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REVIEW ARTICLE

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Cardiac allograft vasculopathy: current concepts and treatment

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Abstract Cardiac allograft vasculopathy (CAV) remains the leading limiting factor of patient and graft survival after the first post-operative year. The pathogenesis involves both immunological and non-immunological factors. Here, we present recent advances and discuss potential preventative and treatment regimens. A review of the current literature of heart transplantation, detailing molecular mechanisms, pharmacological risk factors and novel immunosuppression regimens was performed. Recent findings demonstrate the pivotal role of the endothelium, resulting in release of pro-fibrotic cytokines, recruitment of circulating leucocytes, proliferation of vascular smooth muscle cells, and deposition of extracellular matrix proteins (ECMs). The role of HMG-CoA reductase inhibitors and anti-hypertensives remains controversial, but there is increasing evidence advocating their prophylactic use. We can conclude that novel immunosuppressive agents such as rapamycin, mycophenolate mofetil and FTY-720 are experimental immunosuppressive agents that are undergoing evaluation in clinical trials. The prophylactic use of statins and anti-hypertensive drugs needs to be defined but needs to suggest potential strategies to prolong cardiac allograft survival.

Keywords Allograft vasculopathy · Chronic rejection · Immunosuppression

Introduction

The past two decades have seen considerable advances in short-term patient survival following cardiac transplantation, with reported survival rates between 80% and 90% at 1 year [1]. These commendable outcomes have been largely attributed to the introduction of more potent and selective immunosuppressants, such as cyclosporine and tacrolimus [2], and improvements in the understanding of the immunology of transplantation, tissue typing and organ preservation, and the management of acute rejection and opportunistic infections [3]. Despite improvements in short-term patient survival, long-term survival rates remain largely unchanged, with 60% patient survival at 5 years and 45% at 10 years [4]. An accelerated form of coronary artery disease known as cardiac allograft vasculopathy (CAV) accounts for the majority of these graft losses after the first post-operative year [5].

CAV is angiographically detectable in 20% of grafts at 1 year and 50% at 3 years [6]. It has been demonstrated as early as 3 months post-transplantation [7]. The diffuse nature of CAV is limited to the graft segment, sparing other vessels throughout the body, suggesting predominantly immunological mechanisms associated with non-immunological risk factors of both donor (e.g. pre-existing disease, ischaemia time) and recipient (e.g. diabetes, hypertension, hyperlipidaemia) origin. Cardiac allografts show only partial re-innervation [8], and heart transplant recipients do not experi-

ence symptoms of angina pectoris. Clinical evidence of severe CAV is only apparent with the progression of complications such as congestive cardiac failure, arrhythmias and sudden death [9]. To date, re-transplantation remains the only effective treatment for severe CAV. This chapter reviews the pathogenesis and aetiological risk factors of cardiac allograft vasculopathy and highlights potential therapeutic targets to improve long-term graft survival.

Histological characteristics

CAV is characterised by diffuse and concentric myointimal thickening that affects both intramyocardial and epicardial arteries and veins [10]. It differs from native coronary artery disease, which is characterised by more proximal, focal and eccentric lesions. Furthermore, the internal elastic lamina is rarely broken in CAV, and deposition of calcium is rare. Early histological changes result from proliferation of vascular smooth muscle cells and deposition of extracellular matrix proteins. Unlike classic atheromatous disease, these lesions show an absence of plaques. Conversely, late graft changes show lipid infiltration and calcification within the coronary vessels, representing a spectrum of 'host-versus-graft disease' with superimposed atheroma [11]. The presence of moderate-to-severe proximal or mid-vessel disease is associated with a predictive mortality of 50% at 2 years [12].

Immunology of CAV

Whilst the pathogenesis of CAV is poorly understood, there is considerable evidence to support a primarily immunologically mediated injury. Allograft vasculopathy is confined to the vasculature of the 'donor' segment only, sparing recipient vessels. Veins as well as arteries are also subject to the development of CAV. Cardiac allografts performed in animal models across major histocompatibility complexes have demonstrated the subsequent progression of CAV [13, 14] associated with the presence of immunological inflammatory mediators, namely increased expression of pro-fibrotic cytokines, adhesion molecules and heavy lymphocytic infiltrates [15]. Furthermore, the severity of CAV is associated with a higher mismatch at the human leukocyte antigen-DR locus [16]. There is conflicting evidence regarding the role of acute rejection in the progression of CAV. Several groups have demonstrated a relationship between the number of acute rejection episodes and CAV [17, 18], whilst other authors dispute this association [19, 20].

The initiating injury is probably endothelial in origin. The endothelial cell controls vasomotor activity, inhibits thrombus formation and leukocyte adhesion and also inhibits vascular smooth muscle cell proliferation [21]. Clinical studies have shown that endothelial cells express major histocompatibility complex class-I and class-II antigens [22], anti-human leukocyte antibodies [23] and antibodies to endothelial cell antigens [24]. Circulating T- cells are constantly present in heart transplant recipients, even in the absence of rejection episodes [25]. Upon recognition of endothelial cell antigens, T cells and macrophages infiltrate the endothelium and release a myriad of cytokines, recruiting more inflammatory cells, increasing the expression of endothelial cell antigens and regulating the proliferation of vascular smooth muscle cells [26]. Activated endothelial cells show increased uptake of circulating cytokines and become more permeable to lipids [27]. Activated endothelial cells and macrophages produce oxygen-free radicals that react with low-density lipoproteins and increase monocyte adhesion and T-cell activation [28]. This 'inflammatory response' is perpetuated by lymphocytic cytokine secretion: interleukin-2 (IL-2) stimulates proliferation of alloreactive lymphocytes, interferon-γ upregulates intercellular adhesion molecule-1, thus conscripting more lymphocytes and macrophages to the vessel wall. These macrophages release cytokines and growth factors, including IL-1, IL-2, IL-6, transforming growth factor-β (TGF-β), basic fibroblast growth factor (bFGF), endothelial growth factor (EGF), insulin-like growth factor (IGF) and platelet-derived growth factor (PDGF), all potent mitogens of vascular smooth muscle cells. The result of this 'repair phase' is proliferation of smooth muscle cells and deposition of extracellular matrix proteins, and, ultimately, encroachment of the coronary vasculature.

Metabolic risk factors

Hypertension

Post-transplant hypertension develops in 60–80% of patients in the immediate postoperative period. This is largely attributed to the hypertensive effects induced by prednisolone and cyclosporine [29]. Research into the use of an anti-hypertensive agent that may also have a vascular protective role has advocated the use of calcium channel blockers and angiotensin-converting enzyme inhibitors. The majority of these experimental data has been concluded from balloon injury [30], hypercholesterolaemic and aortic/cardiac allograft models of intimal hyperplasia and vasculopathy [31]. In the clinical setting, several randomised controlled trials have demonstrated a significant reduction in CAV following treatment with calcium channel blockers [32, 33, 34]. At present, there is little evidence supporting

the use of angiotensin-converting enzyme inhibitors [35, 36]. Angiotensin (AT) has potent pro-fibrotic effects beyond its vasoconstrictive effects on glomerular haemodynamics. AT promotes endothelial and vascular smooth muscle cell proliferation and favours the accumulation of extracellular matrix proteins [37, 38]. Downregulation of AT1 and AT2 receptors has been demonstrated in human cardiac transplant biopsies following treatment with angiotensin-converting enzyme inhibitors [39]. However despite this effect, Mehra et al. failed to detect any difference in intimal thickening following prophylactic use of angiotensin-converting enzyme inhibitors [33].

Hyperlipidaemia

Hyperlipidaemia is observed in 60–80% of heart transplant recipients. There is now convincing evidence to show a direct correlation between hypercholesterolaemia and CAV [40, 41]. The development of obesity, a common sequela after transplantation, may be an additional factor [42]. Winters et al. have shown a correlation between BMI and the degree of intimal narrowing in failed cardiac transplants [43]. The cause of hyperlipidaemia after transplantation is multi-factorial: immunosuppressive therapy is certainly an important contributory factor. Indeed, the dose of prednisolone is a strong predictor of increased levels of cholesterol and low-density lipoprotein post-transplantation [44]. Co-administration of cyclosporine increases the steroid-induced effect on hyperlipidaemia by decreasing hepatic lipoprotein lipase activity P1 [45], resulting in a further rise of 20–30% total and LDL cholesterol. Treatment with HMG-CoA reductase inhibitors has been shown to reduce LDL-cholesterol levels, to prolong long-term survival significantly, and to reduce the incidence of angiographically detectable CAV [46]. Furthermore, studies assessing the effect of HMG-CoA reductase inhibitors on native coronary atherosclerosis have shown significant regression of pre-existing lesions [47]. The beneficial effects of statins extend beyond their directly lipid-lowering effect. Simvastatin has been shown to inhibit both rodent and human vascular smooth muscle proliferation in vitro [48], inhibit monocyte chemotaxis and regulate cytotoxicity of T lymphocytes [49]. Kobashigawa et al. have previously shown similar findings with pravastatin. The clinical data from these two trials, and the experimental data, advocate the use of routine HMG-Co, a therapy immediately following heart transplantation [50].

Cytomegalovirus infection

A number of clinical studies have shown an association between cytomegalovirus (CMV) infection and accelerated arteriosclerosis in both transplanted and native coronary vessels [51, 52]. However, other authors dispute this association. Dummer et al. failed to detect any association between CMV seropositivity and CAV in 314 consecutive heart transplant recipients [53]. The possible mechanisms of CMV-associated vasculopathy have been only partly elucidated. CMV has the potential to infect endothelial cells and cause a cytopathic effect, resulting in increased endothelial adherence of granulocytes [54]. Increased expression of inflammatory cytokines such as interleukin-1 and tumour necrosis factor-α initiates vascular smooth muscle cell proliferation [55]. Work by Lemstrom et al. has demonstrated an increase in TGF-β and PDGF-BB in rat aortic allografts infected with cytomegalovirus. Conversely, graft arteriosclerosis was inhibited in those rats treated with ganciclovir [56]. CMV infection also causes an upregulation of HLA class-I antigens and adhesion molecules on the vascular endothelium and smooth muscle cells. Merigan et al. showed a reduced incidence of CMV in seropositive patients treated with prophylactic ganciclovir, but there was no effect on seronegative recipients of seropositive hearts [57]. However, Valantine et al. later showed a significant reduction in the severity of transplant vasculopathy in those patients randomised to ganciclovir [58]. Valantine et al. showed a further reduction in both the incidence of cytomegalovirus infection and the severity of intimal thickening in seronegative patients who had received heart transplants from seropositive donors with the addition of cytomegalovirus hyper-immune globulin to ganciclovir, when compared with those patients administered ganciclovir alone [59].

Diagnosis

Coronary angiography

Coronary angiography has remained the principal investigative tool for diagnosis of the coronary anatomy in most centres. Angiography detects the presence of vasculopathy in 10–20% of recipients at 1 year post-transplant, rising 10% per year to 50% at 5 years [60]. The technique utilises a single projection of the contrast-filled lumen and, therefore, may underestimate the true lesion size, depending on the line of imaging. The technique also relies partly on comparison of lumenal diameter between a 'diseased' and 'normal' segment and, thus, angiography compares poorly with histological findings from post-mortem examinations [61, 62]. Whilst most transplant centres currently perform coronary angiography as part of an annual review programme, due to the diffuse nature of CAV, angiography continues to underestimate the severity of vasculopathy.

Intravascular ultrasound

More recently, intravascular ultrasound (IVUS) has emerged as a useful adjunct to coronary angiography. Its ability to visualise the cross-section of the vessel provides not only accurate information on lumen size, but also quantification of intimal thickening and vessel-wall morphology [63]. Conversely, coronary angiography is still required to image the longitudinal anatomy [64]. Whilst angiography detects an incidence of 10–20% of CAV at 1 year, IVUS detects abnormal intimal thickening in 50% of patients at 1 year [65]. Furthermore, prospective studies using IVUS have demonstrated a relationship between intimal thickening and graft survival. Patients with significant intimal thickening (>5 mm) at 1 year are associated with accelerated progression to CAV and have the poorest prognosis [66].

Immunosuppressive drugs

Over the past few years an overwhelming number of novel immunosuppressive agents, all with differing mechanisms of action, have been investigated for both their short-term and long-term properties. A balance exists between adequate immunosuppression, to prevent rejection, and over-immunosuppression, which would predispose the patient to opportunistic infections, vasculopathy and malignancy. Furthermore, all immunosuppressive agents have a wealth of serious adverse side effects, including nephrotoxicity, hepatotoxicity, diabetes, hypertension, hyperlipidaemia and malignancy [67].

Since its introduction in the early 1980s, cyclosporine has revolutionised short-term graft survival following solid organ transplantation [68]. Triple-drug therapy of steroids, cyclosporine and azathioprine has formed the mainstay of post-transplantation immunosuppression for nearly 20 years. However, this regimen has little effect on the development of allograft vasculopathy. The use of cyclosporine in transplantation is associated with the development of hypertension, hyperlipidaemia and nephrotoxicity. Unfortunately, low-dose cyclosporine protocols (<4 mg/kg per day) are associated with an increased incidence of CAV [69]. To this end, intensive research is in progress to develop more selective drugs in an attempt to prolong long-term allograft survival.

A novel calcineurin inhibitor, tacrolimus (FK506), has a mechanism of action similar to that of cyclosporine, but is a more potent immunosuppressive agent. Tacrolimus binds to an intracellular immunophyllin known as FK-binding protein (FKBP-12). This complex inhibits calcineurin phosphatase, inhibiting cytokine production (IL-2) and halting T-cell cycle progression in Go phase [70]. In the clinical setting, tacrolimus has resulted in a reduction of acute rejection episodes, including steroid-resistant rejection [71, 72]. Its role in the prevention of allograft vasculopathy, however, is unclear [73]. In a prospective randomised controlled trial, Klauss et al. investigated 73 consecutive orthotopic cardiac transplant recipients randomised to receive cyclosporine or tacrolimus-based therapy at 1 year, with intravascular ultrasound; 73% of patients in the tacrolimus group showed a progression of CAV (defined as an increase in intimal index $>5\%$), compared with 38% in the cyclosporine-treated group ($P=0.082$; relative risk: 2.1). Pham et al. [74] however, failed to detect any statistical difference in CAV between patients randomised to receive cyclosporine ($n=80$) and tacrolimus ($n=80$) at 4 years. Further studies are therefore required to define the long-term indications for tacrolimus.

Rapamycin, a fermentation product of *Streptomyces hygroscopicus*, was originally investigated for its antifungal properties. It soon became apparent that its lymphopenic properties limited its potential as an antibiotic and suggested a potential role as an immunosuppressive agent. It has a similar molecular structure to tacrolimus and also binds to FKBP-12. This complex, however, does not inhibit calcineurin phosphatase, but binds to 'mammalian targets of rapamycin' (mTOR), inhibiting DNA and protein synthesis and thereby arresting the T-cell cycle in the G1 phase [75]. Furthermore, rapamycin inhibits antigen and cytokine proliferation of B-cell lymphocytes [76]. Experimental evidence suggests a potential role for rapamycin in both the prevention and treatment of CAV. Rapamycin exhibits a potent anti-proliferative effect on stimulated vascular smooth muscle cells [77]. Additionally, the observed inhibitory effects are greater than those exhibited by cyclosporine, mycophenolate or tacrolimus [78]. Similarly, rapamycin inhibits intimal hyperplasia in both mechanically mediated and immune-mediated rodent models [79]. Meiser et al. demonstrated a significant dose-dependent reduction in allograft vasculopathy in rat cardiac allografts up to 100 days post-transplantation. The administration of rapamycin with an equipotent dose of cyclosporine showed a significant reduction of established CAV in the same model [80]. Similar findings have been reproduced in a non-human primate immune-mediated aortic allograft model: rapamycin was administered 45 days after aortic transplantation [81]. Intravascular ultrasound was used to confirm the presence of allograft vasculopathy prior to treatment being commenced and at the end of the study. Rapamycin-treated animals showed no progression of intimal thickening at day 105 ($P<0.037$, t-test) and greater than 20% regression in four out of the six treated animals. The rate of progression or regression of intimal thickening was directly related to the rapamycin trough level, confirming a dose-dependent effect. Additionally, rapamycin has been shown to act synergistically with calcineurin-inhibitors to prolong rat cardiac

allograft survival [82]. To date, there are no published reports on the role of rapamycin following cardiac transplantation.

Mycophenolate mofetil (MMF/RS-61443) is a potent non-competitive inhibitor of inosine monophosphate dehydrogenase, a rate-limiting enzyme in the de novo synthesis of guanosine. MMF also inhibits proliferation of B and T lymphocytes and downregulates adhesion molecules, lymphocytes and proliferation of vascular smooth muscle cells [83, 84]. A number of trials have advocated the use of MMF as an alternative to azathioprine in the treatment of persistent or refractory rejection [85, 86]. To date, the use of MMF has not shown any improvement in the progression of CAV: Kobashigawa et al. (1998) [87] later reported the findings of 650 heart transplant recipients in a prospective randomised trial of MMF versus azathioprine, in conjunction with cyclosporine and prednisolone. There were no significant differences in the progression or development of IVUS-detected CAV at 1 year post-transplantation. More data are clearly required if its potential role in the longer-term is to be defined.

Finally, an experimental study drug, FTY-720, has been shown to lower the circulating peripheral lymphocyte count by promoting the migration of lymphocytes to secondary lymphoid organs [88]. Research has been restricted to animal models to date, but FTY-720 significantly decreases the development of CAV in rat cardiac allografts given as monotherapy [89] or in combination with cyclosporine or rapamycin [90]. The experimental evidence promises potential for the use of FTY-720 in the clinical setting.

Role of surgical intervention

Traditional re-vascularisation procedures have limited application in patients with CAV. The diffuse nature of CAV contrasts with the discrete proximal lesions that are observed in native coronary vessels. The presence of distal arteriopathy frequently excludes the use of bypass grafting due to the lack of distal 'runoff'. Halle et al. (1995) performed a retrospective meta-analysis of 13 medical centres that had performed palliative coronary re-vascularisation on heart transplant recipients [91]: 66 patients had undergone coronary angioplasty. Technical success was achieved in 153 out of 162 (94%) of stenoses. Two patients had had fatal peri-procedural myocardial infarctions. Re-stenosis occurred in 42 of 76 (55%) lesion at 8 ± 5 months. This compares with a previous study by von Scheidt et al. (1995), who reported a 61% re-stenosis rate [92]. Unsurprisingly, the presence of distal arteriopathy was associated with poor prognosis. Eleven patients had undergone directional atherectomy. Two patients had died during the procedure, and the remaining nine patients were all alive without having undergone transplantation 7 ± 4 months post-procedure. Twelve patients had received coronary artery bypass surgery. Four patients died intra-operatively, and seven patients were alive 9 ± 7 months post-surgery. Wong et al. (1998) have shown a short-term benefit of coronary stenting, with a significant reduction in re-stenosis rate of 44% in stented vessels vs 86% in angioplasty alone at 345 ± 252 days [93]. The long-term efficacy of coronary stenting in cardiac transplants is yet to be reported.

At present, re-transplantation is the only definitive treatment for patients with severe CAV. However, long-term survival rates are significantly poorer than those for primary transplantation [94]. The United States Joint International Society for Heart and Lung Transplantation (ISHLT) and the United Network for Organ Sharing (UNOS) recently published morbidity and mortality data collected retrospectively from their registries. CAV was the primary indication for re-transplantation in $> 50\%$ of patients. Survival for the entire cohort was 65, 59 and 55% for 1, 2 and 3 years respectively. CAV as underlying diagnosis of graft failure, longer intervals between transplant and graft failure, younger recipient age and lack of pre-transplant mechanical ventilation are all univariate predictors of increased survival. In the face of continued organ shortage, re-transplantation will remain a controversial issue and poses considerable ethical problems regarding the allocation of 'precious' organs.

Future implications

Developments in stem-cell research have shown that purified haematopoietic stem cells differentiate into vascular smooth muscle cells in vitro and in vivo [95]. This finding, which indicates that somatic stem cells account for the pathological modelling of distant organs, may provide therapeutic strategies for the treatment of transplant arteriosclerosis through target mobilisation, homing and proliferation of vascular progenitor cells. Lemstrom and Koskinen examined expression and localisation of platelet-derived growth factor ligand (PDGF-AA, PDGF-BB) and receptor proteins (PDGF-R) in rat cardiac allografts by immunohistochemistry [96]. They showed a positive correlation between PDGF-AA, PDGF-R alpha and PDGF-R beta expression in intimal cells with the development of intimal thickening. Furthermore, this study also showed a significant reduction in intimal PDGF-AA and PDGF-R alpha expression and intimal thickening in animals receiving high-dose cyclosporine, compared with those receiving low-dose cyclosporine regimens. The development of PDGF antagonists offers potential novel pathways to inhibit the development of allograft vasculopathy.

The administration of exogenous growth factors to promote angiogenesis in peripheral vascular [97] and native coronary heart disease [98] has resulted in increased collateral vessel development. The findings of these studies have been advocated as a treatment for CAV. However, there are concerns regarding the effect of increased expression and production of growth factors on the development of CAV in transplanted hearts with severe CAV. However, these hearts do not exhibit increased collateralisation [99]. Miller et al. demonstrated that a persistent elevation of acidic fibroblast growth factor in cardiac allografts was associated with intimal neo-capillary formation and intimal hyperplasia [100]. Miller et al. also demonstrated an association between increased production of the matrix glycoprotein thrombospondin-1 (TSP-1) and CAV in these patients. TSP-1 potently inhibits angiogenesis in vivo and blocks microvascular endothelial cell proliferation by angiogenic factors [101]. This finding supports the hypothesis that high levels of TSP-1 inhibit neo-angiogenesis in response to increased expression of growth factors and may accelerate neointimal smooth muscle cell proliferation and CAV. Antibodies to TSP-1 have been shown to reduce intimal proliferation significantly in the balloon-injured rat carotid model [102] and offer further potential targets to prevent the progression of CAV.

Conclusion

The development of cardiac allograft vasculopathy is a complex, poorly understood process that continues to limit patient and graft survival following heart transplantation. More sensitive diagnostic techniques such as intravascular ultrasound allow earlier and more accurate detection of its presence, but currently the only effective treatment is re-transplantation. It is clear that prevention, rather than treatment, is the most efficacious method of prolonging survival. The introduction of prophylactic lipid-lowering agents, and anti-hypertensive and immunosuppressive drugs with an inhibitory effect on vascular injury, would therefore seem appropriate, as increasing evidence from experimental and clinical trials advocates their potential benefits.

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