Université de Montréal

Effets protecteurs du Celsior sur la fonction endothéliale d'artères coronaires porcines dans un modèle de préservation myocardique et de greffe cardiaque hétérotopique

Par

Éric Dumont

Département de Chirurgie

Faculté de Médecine

Mémoire présenté à la Faculté des Études supérieures en vue de l'obtention du grade de Maîtrise en Sciences Biomédicales

Juin, 2003

Université de Montréal

Faculté des études supérieures

Ce mémoire intitulé :

Effets protecteurs du Celsior sur la fonction endothéliale d'artères coronaires porcines dans un modèle de préservation myocardique et de greffe cardiaque hétérotopique

Présenté par :

Éric Dumont

A été évalué par un jury composé des personnes suivantes :

Michel White

Président-rapporteur

Michel Pellerin

Directeur de recherche

Louis P. Perrault

Co-directeur de recherche

Denis Bouchard

Membre du jury

Résumé

La survie des patients après transplantation cardiaque est limitée par l'apparition d'une forme d'athérosclérose accélérée au niveau des artères coronaires menant à des infarctus silencieux et une perte progressive de la fonction du greffon. L'apparition précoce d'une dysfonction endothéliale au niveau des artères coronaires prédit le développement de la maladie du greffon 1 an après la transplantation. Les facteurs impliqués dans l'apparition de cette dysfonction endothéliale incluent le rejet cellulaire et humoral, la dyslipidémie, l'infection au cytomégalovirus, l'exposition des artères coronaires aux solutions cardioplégiques, l'ischémie froide, et le type de solution de préservation utilisé lors du prélèvement cardiaque.

Le premier article rapporte l'effet de l'utilisation du Celsior, une nouvelle solution de préservation spécifiquement conçue pour la greffe cardiaque, sur la fonction endothéliale d'artères coronaires porcines dans un modèle de préservation cardiaque comparé à deux stratégies courantes de cardioplégie et de préservation utilisées en greffe cardiaque. Les résultats obtenus démontrent que l'utilisation du Celsior pour l'induction de la cardioplégie et la préservation myocardique a un effet protecteur à court terme sur les relaxations endothélium-dépendantes médiées par les protéines G comparé aux autres stratégies. Ces résultats pourraient être associés avec

des meilleurs résultats à court et moyen terme en greffe cardiaque avec l'utilisation du Celsior lors du prélèvement.

Le deuxième article rapporte les effets protecteurs du Celsior sur la fonction endothéliale des artères coronariennes épicardiques porcine à 30 jours post-transplantation cardiaque hétérotopique chez le porc. L'utilisation du Celsior pour l'induction de la cardioplégie et la préservation est comparé avec deux stratégies de cardioplégie et préservation utilisées en greffe cardiaque. Les résultats obtenus démontrent une meilleure réactivité endothéliale des artères coronaires épicardiques préservées au Celsior 1 mois après la transplantation. L'utilisation du Celsior pour l'induction de la cardioplégie et la préservation pourrait potentiellement réduire l'incidence d'athérosclérose accélérée du greffon comparé aux autres solutions en préservant les effets protecteurs de l'endothélium sur la paroi vasculaire.

Mots clés : endothélium, athérosclérose précoce, cardioplégie, préservation, greffe cardiaque.

Summary

Survival after heart transplantation is limited by the appearance of an accelerated form of atherosclerosis in the coronary arteries that leads to silent infarcts and progressive lost of graft function. Early appearance of coronary artery endothelial dysfunction is predictive of development of coronary allograft vasculopathy one year after transplantation. Factors which have been incriminated as the cause of this dysfunction include cellular and humoral rejection, hypercholesterolemia, CMV infection, exposure of coronary arteries to cardioplegic solutions, cold ischemia, preservation solutions, and reperfusion after implantation.

The first article evaluates the effects of Celsior (an antioxidant solution specifically designed for cardiac preservation) in a model of heart preservation (4 hours to mimic clinical conditions) compared to two commonly used cardioplegic and preservation strategies on coronary artery endothelial function in a porcine model. The results demonstrate that Celsior cardioplegia and storage allowed for functional recovery of endothelium-dependent relaxations to serotonin compared to saline preservation. The observed effect may be associated with an improvement in both short- ad long-term outcome in heart transplantation.

The second article examined the potential protective effects of Celsior, a new preservation solution specifically designed for heart transplantation, on coronary endothelial reactivity at 1 month post-transplantation compared with crystalloid and blood based cardioplegic solutions. The results demonstrated that the use of crystalloid solution causes a decrease in endothelium-dependent relaxations to agonists as compared to blood and Celsior solutions. The use of Celsior has a protective effect on endothelial function at 1 month and potentially reduces the incidence of coronary allograft vasculopathy compared to other solutions by preserving the protective effects of the endothelium on the vascular wall.

Key words: Endothelium, accelerated atherosclerosis, cardioplegia, preservation, heart transplantation.

TABLE DES MATIÈRES

LIST OF FIGURES AND TABLES

INTRODUCTION

ARTICLE 1

ARTICLE 2

ABBREVIATIONS

5HT	Serotonin
Ach	Acetylcholine
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BK	Bradykinin
Ca++	Calcium
A23187	Calcium ionophore
cAMP	Cyclic adenosine 3,5-monophosphate
cGMP	Cyclic guanosine 3,5-monophosphate
EDCF	Endothelium derived contracting factor
EDHF	Endothelium derived hyperpolarizing factor
EDRF	Endothelium derived relaxing factor
eNOS	Endothelial nitric oxide synthase
ICAM	Intracellular adhesion molecule
iNOS	Inducible nitric oxide synthase
IP3	Inositol triphosphate
ISHLT	International Society for Heart and Lung Transplantation
IVUS	Intravascular ultrasound
L-NMMA	N-monomethyl-L-arginine
MHC	Major histocompatibility complex
NADPH	Nicotinamide adenine dinucleotide phosphate
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
PDGF	Platelet derived growth factor
PGF2α	Prostaglandin F2 alpha
PGI2	Prostaglandin I2 (Prostacyclin)
Gi Protein	Gi-coupled protein (GTPase binding protein)
Gq Protein	Gq-coupled protein
SEM	Standard error of the mean
SMC	Smooth muscle cells
SNP	Sodium nitroprussiate
TXA2	Thromboxane A2
UW	University of Wisconsin solution
VCAM	Vascular cell adhesion molecule

Remerciements

J'aimerais remercier mes directeurs de recherche, Dr Louis P. Perrault et Dr. Michel Pellerin pour m'avoir fait confiance dans ce projet, pour leurs précieux conseils, et pour leur dévouement à me guider et me corriger.

Je ne peux oublier l'aide extraordinaire que m'a apporté Nathalie Desjardins pour les manipulations et greffes, les chambres d'organes et la tabulation des statistiques. Sans elle, mon séjour au laboratoire aurait été différent et cet ouvrage n'aurait jamais vu le jour.

Merci aussi à Olivier et Josie pour leur aide et leur présence dans le laboratoire. Je vous garde une place dans ma mémoire.

Finalement, j'aimerais remercier mes parents qui m'ont toujours supporté durant ces longues études et Leah pour sa patience, son amour et sa générosité. Hang in there, Babe !

Introduction

The Normal Endothelium

The normal endothelium contributes to vasomotor tone and to the maintenance of a non-thrombogenic vascular surface, acts as a selective barrier for the transport of macromolecules and solutes needed for cellular metabolism, contributes to the proliferation of the underlying smooth muscle, and regulates the adhesion and transmigration of neutrophils, monocytes and lymphocytes. These endothelial properties are due to its capacity to sense hormonal and hemodynamic stimuli by three basic mechanisms: the secretion of endothelium-dependent factors, the expression on its surface of cell adhesion molecules and signal transduction coupling molecules, and morphological changes.

The role of the vascular endothelium was first recognized by Furchgott and Zawadski in 1980 (1) following the discovery of the endothelium-dependency of the dilator response to acetylcholine. Since then, three pathways have been described that mediate endothelium-dependent relaxations. Endothelium-dependent vasodilatation can be mediated by the

release of nitric oxide (NO), prostacyclin, or endothelium-dependent hyperpolarizing factor (EDHF) (Figure 1) (1-6).

1) Nitric Oxide

Nitric oxide is a labile molecule and diffuses readily through plasma membranes. It is formed from L-arginine via the constitutive form of nitric oxide synthase (7,8) (NO synthase III). The activation of this enzyme depends on the intracellular concentration of calcium ion in the endothelial cell, on calmodulin, and requires nicotinamide adenine dinucleotide phosphate (NADPH) for optimal activity (9). NO synthase can be competitively inhibited by analogs of L-arginine N-monomethyl-L-arginine (L-NMMA) and N-nitro-L-arginine (Figure 1) (9). There are three types of nitric oxide synthase enzymes responsible for the production of NO. Nitric oxide synthase type I (nNOS) exists in the neuronal cells of the brain in peripheral nonadrenergic noncholinergic neurons (10). It produces NO for the activation of the signal transduction pathways in neurotransmission. Inducible NOS II (iNOS) is produced by macrophages in response to endotoxins, micro-organisms, or cytokine secretion. INOS has shown cytotoxic effects on invading bacteria and tumor cells, is capable of producing much greater quantities of NO than the other isoforms (11), and is responsible for the vasodilatatory effect seen in sepsis and septic shock.

Endothelial cells generate NOS III (eNOS) which produces NO one of several endothelium-derived relaxing factor (EDRF). Most vasoactive stimuli such as bradykinin and shear stress mediate their activities through the production of NO derived from the endothelium. Cell membrane-bound G-proteins transduce signals intracellularly and play a pivotal role in cell biology (12-14). These intracellular signals cause an increase in inositol triphosphate and diacylglycerol which in turn alter calcium levels within the cell and activate Ca++ dependent proteins such as eNOS. NO diffuses into smooth muscle cells within 200 μm and causes relaxation via activation of guanylate cyclase in vascular smooth muscle (15) (Figure 2). Since NO has a short half-life of 6 seconds, its effects are stimulus and dose-dependent. Once entered into the smooth muscle cell (SMC), NO binds to soluble guanylate cyclase which stimulates the conversion of GTP to guanosine 3',5'-monophosphate (cGMP). cGMP is associated with inhibition of SMC contractility by shifting the intracellular calcium (Ca++) to a lower concentration. Since an increased concentration of Ca++ is required for the dephosphorylation of the myosin light chain kinase and thus smooth muscle contraction, rises in the cGMP concentration induce vasorelaxation (16-18). The production of NO significantly contributes to endothelium-dependent relaxations in coronary, systemic, mesenteric, and pulmonary beds. Its

contribution to vasomotor tone is suggested by the fact that inhibitors of NO cause a generalized vasoconstriction and a rise in arterial blood pressure in animals and humans (19,20). Endothelial cells also secrete NO in the lumen of blood vessels which is immediately neutralized in normal situations by the presence of oxyhemoglobin. Thus NO acts as an interface between the blood stream and the endothelium and in this way inhibits platelet and leukocyte adhesion. Physiologically, platelets normally circulate in the peripheral bloodstream in an inactivated state due to the liberation of NO and prostacyclin by the endothelium. Both mediators increase cGMP or cAMP in platelets and thereby prevent platelet adhesion and aggregation (21-24). At sites where platelets are activated, they release active mediators which interact with endothelial receptors (Figure 5) and cause endothelium-dependent relaxations mediated by NO. The presence of endothelium inhibits to a large degree the vasoconstriction induced by thromboxane A2 and serotonin derived from platelets during aggregation. The platelet-derived mediator primarily responsible for the stimulation of NO formation is ADP and to a lesser extent serotonin, which act respectively on Py-purinergic and 5-HT1d receptors (25,26). Serotonin also binds to $5HT_2$ receptors on smooth muscle cells which cause vasoconstriction when stimulated. The action of platelet products and thrombin is crucial for the protective role of the

endothelium against inappropriate coagulation. Not only does the release of NO induce a direct relaxation of smooth muscle thereby inducing vasodilatation and preventing platelet microaggregation, but the endothelial barrier prevents the vasoconstricting substances (thromboxane A2 and serotonin) from acting on the vascular smooth muscle. When the endothelial barrier is damaged, platelet aggregation goes unopposed with the liberation of thromboxane A2 and serotonin and the platelet activator effect of thrombin (25).

Numerous neurohormonal stimuli can cause the release of nitric oxide from endothelial cells (Figure 3). Circulating hormones such as catecholamines or vasopressin, prostanoids such as histamine or bradykinin, mediators derived from platelets (serotonin, ADP) or formed during coagulation (thrombin) can all bind to specific receptors on the surface of endothelial cells and cause the activation of eNOS via distinct signal transduction pathways (Figure 4). For example, α-adrenergic, serotonin, and thrombin receptors are coupled to a Gi-protein pathway which is sensitive to pertussis toxin whereas ADP and bradykinin (which also mediates release EDHF) mediate production of NO via activation of Gq-proteins (27,28). Activation of both these protein pathways by the binding of agonists to their cell surface receptors leads to an intracellular increase in inositol

triphosphate and diacylglycerol, an increase in intracellular calcium and activation of eNOS. The Gi-protein mediated pathway is one of the first pathways affected in many cardiovascular diseases such as acute and chronic cardiac rejection (29,30) and atherosclerosis (31,32), hypercholesterolemia, and ischemia-reperfusion injury and may be an early marker of pathological activation of the endothelium in vascular diseases. The Gq-protein mediated pathway is more robust and is usually affected later in the disease process.

2) Prostacyclin (PGI2)

Prostacyclin is predominantly formed by the endothelium, although vascular smooth muscle cells have a lesser capacity to produce prostanoids. It is a major byproduct of cyclooxygenase and induces SMC relaxation in response to hypoxia and shear forces. Prostacyclin causes vasodilatation by increasing cyclic AMP in vascular smooth muscle cells. In most vessels, the contribution of prostacyclin to endothelium-dependent relaxation is minimal but it acts synergistically with NO to prevent platelet aggregation (33,34).

3) Endothelium-derived Hyperpolarizing Factor

Recent studies have demonstrated that acetylcholine and other endothelium-dependent vasorelaxants cause an endothelium-dependent hyperpolarization from a diffusible factor derived from the endothelium (endothelium derived hyperpolarizing factor) distinct from NO and

prostacyclin (35-39). Indeed, inhibitors of nitric oxide formation or cyclooxygenase such as L-NMMA or indomethacin only partially inhibit endothelium-dependent relaxations to bradykinin. The chemical nature of EDHF remains to be determined but it causes hyperpolarization of vascular smooth muscle cells via ATP-dependent potassium channels (40-42). The exact type of potassium channel is unknown but is probably calcium dependent. The contribution of EDHF to endothelium-dependent relaxation varies according to the size of the vessel but is greatest in resistance arteries (38,43).

4) Endothelium-derived Contracting Factors

The endothelium can also produce contracting factors derived from the cyclooxygenase pathway, namely prostaglandin H2, arachidonic acid, and thromboxane A2 (44), as well as endothelin-1(44-49). Endothelin-1 is more likely to play a role in the long term regulation of vasomotor tone rather than the spontaneous vasoconstriction of the vascular smooth muscle. Endothelin production is stimulated by chemical stimuli such as hypoxia, and mechanical forces, as well as agonists such as thrombin, vasopressin, and angiotensin II.

Coronary Allograft Vasculopathy and Endothelial Dysfunction

Heart transplantation remains the only efficacious treatment for terminal heart failure but has an associated 5-year survival of 75% owing to the development of graft coronary vasculopathy, which leads to silent infarcts, graft failure, and sudden death (50-52). It is estimated that over 50% of patients have significant stenotic disease at 3 years after transplantation using intravascular ultrasound for evaluation and 50% of these will develop graft failure (53). Most of these individuals do not present symptoms of the disease because of cardiac denervation occurring during harvesting. The first clinical manifestations are often arrythmias, heart failure, or sudden death. Graft coronary vasculopathy is manifested by a unique and unusually accelerated form of coronary disease affecting both the smaller intramyocardial vessels and the larger epicardial arteries (54). Cardiac allograft vasculopathy affects only allograft vessels, sparing all native vessels. Intimal hyperplasia is visible in up to 75% of patients at 1 year using IVUS (55).

The pathogenesis and morphological patterns of coronary allograft vasculopathy are yet to be fully elucidated but the histological changes have been described. Pathological examination of coronary arteries from human cardiac allografts have shown that compared with conventional

atherosclerotic plaques, the disease is diffuse rather than segmental, concentric in nature rather than eccentric, develops within months to years, and also occurs in the pediatric population after transplantation (56,57-61). The initial lesion is characterized by an early intimal proliferation which progresses with time to plaques incorporating lipid deposits and late calcification of coronary vessels (54). These histopathological changes are a function of time; early after transplantation a vasculitis predominates characterized by cellular infiltrates consisting of modified smooth muscle cells, T lymphocytes, and monocytes/macrophages which progress to focal atherosclerotic plaques (59,62). The intimal hyperplasia is diffuse which explains the obliteration of the small distal resistance vessels before the larger epicardial coronary arteries (63).

Coronary endothelial dysfunction has been shown to be a precursor to the development of intimal hyperplasia and atherosclerosis in many vascular diseases such as hypercholesterolemia and diabetes. It also precedes the development of coronary allograft vasculopathy as is shown by an impaired endothelium-dependent relaxation to different stimuli at different time points after transplantation: the vasomotor response to acetylcholine (64,65) and the cold pressor test (66) may be abnormal a few weeks to months after transplantation while exercise-induced vasodilatation is typically maintained

for several months to years (67). These abnormalities are predictive of the development of graft vasculopathy and may predict an adverse clinical outcome (50,68-69). Endothelial cell damage also leads to enhanced release of endothelin, a potent vasoconstrictor peptide, with subsequent increase in coronary vascular resistance and diminished myocardial blood flow (70). The time course of coronary endothelial dysfunction in acute untreated rejection has been characterized (30) and begins with an endothelial dysfunction due to a progressive destruction of the endothelial lining 5 days after heart transplantation which initially involves Gi-protein mediated relaxations. The dysfunction worsens over time and comes to affect all endothelial mechanisms and vascular smooth muscle. Contradictory results have been reported in the relationship between endothelial dysfunction and vessel morphology. Thus, an impaired vasomotor response to papaverine-induced increased coronary blood flow has been described in transplant patients with significant abnormalities of the vessel wall (71) while other studies have not confirmed the relationship between the impairment of endothelium-dependent and independent vasodilatation and degree of intimal hyperplasia (71-73). Therefore, it appears there is no simple relationship between coronary artery structure and function in transplanted

patients; some normal appearing vessels do not dilate while others with intimal hyperplasia show good vasodilatation (74).

The endothelium lies in a strategic anatomical position between the circulating blood and the vascular smooth muscle cell. For this reason, the graft's endothelium is the primary target of the immunological response to the allograft due to the expression of alloantigens on its surface in conjunction with MHC molecules (direct response), thereby activating host helper T cells (72). Activated host helper T cells release interleukin-2 that leads to the proliferation of other alloreactive cells with the secretion of cytokines (73). In response, the endothelium expresses cell surface antigens and adhesion molecules (ICAM,VCAM) and secretes chemoattractant factors to macrophages which then enter the vessel wall along with lymphocytes which enhance the migration and proliferation of vascular smooth muscle cells. This leads to an endothelial dysfunction which significantly contributes to the increase of platelet-vessel wall interaction, vasoconstriction, and proliferation of vascular smooth muscle cells. Under these conditions, endothelium-dependent vasodilatation is reduced and endothelium-dependent constrictor responses are augmented.

Although the immunological hypothesis for endothelial dysfunction as a precursor to coronary allograft vasculopathy is a valid one, numerous

factors may trigger endothelial dysfunction (74,75) after transplantation, including cellular and humoral rejection as described above, hyperlipidemia, cytomegalovirus reactivation, and the toxic effects of immunosuppressants such as cyclosporin A. The process of transplantation itself exposes coronary arteries to cardioplegic solutions (76), cold ischemia (77), preservation solutions (78), and reperfusion after implantation (79-81) which may cause pathologic activation of the endothelium rendering it dysfunctional. Cold ischemic storage can be deleterious to the preserved organ by causing cellular swelling, extracellular edema, cellular acidosis, depletion of metabolic substances, reperfusion injury, calcium overload, and endothelial injury. Injury caused by reperfusion is attributed to ischemic cell swelling, the no-reflow phenomenon which is the failure of uniform blood flow to return to all ischemic tissue, reperfusion-induced hemorrhage, and oxygen-derived free radicals (82). The role of ischemia and reperfusion is an early, transient cofactor of endothelial injury after transplantation (83,84). The activation of the microvascular endothelium leads to the production of oxygen free radicals with subsequent activation of leukocytes and macrophages. Activated cells release oxygen radicals and other mediators such as cytokines, proteases, and eicosanoids. These free radicals can scavenge endothelium-derived nitric oxide (NO) or have direct cytotoxic

effects on the endothelium to induce a coronary endothelial dysfunction. The chief reactive oxygen species is the superoxide anion. All cells generate a basic level of superoxide anion, but the main producers are the mitochondria (85). In the setting of acute injury, superoxide anion production is mediated chiefly by the enzymes xanthine oxidase, nitric oxide synthase, and by neutrophils (86). In ischemia-reperfusion, the effects of NO can be both beneficial and detrimental. The vasodilatation induced by NO may be essential for the perfusion of organs during shock (87). The main harmful effects of NO is the production of peroxynitrite. When NO combines with the superoxide anion, the resultant peroxynitrite is a much stronger oxidant than either of its constituents resulting in apoptosis or cytotoxicity. Thus, postischemic reperfusion injury is a network of interactions mediated by a large variety of oxidative molecules and aggressive mediators leading to an early endothelial dysfunction.

Endothelial dysfunction after transplantation is a consequence of initial injury to the endothelium by mechanisms described above. When the endothelial cell is injured, it becomes activated and plays a pivotal role in the development of atherosclerosis and allograft vascular disease. Endothelial activation may lead to the induction of genes which are normally suppressed and inhibition of beneficial genes (88). The activated endothelial

cell can have critical interactions with smooth muscle cells, macrophages, and platelets, all of which play a critical role in the atherosclerotic and coronary artery vasculopathy process (88). It has been hypothesized that a transcription factor, nuclear factor-kappa B (89), and other signaling pathways induce the inappropriate and damaging gene expression by activated endothelial cells. The damaged endothelium results in dysfunctional cellular activities manifested as alterations in endothelial permeability, loss of antithrombogenic characteristics with microthrombi formation, increased adhesion of leucocytes, and release of vasoactive substances and growth factors (88). The endothelium normally provides a barrier to the passage of plasma proteins and growth factors into the media, but in the presence of endothelial injury, proteins pass through the endothelial layer and accumulate in the media (90). Growth factors then stimulate smooth muscle cells to migrate into the intima and proliferate, leading to progressive intimal hyperplasia (91). All of these consequences further promote the development of atherosclerosis and allograft vasculopathy.

Preservation Solutions and the Endothelium

Despite the ongoing research in order to extend the length of preservation of organs for transplantation, the clinically acceptable ischemic

time for heart transplantation remains 4-8 hours (92,93), an amount of time significantly less than for liver (94) and kidney (95) transplantation which are 24 and 48 hours respectively. A retrospective analysis of heart transplant centers in the United Network of Organ Sharing (96) showed that 167 different preservation solutions are used in heart transplantation today and only 11% of centers use University of Wisconsin solution (UW). Less than optimal solutions may be sufficient if the cold ischemic period is kept < 4 hours but this is rarely the case in a clinical setting. A multivariate analysis of data from the International Heart Transplant Registry (97) showed that ischemic time has a significant ($P<0.001$) effect on 1 and 5 year mortality. Improvements in myocardial preservation solutions can increase the safety period of cold ischemic time without increasing the risk of mortality, and may improve short and long-term cardiac function after transplantation. Superior myocardial preservation may also increase the donor pool by allowing the use of suboptimal donors.

Preservation solutions can prevent endothelial cells from injury from oxygen-derived free radicals during the ischemic period. Nilsson et al showed that there was a nonselective decrease in endothelium-dependent relaxations immediately after perfusion with crystalloid cardioplegia with functional recovery after 5 hours of cold storage whereas comparable

changes in function of the endothelium were not observed after perfusion with a blood cardioplegic solution (98). The type of solution used for graft preservation has been suggested to play a role in the subsequent development of graft coronary atherosclerosis: specifically, grafts preserved with an intracellular-type (potassium 125 mEq/L) University of Wisconsin solution were more susceptible to developing accelerated atherosclerosis over a 36-month follow-up period than those preserved in extracellular-type (potassium 30 mEq/L) Stanford solution (99).

As previously mentioned, there is a four-step sequence of potentially damaging events on the endothelium in hearts undergoing the transplantation procedure: (1) donor arrest, (2) cold storage, (3) warm ischemic arrest associated with implantation, (4) reperfusion (100). Two types of solutions have traditionally been used to preserve the allograft. The first solution is used as a flush-out and storage medium during steps 1 through 3 and the second serves as reperfusate at the onset of step 4. There are limitations however to the approaches currently used to preserve cardiac allografts. The first solution used is normally based on the principles of myocardial preservation that have been developed for cardiac procedures such as coronary artery bypass surgery. An example is the use of St- Thomas' Hospital cardioplegic solution as a storage medium. This approach is not

necessarily harmful for the allograft but does not take into account the specificities of the allograft compared to hearts subjected to a single crossclamp period during bypass surgery. These specificities include longer period of ischemic injury, a greater degree of cooling during arrest, and the potential for myocardial injury before harvesting by underlying cerebral injury. The second type of solution utilizes the principles of preservation that have been developed for transplantation of abdominal organs. This is illustrated by the use of solutions such as Euro-Collins and University of Wisconsin (UW) in heart transplant recipients. These solutions have yielded good results but their use does not meet the specificities of an ischemic/reperfused myocardium. Differences between the heart and other organs relates to cation transport (101), membrane permeability to different solutes (102,103), and defense mechanisms against oxygen free radicals (104). A solution which is effective for storage may not be suitable for perfusion during initial arrest (105,106) or subsequent cardioplegia given after reimplantation (107).

These considerations have led Menasché and colleagues (108) to develop an original preservation solution called Celsior which meets the requirements for heart transplantation (Table 1). Celsior combines the principles of organ preservation and allows cold storage specific for

ischemic/reperfused myocardium and permits a single solution to be used throughout all phases of the transplantation procedure. Celsior is a high sodium, low potassium solution which uses glutamate as an energy substrate. It is an extracellular-type solution with considerable antioxidant properties due to its high content in reduced glutathione, which confers superior preservation of myocardial function by preventing production of free radicals during preservation and reperfusion (105-107, 109). The prevention of free radical injury is achieved by the combination of three components: reduced glutathione, mannitol, and histidine. The decrease in production of active oxygen species, which are known to degrade NO, could improve protection of endothelium-derived NO, which exerts a regulatory and protective role on the vascular wall. Celsior also has the ability to prevent cellular swelling by the combination of lactobionate (a chelator of Fe^{2+} ion) and mannitol, giving it a concentration of 140 mmol/L to counterbalance intracellular osmotically active molecules. Experimentally, hearts arrested and stored in Celsior were found to incur significantly smaller losses of compliance after ischemia and greater contractile function during reperfusion compared to hearts exposed to St-Thomas' Hospital solution (108). Another study showed that hearts stored in Celsior for 8 hours at 4°C and reperfused retrogradely in a Langendorff model for 60

minutes had a better left ventricular developped pressure than those stored in St-Thomas' Hospital, University of Wisconsin (UW), and modified (lower potassium concentration) UW (110). Celsior was also found to significantly better preserve high energy phosphate levels and myocardial pH versus UW in canine hearts after 12 hour cold storage with a superior preservation of glycogen granules at histology (111). Warnecke et al recently published a report comparing the UW solution to Celsior in a porcine allogeneic heart transplantation model with measurement of right ventricular function (RV) (112). In this study, myocardial preservation with Celsior solution resulted in significantly better post-ischemic RV function than the UW solution after transplantation (112). In addition endothelial cells incubated for 6 or 24 hours at 4°C with Celsior or UW solutions were significantly better preserved in terms of their viability and proliferative capability compared to those incubated in Euro-Collins or St-Thomas solutions (113). Endothelium-dependent relaxations to acetylcholine were also significantly more impaired in segments of proximal and distal coronary arteries perfused and incubated for 15 hours in Plegisol solution compared to Celsior (114) in an in vitro isolated rat heart model. Clinical efficacy studies are currently underway in Europe and North America (114). Results of a prospective multicenter study evaluating the Celsior solution for cardiac preservation showed an operative

mortality of 8.6% and a 5-year actuarial survival rate of 75%, comparing favorably with the international standards set for heart transplantation (114). Atrial fibrillation and heart block rates were also very low in this cohort, suggesting a role for Celsior in preserving cardiac sinus rhythm (114).

As noted above, there is evidence that early development of endothelial vasomotor dysfunction predicts the subsequent development of cardiac allograft vasculopathy at 1 year post-transplant (115). The first study presented in this memoir suggests that storage with the Celsior solution better preserves endothelial function in cardiac allografts before reimplantation when compared with a saline solution. The effects of the Celsior solution on endothelium-dependent and independent relaxations early (1 month) after cardiac transplantation had not been evaluated. Therefore, the second study presented in this memoir examines the effects of Celsior, blood and crystalloid cardioplegic solutions on endothelium-dependent and independent relaxations in porcine coronary arteries 30 days after transplantation in a porcine heterotopic retroperitoneal transplantation model to demonstrate a potential protective role of Celsior on the endothelium and the development of intimal hyperplasia at a distance in time from graft implantation.

Article #1:

Improved Preservation of Coronary Endothelial Function with Celsior Compared with Blood and Crystalloid Solutions in Heart Transplantation

E. Dumont, L.P. Perrault, O. Malo, M. Pellerin, M. Carrier

Montreal Heart Institute, Montreal, Canada

Background : Endothelial injury from preservation solutions has been implicated in acute coronary vasospasm and pathologic activation of the endothelium, which can contribute to the development of graft coronary vasculopathy after heart transplantation. Preservation solutions with powerful antioxidant capacity may decrease the occurrence of these complications. This study was designed to evaluate the effects of Celsior (an antioxidant solution specifically designed for cardiac preservation) in a model of heart preservation (4 hours to mimic clinical conditions) compared to two commonly used cardioplegic and preservation strategies on coronary artery endothelial function in a porcine model.

Methods : Pig hearts were either immediately explanted and preserved (control) or subjected to crystalloid, blood, or Celsior cardioplegia followed by 4-hour preservation in either saline or Celsior solutions at 4°C (seven experimental groups). Endothelium-dependent relaxation of normal porcine epicardial coronary arteries to serotonin (5-HT, an agonist that activates 5-$HT1_d$ receptors coupled to Gi proteins) and bradykinin (BK, which activates B2 receptors coupled to Gq proteins) was studied in standard organ chamber experiments.

Results : Cardioplegia with blood, crystalloid, and Celsior solutions caused statistically significant decreases in endothelium-dependent relaxations to 5-

HT and BK compared to controls. Arteries submitted to cardioplegia and preservation demonstrated significantly decreased relaxations to 5-HT compared with the control group except the Celsior + Celsior group. Endothelium-dependent relaxations to bradykinin were unaltered in all groups after a 4-hour storage period irrespective of preservation solution used except in one group.

Conclusion : Cardioplegia alone caused a decrease in endothelium-dependent relaxations to Gi and Gq-protein coupled agonists. Celsior cardioplegia and storage allowed functional recovery of endothelium-dependent relaxations to serotonin compared to saline preservation. The observed effect could improve both short- and long-term outcome in heart transplantation.

Introduction

Heart transplantation is the preferred treatment of medically unresponsive terminal cardiac failure but remains associated with a 5-year survival rate of 70% because of the development of graft coronary vasculopathy which leads to silent infarcts, graft failure, and sudden death with no efficacious treatment currently available (1-3). Coronary endothelial dysfunction precedes the development of atherosclerosis in many vascular conditions such as diabetes mellitus, hypercholesterolemia and is predictive in human transplant recipients of the development of intimal hyperplasia and of adverse outcomes (4). Numerous factors may trigger the endothelial dysfunction (5,6) after transplantation including cellular and humoral rejection, hyperlipidemia, cytomegalovirus reactivation, the toxic effects of cyclosporin. The process of transplantation itself with exposure of coronary arteries to cardioplegic solutions (7), cold ischemia (8), preservation solutions (9), and reperfusion after implantation (10-12) may cause pathological activation (13) of the endothelium rendering it dysfunctional. Endothelial dysfunction is associated with an attendant loss of the endothelial cells regulatory and protective properties on the homeostasis of the vascular wall which is achieved, under normal conditions, by the release of endothelium-derived relaxing factors (EDRF) such as nitric oxide (NO),

prostacyclin (PGI_2) and the endothelium-derived hyperpolarizing factor (EDHF). Under pathological conditions, the endothelium may also release endothelium-derived constricting factors (EDCF) such as the superoxide anion (O^{2-}), endoperoxydes, thromboxane A2 and endothelin-1 which may contribute to the development of atherosclerosis by inactivating EDRFs or by a direct effect on the vascular wall. Ischemia and reperfusion lead to the production of free radicals which can scavenge endothelium-derived NO and induce a coronary endothelial dysfunction manifested by reduced dilatory responses to endothelium-dependent agonists (14).

Preservation solutions could prevent endothelial cells from injury due to oxygen derived free radicals during the ischemic period. Nillson et al. showed that preservation for 5 hours in a crystalloid solution caused a reversible acute endothelial dysfunction while no alterations were detected when hypothermic blood cardioplegia was used (15). The type of solution used for graft preservation has been incriminated in the causation of a higher incidence of graft coronary atherosclerosis: specifically, grafts preserved in the intracellular type University of Wisconsin solution were more susceptible to developing accelerated atherosclerosis than those preserved in the extracellular type Stanford solution (16). Moreover, a link has been established between the severity of perioperative ischemic damage, the

duration of cold ischemia and the subsequent development of graft coronary vasculopathy (17,18).

There is substantial experimental evidence which shows that oxygen-derived free radicals contribute to early postoperative failure of cardiac allografts. The Celsior solution (Pasteur-Mérieux, Lyon, France) is an original preservation solution specifically designed for prolonged heart preservation (Table 1). It combines the general principles of preservation solutions as well as aspects specific to the heart such as prevention of the development of ischemic contracture and dysfunction due to edema. It is an extracellular type solution with a great antioxidant capacity due to its high content in reduced glutathion which confers a superior preservation of myocardial function by preventing production of free radicals during preservation and at the time of reperfusion (15,22-24). The Celsior formulation was developed with two main principles in mind: first, the prevention of cellular swelling which is achieved by the combination of lactobionate (a chelator of Fe^{++} ion, implicated in the Findel reaction) and mannitol (which has a smaller antioxidant effect by scavenging O^{-} singlet) which has a concentration of 140 mmol/L to counterbalance intracellular osmotically active molecules. The second guiding principle is the protection from free radical injury which is achieved by the combination of three

components: reduced glutathion, mannitol and histidine. These effects on reactive oxygen species production, which are known to degrade NO, could improve preservation of endothelial-derived NO. Celsior was found to be effective *in vitro* in an isolated rat heart model and *in vivo* in a rabbit heterotopic heart transplantation model (25). Clinical efficacy studies are currently underway in Europe and North America, but the effects of Celsior on endothelial function and on the development of graft vasculopathy are unknown.

This study was designed to investigate the effect of cardioplegia and prolonged hypothermic storage with Celsior, blood or a crystalloid solution (KCl 60 mEq added in) followed by 4 hours of preservation at 4° C in saline (NaCl 0.9%) or Celsior solutions, on the endothelial function of porcine epicardial coronary arteries.

Materials and Methods

A. Animals and Experimental Groups

Forty Landrace piglets with a mean weight of 20.2 ± 2.0 kg (range 14.0 to 22.2 kg), age 10 ± 1 weeks, were used for investigating the effects of arrest and storage with different solutions on coronary artery endothelial

function. Ten groups were studied (Table 2): hearts in the control group were submitted to immediate excision without administration of cardioplegia nor preservation (Group 1); three groups had cardioplegia with either Celsior (Group 2), blood (Group 3), or crystalloid (Group 4) solutions without preservation; two groups had cardioplegia induced with a crystalloid solution and were then stored for 4 hours in a saline solution (0.9% NaCl) (Group 5) or 4 hours in Celsior solution (Group 6) (4°C); two groups had cardioplegia induced with normothermic blood cardioplegia (37 C) and were then stored for 4 hours in saline solution (Group 7), or 4 hours in Celsior solution (Group 8); finally two groups underwent cardioplegia with Celsior and were stored in either saline (Group 9) or Celsior solutions (Group 10) for 4 hours. All animals received humane care in compliance with the recommendations of the guidelines on the care and use of laboratory animals issued by the Canadian Council on Animals and *Guidelines of Animal Care,* and were approved by a local committee.

B. Anesthesia and Surgical Technique

Anesthesia was induced with an intramuscular mixture injection of ketamine (20 mg/kg; Rogarsetic, Montreal, Quebec, Canada) and xylazine (2mg/kg; Rompun, Etobicoke, Ontario, Canada). Swines were artificially

ventilated with an O_2/air mixture (3:2). Respiratory control was maintained by frequent determinations of arterial blood gases and acidosis was balanced with 8.4% sodium bicarbonate (Abbott laboratories, Montreal, Que., Canada). Light anaesthesia was supported by halothane 1% (halocarbon laboratories, Markham, NJ, USA). Hair was shaved at the operative field and the skin was surgically disinfected. A median sternotomy and pericardial incision was performed, and heparin (3 mg/kg, heparin sodium; Leo laboratories, Kingston, Ont., Canada) was given through a central venous catheter. The distal right innominate artery was ligated and the proximal portion cannulated with a polyvinyl chloride catheter positioned in the ascending aorta for administration of cardioplegia. After clamping of the aortic arch between the two innominate arteries, asystole was induced through injection of the cardioplegia solution in the ascending aorta at a maximal pressure of 60 mm Hg. The heart was vented by incision of the right and left atrial appendages and the heart was excised.

C. Cardioplegia and Storage Period

In the control group, the hearts were immediately excised and transferred to organ chambers. The cardioplegic solutions used were crystalloid (12 mL/kg), Celsior (12 mL/kg) or normothermic blood (4:1 blood-Ringer's

lactate ratio 15 mL/kg). In the normothermic blood group, blood was harvested during the initial phase of anaesthesia in a 450 mL bag with 63 mL citrate dextrose phosphate and kept normothermic. Potassium chloride (mean 60 mEq) was then added through a Y-tubing in the crystalloid, blood and Celsior solution groups and administered in the aortic root. After flooding of the pericardium with cold storage solution, the heart was excised and placed in the designated storage solution for 4 hours at 4° C or transferred to organ chambers as described above.

D. Vascular Reactivity

The left anterior descending, left circumflex and the right coronary arteries were excised from the myocardium and dissected free from the epicardium, myocardium and from adventitial tissue and divided in 4 mm wide rings and used randomly in organ chamber experiments. Sixteen rings were used for each animal, eight rings per endothelium-dependent agonists (5-HT and BK).

The endothelial function of control and arteries rings submitted to cardioplegia and storage was studied in organ chambers filled with Krebs-bicarbonate solution (20 mL at 37° C: composition in mmol/L: NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.1, $CaCl_2$ 2.5, $NaHCO_3$ 25 and

calcium ethylenediaminotetraacetic acid 0.026). Oxygenation was insured using a 95% O_2 / 5% CO_2 gas mixture. The rings were suspend between two metal stirrups, one of which was connected to an isometric force transducer. Data were collected with a data acquisition software (IOS3, Emka Inc., Paris, France).

All studies were performed in the presence of indomethacin (10^{-5} mol/L, to exclude the production of endogenous prostanoids), propranolol (10^{-7} mol/L, to prevent the activation of β-adrenergic receptors), and ketanserin (incubated 45 minutes before the addition of serotonin: 10^{-6} mol/L, to block serotonin 5HT-2 receptors which cause contraction of smooth muscle cells in the absence of ketanserin). Each preparation was stretched to the point of its active length curve (usually 3.5 g), as determined by measuring the contraction to potassium chloride (30 mmol/L) at different levels of stretch, and then stabilised for 30 minutes. The maximal contraction was determined with potassium chloride (60 mmol/L) and rings were excluded if they failed to contract to potassium chloride (exclusion rate less than 5%). After washing and 45 minutes of stabilisation, prostaglandin $F_{2\alpha}$ (range, 2×10^{-6} to 10^{-5} mol/L) was added to achieve a contraction averaging 50% of the maximal contraction to KCl (60 mmol/L). Endothelium-dependent relaxations to serotonin (5-HT, 10^{-10} to 10^{-5} mol/L, an agonist which

activates $5HT_{1d}$ receptors coupled to Gi-proteins which lead to the release of NO) and bradykinin (BK, 10^{-12} to 10^{-6} mol/L which activates B2 receptor coupled to Gq-proteins which lead to the release of NO and EDHF) were measured. At the end of the experiments, endothelium-independent relaxations were studied using a single bolus of sodium nitroprusside (SNP 10^{-5} mol/L, a nitric oxide donor). No rings were exposed to more than one endothelium-dependent agonist.

E. Drugs

All solutions were prepared daily. Bradykinin, 5-Hydroxytryptamine creatinin sulfate (serotonin), indomethacin, ketanserin and sodium nitroprusside were purchased from Sigma Chemical Co. (Oakville, Ontario, Canada). Propranolol was purchased from Biomol Research Laboratories Inc. (Plymouth Meeting, PA, USA) and prostaglandin F2α was purchased from Cayman Chemical Company (Ann Arbor, MI, USA).

F. Statistical analysis

Contractions to PGF2α are expressed as the percentage of the maximal contraction to potassium chloride (60 mmol/L) for each group and expressed

as means ± standard error of the mean (SEM); "n" refers to the number of animals studied. Relaxations are expressed as percentage of the maximal contraction to PGF2α for each ring. ANOVA studies were performed to compare dose-response curves. Differences were considered to be statistically significant when the value of $p < 0.05$.

Results

A. Vascular Reactivity

Contractions

There were statistically significant differences in the amplitude of the contraction to potassium chloride (60 mmol/L) between the crystalloid/Celsior cardioplegia groups and the control/blood groups. There were also statistically significant differences in the amplitude of the contraction to potassium chloride (60 mmol/L) among the groups, except in arteries submitted to cardioplegic arrest with Celsior and preservation in saline (group 6) which showed a significant decrease (Figure 1). There were no statistically significant difference in contractions to PGF2α (range 2×10^{-6} to 10^{-5} mol/L) except in the group submitted to cardioplegic arrest with Celsior and preservation in the saline solution (group 6) which showed a significant increase (88.1 ± 4.3%, $p < 0.05$ vs all groups) (Figure 2).

Relaxations

Serotonin. There were no significant difference in endothelium-dependent relaxation to serotonin and bradykinin between arteries submitted to storage in saline only (group1) versus normal porcine coronary arteries freshly explanted and tested (data not shown).

There was a statistically significant decrease in endothelium-dependent relaxations to serotonin in porcine coronary arteries submitted to crystalloid, blood, or Celsior cardioplegia alone (Groups 2-4) (Figure 3).

There were no statistically significant decreases of endothelium-dependent relaxations to serotonin in the Celsior cardioplegia + Celsior preservation group (group 10) compared with controls. When the Celsior and crystalloid solutions were used for cardioplegia , the arteries subsequently preserved in Celsior (group 6 and 10) showed a significantly superior preservation of relaxation to 5-HT compared to those preserved in saline ($p < 0.05$ for Celsior + Celsior group vs Celsior + Saline only) (Figure 4). There was a statistically significant decrease in endothelium-dependent relaxations to serotonin in arteries from all other cardioplegic/preservation groups (groups 5,6,7,8,9) compared with controls (Figure 4).

Bradykinin. There was a statistically significant decrease of endothelium-dependent relaxations to bradykinin in rings from all coronary arteries exposed

to either blood, crystalloid, or Celsior cardioplegia alone (Figure 3). There was a statistically significant decrease of endothelium-dependent relaxations to bradykinin in rings from the Blood + Celsior and Celsior + Saline groups (groups 8 and 9) at one concentration (3×10^{-8} mol/L) ($p < 0.05$ vs control group). There were no statistically significant differences between all other four cardioplegic/storage groups compared with controls (Figure 5).

Endothelium-independent relaxations. There were no statistically significant differences in the maximal relaxation to sodium nitroprusside (SNP; 10^{-5} mol/L) in all groups (data not shown).

Discussion

The major findings of this study are that exposure of porcine coronary arteries to cardioplegic arrest with crystalloid, blood or Celsior cardioplegic solutions cause a significant decrease in endothelium-dependent relaxations to serotonin and bradykinin. Subsequent preservation in saline does not provide recovery of endothelium-dependent relaxations. Induction of arrest and preservation with Celsior allows for functional recovery of endothelium-dependent relaxations to both serotonin and bradykinin and thus better preserves the endothelial function of coronary arteries under the present experimental conditions.

Endothelium-dependent contractions

The significant decrease in the amplitude of contraction to potassium chloride in the arteries submitted to cardioplegia with Celsior and saline preservation may be related to the greater potassium loading or decreased sensitivity to exogenous potassium in this group although it was not observed in the other groups in which Celsior was used for cardioplegia. The greater amplitude of contraction to prostaglandin F2α in the arteries submitted to Celsior cardioplegia and saline preservation may reflect a greater sensitivity of the vascular smooth muscle cell to exogenous prostaglandins. Under certain conditions, endothelium-dependent contractions can be explained either by the withdrawal of the release of endothelium-derived relaxing factors (NO, prostacyclin) or by the production of diffusible vasoconstrictor substances coined endothelium-derived contracting factors (EDCF).

Effect of the cardioplegic solution

Perfusion of coronary arteries with the crystalloid, blood, and Celsior cardioplegic solutions all caused a decrease in endothelium-dependent relaxations to serotonin and bradykinin. Perfusion of coronary arteries with the crystalloid and blood solutions followed by preservation in either saline or Celsior to obtain an electromechanical silence induces a decrease in Gi-protein-mediated relaxations. Perfusion of Celsior for cardioplegic arrest

followed by preservation in saline also causes a similar type of dysfunction. This endothelial dysfunction preferentially involves Gi- protein mediated relaxations as demonstrated by reduced responses to serotonin which acts by binding to 5-HT_{1D} receptors coupled to Gi-proteins in normal porcine coronary arteries. The Gq-protein mediated relaxations are altered to a lesser degree as evidenced by preservation of relaxations to bradykinin (which binds to B2 receptors coupled to Gq-proteins and leads to the release of NO and EDHF in normal porcine coronary arteries) except at one concentration in the group submitted to crystalloid cardioplegia with storage in the saline solution. The G_i protein mediated pathway is one of the first pathways affected in many cardiovascular diseases such as acute and chronic cardiac rejection (19,26), atherosclerosis (27,28) and may be an early marker of pathological endothelial activation implicated in the development of vascular diseases. Preservation of this sensitive endothelial cell signaling pathway, reflecting the final capacity of the vascular wall to produce the vascular protector endothelial NO is important to avoid progression to a generalized endothelial dysfunction associated with advanced atherosclerosis. Endothelium-dependent relaxations mediated by the NO donor, sodium nitroprusside, were unaffected, therefore demonstrating the integrity of vascular smooth muscle cells. This confirms that the reduction of

vascular relaxation observed throughout the groups studied is due to endothelium-dependent mechanisms.

Perfusion of coronaries with a crystalloid solution is known to alter endothelium-dependent relaxations in comparison to perfused control (29) without any morphological alterations of endothelial cells. Cardioplegia with St-Thomas solution delivered at 60 to 65 mm Hg significantly reduces endothelium-dependent relaxations in addition to the cold ischemic period and reperfusion (30). However, others have shown that use of a hyperkalemic crystalloid cardioplegic solution does not irreversibly alter endothelium-dependent relaxations to acetylcholine (G-protein mediated) or adenosine diphosphate (ADP) (31). Perfusion at a pressure of 240 cm H_2O impairs the vasodilatory response to serotonin and results in vasoconstriction (32). These studies suggest that the dysfunction observed are due to a traumatic effect of baroreceptors or to shear stress on the vascular wall of the artery and not from the cardioplegia solution itself. He et al. demonstrated that the relaxation to EDHF is altered following exposure to a hyperkalemic solution due to partial membrane depolarisation of the smooth muscle cells affecting the potassium channels and the release of the endothelium-derived relaxing factors (34,35). Although Celsior is sufficient in itself to induce cardioplegic arrest, potassium was added in the Y-tubing as with the two other cardioplegic

solutions to negate the confounding effect of hyperkaliemia on the induction of coronary endothelial dysfunction in this model.

Effect of the storage solution

The preservation times currently considered safe range between 4 to 6 hours and longer ischemic periods are associated with a reduction of the survival rate of heart transplant recipients (35). Storage at 4° C was superior in preserving the endothelial and myocardial function compared to 20° C in an isolated heart model in rats (36). Accordingly, porcine coronary arteries exposed to 4 hours of storage at 4° C demonstrated similar endothelium-dependent relaxations compared with freshly harvested arteries. This strongly suggests that the endothelial dysfunction observed in the groups exposed to cardioplegia solutions is not a consequence of preservation but a result of perfusion of the coronary arteries during cardiac arrest (37). The preservation time of 4 hours in this study was chosen since it represents the average ischemic time in a clinical heart transplantation setting.

Storage of the heart in a saline solution does not allow recovery of Gi-protein mediated relaxations during the 4 hour storage period as shown by the decrease relaxations to serotonin in the cardioplegia groups stored in saline. Storage in the Celsior solution was associated with a better preservation of Gi-protein mediated relaxations except in the group in which blood was used as

the cardioplegic solution which showed a significant endothelial dysfunction . The fact that storage in saline after Celsior cardioplegia allowed for recovery of endothelial function to Gq-protein mediated relaxations can be explained in a twofold manner. Firstly, it is highly likely that endothelial dysfunction seen in all three groups after cardioplegia alone was due to shear forces and barotrauma and not to the cardioplegic solution itself. It is known that infusion of a hyperkalemic crystalloid cardioplegic solution does not alter the endothelium in a irreversible manner (31) and thus the 4 hours of preservation in saline could have allowed for functional recovery of the endothelium in the more resistant Gq-protein mediated pathway. Secondly, the Celsior cardioplegic solution might cause initial dysfunction but may allow a better recovery of endothelial function regardless of the preservation solution used by its beneficial properties likely related to its antioxydant capacity to eliminate free radicals production during ischemia combined to a reduction of myocardial oedema (25). Use of this new preservation solution could protect grafts from the deleterious effects of preservation (38) as suggested by a clinical study in which use of Celsior was associated with an increased inotropic and vasodilatory response in transplanted patients in contrast to the University of Wisconsin solution (39). This is consistent with the observation that the superior arrest and storage strategy under the present experimental

conditions is use of Celsior for induction of cardioplegia and storage in Celsior. Indeed, endothelium-dependent relaxations of coronary arteries exposed to cardioplegia and preservation with Celsior solution are similar to the control group. Storage of the heart for 24 hours in a crystalloid solution with the antioxydant indonoindole also preserves endothelium-dependent relaxations in comparison to crystalloid alone (40) suggesting that improvement in the formulation of preservation solutions may lengthen the safe ischemic time for heart transplantation. Lung preservation, using Celsior, (41,42) has also yielded favorable initial results.

Limitations

Damage by production of free radicals by leukocytes at the time of reperfusion is a significant contributing factor in the development of pathological activation of the endothelium (14). The present study solely addresses the effects of arrest and preservation solutions on coronary endothelial function. Further studies evaluating the consequences of myocardial reperfusion following storage on coronary endothelial function are needed since the endothelial dysfunction observed with arrest and storage are likely to be compounded by reperfusion. The benefits of using Celsior may become even more evident considering its proven capacity for capture of oxygen derived free radicals occurring during reperfusion. Use of

normal swine does not reflect the clinical condition of organ harvest associated with brain death which may be associated with massive cathecolamines release and functional disturbances depending on the mechanism of death. Experimental studies evaluating the effects of this condition on myocardial and coronary endothelial function may yield further insight on the problematic of solid organ procurement and storage. Finally, studies of the effects of preservation solutions and storage on coronary microvascular function should also be undertaken considering the central role of the microcirculation in autoregulation of coronary blood flow.

Conclusion

In summary, cardioplegic arrest and storage with Celsior constitutes the best strategy permitting the preservation of endothelium-dependent relaxation in porcine epicardial coronary arteries. Superior preservation of coronary endothelial function at the time of implantation may reduce early graft failure by improving coronary blood flow and limit the development of graft coronary vasculopathy by maintaining the protective effects of the endothelium on the vascular wall.

Bibliography

1. Davis S, Yeung A, Meredith I, Charbonneau F, Selwyn P, Anderson T. Early endothelial dysfunction predicts the development of transplant coronary artery disease at 1 year posttransplant. *Circulation* 1996;93:457-62.
2. Billingham M. Cardiac transplant atherosclerosis. *Transplant Proc* 1987;19(suppl 5):19-25.
3. Young J. Allograft vasculopathy: diagnosing the nemesis of heart transplantation. *Circulation* 1999;3:458-60.
4. Libby P, Salomon R, Payne D, Schoen F, Pober J. Functions of vascular wall cells related to development of transplantation-associated coronary arteriosclerosis. *Transplant Proc* 1989;21:3677-84.
5. Drexler H, Fischell TA, Pinto FJ, Chenzbraun A, J B, Cooke JP, Alderman EL. Effect of L-arginine on coronary endothelial function in cardiac transplant recipients, relation to vessel wall morphology. *Circulation*. 1994:1615-1623.
6. Ross R. The pathogenesis of atherosclerosis an update. *N Engl J Med*. 1986;314:488-500.

7. Lücher TF, Vanhoutte PM. The endothelium: Modulator of cardiovascular function. In: Press C, ed. *Boca Raton, FL*; 1990:1-228.

8. Furchgott R, Vanhoutte P. Endothelium-derived relaxing and contracting factors. *FASEB J.* 1989;3:2007-2018.

9. Dorphin A. Nucleotide binding proteins in signal transduction and disease. *Trends Neurosc.* 1987;10:53-57.

10. Rickenbacher P, Pinto F, Lewis N, Hunt S, Alderman E, Schoroeder F, Stinson E, Brown B, Valantine H. Prognostic importance of intimal thickness as measured by intracoronary ultrasound after cardiac transplantation. *Circulation.* 1995;92:3445-3452.

11. Mugge A, Brandes R, Heublien B, Nolte C, Haverich A, lichtlen P. Endothelial dysfunction in heart transplanted patients with graft vasculopathy. *Eur Heart J.* 1995;16 (Suppl J):78-83.

12. Flavahan NA, Shimokawa H, Vanhoutte PM. Pertussis Toxin Inhibits Endothelium-dependent RElaxations to Certain Agonists in Porcine Coronary Arteries. *Journal of Physiology.* 1989;408:549-560.

13. Flavahan N, Vanhoutte P. Endothelial cell signaling and endothelial dysfunction. *Am J Hypertension.* 1995;8:28S-41S.

14. Sellke FW, Shafique T, Ely DL, Wientraub RM. Coronary Endothelial Injury After Cardiopulmonary Bypass and Ischemic

Cardioplegia Is Mediated By Oxygen-Derived Free Radicals. *Circulation*. 1993;88 part 2:395-400.

15. Nilsson FN, Miller VM, Vanhoutte PM, McGregor CGA. Methods of cardia preservation alter the function of the endothelium in porcine coronary arteries. *The Journal od Thoracic and Cardiovascular Surgery*. 1991;102 No 6:923-30.

16. Drinkwater DC, Rudis E, Laks H, Marino J, Stein D, Ardehali A, Aharon A, Moriguchi J. University of Wisconsin Solution versus Stanford Cardioplegic Solution and the Development of Cardiac Allograft Vasculopathy. *J Heart Lung Transplant*. 1995;14 No 5:891-6.

17. Gaudin P, Ryburn K, Hutchins. Peritransplant injury to the myocardium associated with the development of accelerated arteriosclerosis in heart transplant recipients. *Am J Surg Pathol*. 1994;18:338-346.

18. Veinot J, Walley V. Cardiac transplant arteriopathy. *Am J Surg Pathol*. 1995;19:727-734 (Letter).

19. Perrault L, Bidouard J, Janiak P, Villeneuve N, Bruneval P, Vilaine J, Vanhoutte P. Impairment of G-protein-mediated signal transduction in

the porcine coronary endothelium during rejection after heart transplantation. *Cardiovasc Res*. 1999;43:457-470.

20. Perrault L, Bidouard J-P, Janiak P, Villeneuve N, Bruneval P, Vilaine J-P, Vanhoutte P. Time Course of Coronary Endothelial Dysfunction in Acute Untreated Rejection After Heterotopic Heart Transplantation. *J Heart Lung Transplant*. 1997;16:643-657.

21. Rao V, Feindel C, Weisel R, Boylen P, Cohen G. Donor blood perfusion improves myocardial recovery after heart transplantation. *J J Heart Lung Transplant*. 1997;16:667-73.

22. Anderson T, Meredith I, Uehata A, Mudge G, Selwyn A, Yeung PGA. Functional significance of intimal thickening as detected by intravascular ultrasound early and late after cardiac transplantation. *Circulation*. 1993;88:1093-1100.

23. Evora P, Pearson P, Schaff H. Impaired endothelium-dependent relaxation after coronary reperfusion injury: evidence for G-protein dysfunction. *Ann Thorac Surg*. 1994;57:1550-1556.

24. Pearson P, Schaff H, Vanhoutte P. Long term impairment of endothelium-dependent relaxations to aggregating platelets after reperfusion injury in canine coronary arteries. *Circulation*. 1990;81:1921-1927.

25. Menasché P, Termignon JL, Pradier F, Grousset C, Mouas C, Alberici G, Weiss M, Piwnica A, Bloch G. Experimental evaluation of celsior, a new heart preservation solution. *Eur J Cardio-thorac Surg.* 1994;8:207-213.

26. Perrault LP, Bidouard J-P, Janiak P, Villeneuve N, Bruneval P, Vilaine J-P, Vanhoutte PM. Time Course of Coronary Endothelial Dysfunction in Acute Untreated Rejection After Heterotopic Heart Transplantation. *J Heart Lung Transplant.* 1997;16 No 6:643-57.

27. Flavahan N, Vanhoutte P. Pertussis toxin inhibits endothelium-dependent relaxations evoked by fluoride. *Eur J Pharmacol.* 1990;178:121-124.

28. Shimokawa H, Flavahan NA, Vanhoutte PM. Loss of Endothelial Pertussis Toxin-Sensitive G Protein Function in Atherosclerotic Porcine Coronary Arteries. *Circulation.* 1991;83:652-60.

29. Nilsson F, Miller V, Johnson C, Tazelaar H, McGregor C. Cardioplegia alters porcine coronary endothelial cell growth and responses to aggregating platelets. *J Vasc Res.* 1993;30 (1):43-52.

30. Budrikis A, Liao Q, Bolys R, Westerlaken B, Steen S. Effects of Cardioplegic Flushing, Storage, and Reperfusion on Coronary Circulation in the Pig. *Ann Thorac Surg.* 1999;67:1345-9.

31. Evora PRB, Pearson PJ, Schaff HV. Crystalloid cardioplegia and hypothermia do not impair endothelium-dependent relaxation or damage vascular smooth muscle of epicardial coronary arteries. *J Thorac Cadiovasc Surg*. 1992;104:1365-74.

32. Katayama O, Amrani M, Ledingham S, Jayakumar J, Smolenski RT, Severs N, Rothery S, Yacoub MH. Effect of cardioplegia infusion pressure on coronary artery endothelium and cardiac mechanical function. *European Journal of Cardio-thoracic Surgery*. 1997;11:751-762.

33. He G. Effect and mecanism of cardioplegic arrest on the coronary endothelium-smooth muscle interaction. *Clin Exp Pharmacol Physiol*. 1998;25 (10):831-835.

34. He G. Coronary endothelial function in open heart surgery. *Clin Exp Pharmacol Physiol*. 1997;24 (12):955-957.

35. Keck B, White R, Breen T, Daily O, Hosenpud J. Thoracic organ transplants in the United States: a report from the UNOS/ISHLT Scientific Registry for Organ Transplants, United Network for Organ Sharing, International Society for Heart and Lung Transplantation. *Clin Transplant*. 1994:37-46.

36. Amrani M, Mankad PS, Yacoub MH. Improved preservation of endothelial function at 4 C. *Eur J Cardio-thorac Surg*. 1992;6:72-78.

37. Wheeldon D, Sharples L, Wallwork J. Donor heart preservation survey. *J Heart Lung Transplant*. 1992;11:986-993.

38. Wieselthaler G, Chevtchik O, Konetschny R, Moidl R, Mallinger R, Mares P, Griessmacher A, Grimm M, Wolner E, Laufer G. Improved graft function using a new myocardial preservation solution: Celsior. Preliminary data form randomized prospective study. *Transpl Proc*. 1999;31:2067-2068.

39. Wildhirt SM, Weis M, Schulze C, Conrad N, Rieder G, Boehm DH, Meiser B, Kornberg A, Reichenspurner H, Scheidt Wv, Reichart B. Myocardial Preservation in Clinical Cardiac Transplantation. *Transplantation Proceedings*. 1999;31:147-148.

40. Wiklund L, Miller V, McGregor C, Sjoquist P, Berggren H, Nilsson F. Protective effects of an indenoindole antioxidant on coronary endothelial function after long-term storage. *Transplantation*. 1995;60 (8):774-778.

41. Barr ML, Nishanian GP, Sakamaki Y, Carey JN, Chang J, Starnes VA. A New Organ Preservation Solution, Celsior, Is Superior to Euro-Collins, and University of Wisconsin Solutions in Decreasing

Lung Reperfusion Injury. *Transplantation Proceedings*. 1997;29:1357-1358.

42. Roberts RF, Nishanian GP, Carey JN, Sakamaki Y, Barr VASML. A Comparison of the New Preservation Solution Celsior to Euro-Collins and University of Wisconsin Solutions in Lung Reperfusion Injury. *Transplantation*.1999;67(1):152-155.

Figures and Tables Legend

Table 1: Composition and characteristics of Celsior solution.

Table 2: Experimental groups.

Figure 1: Amplitude of contractions to potassium chloride (60 mmol/liter). *p<0.05 vs control.

Figure 2: Amplitude of contractions to PGF2α (range 2 X 10-6 to 10-5 mol/liter) *p<0.05 vs control.

Figure 3: Concentration-response curve to serotonin (5-HT) (a) and bradykinin (b) of porcine epicardial coronary arteries after crystalloid cardioplegia, blood cardioplegia, and Celsior cardioplegia. *p<0.05 vs control.

Figure 4: Concentration-response curve to serotonin (5-HT) of porcine epicardial coronary arteries after crystalloid cardioplegia (a), blood cardioplegia (b), and Celsior cardioplegia (c) and subsequent preservation. *p<0.05 vs control.

Figure 5: Concentration-response curve to bradykinin (BK) of porcine epicardial coronary arteries after crystalloid cardioplegia (a), blood cardiolegia (b), and Celsior cardioplegia (c) and subsequent preservation. *$p<0.05$ vs control.

Table 1 : Composition and Characteristics of Celsior solution [a]

Component	Concentration
Mannitol	60
Lactobionate	80
Glutamic acid	20
Histidin	30
Reduced glutathione	3
Potassium	15
Sodium	100
Magnesium	13
Calcium	0.25
Chloride	41.5
PH (at 20°C)	7.3
Osmolarity (mOsm/L)	360

[a] Concentrations are given as millimoles per liter.

Table 2 - Characteristics of the experimental groups.

Group	Cardioplegic Solution	Storage solution (4 hours at 4 C)
1 Control (n=4)	None	Saline
2 (n=6)	Celsior	None
3 (n=5)	Blood	None
4 (n=4)	Crystalloid	None
5 (n=4)	Crystalloid	Saline
6 (n=3)	Crystalloid	Celsior
7 (n=4)	Blood	Saline
8 (n=4)	Blood	Celsior
9 (n=3)	Celsior	Saline
10 (n=4)	Celsior	Celsior

FIGURE 1:

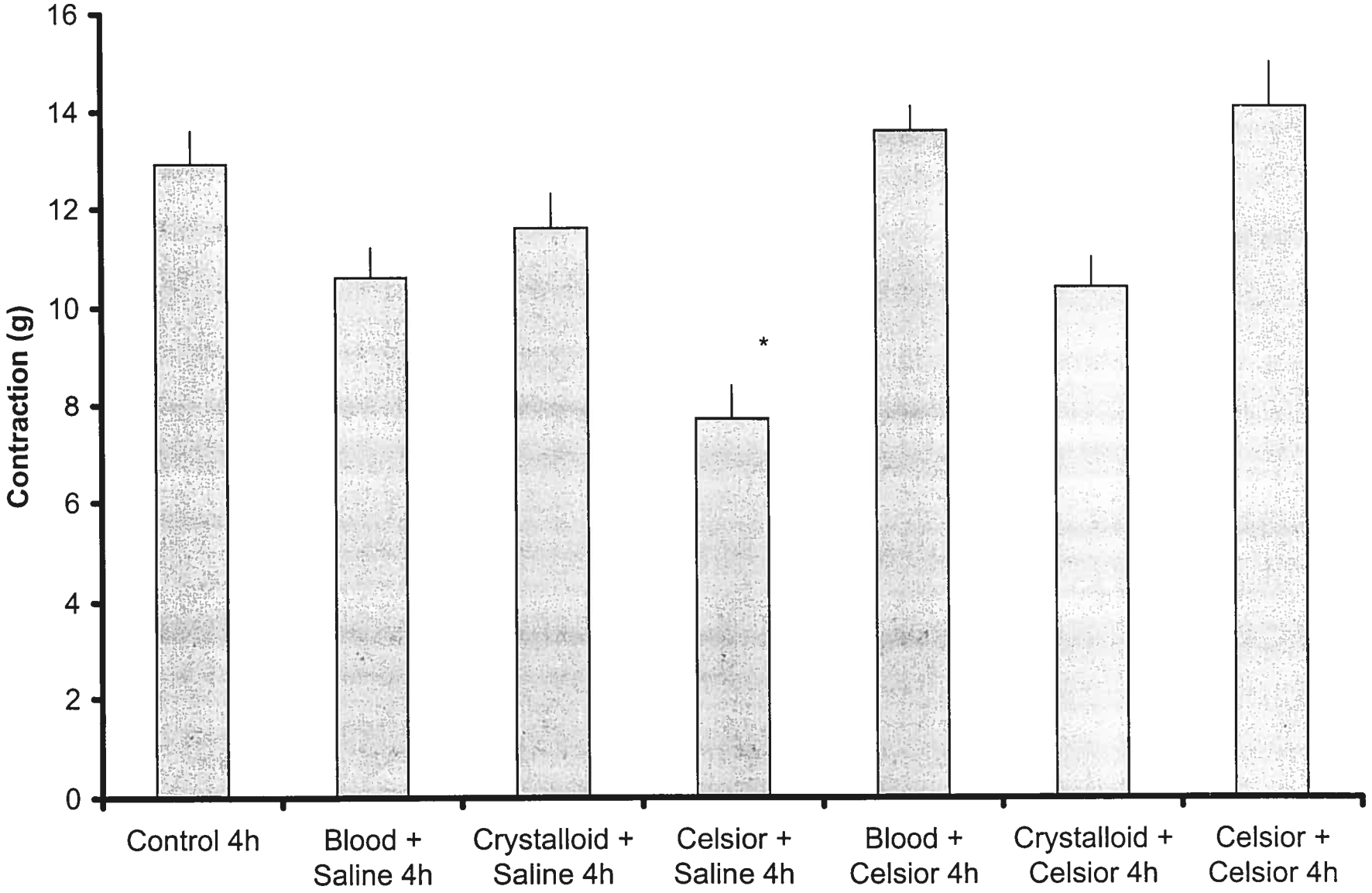

16
14
12
10
8
6
4
2
0
Contraction (g)
*
Control 4h
Blood + Saline 4h
Crystalloid + Saline 4h
Celsior + Saline 4h
Blood + Celsior 4h
Crystalloid + Celsior 4h
Celsior + Celsior 4h

FIGURE 2:

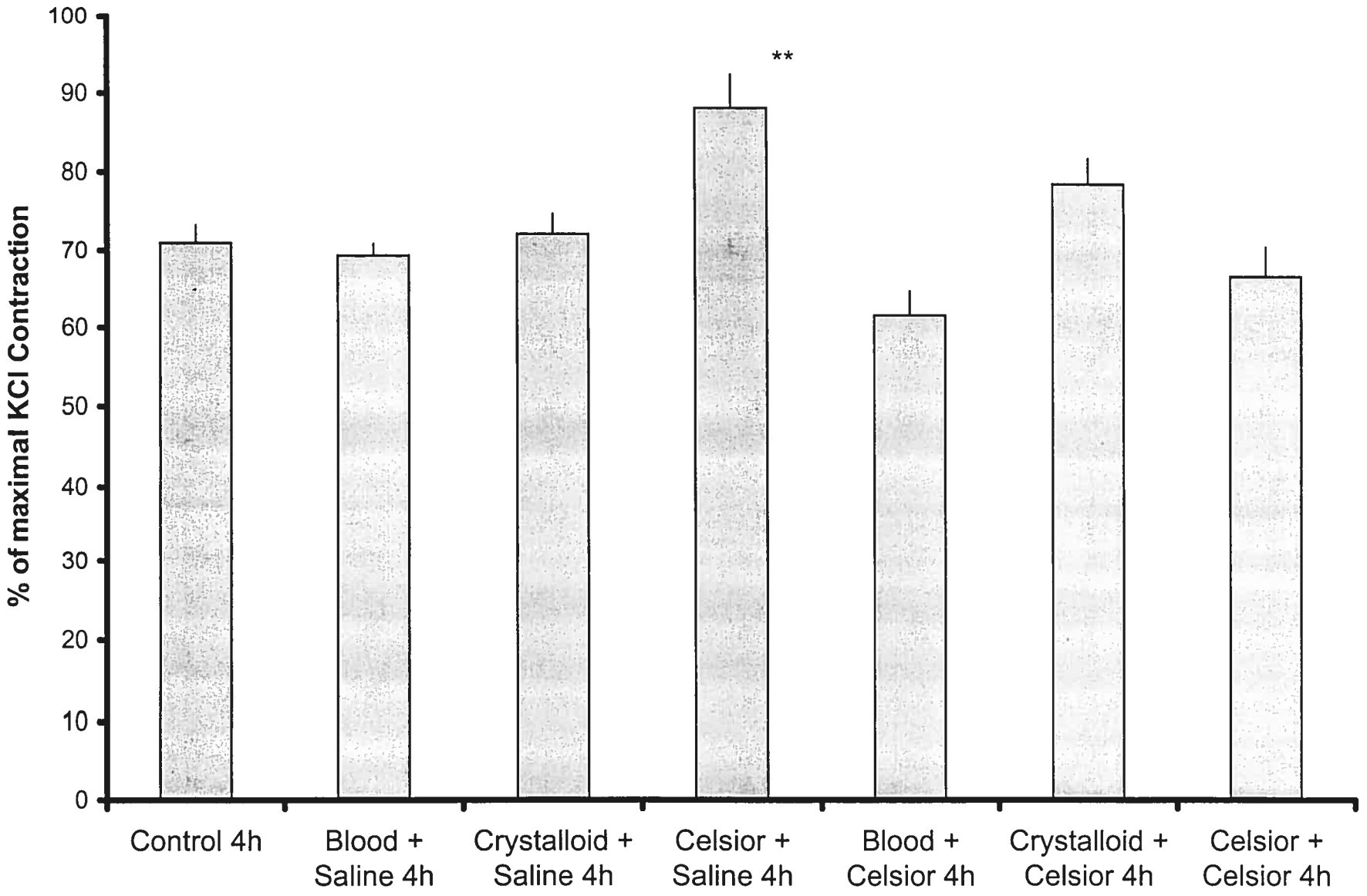

100
90
80
70
60
50
40
30
20
10
0
% of maximal KCl Contraction
**
Control 4h
Blood + Saline 4h
Crystalloid + Saline 4h
Celsior + Saline 4h
Blood + Celsior 4h
Crystalloid + Celsior 4h
Celsior + Celsior 4h

FIGURE 3:

a)

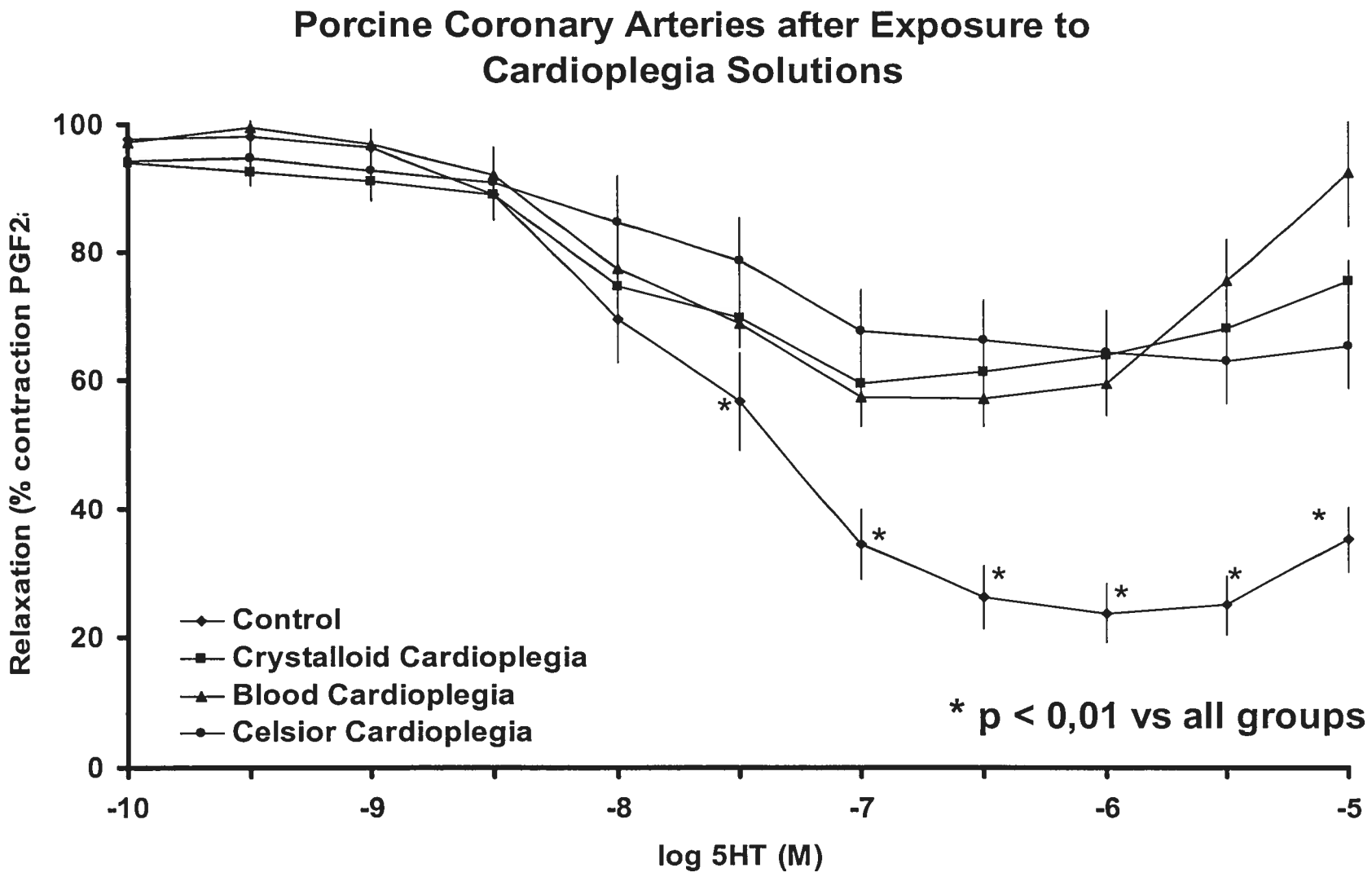

Porcine Coronary Arteries after Exposure to Cardioplegia Solutions
Relaxation (% contraction PGF2:
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
log 5HT (M)
Control
Crystalloid Cardioplegia
Blood Cardioplegia
Celsior Cardioplegia
* p < 0,01 vs all groups

Figure 3:

b)

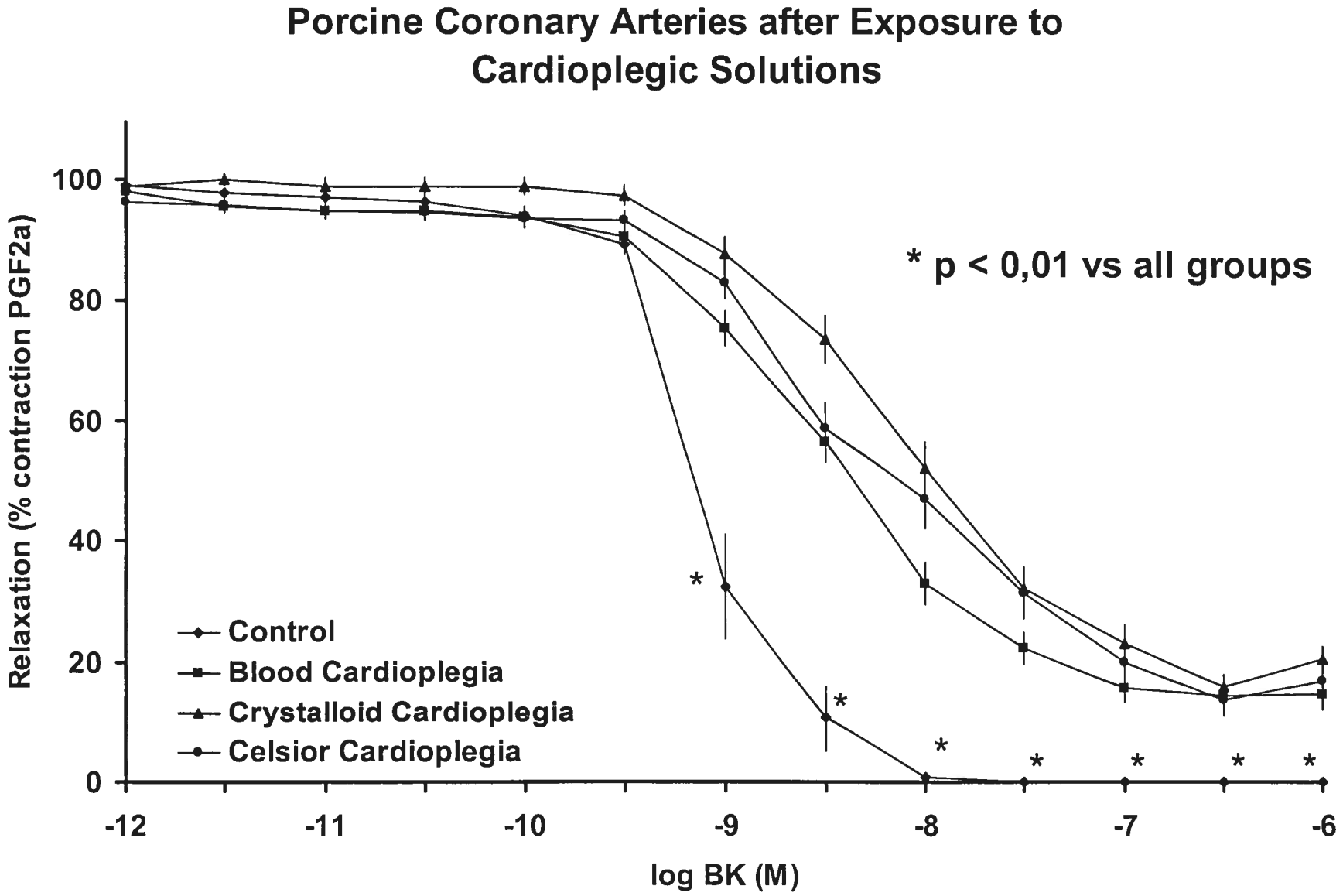
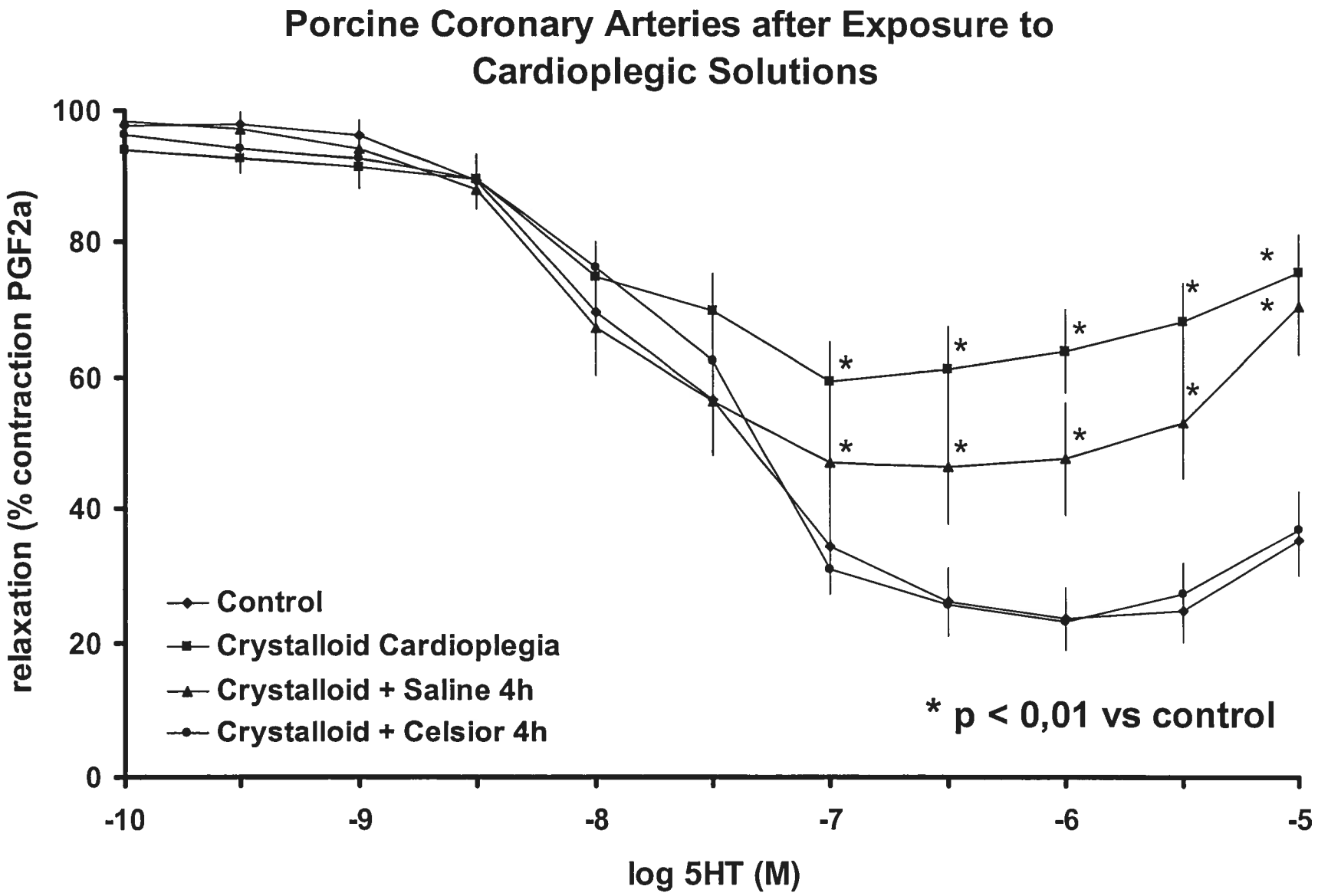
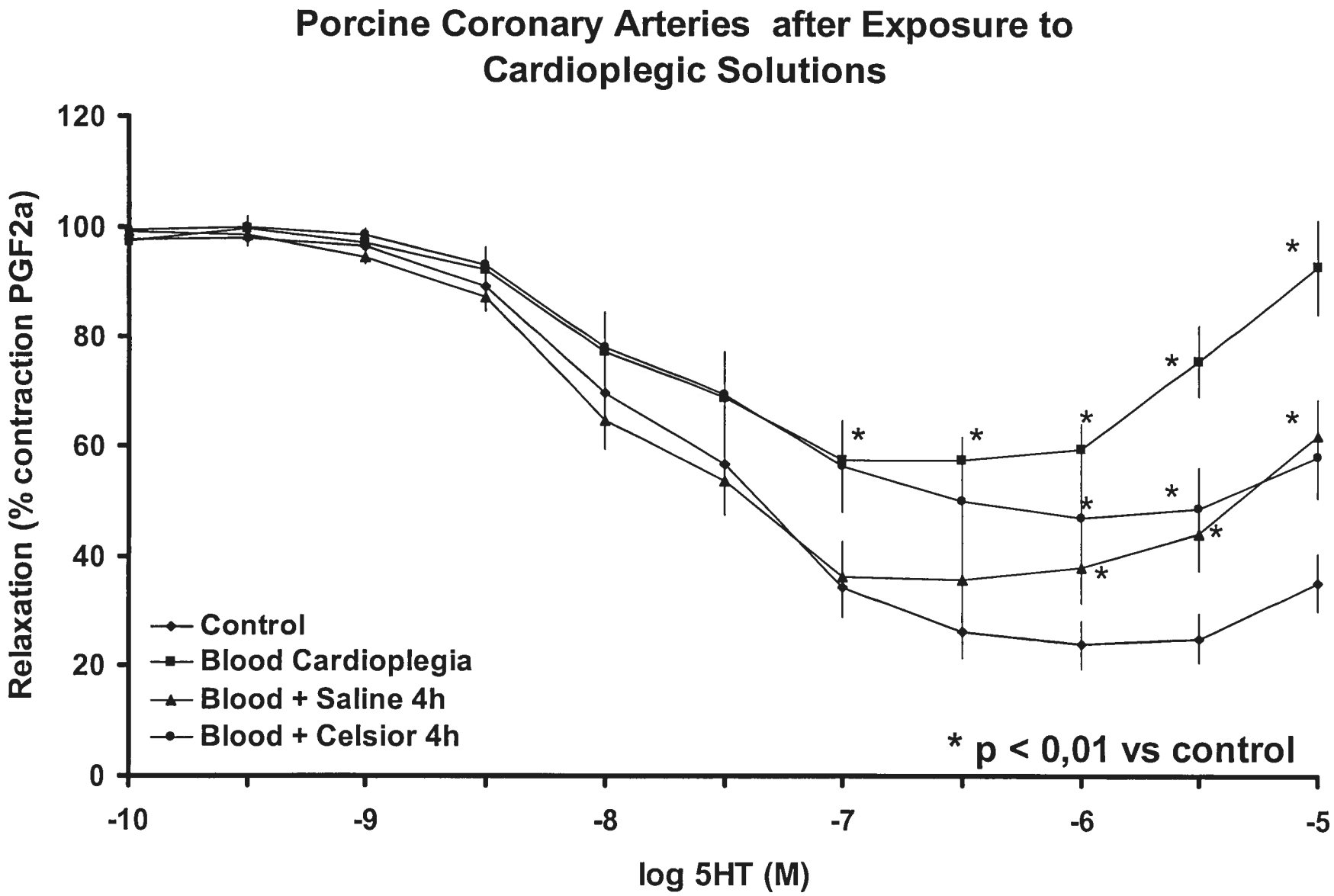

Porcine Coronary Arteries after Exposure to Cardioplegic Solutions
* p < 0,01 vs all groups
Relaxation (% contraction PGF2a)
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
log BK (M)
Control
Blood Cardioplegia
Crystalloid Cardioplegia
Celsior Cardioplegia

FIGURE 4:

a)


Porcine Coronary Arteries after Exposure to Cardioplegic Solutions
relaxation (% contraction PGF2a)
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
log 5HT (M)
Control
Crystalloid Cardioplegia
Crystalloid + Saline 4h
Crystalloid + Celsior 4h
* p < 0,01 vs control

b)


Porcine Coronary Arteries after Exposure to Cardioplegic Solutions
Relaxation (% contraction PGF2a)
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
log 5HT (M)
Control
Blood Cardioplegia
Blood + Saline 4h
Blood + Celsior 4h
* p < 0,01 vs control

c)

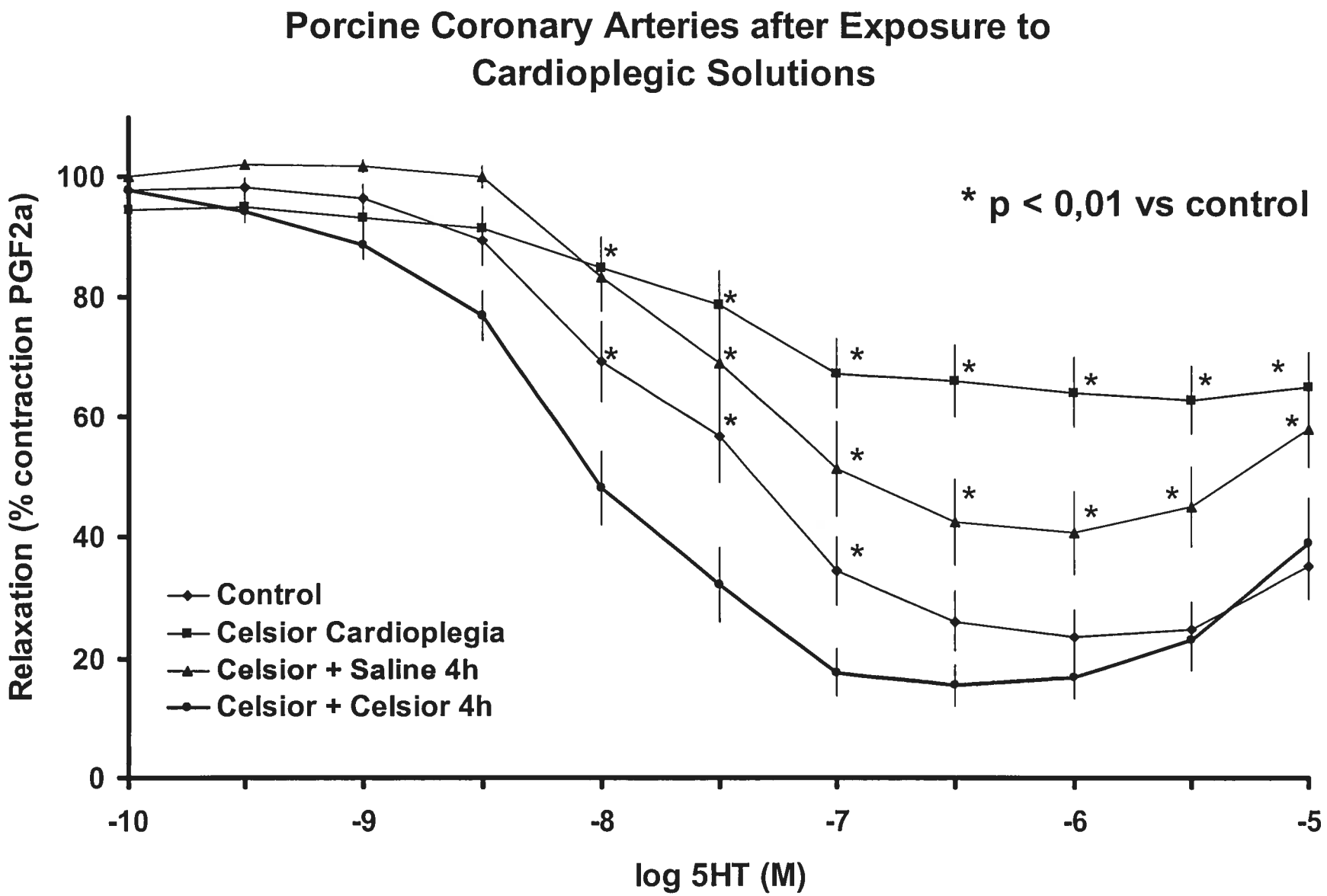
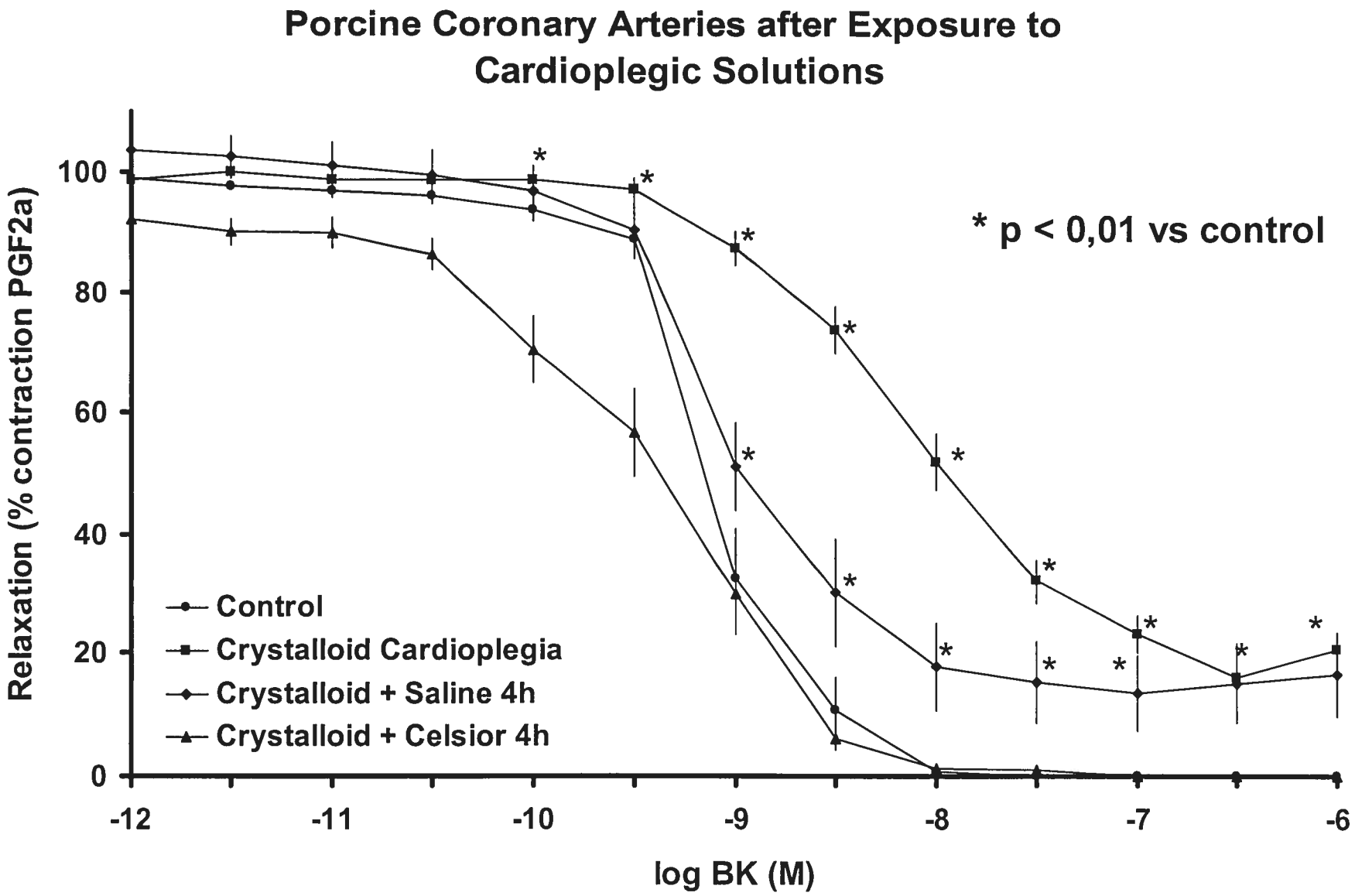
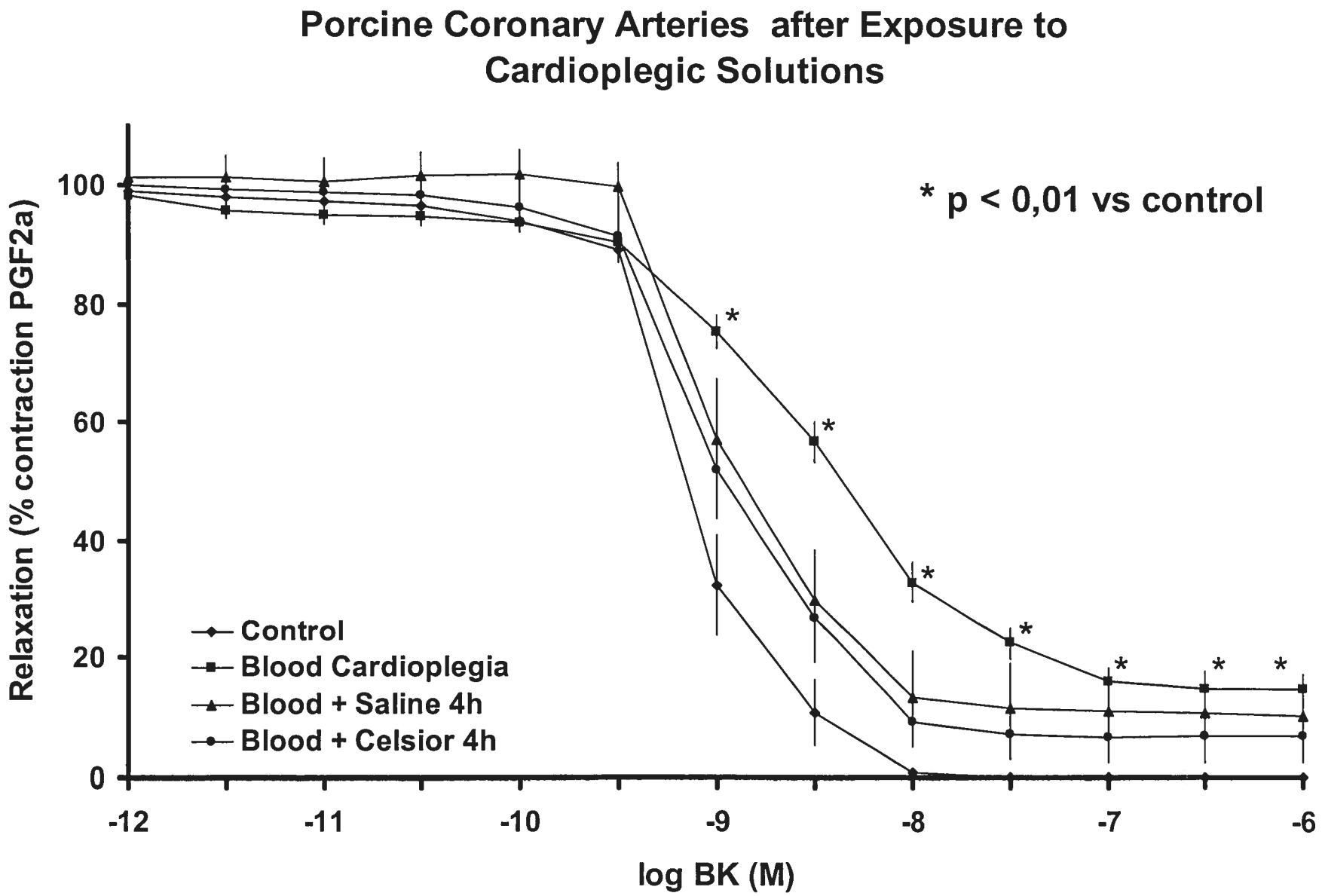

Porcine Coronary Arteries after Exposure to Cardioplegic Solutions
* p < 0,01 vs control
Relaxation (% contraction PGF2a)
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
log 5HT (M)
Control
Celsior Cardioplegia
Celsior + Saline 4h
Celsior + Celsior 4h

Figure 5:

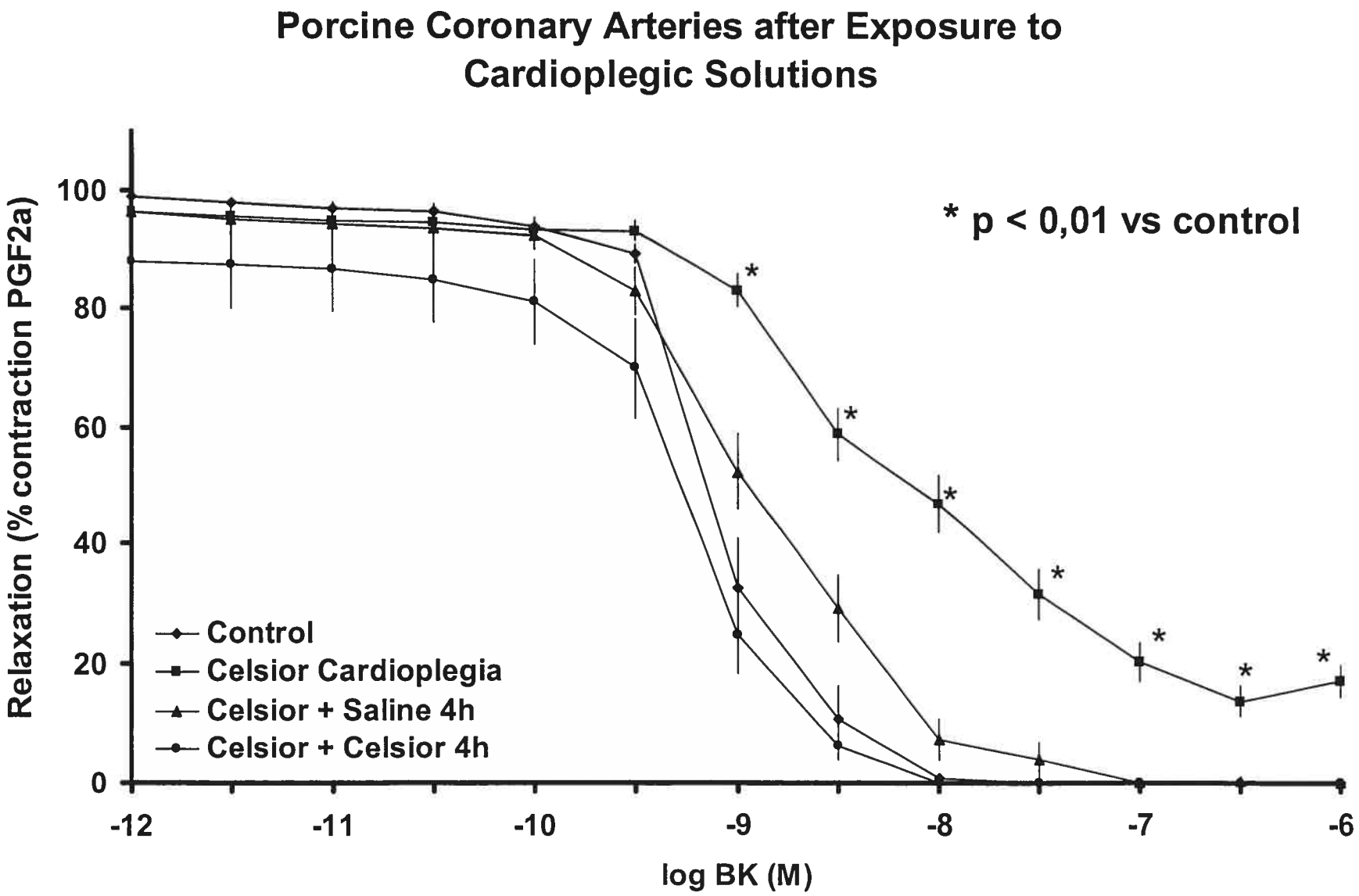
a)


Porcine Coronary Arteries after Exposure to Cardioplegic Solutions
* p < 0,01 vs control
Relaxation (% contraction PGF2a)
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
log BK (M)
Control
Crystalloid Cardioplegia
Crystalloid + Saline 4h
Crystalloid + Celsior 4h

b)


Porcine Coronary Arteries after Exposure to Cardioplegic Solutions
* p < 0,01 vs control
Relaxation (% contraction PGF2a)
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
log BK (M)
Control
Blood Cardioplegia
Blood + Saline 4h
Blood + Celsior 4h

c)


Porcine Coronary Arteries after Exposure to Cardioplegic Solutions
* p < 0,01 vs control
Relaxation (% contraction PGF2a)
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6
log BK (M)
Control
Celsior Cardioplegia
Celsior + Saline 4h
Celsior + Celsior 4h

Article #2:

Protective effects of Celsior on coronary endothelial function one month after transplantation in a porcine model.

Eric Dumont, MD, Olivier Malo, MSc, Michel Pellerin, MD, Michel Carrier, MD, Louis P. Perrault, MD, PhD

ABSTRACT

Background: The development of coronary allograft vasculopathy is preceded by endothelial dysfunction leading to abnormal vascular response to agonists. Endothelial injury from preservation solutions used at the time of organ harvesting has been implicated in acute coronary vasospasm and pathologic activation of the endothelium. The present study was designed to examine the potential protective effects of Celsior, a new preservation solution specifically designed for heart transplantation, on coronary endothelial reactivity at 1 month after transplantation compared with crystalloid and blood based cardioplegic solutions with saline preservation.

Methods: A porcine model of heterotopic heart transplantation was used. Preoperative serum typing for the class I antigen of the swine lymphocyte alloantigen was performed to ensure compatibility for this antigen which permitted survival of the graft with a slow kinetic of rejection without immunosuppression. Three experimental groups were studied: Donor hearts were arrested with hyperkalemic crystalloid, blood or Celsior cardioplegia (12 cc/kg) and immediately implanted in the recipient. Swine were sacrificed at 1 month and rings of epicardial coronary arteries of native (control) and transplanted hearts were studied in organ chambers filled with modified

Krebs-Ringer bicarbonate solution. Endothelium-dependent relaxations to serotonin (5-HT, an agonist that activates 5-HT1d receptors coupled to Gi-proteins), bradykinin(BK, which activates B2 receptors coupled to Gq-proteins), and calcium ionophore were assessed. Neointimal proliferation was evaluated using histomorphometric studies.

Results: There was a significant decrease in endothelium-dependent relaxations to serotonin in all three groups submitted to preservation solutions versus controls. Endothelium-dependent relaxations to bradykinin were significantly reduced in the crystalloid group but not in the blood or Celsior groups versus controls at 1 month. Relaxation to calcium ionophore was significantly decreased in the three groups versus controls, and the crystalloid group had a significant decrease in relaxation versus the blood and Celsior groups. There was a trend towards a diminished neointimal proliferation in the Celsior group at 1 month.

Conclusion: The type of cardioplegic solution used for harvesting hearts significantly influences endothelial reactivity 1 month after transplantation. The use of crystalloid solution causes a decrease in endothelium-dependent relaxations to agonists as compared to blood and Celsior solutions. The use of Celsior and blood has a protective effect on endothelial function at 1

month which could potentially reduce the incidence of coronary allograft vasculopathy compared to other solutions.

Introduction

Although heart transplantation remains the treatment of choice for medically unresponsive terminal heart disease and is associated with a 5-year survival of 70%, graft coronary vasculopathy develops in a majority of transplant recipients and is the main cause of death beyond the first year after transplantation (1-3). The appearance of accelerated atherosclerosis after heart transplantation is preceded by reduced dilatations of the coronary artery to endothelium-dependent agonists, suggesting the presence of an early endothelial dysfunction. When identified, this dysfunction is predictive of the development of graft coronary disease one year after graft implantation and of the occurrence of cardiac events and death (1). The exact mechanisms underlying the coronary endothelial dysfunction that occurs after heart transplantation remain elusive. Patients studied one to five months after graft implantation demonstrate a selective impairment of endothelium-dependent dilatation to acetylcholine, while the dilatation to bradykinin is maintained (4). The time course of coronary endothelial dysfunction in acute untreated rejection is characterized by an endothelial dysfunction initially involving Gi-protein mediated relaxations (5)

developping beyond 5 days after heart transplantation which is due to progressive loss of endothelial cells. The dysfunction worsens over time to affect all endothelial mechanisms and vascular smooth muscle. Other pathways of endothelium-dependent relaxations and vascular smooth muscle cell function also become affected with an increase in the duration of rejection (6).

The numerous factors which may trigger endothelial dysfunction (11,12) after transplantation include cellular and humoral rejection, hyperlipidemia, cytomegalovirus reactivation, and the toxic effects of immunosuppressants such as Cyclosporin A. The process of transplantation itself with exposure of coronary arteries to cardioplegic solutions (13), cold ischemia (14), preservation solutions (15), and reperfusion after implantation (16-18) may cause pathologic activation of the endothelium rendering it dysfunctional.

A retrospective analysis in heart transplant centres in the United Network of Organ Sharing(19) showed that approximately 167 different preservation solutions are used in heart transplantation today. The type of solution used for cardioplegia and preservation has been suspected to play a role in the development of subsequent graft coronary atherosclerosis: specifically, grafts preserved in the intracellular-type (potassium 125

mEq/L) University of Wisconsin solution were more susceptible to developing accelerated atherosclerosis over a 36-month follow-up period than those preserved in extracellular-type (potassium 30 mEq/L) Stanford solution (20). These considerations led Menasché and colleagues (21) to develop an original preservation solution called Celsior which addresses specific issues in heart transplantation. It combines the principles of organ preservation and allows cold storage specific for ischemic/reperfused myocardium and permits a single solution to be used throughout all phases of the transplantation procedure. It is an extracellular type solution with a great antioxidant capacity due to its high content in reduced glutathion which confers a superior preservation of myocardial function by preventing production of free radicals during preservation and at the time of reperfusion (15,22-24). The Celsior formulation was developped with two main principles in mind: first, the prevention of cellular swelling which is achieved by the combination of lactobionate (a chelator of Fe^{++} ion, implicated in the Findel reaction) and mannitol (which also has a small antioxidant effect by scavenging O^{-} singlet) which has a concentration of 140 mmol/L to counterbalance intracellular osmotically active molecules. The second guiding principle is protection from free radical injury which is achieved by the combination of three components: reduced glutathion,

mannitol and histidine. The protective effects on reactive oxygen species production, known to degrade NO, could improve preservation of endothelial-derived NO. Celsior was found to be effective in vitro in an isolated rat heart model and in vivo in a rabbit heterotopic heart transplantation model (25). Clinical efficacy studies are currently underway in Europe and North America but the effects of Celsior on endothelial function and on the development of graft vasculopathy are unknown.

Early development of endothelial vasomotor dysfunction predicts the subsequent development of cardiac allograft vasculopathy 1 year after transplantation (1). Preliminary studies suggest that storage with Celsior solution preserves endothelial function in the cardiac allograft before reimplantation. The effects of Celsior solution on endothelium dependent and independent relaxation early (1 month) after cardiac transplantation have not been evaluated. This study was designed to determine whether a protective effect on donor porcine epicardial coronary artery endothelium is conferred by Celsior cardioplegia versus blood or crystalloid solutions 30 days after heterotopic retroperitoneal transplantation when rejection of the vascular wall affects endothelial reactivity. A period of 30 days was used arbitrarily to allow for early endothelial dysfunction and intimal hyperplasia to occur.

Material and Methods

A. Animals and Immunologic Studies

All experiments were performed using Landrace piglets of either gender, aged 15 ± 2 weeks and weighing 36.6 ± 5 kg. They were conducted in compliance with the "Guide for the care and use of Laboratory Animals" published by the National Institute of Health (NIH publication no. 85-23, revised 1985). All procedures used in this study were approved by the local ethics committee on animal care. Preoperative blood samples were taken for blood typing and determination of the class I antigen of the Swine Lymphocyte Alloantigen (SLA) system by the microlymphocytotoxicity technique. Swine from the same litter were used to ensure a rate of recombination between the class I and class II regions of the major histocompatibility complex of < 1% (Institut National de Recherches Agronomiques). The transplantations were performed between animals compatible for blood type and for the SLA class I antigen.

B. Anesthesia

Premedication was achieved with a mixture of atropine sulfate (0.04 mg/kg) and xylazine (2 mg/kg) injected intramuscularly. Anesthesia was supported by continuous inhalation of 1% isofluorane via orotracheal intubation.

Ventilatory support was established by orotracheal intubation in both the donor and recipient swines with a cuffed tube. Ventilation was maintained with a respirator (Mark 8, Bird Co., Palm Springs, CA. USA) with oxygen supplementation to maintain an arterial oxygen saturation of 95%. Venous access was obtained through the right saphenous vein and right internal jugular vein for volume replacement with Ringer's lactate. Venous blood samples were taken for complete hematological and biochemical profiles. Arterial cannulation was performed in the recipient swine through the right internal carotid artery for blood pressure monitoring and arterial gas analysis. The serum pH was maintained between 7.35 and 7.45 by adjustment of the ventilatory rate and intravenous administration of sodium bicarbonate as needed. Antibiotic prophylaxis was provided with a single intravenous dose of teramycine (10 mg/kg) prior to incision and intramuscular injection in the post-operative period as needed.

C. Cardioplegia

Cardioplegia protocols were established according to three different experimental groups (Table 1). In the first group (n=4), a crystalloid (15 mEq) solution (0.9% saline solution with 15 mEq/L KCl added) was used to induce cardioplegic arrest in the donor via the ascending aorta at a perfusion pressure of 60 mmHg. In Group 2 (n=8), Celsior solution was used to induce

arrest as described above and finally in Group 3 (n=3), donor blood (12 cc/kg) was harvested during the initial phase of anesthesia in a 450 cc bag with 63 cc of citrate dextrose phosphate and kept normothermic. Ringers' Lactate (3 cc/kg) was added to the donor blood for a final blood cardioplegia solution composition of 4:1 blood/crystalloid ratio. Potassium chloride was then added through a Y-tubing to achieve cardioplegia (mean 12 ± 3 mEq) and this solution infused in the ascending aorta as described. The control group were the native hearts harvested from the recipient at the time of sacrifice.

D. Heterotopic Heart Transplantation

Donor: After median sternotomy and pericardial incision, the heart was prepared by dissection and suture control without ligation of the superior and inferior vena cavae, the right and left superior and inferior pulmonary veins and the left hemi-azygous vein (which enters the coronary sinus in the swine). After systemic heparinisation (3 mg/kg), the distal right innominate artery was ligated and proximal portion cannulated with a polyvinyl chloride catheter positioned in the ascending aorta for administration of cardioplegia. The heart was vented by incision of the right and left atrial appendages and the blood vessels isolated previously were ligated. After clamping of the aortic arch between the two innominate arteries, asystole was induced

through injection of cardioplegic solutions according to experimental groups (Table 1) in the ascending aorta at a maximal pressure of 60 mmHg. The heart was vented by incision of the right and left atrial appendages and the blood vessels controled previously were ligated. Local hypothermia was added by flooding the pericardium with cold physiological saline solution (4°C). After excision, the heart was prepared for implantation by ligation of the atrial incisions and dissection of the adventitial tissue between the ascending aorta and the main pulmonary artery and placed in a cold (4°C) solution.

Recipient: After a left subcostal transverse incision, the infrarenal abdominal aorta and vena cava were approached retroperitoneally and dissected free of lymphatic tissue. After systemic heparinisation (3 mg/kg), the infrarenal aorta was clamped by application of a side clamp. End-to-side anastomosis was performed between the donor ascending aorta and recipient abdominal aorta using a running suture of 5-0 polypropylene. After clamping of the donor aorta to avoid immediate reperfusion, the side clamp was released and hemostasis secured. Using the same technique, an end-to-side anastomosis was performed between the donor main pulmonary artery and the inferior vena cava using 6-0 polypropylene. After reperfusion (ischemic time 66.7 ± 15 min), deairing was performed by needle insertion

into the apex and left atrial decompression as needed and normal sinus rhythm reestablished with direct defibrillation as needed. The retroperitoneal cavity and subcutaneous tissues were closed with a 0 vicryl suture.

E. Postoperative care

After standard ventilatory weaning, the animals were left to recover in temperature controlled quarters and fed piglet chow and water *ad libitum.* The animals were examined daily for signs of infection or malaise and were given antibiotics for 5 days if wound infection or core temperature was greater than 38.0°C. The recipients were sacrificed electively on day 30 after transplantation.

F. Explantation protocol and experimental groups

Allograft hearts: After induction of anesthesia with a mixture of ketamine (20 mg/kg) and xylazine (2 mg/kg) injected intramuscularly, venous access, volume replacement and ventilatory support were established as described above. The abdomen was reentered and complete mobilisation of the allograft was performed taking care not to injure the epicardial surface of the heart. The aorta and main pulmonary artery were transected and the allograft explanted and placed in a cold saline solution.

Native hearts were excised through a median sternotomy in the same fashion and placed in a cold physiological solution.

Exclusion criteria: Hearts were excluded if the contractility was absent or if the coronary arteries were thrombosed (n=2 at day 30).

G. Vascular Reactivity

The native and allograft left anterior descending, circumflex, and right coronary arteries were dissected free from the epicardium, myocardium, and adventitial tissue and divided into 4 mm wide rings in a random but matched fashion. Sixteen rings were used for each animal, eight rings for endothelium-dependent agonists serotonin (5-HT) and bradykinin, and eight rings for endothelium-dependent receptor-independent calcium ionophore (A23187). The endothelial function of control and allograft arteries were studied in organ chambers filled with Krebs bicarbonate solution (20 cc at 37°C in the following composition in mmol/L: NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.1, $CaCl_2$ 2.5, $NaHCO_3$ 25 and calcium ethylene-diamine tetraacetic acid 0.026). Oxygenation was insured using a 95% O_2 /5% CO_2 gas mixture. The rings were mounted between two metal stirrups, one of which was connected to an isometric force tansducer. Data were collected with a data acquisition software (IOS3, Emka, Inc., Paris, France). All studies were performed in the presence of indomethacin (10^{-5} mol/liter, to exclude the production of endogenous prostanoids), propranolol (10^{-7} mol/liter, to prevent the activation of β-adrenergic receptors), and ketanserin (incubated 45 minutes before the addition of serotonin: 10^{-6}

mol/liter, to block serotonin 5-HT_2 receptors, which cause contraction of smooth muscle cells in the absence of ketanserin).

Each preparation was stretched to the optimal point of its active length-tension curve (usually 3.5 g), as determined by measuring the contraction to potassium chloride (30 mmol/L) at different levels of stretch, and then stabilized for 30 minutes. The maximal contraction was determined with potassium chloride (60 mmol/L) and rings were excluded if they failed to contract.

After washing and 45 minutes stabilization, prostaglandin F2α (range 2 X 10^{-6} to 10^{-5} mol/L) was added to achieve a contraction averaging 50% of the maximal contraction to KCl. Endothelium-dependent relaxations to serotonin (5-HT; 10^{-10} to 10^{-5} mol/L, an agonist that activates 5-$HT1_d$ receptors coupled to Gi proteins, leading to an increase in NO), bradykinin (BK; 10^{-12} to 10^{-6} mmol/L an agonist that activates B2 receptors coupled to Gq proteins, leading to the production of NO and EDHF), and calcium ionophore (A23187; 10^{-9} to 10^{-6} mol/L an endothelium-dependent receptor-independent agonist, which leads to the release of NO, were compared between native, the three allograft groups, and control coronary rings at 30 days after transplantation. At the end of the organ chamber protocol,

endothelium-independent relaxations were studied using a single bolus of sodium nitroprussiate (SNP; 10-5 mol/L, a NO donor).

H. Morphometric Analysis

After each organ chamber experiments, coronary rings were fixed in 10% formaldehyde for 20 minutes at their optimal tension. All formalin-fixed tissue sections were embedded in paraffin, and five micron sections were stained with orcein. Each section was examined for the presence, extent, and distribution of intimal thickening, luminal narrowing, and inflammatory infiltrates. Histomorphometric studies were performed to evaluate the intima to media surface ratio by light microscopy. Abnormal intimal hyperplasia was associated with an intima to media surface ratio greater than 5%. All histological studies were read in a blinded fashion by an independent observer.

I. Myocardial biopsies and grading of rejection

Surgical myocardial biopsies of allografts and native hearts were taken from the septum, the right and left ventricles of fresh specimens at the time of explantation in allografts and native hearts and fixed in formaldehyde (10%). Hematoxylin eosin-safran staining was performed and the biopsies evaluated for rejection grade, extent of necrosis and ischemic times.

J. Drugs

All solutions were prepared daily. Bradykinin, 5-hydroxytryptamine creatinine sulfate (serotonin), calcium ionophore A23187, indomethacin, ketanserin, and sodium nitroprussiate were purchased from Sigma Chemical Co. (Oakville, Ontario, Canada). Propranolol was purchased from Biomol Research Laboratories, Inc. (Plymouth Meeting, PA) and prostaglandin F2α was purchased from Cayman Chemical Co. (Ann Arbor, MI).

K. Statistical analysis

Contractions to PGF2α are given as a percentage of the maximal contraction to potassium chloride (60 mmol/L) for each group and expressed as mean ± SEM; n refers to the number of animals studied. Relaxations are expressed as percentage of the maximal contraction to PGF2α for each ring. Analysis of variance (ANOVA) studies were performed to compare dose-response curves and differences considered statistically significant at $p<0.05$. Student's *t*-test for paired/unpaired observations was used for statistical analysis in the comparison of the incidence of intimal hyperplasia between different experimental groups and controls.

RESULTS

Vascular Reactivity

Contraction: There were no statistically significant differences in amplitude of contraction to potassium chloride (60 mmol/L) between the different groups (data not shown). There were no statistically significant differences in contraction to PGF2α (range 2 X 10-6 to 10-5 mol/L) between all groups (data not shown).

Endothelium-Dependent Relaxations

Serotonin. There was a statistically significant decrease in endothelium-dependent relaxation to serotonin in the crystalloid, blood, and Celsior cardioplegia groups versus native coronary arteries 1 month after transplantation (Figure 1). There were no significant differences in relaxation between the different study groups.

Bradykinin. There was a statistically significant decrease in endothelium-dependent relaxation to bradykinin in the crystalloid versus the native group 1 month after transplantation. No significant difference in endothelium-dependent relaxation was observed in the blood and Celsior cardioplegia groups versus at 1 month (Figure 2).

Calcium Ionophore. There was a statistically significant decrease in endothelium-dependent relaxation to the calcium ionophore in the

crystalloid versus all other groups in coronary arteries 1 month after transplantation. There was also a statistically significant decrease in endothelium-dependent relaxation to calcium ionophore in the crystalloid, blood, and Celsior groups versus controls (Figure 3).

Endothelium-Independent Relaxations

There were no statistically significant differences in maximal relaxation to sodium nitroprussiate (SNP) (10-5 mol/L) among the groups (data not shown).

Myocardial biopsies

The myocardium from native hearts was normal. Allografted hearts thirty days after implantation showed extensive lymphocytic infiltration with prominent fibrosis and moderate to severe amount of necrosis all compatible with the ISHLT classification grade 3B rejection. There were no significant differences in the rejection grade between all three experimental groups.

Histomorphometric Studies

There were no significant differences in the intima to media ratio between the three cardioplegia groups and controls. There was a trend for the native arteries and the Celsior groups to have less intimal hyperplasia than the blood and crystalloid groups but this did not reach statistical significance.

DISCUSSION

The major findings of this study are that 1) exposure of porcine coronary arteries to cardioplegic arrest with Celsior or sanguineous solutions before heterotopic heart transplantation better preserves endothelial reactivity of epicardial coronary arteries at 30 days under the present experimental conditions compared to crystalloid solution. 2) All three cardioplegic solutions studied induce a significant decrease of endothelium-dependent relaxations to serotonin 30 days after transplantation. 3) Crystalloid cardioplegia induce a generalized endothelial dysfunction to all three agonists and is clearly the most detrimental to the endothelium. There were no significant differences in intimal hyperplasia between experimental and control groups at 30 days.

The type of cardioplegic solution used for graft harvesting has been suggested to play a role in the development of subsequent graft coronary atherosclerosis (18). Celsior combines the principles of organ preservation and allows cold storage specific for ischemic/reperfused myocardium and permits a single solution to be used throughout all phases of the transplantation procedure. Celsior used as a preservation solution has been shown to improve post-operative right ventricular function compared to UW (19) in the allograft, to better preserve ventricular compliance and contractile

function compared to St-Thomas' Hospital solution, and to preserve endothelium-dependent relaxations to acetylcholine compared to Plegisol solution after incubation for 15 hours at 4^0 C (20). A recent study has demonstrated that blood cardioplegia and controlled cardiac graft reperfusion was associated with less post-operative myocardial injury and earlier recovery of function (21). Previous experiments from our group have shown that exposure of porcine coronary arteries to cardioplegic arrest with crystalloid, blood, or Celsior cardioplegic solutions all caused significant decreases in endothelium-dependent relaxations to serotonin and bradykinin but that preservation for 4 hours at 4^0 C with the Celsior solution after induction of cardioplegia with crystalloid or Celsior allowed for functional recovery of endothelium-dependent relaxations to serotonin and bradykinin. No studies have compared Celsior to other cardioplegic solutions in terms of a possible protective effect on coronary endothelial function remote from transplantation.

Effect of Cardioplegic Solution

Perfusion of coronary arteries with either crystalloid, blood, or Celsior solutions to obtain electromechanical silence followed by transplantation in the heterotopic position and explantation at 30 days causes a decrease in Gi-protein mediated relaxations, as demonstrated by reduced responses to

serotonin, which acts by binding to 5-$HT1_d$ receptors in normal coronary arteries. The Gi-protein mediated pathway is the most sensitive pathway to injury and one of the first affected in numerous cardiovascular diseases such as acute and chronic cardiac rejection (10,22), atherosclerosis (23,24), hypercholesterolemia, and ischemia-reperfusion injury and serve as an early marker of pathological activation of the endothelium. The Gq- protein mediated pathway is more resistant to injury, presumably because endothelium-dependent relaxations to agonists such as BK are mediated by the release of both NO and EDHF. Consequently, no significant decrease in endothelium-dependent relaxation to bradykinin was noted at 30 days in the groups arrested with blood or Celsior versus controls. Calcium ionophore-mediated relaxations, exerting their effects via a receptor-independent pathway and representing the final capacity of the endothelium to release NO after eNOS stimulation, were significantly altered in the crystalloid group versus the blood and Celsior groups. Endothelium-dependent relaxations to calcium ionophore were also significantly altered in all groups versus controls. Diminished endothelium-dependent relaxations to the calcium ionophore in all experimental groups may be due to either alterations in the calcium pool or membrane permeability to calcium.

All endothelium-dependent relaxations were impaired in the crystalloid arrest group with reduced responses to serotonin, bradykinin, and the calcium ionophore. This pattern of alteration in endothelial vasodilatory capacity could be due to decreased production of NO, increased degradation of nitric oxide by free radicals, or loss of endothelial cell coverage. Hearts arrested with Celsior showed intact endothelium-dependent relaxations to bradykinin and partially to calcium ionophore probably due to its antioxidant capacity combined with its ability to reduce cell edema (25). These properties however did not prevent the occurrence of a selective endothelial dysfunction of Gi-protein mediated relaxations which is likely due to the effect of immune injury operating in this model (10). Some studies have shown however that ischemia and reperfusion during transplantation may cause a selective decrease in G-protein function that may persist up to 3 months after initial injury (6,26) without loss of endothelial cell lining. This may be due to barotrauma by high perfusion pressures (> 100 mmHg) during cardioplegia, mechanical trauma, or immune injury followed by subsequent endothelial regeneration (26). Regenerated endothelial cells from porcine coronary arteries have a selective impairment of G-protein mediated vasodilatation (24). In some long term transplant survivors (> 5 years) the endothelial function may recover even in the presence of moderate intimal

disease, which suggests that the state of endothelial function may vary with the episodic nature of the immune or other injurious factors (5). Endothelium-independent relaxations mediated by the NO donor sodium nitroprussiate were unaffected attesting to the integrity of the vascular smooth muscle cells which confirms that the reduction of vascular relaxation observed in this study is due to endothelium-dependent mechanisms.

Histomorphometric Studies

Histomorphometric studies comparing native and allograft coronary arteries showed that focal sites of intimal thickening exist constitutively in coronary arteries of young swine and represent physiological intimal cushions at the site of flow turbulence and bifurcations. There was no significant difference in the degree of intimal hyperplasia between coronary arteries in the control versus allograft groups at 30 days although there was a tendency towards a decrease in intimal hyperplasia in the Celsior group.

Limitations

A number of limitations are inherent to this study. In this study, we used a model of porcine heterotopic retroperitoneal "non working" type of cardiac graft in which the coronary arteries are perfused retrogradely through the recipient aorta. No left ventricular filling occurs in the presence of a competent aortic valve because of ligation of the pulmonary veins.

However, non working heterotopic allografts develop an endothelial dysfunction comparable to that of working allografts 60 days after transplantation, and has been validated for the study of coronary endothelial dysfunction (27). Although Celsior in itself is sufficient to induce cardioplegic arrest, potassium was added in the Y tube during arrest, as with the two other cardioplegic solutions, to negate the confounding effect of hyperkalemia on the induction of coronary endothelial function. Endothelial function was evaluated in terms of its ability to vasodilate porcine coronary arteries in response to agonists but the presence of normal endothelium was not ascertained in this study. However intact relaxation to bradykinin in the blood and Celsior groups confirms the presence of an endothelial lining. The ability of blood and Celsior cardioplegia to increase resistance to graft rejection was not investigated in this study. The degree of iNOS expression was not evaluated in this study. NO produced by the inducible form may lead to free radical damage due to the production of peroxynitrite contributing to allograft dysfunction. Clinically relevant ischemic times and preservation conditions were not duplicated in this study but analysis of the effects of the Celsior solution on endothelial function after longer preservation times is warranted. No immunosuppressive drugs were used to minimize potential interactions with endothelial function (28) which enabled

us to isolate the effect of the preservation solution and of immune injury. Finally, studies of the effects of different cardioplegic solutions on coronary microvascular function after transplantation were not evaluated.

Conclusion

Cardioplegic arrest and storage with Celsior or blood constitutes the best strategies for preservation of endothelial function of porcine epicardial arteries after heart transplantation in this model. Crystalloid cardioplegia represents the worst modality in terms of endothelial reactivity in this model. Superior preservation of coronary endothelial function may reduce early graft failure by improving coronary blood flow and serve to limit the development of graft coronary vasculopathy by preserving the protective effects of the endothelium on the vascular wall.

Bibliography

1. Davis S, Yeung A, Meredith I, Charbonneau F, Selwyn P, Anderson T. Early endothelial dysfunction predicts the development of transplant coronary artery disease at 1 year posttransplant. *Circulation* 1996;93:457-62.
2. Billingham M. Cardiac transplant atherosclerosis. *Transplant Proc* 1987;19 (suppl 5):19-25.
3. Young J. Allograft vasculopathy: diagnosing the nemesis of heart transplantation. *Circulation* 1999;3:458-60.
4. Gao SZ, Alderman EL, Schroeder JS, Silverman JF, Hunt SA. Accelerated coronary vascular disease in the heart transplant patient: coronary arteriographic findings. *J Am Coll Cardiol* 1988;12:334-40.
5. Billinham ME. Histopathology of graft coronary disease. *J Heart Lung Transplant* 1992;11:S38-S44.
6. Nitenberg A, Benvenuti C, Aptecar E et al. Acetylcholine-induced constriction of angiographically normal coronary arteries is not

time dependent in transplant recipients. *J Am Coll Cardiol* 1993;22:151-8.

7. Mills RM Jr, Billett JM, Nichols WW. Endothelial dysfunction early after heart transplantation: Assessment with intravascular ultrasound and Doppler. *Circulation* 1992;86:1171-4.
8. Kerber S, Rahmel A, Heinemannvechtel O et al. Angiographic, intravascular ultrasound and functional findings early after orthotopic heart transplantation. *Int J Cardiol* 1995;49:119-29.
9. Anderson TJ, Meredith IT, Uchata A et al. Functional significance of intimal thickening as detected by intravascular ultrasound early and late after cardiac transplantation. *Circulation* 1993;88:1093-100.
10. Perrault LP, Bidouard JP, Janiak P et al. Time course of coronary endothelial dysfunction in acute untreated rejection after heterotopic heart transplantation. *J Heart Lung Transplant* 1997;16:643-57.
11. Drexler H, Fischell TA, Pinto FJ et al. Effect of L-arginine on coronary endothelial function in cardiac transplant recipients, relation to vessel wall morphology. *Circulation* 1994;89:1615-23.

12. Ross R. The pathogenesis of atherosclerosis an update. *N Engl J Med* 1986;314:488-500.

13. Luscher TF, Vanhoutte PM. The endothelium: modulator of cardiovascular function. Boca Raton, FL: CRC Press, 1990:1-228.

14. Furchgott R, Vanhoutte PM. Endothelium-derived relaxing and contracting factors. *FASEB J* 1989;3:2007-18.

15. Dorphin A. Nucleotide binding proteins in signal transduction and disease. *Trends Neurosci* 1987;10:53-7.

16. Rickenbacher P, Pinto F, Lewis N et al. Prognostic importance of intimal thickness as measured by intracoronary ultrasound after cardiac transplantation. *Circulation* 1995;92:3445-52.

17. Mugge A, Brandes R, Heublien B, Nolte C, Haverich A, Lichtlen P. Endothelial dysfunction in heart transplanted patients with graft vasculopathy. *Eur Heart J* 1995;16(suppl J):78-83.

18. Flavahan NA, Shimokawa H, Vanhoutte PM. Pertussis toxin inhibits endothelium-dependent relaxations to certain agonists in porcine coronary arteries. *J Physiol* 1989;408:549-60.

19. Demmy TL, Biddle JS, Bennett LE, Walls JT, Schmaltz RA, Curtis JJ. Organ preservation solutions in heart transplantation:

patterns of usage and related survival. *Transplantation* 1997;63:262.

20. Drinkwater DC, Rudis E, Laks H et al. University of Wisconsin solution versus Stanford cardioplegic solution and the development of cardiac allograft vasculopathy. *J Heart Lung Transplant* 1995;14:891-6.
21. Menasché P, Termignon JL, Pradier F et al. Experimental evaluation of Celsior, a new heart preservation solution. *Eur J Cardiothorac Surg* 1994 ;8 :207-13.
22. Warnecke G, Schulze B, Haverich A, Klima U. Celsior solution provides superior post-ischemic right ventricular function as compared with UW solution in a porcine heart transplantation model. J *Heart Lung Transplant* 2002;21:586-89.
23. Kevalaitis E, Nyborg NC, Menasche P. Coronary endothelial dysfunction of isolated hearts subjected to prolonged cold storage: patterns and contributing factors. *J Heart Lung Transplant* 1999;18:239-47.
24. Cerny S, Pavel P, Pirk J, Hola I et al. Donor myocardial protection using secondary blood cardioplegia during heart transplantation:

prospective randomized study in 100 patients. *J Heart Lung Transplant* 2002;21:61-62.

25. Perrault LP, Bidouard JP, Janiak P et al. Impairment of G-protein mediated signal transduction in the porcine coronary endothelium during rejection after heart transplantation. *Cardiovasc Res* 1999;43:457-70.
26. Flavahan N, Vanhoutte P. Pertussis toxin inhibits endothelium-dependent relaxations evoked by fluoride. *Eur J Pharmacol* 1990 ;178 :121-4.
27. Shimokawa H, Flavahan NA, Vanhoutte PM. Loss of endothelial pertussis toxin sensitive G protein function in atherosclerotic porcine coronary arteries. *Circulation* 1991;83 :652-60.
28. Menasché P, Pradier F, Grousset C et al. Improved recovery of heart transplants with a specific kit of preservation solutions. *J Thorac Cardiovasc Surg* 1993;105:353-63.
29. Evora PRB, Pearson PJ, Schaff HV. Impaired endothelium-dependent relaxation after coronary reperfusion injury: evidence for G-protein dysfunction. *Ann Thor Surg* 1994;57:1550-56.

30. Gohra H, McDonald TO, Verrier E, Aziz S. Endothelial loss and regeneration in a model of transplant arteriosclerosis. *Transplantation* 1995;60:96-102.

31. Perrault LP, Bidouard JP, Desjardins N, Villeneuve N, Vilaine JP, Vanhoutte PM. Comparison of coronary endothelial dysfunction in the working and nonworking graft in porcine heterotopic heart transplantation. *Transplantation* 2002;74(3):1-9.

32. Sudhir K, MacGregor JS, DeMarco T et al. Cyclosporin impairs release of endothelium-derived relaxing factors in epicardial and resistance coronary arteries. *Circulation* 1994;90:3018-23.

Figures and Tables Legends

Table 1: Experimental groups

Figure 1: Cumulative concentration-response curves to serotonin (5-HT) in rings of porcine coronary arteries with endothelium after heterotopic heart transplantation. Responses are given as the percent of relaxation to the contraction induced by prostaglandin F2α. Results are presented as means ± SEM.

Figure 2: Cumulative concentration-response curves to bradykinin (BK) in rings of porcine coronary arteries with endothelium after heterotopic heart transplantation. Responses are given as the percent of relaxation to the contraction induced by prostaglandin F2α. Results are presented as means ± SEM.

Figure 3: Cumulative concentration-response curves to calcium ionophore (A23187) in rings of porcine coronary arteries with endothelium after heterotopic heart transplantation. Responses are given as the percent of relaxation to the contraction induced by prostaglandin F2α. Results are presented as means ± SEM.

Table 1:

Groups	Cardioplegia	Preservation
1 Control (n=12)	None	Saline
2 (n=4)	Crystalloid	Saline
3 (n=3)	Blood	Saline
4 (n=8)	Celsior	Celsior

FIGURE 1

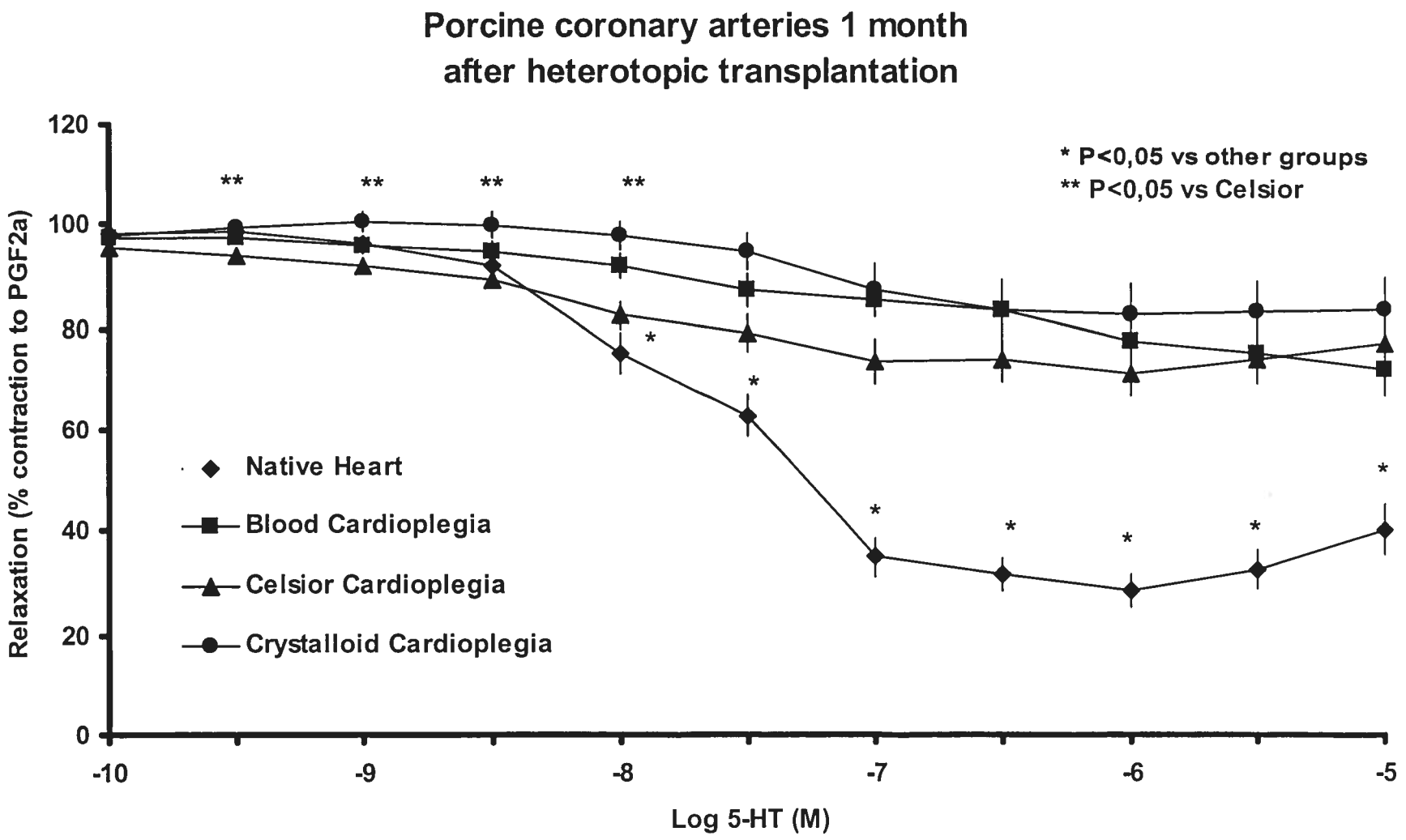

Porcine coronary arteries 1 month
after heterotopic transplantation
* P<0,05 vs other groups
** P<0,05 vs Celsior
Relaxation (% contraction to PGF2a)
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
Log 5-HT (M)
Native Heart
Blood Cardioplegia
Celsior Cardioplegia
Crystalloid Cardioplegia

FIGURE 2

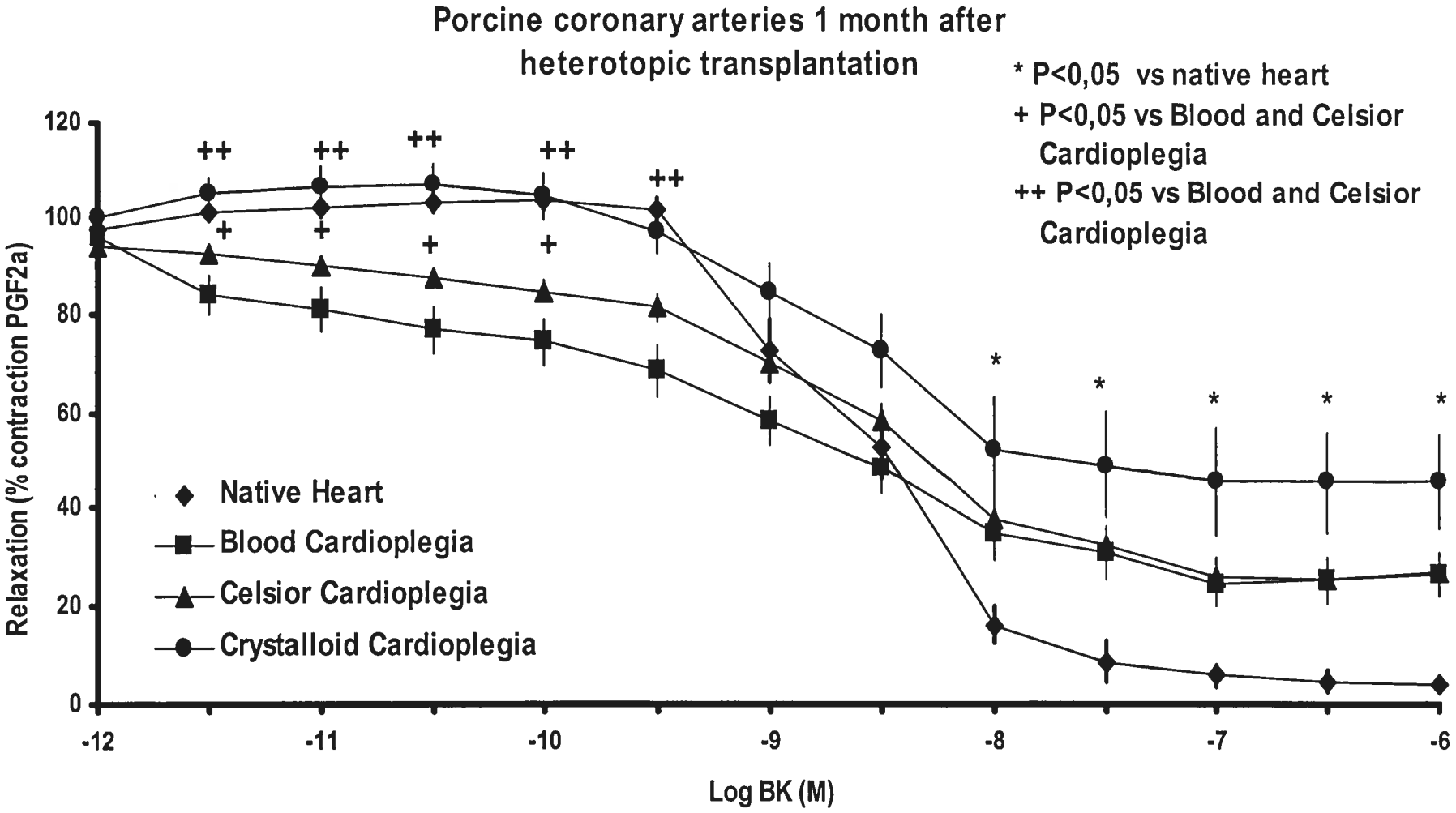
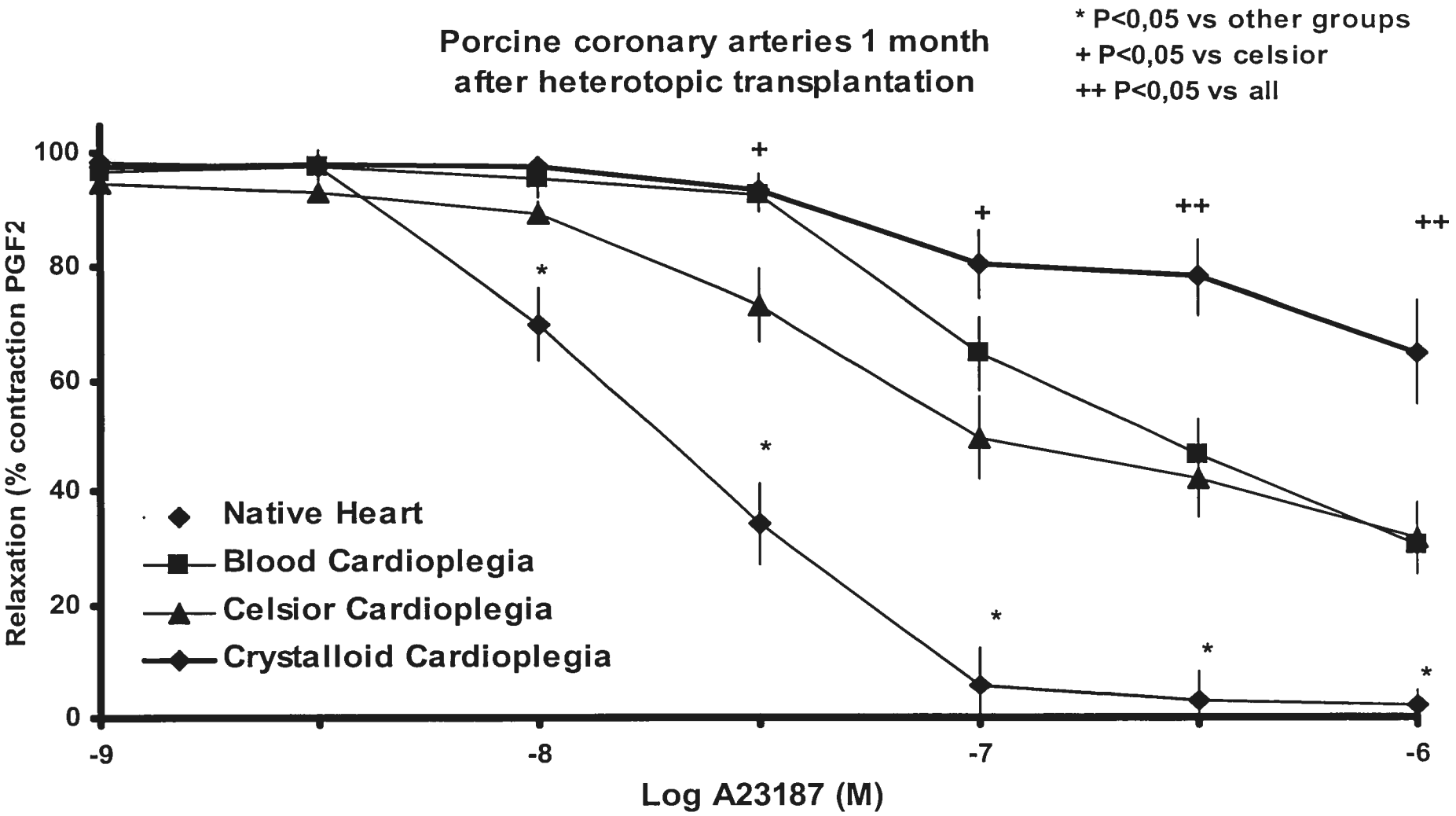

Porcine coronary arteries 1 month after heterotopic transplantation
* P<0,05 vs native heart
+ P<0,05 vs Blood and Celsior Cardioplegia
++ P<0,05 vs Blood and Celsior Cardioplegia
Native Heart
Blood Cardioplegia
Celsior Cardioplegia
Crystalloid Cardioplegia
Relaxation (% contraction PGF2a)
Log BK (M)
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
-6

FIGURE 3


Porcine coronary arteries 1 month
after heterotopic transplantation
* P<0,05 vs other groups
+ P<0,05 vs celsior
++ P<0,05 vs all
Relaxation (% contraction PGF2
100
80
60
40
20
0
-9
-8
-7
-6
Log A23187 (M)
Native Heart
Blood Cardioplegia
Celsior Cardioplegia
Crystalloid Cardioplegia

Conclusion

Although heart transplantation remains the treatment of choice for medically unresponsive terminal heart disease and is associated with a 5-year survival of 70%, coronary allograft disease develops in a majority of transplant recipients and is the main cause of death beyond the first year after transplantation. The appearance of accelerated atherosclerosis after heart transplantation is preceded by reduced dilatations of the coronary artery to endothelium-dependent agonists, suggesting the presence of an early endothelial dysfunction. When identified, this dysfunction is predictive of the development of graft coronary disease one year after graft implantation and of the occurrence of cardiac events and death. The exact mechanisms underlying the coronary endothelial dysfunction occurring after heart transplantation remain elusive. The time course of coronary endothelial dysfunction in acute untreated rejection has been characterized and is due to a progressive destruction of the endothelial lining 5 days after heart transplantation which initially involves Gi-protein mediated relaxations. The dysfunction worsens over time to affect all endothelial mechanisms and the vascular smooth muscle. The process of transplantation itself exposes coronary arteries to cardioplegic solutions, cold ischemia, preservation solutions, and reperfusion after implantation which may cause pathologic

activation of the endothelium rendering it dysfunctional. Preservation solutions have been incriminated in the development of allograft vasculopathy; grafts preserved in intracellular-type (potassium 125 mEq/L) University of Wisconsin solution were more susceptible to developing accelerated atherosclerosis over a 36-month follow-up period than those preserved in extracellular-type (potassium 30 mEq/L) Stanford solution. Hyperkalemic preservation solutions can be injurious to the vascular endothelium by causing direct endothelial denudation and long lasting effects with the regenerated endothelium failing to recover normal reactivity and exhibiting an increased tendency to vasospasm. Other mechanisms involved in initial endothelial injury during cardiac allograft harvesting include cellular swelling and direct free radical injury on the endothelium. Celsior solution could potentially protect the endothelium against these injurious stimuli through its antioxidant properties. The two studies presented in this manuscript address this hypothesis by examining the protective role of Celsior on porcine coronary artery endothelial reactivity following cardioplegia and preservation and the chronic effects of Celsior on endothelial function 1 month after heterotopic heart transplantation in a porcine model.

The first study was designed to assess the acute effects of different cardioplegic (Celsior, blood and crystalloid) and preservation (saline, Celsior) solutions on porcine coronary endothelial function. The findings indicate that cardioplegia alone using either Celsior, blood, or crystalloid solutions, causes a decrease in endothelium-dependent relaxations to both serotonin, 5-HT, an agonist that activates 5-$HT1_d$ receptors coupled to Gi-proteins, and bradykinin, BK, which activates B2 receptors coupled to Gq-proteins in porcine coronary arteries. Subsequent preservation in saline does not produce a recovery of endothelium-dependent relaxations. Induction of arrest and preservation with Celsior allows for functional recovery of endothelium-dependent relaxations to both serotonin and bradykinin and thus better preserves the endothelial function of coronary arteries under these experimental conditions. The beneficial properties of Celsior are likely related to its antioxidant capacity, limiting free radical injury during ischemia and reducing cell edema. Use of this new preservation solution could protect grafts from the deleterious effects of preservation as suggested by a clinical study in which use of Celsior was associated with an increased inotropic and vasodilatory response in transplanted patients in contrast to the University of Wisconsin solution. The initial endothelial dysfunction to cardioplegic agents observed was not caused by irreversible cell injury and denudation but was

most likely the result of barotrauma and cellular edema caused by the injection of solution in the coronary arteries. Celsior preservation allowed functional recovery of endothelium-dependent relaxations to bradykinin, an agonist whose vasodilatatory action is mediated by a Gq-protein pathway known to be more resistant to injury, but also to serotonin, one of the first pathways affected in many cardiovascular diseases, such as acute and chronic rejection and atherosclerosis. Therefore use of the Celsior solution preserves endothelial function when used during cardioplegia and preservation compared to saline in a porcine model.

Whether use of Celsior for harvesting during transplantation preserved graft coronary endothelial function and limited intimal hyperplasia remotely from the time of graft implantation remained to be determined. Specifically, does Celsior protect the endothelium against reperfusion injury and do these effects persist 1 month after transplantation ? The second study was designed to answer this question and examined the effects of Celsior, crystalloid and sanguineous cardioplegic solutions on porcine coronary endothelial reactivity and the development of graft intimal hyperplasia one month after heterotopic transplantation. The major findings of this study are that 1) use of Celsior or blood-based cardioplegic solutions confer a superior protection of epicardial coronary endothelial function one month after heart

transplantation compared to a crystalloid solution. 2) All three preservation strategies are associated with a significant decrease of Gi-protein mediated (5HT) endothelium-dependent relaxations. 3) The use of a crystalloid solution is associated with a significant decrease of relaxation to the receptor-independent endothelium-dependent agonist A23187. 4) There were no significant differences in allograft coronary intimal hyperplasia between experimental groups. Hearts arrested with Celsior showed intact endothelium-dependent relaxations to bradykinin and partially to calcium ionophore probably due to its antioxidant capacity combined with its ability to reduce cellular edema, although these properties did not prevent the occurrence of an endothelial dysfunction to Gi-protein mediated relaxation. The decreased endothelium-dependent relaxations to serotonin are likely due to graft rejection that occurs in the grafts as the swine were not treated with immunosuppressants during the study period as previously published (30). However, some studies have shown that ischemia and reperfusion during transplantation may cause a selective decrease in Gi-protein function that may persist up to 3 months after initial injury without loss of endothelial cell lining which may be due to barotrauma by high perfusion pressures (> 100 mmHg) during cardioplegia, mechanical trauma, or immune injury followed by subsequent endothelial regeneration since regenerated endothelial cells

from porcine coronary arteries have a selective impairment of G-protein mediated vasodilatation.

To date, only one study has examined the comparative role of cardioplegic solutions on coronary endothelial function one month after heart transplantation. The authors found similar results when comparing Celsior and UW solutions. However, immunosuppressive regimens were different and no data was available regarding the degree of rejection in both groups. In the present study, there were no significant differences in the degree of rejection between the three experimental groups which showed grade 3B rejection at myocardial biopsy. Consequently, observed differences in endothelial function are due to the type of arrest and preservation solutions used at the time of implantation and not due to differences in the degree of immune injury. Clinically relevant ischemic times and preservation conditions were not duplicated in this study to guarantee maximum graft survival at 30 days but analysis of the effect of different solutions on coronary endothelial function and intimal hyperplasia after longer preservation times are warranted. No immunosuppressive drugs were used to avoid interactions on coronary endothelial function. Since agents currently used in clinical heart transplantation such as cyclosporin A, tacrolimus, sirolimus and mycophenolate mofetil have direct effects on

coronary endothelial function, experimental protocols integrating use of these agents should be devised. Also, the impact of statins and calcium channel antagonists, both used clinically in all patients after heart transplantation and proven to diminish the long term occurrence of intimal hyperplasia and coronary allograft vasculopathy, was not evaluated in this study. These drugs could have a beneficial effect on endothelial function even one month after the initial transplant procedure and these agents should be evaluated in future studies.

In conclusion, cardioplegic arrest with Celsior solution or blood and preservation with Celsior constitutes the best strategy for preservation of endothelium-dependent relaxations in porcine epicardial coronary arteries compared to crystalloid solutions 30 days after heart transplantation despite similar degrees of rejection. Superior preservation of coronary endothelial function may reduce early graft failure and limit the development of CAV by maintaining the protective homeostatic effects of the endothelium on the vascular wall. This in turn could lead to a better long term survival of patients after heart transplantation and less complications related to accelerated atherosclerosis. Better cardioplegic and preservation solutions are one modality on which focus can be given in order to better preserve coronary endothelial function and diminish the incidence of CAV but one

must not forget that CAV is a multifactorial process that involves preoperative, intraoperative, and postoperative factors and each of these elements must be targeted for a more efficacious prevention of CAV.

Bibliography

1. Furchgott, RF, Zawadski JV. The obligatory role of the endothelial cells in relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; 288: 373-376
2. Luscher TF, Vanhoutte PM. *The endothelium modulator of cardiovascular function.* Boca Raton, Florida: CRC Press, 1990: 1-215
3. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987; 327: 524
4. Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988; 333: 664
5. Yang Z, Von Segesser L, Bauer G, Stulz P, Tschudi M, Luscher TF. Different activation of endothelial L-arginine and cyclooxygenase pathway in human internal mammary artery and saphenous vein. *Circ Res* 1991; 68: 52
6. Linder L, Kiowski W, Buhler FR, Luscher TF. Indirect evidence for release of endothelium-derived relaxing factor in human forearm circulation in vivo: blunted response in essential hypertension. *Circulation* 1990; 81: 1762

7. Bredt DS, Hwang PM, Glatt CE, Lowenstein C, Reed RR, Snyder SH. Cloned and expressed nitric oxide synthase structurally resembles cytochrome P-450 reductase. *Nature* 1991; 351: 714

8. Luscher TF, Noll G. Endothelium dysfunction in the coronary circulation. *J Cardiovasc Pharm* 1994;24(suppl 3):S16-S26.

9. Sanders DB, Kelley T, Larson D. The role of nitric oxide synthase/nitric oxide in vascular smooth muscle control. *Perfusion* 2000;15:97-104.

10. Sanders DB, Larson DF, Jablonowski C, Olson L. Differential expression of inducible nitric oxide synthase in septic shock. *J Extracorp Technol* 1999;31:118-24.

11. Redmond EM, Cahill PA, Sitzman JV. Flow-mediated regulation of G-protein expression in cocultured vascular smooth muscle and endothelial cells. *Arterioscler Thromb Vasc Biol* 1998;18:75-83.

12. Levitski A, Bar-Sinai A. The regulation of adenyl cyclase by receptor G proteins. *Pharmacol Ther* 1991;50:271-83.

13. Helmreich EJ, Hofmann KP. Structure and function of proteins in G-protein coupled signal transfer. *Biochem Biophys Acta* 1996;1286:285-322.

14. Rapoport RM, Murad F. Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. *Circ Res* 1983;52:352.

15. Pallas F, Larson DF. Cerebral blood flow in the diabetic patient. *Perfusion* 1996;11:363-70.

16. Schmidt HW, Lohmann SM, Walter U. The nitric oxide and cGMP signal transduction system: regulation and mechanism of action. *Biochim Biophys Acta* 1993;1178:153-75.

17. Lincoln TM, Cornwell TL. Intracellular cyclic GMP receptor proteins. *FASEB J* 1993;7:328-38.

18. Janiak P, Perrault LP, Gransagne D, Bidouard JP, Villeneuve N, Vilaine JP, Vanhoutte PM. Expression of matrix metalloproteinases in coronary arteries after balloon denudation and heart transplantation. *Circulation* 1996;(suppl I):I-586.

19. Perrault LP, Mahlberg F, Breugnot C, Bidouard JP et al. Hypercholesterolemia increases coronary endothelial dysfunction, lipid content, and accelerated atherosclerosis after heart transplantation. *Arterioscler Thromb Vasc Biol* 2000;20:728-736.

20. Busse R, Luckhoff A, Bassenge E. Endothelium-derived relaxant factor inhibits platelet activation. *Naunyn Schmiedebergs Arch Phamacol* 1987;336:566.

21. Radomski MW, Palmer RMJ, Moncada S. The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br J Pharmacol* 1987;92:639-46.

22. Radomski MW, Palmer RMJ, Moncada S. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet* 1987;2:1057-68.

23. Macdonald PS, Read MA, Dusting GJ. Synergistic inhibition of platelet aggregation by endothelium-derived relaxing factor and prostacyclin. *Thromb Res* 1988;49:437.

24. Forstermann U, Mugge A, Alheid U et al. Selective attenuation of endothelium-mediated vasodilatation in atherosclerotic human coronary arteries. *Circ Res* 1988;62:185.

25. Yang Z, von Segesser L, Bauer E et al. Different interactions of platelets with arterial and venous coronary bypass vessels. *Lancet* 1991;337:939.

26. Flavahan NA, Vanhoutte PM. G-protein and endothelial responses. *Blood Vessels* 1990;27:218-229.

27. Flavahan NA, Vanhoutte PM. Endothelial cell signaling and endothelial dysfunction. *Am J Hypertens* 1995;8:S28-S41.

28. Perrault LP, Bidouard JP, Janiak P et al. Impairment of G-protein mediated signal transduction in the porcine coronary endothelium during rejection after heart transplantation. *Cardiovasc Res* 1999;43:457-70.

29. Perrault LP, Bidouard JP, Janiak P et al. Time course of coronary endothelial dysfunction in acute untreated rejection after heterotopic heart transplantation. *J Heart Lung Transplant* 1997;16:643-57.

30. Flavahan N, Vanhoutte PM. Pertussis toxin inhibits endothelium-dependent relaxations evoked by fluoride. *Eur J Pharmacol* 1990;178:121-4.

31. Shimokawa H, Flavahan N, Vanhoutte PM. Loss of endothelial pertussis toxin-sensitive G protein function in the atherosclerotic porcine coronary arteries. *Circulation* 1991;83:652-60.

32. Vane JR. The Croonian Lecture, 1993. The endothelium: maestro of the blood circulation. *Phil Trans R Soc Lon B* 1994;348:225-246.

33. Moncada S, Vane JR. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A2 and prostacyclin. *Pharmacol Rev* 1979;30:293-331.

34. Feletou M, Vanhoutte PM. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. *Br J Pharmacol* 1988;93:515-524.

35. Komori K, Vanhoutte PM. Endothelium-derived hyperpolarizing factor. *Blood Vessels* 1990;27:238-45.

36. Nagao T, Vanhoutte PM. Endothelium-derived hyperpolarizing factor and endothelium-dependent relaxations. *Am J Resp Cell Mol Biol* 1993;8:1-6.

37. Nakashima M, Mombouli JV, Taylor AA, Vanhoutte PM. Endothelium-dependent hyperpolarization caused by bradykinin in human coronary arteries. *J Clin Invest* 1993;92:2867-2871.

38. Vanhoutte PM. Endothelium-derived hyperpolarizing factor, Ed. Harwood Academic Publishers, The Netherlands,1996.

39. Vanhoutte PM. The end of the quest? *Nature* 1989.

40. Tare M, Parkington HC, Coleman HA, Neild TO, Dusting GJ. Hyperpolarization and relaxation of arterial smooth muscle caused by NO derived from the endothelium. *Nature* 1990;346:69.

41. Standen NB, Quayle JM, Davies NW et al. Hyperpolarizing vasodilators activate ATP-sensitive potassium channels in arterial smooth muscles. *Science* 1989;245:177.

42. Campbell WB, Gebremedhin D, Pratt PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing. *Circ Res* 1996;78:415-23.

43. Luscher TF, Boulanger CM, Dohi Y, Yang Z. Endothelium-derived contracting factors. *Hypertension* 1991;19:117.

44. Yanagisawa M, Kurihara H, Kimura S et al. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988;332:411.

45. Kimura S, Kasuya Y, Sawamura T et al. Structure-activity relationships of endothelin : importance of the c-terminal moiety. *Biochem Biophys Res Commun* 1988;156:1182.

46. Boulanger C, Luscher TF. Release of endothelin from the porcine aorta: inhibition by endothelium-derived nitric oxide. *J Clin Invest* 1990;85:587.

47. Kasuya Y, Ishikawa T, Yanagisawa M et al. Mechanism of contraction to endothelin in isolated porcine coronary artery. *Am J Physiol* 1989;257:H1828.

48. Luscher TF, Yang Y, Tschudi M et al. Interaction between endothelin-1 and endothelium-derived relaxing factor in human arteries and veins. *Circ Res* 1990;66:1088.

49. Davis S, Yeung A, Meredith I, Charbonneau F, Selwyn P, Anderson T. Early endothelial dysfunction predicts the development of transplant coronary artery disease at 1 year posttransplant. *Circulation* 1996;93:457-62.

50. Billingham M. Cardiac transplant atherosclerosis. *Transplant Proc* 1987;19(suppl 5):19-25.

51. Young J. Allograft vasculopathy: diagnosing the nemesis of heart transplantation. *Circulation* 1999;3:458-60.

52. Gao SZ, Alderman EL, Schroeder JS, Silverman JF, Hunt SA. Accelerated coronary vascular disease in the heart transplant patient: coronary arteriographic findings. *J Am Coll Cardiol* 1988;12:334-40.

53. Billingham ME. Histopathology of graft coronary disease. *J Heart Lung Transplant* 1992;11: S38-S44.

54. Yeung AC, Davis SF, Hauptmann PJ et al. Incidence and progression of transplant coronary artery disease over 1 year : results of a multicenter trial with the use of intravascular ultrasound. *J Heart Lung Transplant* 1995;14:S215-S220.

55. Pucci AM, Forbes RDC, Billingham ME. Pathologic features in long term cardiac allograft. *J Heart Transplant* 1990;9:339-345.

56. Billingham ME. Histopathology of graft coronary disease. *J Heart Lung Transplant* 1992;11(3):S38-S44.

57. Billingham ME. Pathology and etiology of chronic rejection of the heart. *Clin Transplant* 1994;8:289-92.

58. Billingham ME. Pathology of graft vascular disease after heart and heart-lung transplantation and its relationship to obliterative bronchiolitis. *Transplant Proc* 1995;27(3):2013-16.

59. Billingham ME. Cardiac transplant atherosclerosis. *Transplant Proc* 1987;19(4):19-25.

60. Billingham ME. Graft coronary disease: lesions and the patients. *Transplant Proc* 1989;21(4):3665-6.

61. Johnson DE, Gao SZ, Schroeder JS et al. The spectrum of coronary artery pathologic findings in human cardiac allografts. *J Heart Transplant* 1989;8:349-59.

62. Mc Manus BM, Lin H, Wilson JE, Kendall TJ. Comparable proximal and distal severity of intimal thickening and size of epicardial coronary arteries in transplant arteriopathy of human cardiac allografts. *J Heart Lung Transplant* 1994;13(5):824-33.

63. Nitenberg A, Benvenuti C, Aptecar E et al. Acetylcholine-induced constriction of angiographically normal coronary arteries is not time dependent in transplant recipients. *J Am Coll Cardiol* 1993;22:151-8.

64. Mills RM, Billett JM, Nichols WW. Endothelium dysfunction early after heart transplantation: Assessment with intravascular ultrasound and Doppler. *Circulation* 1992;86:1171-4.

65. Benvenuti C, Aptecar E, Mazucotelli JP et al. Coronary artery response to cold-pressure test is impaired early after operation in heart transplant recipients. *J Am Coll Cardiol* 1995;26:446-51.

66. Vassalli G, Gallino A, Kiowski W et al. Reduced coronary flow reserve during exercise in heart transplant recipients. *Circulation* 1997;95:607-13.

67. Kerber S, Rahmel A, Heinemannvechtel O et al. Angiographic, intravascular ultrasound and functional findings early after orthotopic heart transplantation. *Int J Cardiol* 1995;49:119-29.

68. Anderson TJ, Meredith IT, Uchata A et al. Functional significance of intimal thickening as detected by intravascular ultrasound early and late after cardiac transplantation. *Circulation* 1993;88:1093-1100.

69. Evora PRB, Pearson PJ, Schaff HV. Impaired endothelium-dependent relaxation after coronary reperfusion injury: evidence for G-protein dysfunction. *Ann Thorac Surg* 1994;57:1550-6.

70. Heroux AL, Silverman P, Costanzo MR et al. Intracoronary ultrasound assessment of morphological and functional abnormalities associated with cardiac allograft vasculopathy. *Circulation* 1994;89:272.

71. Libby P, Tanaka H. The pathogenesis of coronary arteriosclerosis (chronic rejection) in transplanted hearts. *Clin Transplant* 1994;8:313-18.

72. Hosenpud JD, Shipley GD, Wagner CR. Cardiac allograft vasculopathy: Current concepts, recent developments, and future directions. *J Heart Lung Transplant* 1992;11:9-23.

73. Schutz A, Kemkes BM, Kugler CH, Angermann C et al. The influence of rejection episodes on the development of coronary artery disease after heart transplantation. *Eur J Cardiothorac Surg* 1990;4:300-308.

74. Ross R. The pathogenesis of atherosclerosis an update. *N Engl J Med* 1986;314:488-500.

75. Luscher TF, Vanhoutte PM. The endothelium: modulator of cardiovascular function. Boca Raton, FL: CRC Press, 1990: 1-228.

76. Furchgott R, Vanhoutte PM. Endothelium-derived relaxing and contracting factors. *FASEB J* 1989;3:2007-18.

77. Dorphin A. Nucleotide binding proteins in signal transduction and disease. *Trends Neurosci* 1987;10:53-7.

78. Rickenbacher P, Pinto F, Lewis N et al. Prognostic importance of intimal thickness as measured by intracoronary ultrasound after cardiac transplantation. *Circulation* 1995;92:3445-52.

79. Mugge A, Brandes R, Heublien B et al. Endothelial dysfunction in heart transplanted patients with graft vasculopathy. *Eur Heart J* 1995;16(suppl J):78-83.

80. Flavahan NA, Shimokawa H, Vanhoutte PM. Pertussis toxin inhibits endothelium-dependent relaxations to certain agonists in porcine coronary arteries

81. Braunwald E, Kloner RA. Myocardial reperfusion: a double-edged sword? *J Clin Invest* 1985;76:1713.

82. Ardehali A, Laks H, Drinkwater DC, et al. Expression of major histocompatibility antigens and vascular adhesion molecules on human cardiac allografts preserved in University of Wisconsin solution. *J Heart Lung Transplant* 1993;12:1044-1052.

83. Day JD, Rayburn BK, Gaudin PB et al. Cardiac allograft vasculopathy: the central role of ischemia-induced endothelial cell injury. *J Heart Lung Transplant* 1995;14:S142-S149.

84. Schacter M, Foulds S. Free radicals and the xanthine oxidase pathway, in Grace PA, Mathie RT (eds): Ischemia-reperfusion injury. Oxford, Blackwell Science, 1999, p137.

85. Moore F, Caine WT. Emerging immunologic basis of reperfusion injury. *Transplantation Reviews* 2000;14(3):158-167.

86. Johnson ML, Billiar TR. Roles of nitric oxide in surgical infection and sepsis. *World J Surg* 1998;22:187.

87. Briganti EM, Bergin PJ, Rosenfeldt FL, et al. Successful long-term outcome with prolonged ischemia time cardiac allografts. *J Heart Lung Transplant* 1995;14:840.

88. Kirklin JK, Young JB, McGiffin DC. Heart Transplantation (Chapter 17). Churchill Livingstone. 2002;615-665.

89. Collins T. Endothelial nuclear factor-kappa B and the initiation of the atherosclerotic lesion. Lab Invest 1993;68:499-508.

90. Ross R, Glomset J. Atherosclerosis and the arterial smooth muscle cell. Science 1973;180:1332.

91. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801-809.

92. Kawai A, Morita S, Kormos RL, et al. A clinical trial comparing University of Wisconsin solution and cold cardioplegic solution with

load-independent mechanical parameters. *J Heart Lung Transplant* 1994;13:150.

93. Todo S, Nery J, Yanaga K, et al. Extended preservation of human liver grafts with UW solution. *JAMA* 1989;261:711.

94. Ploeg RJ, van Bockel JH, Langendijik PT et al. Effect of preservation solution on results of cadaveric kidney transplantation. *Lancet* 1992;340:129.

95. Demmy TL, Biddle JS, Bennett LE, et al. Organ preservation solutions in heart transplantation : patterns of usage and related survival. *Transplantation* 1997;63:262.

96. Hosenpud JD, Bennett LE, Keck BM, et al. The registry of the International Society for Heart and Lung Transplantation: Fifteenth Official Report-1998. *J Heart Lung Transplant* 1998;17:656.

97. Nilsson FN, Miller VM, Vanhoutte PM, McGregor CG. Methods of cardiac preservation alter the function of the endothelium in porcine coronary arteries. *J Thorac Cardiovasc Surg* 1991;102:923-30.

98. Drinkwater DC, Rudis E, Laks H et al. University of Wisconsin versus Stanford cardioplegic solution and the development of cardiac allograft vasculopathy. *J Heart Lung Transplant* 1995;14:891-6.

99. Menasché P, Pradier F, Grousset C, et al. Improved recovery of heart transplants with a specific kit of preservation solutions. *J Thorac Cardivasc Surg* 1993;105:353-63.

100. Martin DR, Scott DF, Downes GL, Belzer FO. Primary cause of unsuccessful liver and heart preservation: cold sensitivity of the ATPase system. *Ann Surg* 1972;175:111-7.

101. Belzer FO, Hoffman RM, Southard JH. Kidney preservation. *Surg Clin North Am* 1978;58:261-71.

102. Wicomb WN, Collins AB, Tokunaga Y, Esquivel C. Choice of cation in solutions for hypothermic storage of liver and heart: high-sodium versus high-potassium. *Transplantation* 1991;51:281-2.

103. Kohno H, Shiki K, Ueno Y, Tokunaga K. Cold storage of the rat heart for transplantation. Two types of solution required for optimal preservation. *J Thorac Cardiovasc Surg* 1987;93:86-94.

104. Toshima Y, Kohno H, Matsuzaki K, et al. Collins solution for cold storage of the heart for transplantation must be reversed with cardioplegic solution before reperfusion. A functional and metabolic study in the rat heart. *J Thorac Cardiovasc Surg* 1992;104:1572-81.

105. Swanson DK, Myerowitz D, Watson KM, et al. A comparison of blood and crystalloid cardioplegia during heart transplantation after 5 hours of cold storage. *J Thorac Cardiovasc Surg* 1987;93:687-694.

106. Menasché P, Termignon JL, Pradier F, et al. Experimental evaluation of Celsior, a new heart preservation solution. *Eur J Cardiothor Surg* 1994;8:207-213.

107. Nilsson FN, Miller VM, Vanhoutte PM, McGregor GA. Methods of cardiac preservation alter the function of the endothelium in porcine coronary arteries. *J Thorac Cardiovasc Surg* 1991;102:923-30.

108. Michel P, Hadour G, Rodriguez C, et al. Evaluation of a new preservative solution for cardiac graft during hypothermia. *J Heart Lung Transplant* 2000;19:1089-97.

109. Mohara J, Takahashi T, Oshima K,et al. The effect of Celsior solution on 12-hour cardiac preservation in comparison with University of Wisconsin solution. *J Cardiovasc Surg* 2001;42:187-92.

110. Warnecke G, Schulze B, Haverich A, Klima U. Celsior solution provides superior post-ischemic right ventricular function as compared with UW solution in a porcine heart transplantation model. *J Heart Lung Transplant* 2002;21:586-589.

111. Alamanni F, Parolari A, Visigalli R, et al. Endothelial cell injury induced by preservation solutions : a confocal microscopy study. *Ann Thorac Surg* 2002;73:1606-15.

112. Kevelaitis E, Nyborg N, Menasche P. Coronary endothelial dysfunction of isolated hearts subjected to prolonged cold storage : Patterns and contributing factors. *J Heart Lung Transplant* 1999;18:239-47.

113. Keck B, White R, Breen T, et al. Thoracic organ transplants in the United States: a report from the UNOS/ISHLT Scientific Registry for Organ Transplants, United Network for Organ Sharing, International Society for Heart and Lung Transplantation. *Clin Transplant* 1994;37-46.

114. Remadi JP, Baron O, Roussel JC, Habash O, et al. Myocardial preservation using Celsior solution in cardiac transplantation : Early results and 5-year follow-up of a multicenter prospective study of 70 cardiac transplantations. *Ann Thorac Sur*g 2002;73:1495-9.

115. Davis SF, Yeung AC, Meredith IT, et al. Early endothelial dysfunction predicts the development of transplant coronary artery disease at 1 year post-transplant. *Circulation* 1996;93:457-62.

Table 1 : Composition and Characteristics of Celsior solution [a]

Component	Concentration
Mannitol	60
Lactobionate	80
Glutamic acid	20
Histidin	30
Reduced glutathione	3
Potassium	15
Sodium	100
Magnesium	13
Calcium	0.25
Chloride	41.5
PH (at 20°C)	7.3
Osmolarity (mOsm/L)	360

[a] Concentrations are given as millimoles per liter.

ENDOTHELIUM-DEPENDENT RESPONSES

(not present in all blood vessels)

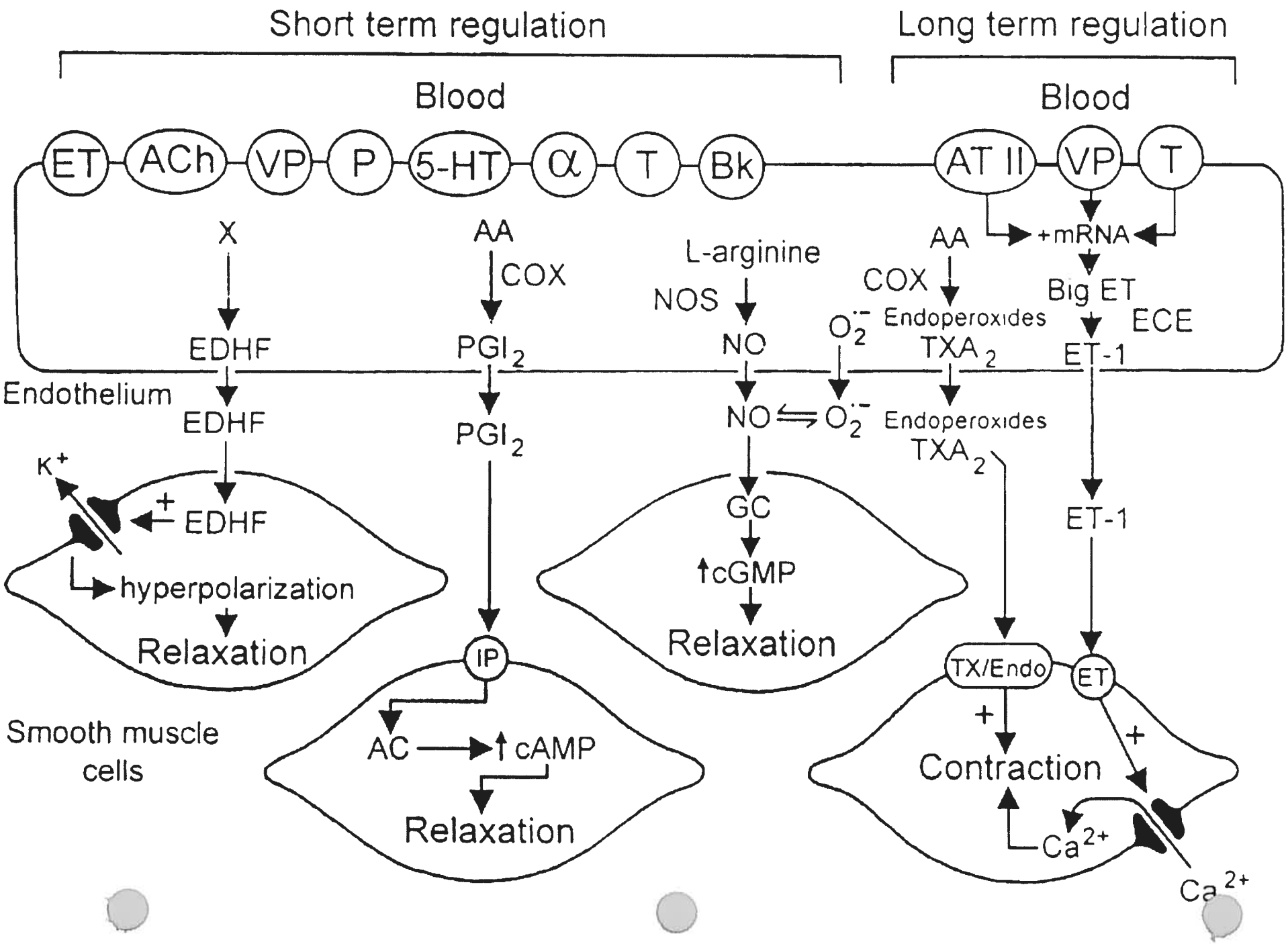


Figure 2

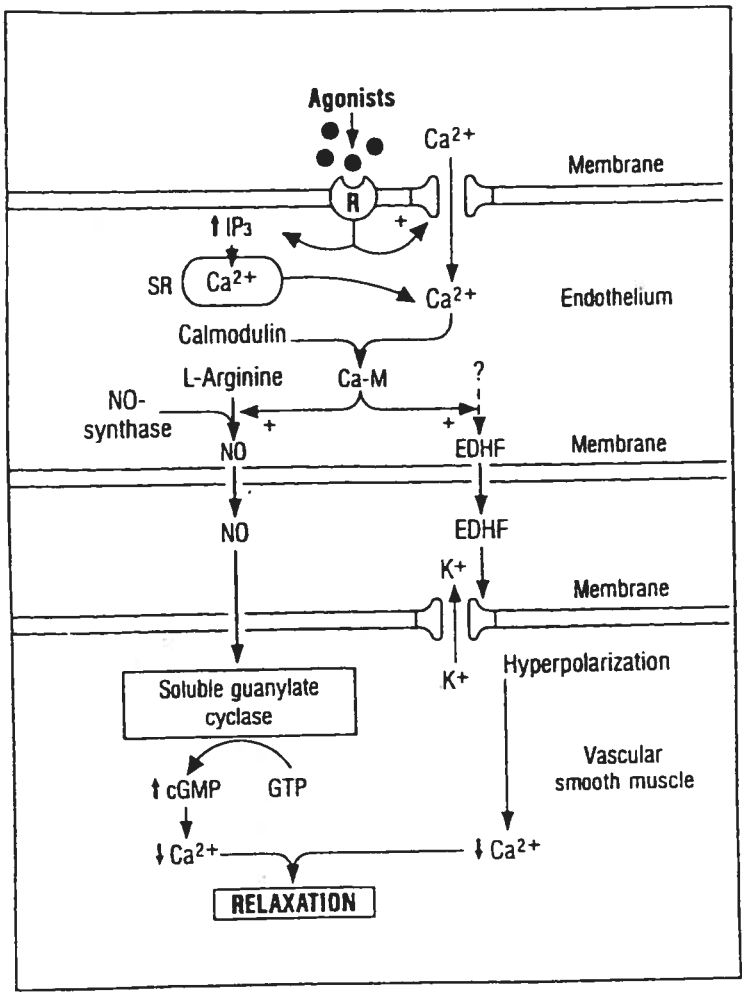


Figure 3

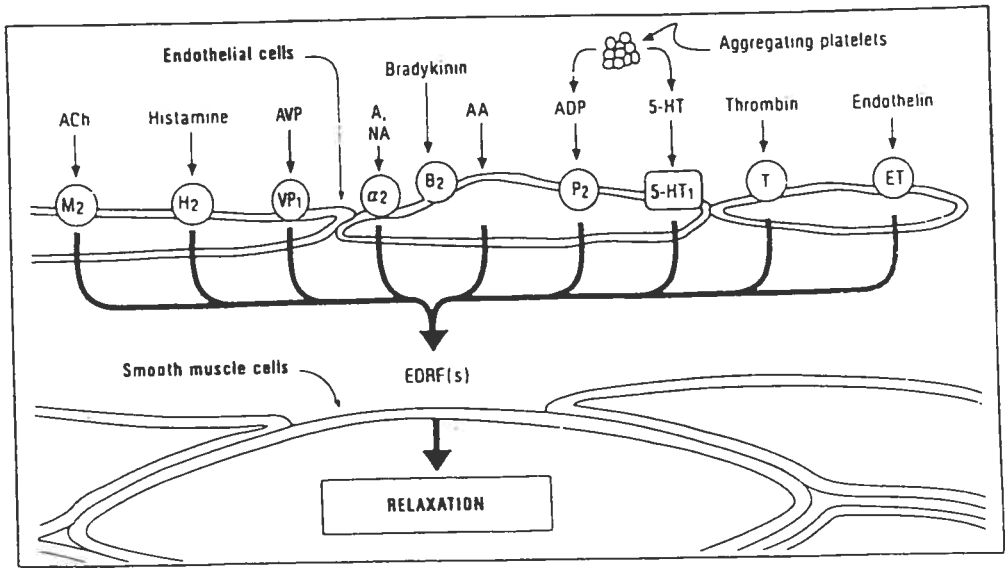


Figure 14

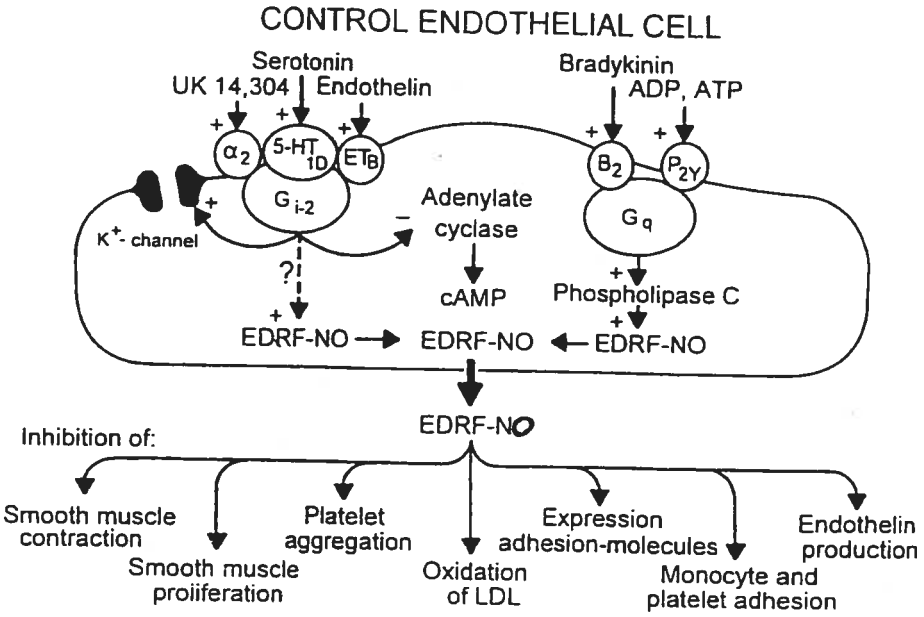


Figure 15