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# Heart transplantation: A magnified model of heart-brain interactions

**T**he heart-brain interaction is a burgeoning area of science that has been gaining visibility among researchers interested in the relationship between the central nervous system and cardiovascular system. This review explores heart transplantation as a model for providing insight into the heart-brain link, with an emphasis on findings from recent human investigations.

## ■ PROGRESS IN TRANSPLANT OUTCOMES

Cardiac transplantation has become a widely accepted therapy for patients with end-stage heart failure. Approximately 2,000 heart transplants are performed annually in the United States. Long-term outcomes after transplantation have improved with advances in transplant candidate selection, surgical techniques, immunosuppressive medications, and postoperative care.

The current survival rate after heart transplantation has been reported as approximately 50% at 10 years by the International Society for Heart and Lung Transplantation registry.<sup>1</sup> Primary graft failure is the most common cause of early death (within 30 days after transplantation), and transplant coronary artery vasculopathy is the most common cause of late death (> 1 year after transplantation).<sup>1</sup>

## ■ TRANSPLANT RECIPIENT SURVIVAL AND MODE OF DONOR BRAIN DEATH

The mode of donor brain death has recently been found to contribute to the pathophysiology of coronary allograft vasculopathy and graft ventricular dysfunction.<sup>2-5</sup> Spontaneous intracranial bleeding in the donor culminated in progression of vasculopathy in the heart recipient, as confirmed by intravascular ultrasonography,<sup>5</sup> a highly sensitive technique for measuring the thickness of the inner lining (intima and media) of the coronary arteries in humans. Several animal models

have demonstrated impairment of myocardial function and hemodynamic performance after brain death.<sup>6-8</sup> Atraumatic intracranial bleeding, which occurs in approximately 39% of donors suffering brain death, is a potential independent risk factor for death after cardiac transplantation.<sup>2,3</sup>

## Proposed mechanisms

Although the exact causes of cardiac dysfunction after brain death remain unknown, one proposed mechanism is the excessive catecholamine surge that accompanies the endocrine perturbations associated with intracranial bleeding.<sup>9-11</sup> Further, spontaneous intracranial bleeding has been associated with an increased incidence of post-transplant ischemic injury complicated by myocardial fibrosis.<sup>2</sup> The myocardial dysfunction accompanying brain injury is associated with marked alterations in beta-adrenergic signal transduction as well as changes in the contractile apparatus.<sup>10</sup>

The type and extent of myocardial injuries are related to the type of brain injury. In a study of 27 patients whose hearts were systematically examined after an acute fatal episode of intracranial brain hemorrhage, Baroldi et al found evidence of myocardial necrosis in up to 89% of these patients, as compared with only 4% of 45 control cases of fatal head trauma.<sup>8</sup> Increased donor age is another confounding factor among heart donors who die from brain injury caused by intracranial bleeding.<sup>3</sup> This may potentially contribute to the higher incidence of post-transplant ischemic injury complicated by fibrosis.

The increased risk of coronary allograft vasculopathy, myocardial fibrosis, and worse survival has prompted us to further evaluate the link between the brain and the heart at the tissue level.

## ■ INTEGRIN $\alpha V\beta 3$ AND THE HEART-BRAIN LINK

We have recently shown that the vitronectin receptor ( $\alpha V\beta 3$ ), a member of the integrin family,<sup>12</sup> is upregulated in coronary allograft vasculopathy.<sup>13</sup> Integrin  $\alpha V\beta 3$  is expressed by many cells, including platelets,

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lymphocytes, monocytes, macrophages, smooth muscle cells, and vascular endothelial cells,<sup>14</sup> and it interacts with several ligands, including metalloproteinase, fibronectin, osteopontin, thrombospondin, vitronectin, von Willebrand factor, prothrombin, and fibrinogen,<sup>15</sup> thus playing a significant role in bone resorption, angiogenesis, endothelial cell migration, tumor invasion, atherosclerosis, apoptosis, and the cellular immune response.<sup>16,17</sup> Integrin  $\alpha\beta3$  also mediates transmembrane signaling,<sup>18</sup> regulating gene expression and contributing to vascular cell survival.<sup>19</sup>

### Systemic activation in donors with intracranial bleeding

We recently demonstrated the presence of systemic activation of  $\alpha\beta3$  in hearts from donors with spontaneous intracranial hemorrhage (ICH).<sup>20</sup> We evaluated mRNA expression of  $\alpha\beta3$  (using TaqMan polymerase chain reaction) in endomyocardial biopsies at 1 week following transplant in 20 recipients of hearts from ICH donors and 20 recipients from trauma donors. To investigate whether systemic activation of  $\alpha\beta3$  was present in the donor before transplantation,  $\alpha\beta3$  expression was also evaluated in the corresponding donor spleen lymphocytes. All patients underwent serial coronary intravascular ultrasonography to evaluate for coronary vasculopathy. Compared with the trauma group, the ICH group showed a significant increase in mRNA expression of  $\alpha\beta3$  in the heart biopsies (3.8-fold,  $P = .012$ ) and in the corresponding donor spleen lymphocytes (3.5-fold,  $P = .014$ ). At 1 year, the ICH group also showed increased progression of coronary vasculopathy.

### Resulting hypotheses

Our findings of significantly increased mRNA expression of  $\alpha\beta3$  in heart biopsies 1 week after transplantation in recipients of hearts from donors with ICH highlights the potential impact of donor cause of death on postcardiac transplant outcomes. Further, our findings of increased mRNA expression of  $\alpha\beta3$  in the corresponding donor lymphocytes in the presence of ICH suggests systemic activation of  $\alpha\beta3$  and therefore indicates that the index insult occurred prior to transplantation.

We hypothesized that disruption of the blood-brain barrier as a result of ICH is associated with systemic activation of  $\alpha\beta3$ . It is unknown whether this activation serves as a protective counterregulatory effect in the donor. Recently, the importance of alpha v integrins in vascular function has been demonstrated in knockout mouse models in which alpha v null mice have been noted to exhibit intracerebral hemorrhage.<sup>21</sup> Since we have shown that this effect is sys-

temic, we also hypothesized that the donor heart is affected prior to transplantation and in response to vascular injury; thus, smooth muscle cells migrate from the media into the intima, where myointimal proliferation leads to the development of vasculopathy, as confirmed by intravascular findings we have reported.<sup>20</sup>

Our clinical observation shed light on the relationship between  $\alpha\beta3$  and allograft vasculopathy in relation to donor cause of death. Animal transplant models are needed to further explore mechanistic cause-and-effect relationships. Such models may be difficult to design, however, since alpha v knockout mouse models may result in lethal complications, with the mice not surviving transplantation, which would preclude evaluation of the relationship to vasculopathy.

## ■ THE METALLOPROTEINASE SYSTEM AND THE HEART-BRAIN LINK

We have observed that heart transplant recipients who develop myocardial ischemic injury or interstitial fibrosis following transplantation are more likely to have received their transplants from a donor whose death was related to brain injury.<sup>22</sup> We have also shown that myocardial ischemic injury following cardiac transplantation is associated with activation of the matrix metalloproteinase (MMP) induction system.<sup>23</sup> Heart biopsies from ICH donors show a significant increase in mRNA expression of MMP-2 (17-fold,  $P < .0001$ ) and MMP-9 (20-fold,  $P < .0001$ ) compared with biopsies from trauma donors.<sup>24</sup> This upregulation is associated with increased myocardial fibrosis ( $29\% \pm 10\%$  vs  $19\% \pm 6\%$ ,  $P = .003$ ), as shown by picrosirius staining, and subsequent development of coronary vasculopathy, as evidenced by intravascular ultrasonography.<sup>24</sup>

### Evidence supporting systemic activation

The extracellular matrix molecules, such as type IV collagen, laminin, and fibronectin, constitute the basement membrane underlying the vasculature and play a critical role in maintaining integrity of the blood-brain barrier.<sup>25</sup> MMP-2 and MMP-9 have been shown to degrade the extracellular matrix components of the basement membrane and to be involved in the progression of hemorrhagic strokes.<sup>26</sup> We have also shown that there is increased mRNA expression of MMP-2 and MMP-9 in the corresponding donor spleen lymphocytes in the presence of intracranial bleeding, which suggests systemic activation of the metalloproteinase system and that the precipitating insult occurs prior to transplantation.<sup>24</sup>

We have thus postulated that intracranial bleeding is associated with MMP release and subsequent disruption of the blood-brain barrier, resulting in a systemic activation process as evidenced by the splenic upregulation of MMP expression. Subsequently the donor heart coronary vasculature is subject to the effects of systemic activation of MMP, resulting in vascular injury prior to transplantation. After transplantation, smooth muscle cells migrate from the media into the intima, where they contribute to the development of neointimal lesions. Increased MMP expression contributes to the migratory response of smooth muscle cells by releasing them from their surrounding extracellular matrix.<sup>27</sup> In the presence of intracranial bleeding, this injury is translated into increased vasculopathy and myocardial fibrosis. In fact, using multivariate regression analysis, MMP-9 in donor spleen lymphocytes was found to be an independent risk factor for vasculopathy (odds ratio = 2.41,  $P = .01$ ).<sup>24</sup>

Of course, cardiac allograft vasculopathy is a multifactorial process mediated by immune and nonimmune factors, so we acknowledge that these isolated findings are merely part of a more complex process.

### ■ THE RENIN-ANGIOTENSIN SYSTEM AND THE HEART-BRAIN LINK

The renin-angiotensin system is activated during transplantation of the heart and other organs and promotes ischemia-reperfusion injury and fibrosis. Inflammatory effects of the renin-angiotensin system may be caused by the production of tumor necrosis factor- $\alpha$ , transforming growth factor- $\beta$ , and monocyte chemoattractant protein-1.<sup>28-30</sup> Angiotensin II may mediate T-cell proliferation and thus may contribute to alloimmune responses.<sup>31</sup> The interplay between angiotensin II receptor subtype 1 (AT1R) and metalloproteinases has been illustrated by the modulatory impact of AT1R blockade on the extracellular matrix regulatory system in animal experiments.<sup>32,33</sup>

Both clinical and experimental investigations suggest that activation of the renin-angiotensin system occurs along with increased sympathetic drive in patients with spontaneous intracranial bleeding.<sup>34</sup> Angiotensin II interacts with the sympathetic nervous system to maintain adequate cerebral perfusion.<sup>35</sup> It seems plausible that activation of the renin-angiotensin system, which exerts a protective effect by counteracting the elevation in intracranial pressure that occurs following intracranial bleeding (such as subarachnoid hemorrhage),<sup>36</sup> may have a detrimental effect on the donor heart.

We have recently shown that AT1R is upregulated in heart biopsies from recipients of cardiac transplants from donors with spontaneous intracranial bleeding compared with those from trauma donors (4.7-fold increase in mRNA expression of AT1R,  $P < .0001$ ).<sup>37</sup> We have also shown that increased expression of AT1R was present in the corresponding donor spleen lymphocytes, suggesting a generalized activation of the renin-angiotensin system and suggesting that the index insult of angiotensin activation occurred in the donor prior to transplantation.<sup>37</sup> Further, we have shown mRNA expression of AT1R in the donor spleen lymphocytes to be a strong independent predictor of transplant vasculopathy (odds ratio = 4.39,  $P = .02$ ).<sup>37</sup>

### ■ SUMMARY

The human heart transplant model unmasks the heart-brain link as an active process that is clinically demonstrated and confirmed at the tissue level. Further studies are needed to elucidate the relative contribution of each of these isolated observations to the pathogenesis of coronary allograft vasculopathy, which remains enigmatic. Recent studies have suggested that mTOR inhibitors may have the ability to attenuate this lethal process that limits the long-term survival of cardiac transplant recipients.<sup>38</sup>

The observations we have discussed here suggest that other targeted therapies, including glycoprotein IIb/IIIa inhibitors, tissue metalloproteinase inhibitors, and angiotensin receptor blockers, may facilitate the attenuation of cardiac transplant vasculopathy, but clinical trials are difficult to conduct in this relatively small population of patients. These observations may shed insight, however, into the pathophysiology of hypertension and its impact on the vascular system, as cardiac transplantation provides a setting in which heart-brain interactions are magnified and the pathophysiology occurs over years rather than decades.

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