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Human Leukocyte Antigen Mismatch and Other Factors Affecting Cryopreserved Allograft Valve Function

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ABSTRACT

The causes of cryopreserved allograft heart valve degeneration are poorly understood. We investigated HLA mismatch and other factors implicated in allograft valve degeneration. For this study we recruited 110 adult recipients of allograft heart valves who underwent surgery between June 1998 and March 2003 in the state of Victoria, Australia. Recipients and donors were HLA typed using serological and molecular methods. Valve function at most recent echocardiographic follow-up was examined for an association with the following variables using univariate and multivariate methods: HLA-A,-B, and -DR donor-recipient mismatch; HLA class I mismatch; total HLA mismatch; valve ischemic time; recipient age; donor age; ABO blood group donor-recipient match; and allograft size. Mean recipient age was 45 years (18-75 years), 75% were men. Seventy-four pulmonary (62 Ross procedure) and 36 aortic allografts were examined. Median valve ischemic time was 31 hours, range 20-48 hours. Echocardiographic follow-up was complete at a mean of 41 (±18) months, range 6-85 months. At univariate analysis longer ischemic time and younger recipient age were associated with valve dysfunction. HLA-A, -B, or DR mismatch, HLA class I mismatch, total HLA mismatch, donor age, ABO mismatch, and allograft size were not associated with valve dysfunction. Only younger recipient age remained significant at multivariate analysis. In conclusion, longer ischemic times and younger patient age predicted valve dysfunction at a mean of 3 years follow-up. Recipient age remained the strongest predictor of valve dysfunction. These results indicate that allograft ischemic times should be minimized.

INTRODUCTION

An ideal valve prosthesis has not yet been found. The allograft valve has been used for more than 40 years and remains an important prosthesis with many advantages. However, as with other biological valve prostheses, the durability of the allograft is finite. The cause of long-term allograft valve degeneration remains unclear. Various factors have been implicated, including younger recipient age [Lund 1999; O'Brien 2001], older donor age [Lund 1999], surgical technique [O'Brien 1995; Lund 1999; O'Brien 2001], valve processing variables [Gall 1998; Lund 1999], valve size [Tweddell 2000; Forbess 2001; Baskett 2003], and a host immunological response [Dignan 2000; Baskett 2003; Dignan 2003]. We aimed to investigate various factors implicated in allograft valve degeneration.

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MATERIALS AND METHODS

The Donor Tissue Bank of Victoria has collected blood samples from cadaveric valve donors routinely since 1997. This historical cohort study was designed to utilize the availability of these specimens for donor HLA typing. The study included adult patients who underwent treatment during the time period between June 1998 and March 2003, received an allograft valve supplied by the Donor Tissue Bank of Victoria, and were operated on by 7 participating valve surgeons in the state of Victoria. HLA and ABO matching were not performed presurgically. Patients were excluded if informed consent was not obtained or echocardiographic follow-up results were not available. Postoperative valve function at the most recent echocardiographic follow-up was examined for an association with the following variables by univariate and multivariate analyses: HLA-A, -B, and -DR donor-recipient mismatch (0-1 versus 2 mismatches), HLA class I mismatch (0-2 versus 3-4 mismatch), total HLA mismatch (0-4 versus 5-6 mismatch), valve ischemic time, recipient age, donor age, ABO blood group donor-recipient match, and allograft size. The study was approved by the ethics committees of both St. Vincent's Hospital–Melbourne and the Victorian Institute of Forensic Medicine and was conducted in accordance with National Health and Medical Research Council of Australia guidelines.

HLA Typing

Blood samples were obtained from valve recipients at the time of study recruitment. Recipient HLA class I typing

was performed by standard complement-dependent lymphocytotoxicity assay using a panel of antisera from 140 blood donors defining all the known class I specificities [Mittal 1968]. Recipient T lymphocytes were isolated with Dynabeads® coated with antibody specific for CD8 receptor (Dynal Biotech, Oslo, Norway), then incubated with antisera at 20°C-25°C for 40 minutes. Rabbit complement was added, followed by a further incubation period of 60 minutes. Lymphocyte viability was assessed with the addition of ethidium bromide/acridine orange solution. A positive reaction was defined as >10% dead cells per well. Recipient HLA class II typing was performed by polymerase chain reaction (PCR) amplification sequence-specific oligonucleotide (SSO) typing methods. PCR amplification of exon 2 was performed, followed by detection of hypervariable regions by a set of oligo-probes specific for sequence differences.

DNA was extracted from cadaveric blood by conventional salting out methods [Miller 1988]. Donor HLA class I typing was performed by PCR amplification and sequencing methods [Kurz 1999], using in-house PCR primers, BigDye terminator chemistry and an ABI 3730 48-capillary sequencer (Applied Biosystems Inc., Foster City, CA, USA). Donor HLA class II typing was performed by PCR SSO methods as above.

Recipient and donor HLA types were recorded by converting SSO and sequencing designations back to the corresponding serological equivalents. The degree of HLA mismatch between donor and corresponding recipient was graded as a whole number value between 0 and 2 for each HLA locus. When a donor was homozygous at an A, B, or DR locus for an HLA antigen for which the recipient was heterozygous, the degree of mismatch at this locus was designated as 0, and not 1, because there is no functional immune recognition of the donor antigens at this locus by the recipient.

Valve ischemic time was defined as the interval between time of death and time of cryopreservation. Because the donors were coronial cadavers, this interval included the time required for transport of the cadaver to the coronial office, valve harvest, and antibiotic sterilization.

Outcome

Postoperative echocardiography was used to assess valve function. It was performed routinely before hospital discharge and at the discretion of treating physicians during follow-up. The outcome measured was the valve function at the most recent postoperative echocardiographic study, with regard to transvalvular mean gradient and degree of allograft valve regurgitation (trivial, mild, moderate, or severe). The degree of allograft valve dysfunction was categorically classified as nil (nil or trivial valvular regurgitation, mean gradient ≤10 mm Hg), mild (mild valvular regurgitation or mean gradient 11 to 30 mm Hg), moderate (moderate valvular regurgitation, or mean gradient 31 to 50 mmHg), or severe (severe valvular regurgitation or mean gradient >50 mm Hg).

Statistical analysis

Data analysis was performed using SPSS version 11.5 (SPSS, Chicago, IL, USA). Continuous data were expressed as mean (±SD). Univariate analysis was performed to assess for a relationship between each variable and the outcomes. Continuous data were compared using the unpaired *t*-test or Mann-Whitney log-rank test where appropriate. Nominal and categorical data were compared with the χ^2 square test or Fisher exact test. A correlation between 2 numerical variables was assessed by calculating the Pearson correlation coefficient (*r*). Multivariate analysis was performed using linear multiple regression analysis for continuous outcomes (mean valve gradient) and logistic regression analysis for categorical outcomes (valve dysfunction, valve regurgitation). Variables were entered simultaneously; $P < .05$ was considered significant.

RESULTS

A total of 162 valve recipients met the inclusion criteria of the study and, of these, 110 (68%) participated in the study. The remainder were excluded mainly because they were lost to follow-up or declined to participate, or echocardiographic follow-up was not available. Participant age range was 18-75 years at time of surgery, and 82 (75%) were men. Allograft valve implantation was performed between June 1998 and March 2003. Thirty-six aortic allografts were implanted in the aortic position and 74 pulmonary allografts implanted in the pulmonary position (62 of these as part of a Ross procedure). Valve disease was predominantly stenosis in 46 patients, regurgitation in 45, and mixed in 19. Etiology of valve disease was predominantly attributable to congenital bicuspid or unicuspid in 83 (75%) of patients. Other etiology included endocarditis in 14, congenital heart disease in 7, replacement of right ventricular outflow tract conduit or valve prosthesis in 4, and degenerative aortic valve disease in 2 patients. Previous cardiac surgery had been performed in 20 participants, with 5 having received allograft valve or material implantations.

Mean postoperative interval of echocardiographic follow-up was 40.8 (±18.4) months, range 6.2-84.7 months. Mean valve gradient was 8.35 (±5.6) mm Hg, range 1-31 mm Hg. Mild valve regurgitation was present in 23 (21%) of patients and moderate regurgitation in 4 (4%), and no valve showed severe regurgitation. Allograft valve dysfunction was absent in 66 (60%) of the patients, mild in 38 (35%), and moderate in 6 (5%).

Donor HLA-typing was incomplete because DNA extraction from the donor samples failed in approximately 1/3 of donor samples. Failure was attributed to the poor quality of cadaveric blood samples caused by delayed collection and prolonged storage. HLA match data was available for 84 donor-recipient pairs at the HLA-A locus, for 81 at the HLA-B locus, and for 80 at the HLA-DR locus. As expected in a random population, the degree of HLA match was low: 52%-64% of donor-recipient pairs had 2 mismatches, 29%-39% had 1 mismatch, and 7%-8% of donor-recipient pairs were completely matched at each HLA locus. The presence of allograft dysfunction (nil versus mild/moderate) was not associated with the degree of HLA-A, -B, or DR mismatch (0-1 versus 2, $P = .58, .96, .25$); HLA class I mismatch (0-2 versus 3-4 mismatch, $P = .40$); or total HLA

mismatch (0-4 versus 5-6, $P = .29$). Neither mean valve gradients nor the presence of valvular regurgitation (nil versus mild/moderate) was associated with the degree of HLA mismatch (A, B, DR, class I, or total).

ABO match data were available for 104 recipients; 73 (70%) were matched and 31 (30%) mismatched. ABO match status was not associated with allograft valve dysfunction ($P = .52$), mean valve gradient ($P = .64$), or valvular regurgitation ($P = .54$).

Median allograft ischemic time was 31.1 hours, range 20.0-47.6 hours. Ischemic time was higher in patients with valve dysfunction compared to those without (median 32.1 vs 30.0 hours). This difference was significant by log-rank testing ($P = .03$). Similarly, longer ischemic time was associated with valve regurgitation (median 32.3 versus 30.3 hours); $P = .04$ by log-rank testing. It did not correlate with mean valve gradient ($r = 0.04$, $P = .67$).

Mean recipient age at time of surgery was 44.9 (±14.0) years, range 18-75 years. Younger recipient age was significantly associated with valve dysfunction. Mean recipient age for valves with at least mild-moderate dysfunction was 40 years compared to 47 years for the group with no dysfunction ($P = .02$). Recipient age inversely correlated with mean valve gradient ($r = –0.41$, $P = .001$). Mean recipient age was similar in those with and without valve regurgitation (41 versus 45 years, $P = .22$).

Mean donor age was 37.1 (±12.4) years, range 14-55 years. Mean allograft size was 24 (±2.4) mm, range 19-32 mm. Donor age and allograft size were not associated with valve dysfunction, mean valve gradient, or valve regurgitation.

In the multivariate analyses, young recipient age was associated with valve dysfunction ($P = .005$), higher mean valve gradient ($P = .004$), and valve regurgitation ($p = 0.02$). All other variables were not identified as significant predictors.

DISCUSSION

Several factors have been implicated in long-term allograft degeneration. There is increasing evidence that a host immune response may be significant [Hoekstra 1996; Hawkins 2000]. Until recently, the clinical significance of any such host immune response has been unclear. However, recent HLA-typing studies and a study examining anti-HLA antibodies directed against the allograft have shown a link between the immune response and clinically relevant valve dysfunction [Dignan 2000; Baskett 2003; Dignan 2003]. It is likely that any degenerative host immune response will interact with other nonimmunological factors in causing valve degeneration. Because the causes of valve degeneration are likely to be multifactorial, we sought to examine a host of factors in our cohort of allograft valve recipients.

The current study found that longer ischemic time was associated with allograft valve dysfunction and valvular regurgitation. No association was found with valve stenosis. This finding occurred in the context of a fairly narrow range of time between death and cryopreservation of between 20 and 48 hours. Median ischemic time was 31 hours, which encompassed the time required for the cadaver to be transported to the coronial offices, to obtain permission from the family to harvest tissue, to harvest, to complete an antibiotic sterilization process of 6-8 hours and to initiate the cryopreservation process. Other investigators have found that both long [Lund 1999; Tweddell 2000] and very short [Dignan 2000; Baskett 2003] ischemic times may be deleterious to allograft valve function. However, interpretation of these studies is difficult because the total ischemic time (ie, time between death and cryopreservation or implantation) is not stated. Rather, a subset of intervals such as time between harvest and cryopreservation [Dignan 2000], duration of antibiotic preservation [Baskett 2003], duration between death and harvest [Tweddell 2000], or duration between harvest and implantation [Lund 1999] were reported as significant. Based on our findings and the above studies, we conclude that there may exist an optimal duration of allograft ischaemia. This duration should allow down-regulation or degradation of the antigenic elements within the allograft while allowing the structural and cellular elements required for long-term valve durability to be preserved. The exact duration of the "desirable" allograft ischemic time is difficult to define and may lie within the 20-48 hour range studied here.

We identified an association between young recipient age and valve dysfunction by both univariate and multivariate analysis. Young recipient age has long been known to be related to aortic [Lund 1999; O'Brien 2001] and pulmonary [Albert 1993; Daenen 1997; Schorn 1997; Niwaya 1999; Tweddell 2000; Forbess 2001, Gerestein 2001, Baskett 2003] allograft valve degeneration, and bioprosthesis [Frater 1998] degeneration. The mode of failure in the young is thought to be by leaflet and annular calcification leading to valvular stenosis or cuspal rupture leading to regurgitation. In this study, patients were found to have allograft dysfunction by both stenosis and regurgitation. No valves in this series were explanted, and thus direct examination of the allograft to determine the mechanism of dysfunction was not performed. The cause of this age-related phenomenon is unclear. Factors such as a more severe immune response and differences in calcium metabolism have been postulated. Increased hemodynamic demands placed on the allograft by younger patients who are both more active and have greater metabolic needs may also be important.

Historically, older donor age has been found to be significant in predicting allograft valve failure [Yacoub 1995; Lund 1999; Palka 2002]. For instance, Yacoub and coworkers, in their series of 275 homovital aortic allografts, identified donor age of more than 55 years as predicting valve failure [Yacoub 1995]. Hence, most allograft valve banks, including ours, apply a donor age limit of 55-60 years. It is not surprising, therefore, that donor age was not associated with valve dysfunction in this study.

We did not find ABO mismatch predictive of valve dysfunction. It has not been our routine practice to perform prospective ABO match of recipients of allograft valves, because most large series in the literature have not identified ABO mismatch as a significant factor in predicting allograft dysfunction. However, some authors have found that ABO mismatch is significant in pediatric valve recipients

[Yankah 1995; Baskett 2003].

We did not find a significant association between HLA mismatch and allograft dysfunction. The probability of HLA matching was low because of the high diversity of HLA types in a random population and the lack of preoperative HLA matching. These features are an inherent limitation in the study design, which can be overcome only by conducting large international registry studies or prospective randomized matching studies, neither of which has been performed to date in the field of allograft valve implantation. Four studies have investigated this issue. Baskett and colleagues studied 47 pediatric pulmonary allograft valve recipients and found that HLA-DR mismatch was associated with echocardiographic valve failure by multivariate analysis, after a mean follow-up period of 4.6 years (maximum 11 years) [Baskett 2003]. Dignan and associates studied 162, mainly adult, recipients of cryopreserved aortic allografts. They found that HLA-A,-B, and -DR were not associated with echocardiographic allograft failure in the overall analysis of the entire cohort. However, when the subset of 92 patients, with at least 5 years of follow-up, was analyzed separately, HLA-DR mismatch was associated with allograft valve failure by bivariate analysis. It is possible that a type II error has occurred in our study and the 2 studies [Smith 1998; Bechtel 2001] that failed to reveal an association between HLA match and valve performance, because of the bias of small patient number, short follow-up [Bechtel 2001], older recipient cohort, and long durations between donor death and cryopreservation or implantation (in the case of homovital valves) [Smith 1998].

In conclusion, we found that longer ischemic times and younger patient age predicted valve dysfunction at a mean of 3 years follow-up. Younger recipient age remained the strongest predictor of valve dysfunction by univariate and multivariate analysis. We recommend that allograft valve ischemic times be minimized, and when possible, kept below 48 hours.

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