 days, P = 0.03). Hearts from BD donors experienced an accelerated inflammatory response, resulting in rapid leukocyte infiltration and production of cytokines, chemokines, and adhesion molecules. The purpose of this study was to investigate the effect of donor brain death on long-term intragraft changes in a model of chronic rat cardiac allograft rejection.

Material and Methods
Brain death was induced in Lewis (LEW) rats by inflation of an intracranially inserted Fogarty catheter. The condition was validated by apnea, absence of brain stem reflexes, and flat-line EEG. Subsequently, the animals were ventilated for 6 h. A mean arterial blood pressure > 80 mmHg was maintained throughout this period. CON LEW rats underwent the same surgical procedure, except for the inflation of an intracranial catheter, and were also ventilated over a period of 6 h. Subsequently, the hearts of such animals were harvested and transplanted heterotopically in F344 rats. The
recipient F344 rats were treated with cyclosporine 5 mg/kg daily for 30 days, and thereafter every other day. The grafts were explanted 15, 30, 60, 90, and 120 days after transplantation and analyzed by histology and immunohistology.

Results
At the time of explantation, BD and CON hearts exhibited normal morphology. Fifteen days after transplantation, hearts from CON showed minor, focal mononuclear cell (MNC) infiltrates, as opposed to a 4-fold increase of MNC infiltration in BD hearts \( (P < 0.005) \). Subpopulations of MNC included CD4\(^+\) \( (P < 0.005) \) and CD8\(^+\) \( (P < 0.001) \) T cells, as well as macrophages \( (P < 0.01) \), all of which were significantly enhanced in hearts from BD compared with hearts from CON. This was paralleled by an increased immune activation in BD compared with CON hearts, indicated by the number of IL-2R\(^+\) cells \( (P < 0.01) \). Similarly, production of proinflammatory mediators such as IL-2, IFN-\(\gamma\), and TNF-\(\alpha\) (each \( P < 0.01 \)), as well as expression of profibrotic cytokines (TGF-\(\beta\), PDGF, FGF, each \( P < 0.01 \)), were up-regulated significantly in hearts from BD compared with hearts from CON. Thirty and 60 days after engraftment, density of MNC infiltrates further increased in BD hearts \( (P < 0.001 \text{ vs. CON}) \). Moreover, interstitial fibrosis developed more intensely in BD hearts as compared with hearts from CON \( (P < 0.01) \). Ninety and 120 days following engraftment, fibrosis in BD hearts was widespread throughout the graft, whereas in CON hearts, small areas of fibrosis were detected predominately in the subendocardium and subepicardium, with the myocardium, in general, being well preserved \( (P < 0.001) \).

Discussion
Brain death has been described as severely affecting hemodynamic stability, neuroendocrine balance, and metabolism of the subject experiencing this condition.\(^3\) In the early period following this event, a tremendous catecholamine storm occurs, leading to an imbalance in myocardial oxygen delivery and consumption.\(^4\) This might cause an ischemic injury to the donor heart, making it more susceptible to additional inflammatory stimuli, such as the ischemia/reperfusion injury during the transplant procedure (as shown in acute rat cardiac allograft rejection).\(^5\) In the present experiments, immunosuppression of the recipients prevented acute rejection of grafts from both BD and CON donors. However, despite the same amount of immunosuppression in both groups, the inflammatory response in BD grafts was more pronounced as compared with CON grafts. Subsequently, fibrosis, which is known to occur in the chronic rejection process, developed more intensely in BD grafts than it was observed in CON hearts.\(^6,7\) The augmentation of interstitial fibrosis in hearts from BD donors might be attributed to the enhanced ischemia/reperfusion injury affecting such organs following transplantation.\(^2\) Similarly, ischemic insults, induced by coronary artery stenosis and intracoronary microembolization, respectively, have been shown to result in the development of fibrosis.\(^8,9\) Profibrotic cytokines, such as TGF-\(\beta\), might have mediated the fibrotic remodeling process. In particular, TGF-\(\beta\) has been shown to be inducible by ischemic injury preceding the development of fibrosis.\(^10\) In summary, the insult to the donor heart induced by brain death may trigger an increased host allore sponsiveness toward such organs, which in the long term, ultimately results in fibrotic remodeling and loss of functional integrity.

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References


