Two-decade analysis of cardiac storage for transplantation

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Abstract

Objective: Cardiac storage solutions and methods remain unstandardized. We have surveyed the literature to establish how the subject has progressed, addressing models of preservation and measures of outcome. Since a lot of the literature on cardiac storage is generated in the laboratory, we were particularly interested to evaluate to what extent bench work finds its way into and clinical practice. The discussion focuses in addition to new areas of research and introduces the concept of integrated organ preservation.

Methods: Five representative journals (J Thorac Cardiovasc Surg, Circulation, J Heart Lung Transplant, Eur J Cardio-thorac Surg and Ann Thorac Surg) were searched by hand for papers published between 1980–1999. All laboratory, animal experimental and clinical studies focused on prolonged cardiac preservation and storage were selected.

Results: Two hundred and forty-nine publications were identified using preset criteria. Of these, 196 (79%) were studies performed in animal models and 10 (4%) were experiments carried out on animal tissue. One hundred and five experiments (42% of all studies) were performed in small animals. The most common animal model was of ischemia followed by ex vivo reperfusion (121 studies, 49% of publications). The measures of outcome were classified as biochemical, functional, morphologic and endothelial; the majority of studies had one (48%) or two (40%) end-points. Twenty-five studies (10%) had endothelial measures of outcome, alone or in combination with other types of outcomes. Human clinical work was represented by 34 (14%) studies of clinical transplantation and nine (4%) experiments on human tissue only. There were five randomized clinical trials, representing 2% of all papers and 15% of all clinical research.

Conclusion: In conclusion, most of the surgical publications on prolonged cardiac preservation result from animal research. Small animal models of ex vivo ischemia and reperfusion are predominant.

Keywords: Cardiac transplantation; Storage; Preservation; Myocardium; Endothelium

1. Introduction

The need to expand the donor pool together with organ procurement at distant sites are the main factors stimulating continued interest in better organ preservation techniques. The above situation is described in the opening statement of a vast number of publications dealing with the subject in the last two decades. We undertook a search of heart preservation-related papers published between 1980 and 1999 in five principal journals. The focus of this literature search was on the methods employed in experimental, animal and human studies and on types of outcome measures. In 1978 Hearse and colleagues published their observations on the oxygen and calcium paradox in reperfusion [1]. This and other studies set the scene for unprecedented developments over the subsequent years on the role of ischemia-reperfusion injury in models of regional and global myocardial ischemia. For example, the deleterious ultrastructural effect of prolonged storage and reperfusion on the cardiac allograft was highlighted by Billingham et al. as early as 1980, but the significance of this phenomenon remained unclear for years afterwards [2]. All studies including measures of endothelial function and reperfusion damage after prolonged cardiac preservation were therefore included in our survey.

2. Material and methods

All issues of The Annals of Thoracic Surgery, The European Journal of Cardio-thoracic Surgery, The Journal of Thoracic and Cardiovascular Surgery and The Journal of Heart and Lung Transplantation between 1980 and end of 1999 were searched by hand for papers on preservation methods in cardiac transplantation. Circulation was searched over the same interval using a combination of hand search for the journal supplements and electronic search with the journal’s own search engine at http://www.circulationaha.org. The inclusion criteria were all laboratory, animal and human studies focused on heart preservation and storage, and studies applying statistical methods to retrospective series of human cardiac transplantation. The following types of studies and publications were
3. Results

Three issues from volumes 2, 3 and 4 of The Journal of Heart and Lung Transplantation were not available. Two hundred and forty-nine publications were identified according to the preset criteria. Of these, 206 (83%) reported results of experiments performed on animals and 10 (4%) involved animal tissue only. There were 34 (14%) clinical studies and 9 (4%) studies on human tissue (Fig. 1). Of the 196 animal experiments, over a half (105) were carried out in small animals (i.e. rats and rabbits). Only in a minority (47 papers) of the animal studies was it directly apparent from the title that the results were obtained in animal models. The animal experiments utilized most often a model of global ischemia with ex vivo reperfusion (121 publications, 49%) (Fig. 2). Endothelial function was used as an outcome measure, alone or in combination with other descriptors, in one paper only in the 1980’s and in 24 papers in the 1990’s (10% in total). The majority of experiments had one (48%) or two (40%) measures of outcome (Fig. 3). Five clinical studies were randomized trials, representing 15% of the clinical research and 2% of all publications identified. A classification of the animal experiments and of the types of outcome measures for all studies is shown in Tables 1 and 2.

4. Discussion and future perspectives

4.1. Types of preservation

The report of Thomas et al. in 1978 on long distance transportation of human hearts for transplantation fuelled the interest of many research groups on the best methods.

Table 1
Types of experimental studies in animals

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<td>Cold ischemia</td>
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<td>Continuous perfusion</td>
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<td>Ischemia, storage and reperfusion</td>
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<td>Different species</td>
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<td>Paracorporeal circulation (support animal)</td>
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<td>Transplantation</td>
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of organ preservation [3]. The cold storage techniques evolved from two schools of thought. In the first, use of the cardioplegia solutions such as St Thomas’ was extended to cardiac preservation [4]. In contrast, the second approach is derived from experience with intra-abdominal organ preservation and is centered on the Euro-Collins and University of Wisconsin (UW) solutions [5,6]. Both these methods have proved successful, despite the fact that the solutions were not specifically designed for cardiac storage during global ischemia. The rationales of ‘intracellular’ and ‘extracellular’ solutions are reviewed elsewhere [7,8]. The Histidin-Tryptophan-Ketoglutarat (HTK) solution and Celsior were storage media developed with the specific purpose of integrating the principles of solid organ preservation with those of myocardial metabolism [9,10]. These solutions still undergo experimental and clinical trials [11,12]. Despite the differences in their composition and underlying philosophies, the weight of evidence so far is that the effectiveness of UW, HTK and Celsior is closely comparable [12,13]. Novel strategies are therefore required to improve results and extend the time of graft preservation, such as donor and/or recipient cardioplegia modifications. Evidence from small randomized clinical trials is now available to show that blood cardioplegia is beneficial in arresting the donor heart [14] and also during implantation [15]. An attractive option to anoxic storage is aerobic preservation [16]. Continuous perfusion techniques lend themselves to interesting experimental variations, but the lack of clear clinical advantage and the cumbersome addition of equipment during transport makes them virtually absent from clinical practice [17]. After more than two decades our understanding of the cellular phenomena has progressed, but the inherent complexity unfolded leads to even more variability in clinical practice. It is disturbing to note that a survey showed 167 types of heart preservation solutions to be utilized by 147 transplant centers in the United States [18]. Practice at our institution did not change until results of a survey showed that cold storage with cardioplegia solution was associated with a 2.5 risk of increase in deaths [17]. Since 1992 we have therefore been using normal saline as the transit milieu. Better cardiac preservation remains an elusive goal, and the safe ischemic time still does not exceed 4 h. With equal short-term outcomes, a certain preservation strategy will emerge as superior when it prolongs graft survival.

4.2. Preservation physiology

A better perspective for this situation is obtained if we summarize the factors affecting the cardiac allograft in a transplant operation. By analyzing the sequence of events in Fig. 4, it can be seen how even a healthy organ sustains a cumulative injury with consequences ranging from immediate to long term. Recent data from the International Registry of Heart and Lung Transplantation show a clear relationship between ischemic time and mortality at 1 and 5 years postoperatively [19]. Storage has a central position in the time sequence described. However, in experimental models of global ischemia or transplantation, storage is either studied as a primary variable or is included in a protocol which has another variable as the primary exposure.

The endothelium emerged in recent years as an essential component in models of ischemia and can be seen as an ‘organ’ per se. In cardiac transplantation it is the interface between the recipient and the donor heart and is a key player in cardiac allograft vasculopathy (CAV) [20,21]. Early endothelial dysfunction is predictive of CAV at 1 year after the operation [22]. The importance of the endothelium is substantiated by recent findings of proinflammatory gene activation in solid organs in association with brain death [23,24]. In other words brain death primes the endothelium for more subsequent injury during transplantation. Ischemia and reperfusion, by upregulating MHC class I and II expression, further increase the immunoreactivity of the graft and set the scene for unfavourable interaction with alloactivated T-cells [25]. While the concept of endothelial preservation...
is evolving, a loose usage of terms tends to confuse issues further. We feel that the term cardiac preservation should describe the whole strategy to maintain myocardial and endothelial function across transplantation, whereas storage should be reserved for the specific stage in the middle. The events in Fig. 4 illustrate how endothelial injury can be a continuum, both in severity and over time. Pinsky’s group used a murine heterotopic model and elegantly demonstrated that in an isograft milieu IR is in itself sufficient to induce CAV, whereas in allografts IR exacerbates the vasculopathy [26]. This is in line with the notion that CAV results from subclinical graft injury, and is engineered by intricate immunological mechanisms operating in a milieu of multiple non-immunologic risk factors [27].

4.3. Methodology of research

The most common publications in our search are by far from small animal work. An arbitrary classification of the surveyed animal experiments involving cardiac storage is shown in Table 1. The time trends demonstrate that animal research maintains its preponderance (Figs. 1–3), illustrating the fact that many new ideas emerge all the time and are tested in these ‘screening’ experiments. Only a few dedicated groups follow a research hypothesis through to large animal transplant models and clinical trials. Direct clinical implementation of results obtained in isolated tissue or small animal models sometimes meets with failure and publication biases help these events to pass largely unknown. An example of perseverance in relating bench work to clinical results is available from Rosenfeldt’s group. After an initial favourable experience with UW solution in a rat model, a short series of unexpected clinical failures was encountered [11,30]. They returned to the isolated rat heart model and demonstrated elegantly how the UW solution loses its protective action at increased temperatures and has deleterious effects on the endothelium [11]. This research confirms once again that the rewarming associated with implantation is a particularly vulnerable phase.

The most common isolated heart models are the beating heart preparation pioneered by Langendorff and the working heart model, in which afterload conditions and the perfusion method can be adjusted [31,32]. Crystalloid reperfusion negates the effect of a number of key factors in the reperfusion injury (complement, leukocytes, platelets) [33]. Reperfusion with autologous blood does not effectively control variables of rejection-induced dysfunction. Many animal studies of ischemia with or without reperfusion employ live donors and thus exclude the considerable effect of brain death on the heart [34]. This is an important observation, especially when Shivalkar et al. showed that the mode of brain death itself (gradual vs. explosive increase in intracranial pressure) is directly related to the amount of myocardial damage [35]. Meanwhile, the exact mechanism of cardiac dysfunction in brain-dead donors remains to be deciphered [36–38]. The chain of events is bound to be complex, as Yeh and colleagues showed that brain death alters the left ventricular gene phenotype [39].

Animal transplantation models provide more physiologic reperfusion but rejection as a variable is generally poorly controlled. The following observations apply to many of the earlier and some of the later animal studies. The number of study subgroups in I or IR models can range from one to ten and it is not readily apparent which subgroups are study groups and which ones are controls. Steps taken in the

![Diagram](https://academic.oup.com/ejcts/article-abstract/20/4/792/376297)

Fig. 4. The initial cumulative injury sustained by the cardiac allograft. The downstream events resulting from reperfusion with non-autologous blood and the interaction with the recipient’s immune system are not depicted.
study design to eliminate bias are not made explicit or, alternatively, a section on limitations of the study is the exception rather than the rule. Small animal transplant models generally used homozygous subjects but this is not always clear in the protocol. Use of syngeneic subjects is not feasible in large animal models, although attempts to control acute rejection are sometimes noted, by ABO matching for instance [40].

The types of outcome measures for human and animal studies are summarized in Table 2. Comparisons were undertaken between study groups but rarely between methods to validate new ones [41]. It is prudent to observe that the same measure of outcome applied to different species in the same study can yield different results [42,43]. Endothelial changes were documented by surrogate markers of contraction, coronary flow reserve, or by immunohistochemistry to detect adhesion molecules.

4.4. Future directions

In the last decades cardiovascular physiology has been somewhat a victim of its own success. Myocytes represent only one third of the heart cells, and the paradigm that cardiomyocyte preservation automatically leads to good pump function and good outcomes is about to change. It is now clear that the acute injury propagates beyond the endothelium to the smooth muscle cell and the matrix, an area that has received virtually no attention so far in preservation strategies. The in vitro stretch experiments are clearly unable to provide the complex environment in which myocytes and fibroblasts interact in autocrine, paracrine and direct fashion [44]. That these humoral mediators are important is illustrated by research on transforming factor-β1 (TGF-β1), known for its ability to regulate the matrix metalloproteinases and collagen deposition: the TGF-β1 C allele (low TGF producers) in either the donor or the recipient genotype prolongs the time to first diagnosis of CAV [45].

As the kaleidoscope of subcellular controllers is better defined, our clinical possibilities will benefit and expand. The nuclear transcription factor kappa B (NF-κB) is now known to hold a central position in the regulation of many genes expressed by the activated endothelium [46,47]. Therapeutic inhibition of NF-κB, by sulfasalazine for example, prolongs rat allograft survival [48]. Gene polymorphism analysis, gene transfection, transgenic and knock-out mouse technologies have opened even more research horizons. Parallel developments are also seen in the area of programmed cell death [47,49,50]. A novel theory of response to ischemia proposes that myocardial ‘programmed cell survival’ can take the form of reconditioning, stunning or hibernation [50]. The relationship with earlier studies of myocardial metabolic viability also becomes clearer: apoptosis is an energy-consuming process, therefore severe ATP depletion leads directly to necrosis [51]. Anti-apoptotic genes, of which bcl are the most prominent group [47], have established protective roles in the myocardium [52] and liver [53]. Endothelial programmed cell death is equally important, especially in relation to CAV, as Fas-mediated endothelial apoptosis is implicated in allograft vasculopathy [54]. It becomes apparent that different pathways of injury – complement [55], cytokine [56], lymphocyte [25], neutrophil [57], or reactive oxygen species-mediated [58] – meet at closely linked subcellular levels. Simplicity in any of these models remains out of sight, especially when some cytokines or cytoprotective pathways were shown to have a dual role. For example, the effect of tumour necrosis factor on post-ischemic cardiomyocytes is concentration-dependent and ranges from adaptive reduction in contractility to deleterious inflammation [59]. Likewise, induction of heat shock proteins (hsp) may have dual consequences, as the highly immunogenic and crosreactive hsp60 is released into the circulation following cardiomyocyte ischemia [60]. Finally, more clinical studies into peritransplant cytokine biology may pave the way for pharmacological modulation. Yacoub’s group has recently provided the demonstration of a relationship between TNF-α expression and postoperative right ventricular dysfunction [61]. The next decade is therefore faced with a two-fold challenge: (1) elucidation of the mechanisms involved in the convergent pathways, which will permit better cytoprotective interventions, and (2) understanding the contribution of each of these mechanisms at the stages between brain death and reperfusion; this will enable the development of comprehensive strategies to preserve whole organ function.

We are aware of the limitations of this exercise. First of all, other prominent journals with an interest in this area were not included. It was felt however that extending the search to the whole literature would add little to our purpose of showing a publication trend. Moreover, an electronic search of the whole medical literature would be fraught with error due to the extremely diverse nature of the subject and associated keywords. Using the WebSpirs 4.01 software for example, an advanced search of the same journal (The Journal of Thoracic and Cardiovascular Surgery) over 20 years yielded incomplete and conflicting results.

5. Conclusions

The literature on cardiac storage for transplantation is dominated by animal work. In spite of the inherent limitations, this body of bench research contributed considerably to further our understanding of transplantation biology but helped us little in clinical cardiac preservation. Individual results should be interpreted with caution due to departure from the pathophysiology of clinical situations. There is a strong argument for this type of research: it is unethical to submit patients to poorly tested hypotheses. However, a degree of standardization in animal experiments is highly desirable to allow meaningful and worthy comparisons.
Human studies have a reduced prevalence but are the most likely to bring about more uniformity in practice and to improve results. Transplantation is one of the most complex processes of modern medicine and its many unravelled mysteries do deserve a multidisciplinary approach.

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References

Densem CG, Hutchinson IV, Yon, N, Sheldon S, Brooks NH. A low
MacKenna D, Summerour SR, Villarreal FJ. Role of mechanical
Minten J, Segel L, Van Belle H, Wynants J, Flameng W. Differences
Masuda M, Sukehiro S, Mollhoff T, Lu HR, Van Belle H, Flameng W.
Fremes SE, Furukawa RD, Li RK, Weisel RD, Mickle DA, Tumiati
Jeevanandam V, Auteri JS, Sanchez JA, Hsu D, Marboe C, Smith CR,
Yeh T, Wechsler AS, Graham LJ, Loesser KE, Sica DA, Wolfe L,
Szabo G, Hackert T, Sebening C, Melnitchuk S, Vahl CF, Hagl S. role
Shivalkar B, Van Loon J, Wieland W, Tjandra-Maga TB, Borgers M,