Evaluation of cyropreserved internal thoracic artery as an alternative coronary graft: evidence for preserved functional, metabolic and structural integrity

Abstract

The internal thoracic artery (ITA) is the conduit of choice for coronary artery bypass grafting (CABG). This study, utilizing a canine model, evaluates cryopreserved ITA. Sixteen ITAs were harvested and cryopreserved according to United CryoInstitute protocol. Test conduits, 5 cm long and 4 mm mean diameter, were anastomosed to the ligated carotid artery of an unmatched mongrel recipient, above and below the site of native artery ligation. Graft patency was assessed by angiography at 14 days (early) and 980 days (late) postoperatively. Catheterization of the 16 vessels identified three (18%) early and one (6%) late graft occlusion. Ninety days postoperatively, each dog was killed and the graft harvested for histopathological and functional evaluation. Morphologic evaluation, using conventional staining, showed preserved cellular structure, decrease in smooth muscle cells and distorted endothelial layer. Immunocytochemistry, using an antibody against prostacyclin (PGI2), detected PGI2 immunoreactivity in the ITA smooth muscle cells. An in vitro assay performed on the arterial rings confirmed preserved functional integrity of the vascular endothelium and smooth muscle. These findings suggest that cryopreserved ITA may have potential as a substitute graft, in devising conduit strategies for primary or reoperative coronary bypass surgery.

Key words

Coronary bypass surgery · Conduit · Cryopreservation

Introduction

Surgical myocardial revascularization has proven to be a beneficial and safe procedure for patients with coronary occlusive disease [8–11]. Autologous vessels such as saphenous veins and internal thoracic artery (ITA) have been used most often for this operation. However, these native vessels are not always suitable or available as graft materials. About 10–15% of all coronary surgery patients require graft substitutes because of previous bypass surgery, prior surgical injury to native arterial conduits, surgical intervention into the venous system, deep venous insufficiency, deep venous thrombosis, or peripheral arterial circulation disorders [2, 11]. Various biological or artificial graft materials have been used with mixed results [21, 22]. The ITA has become the “gold” standard for bypassing the left anterior descending coronary artery (LAD) because of its superior long-term patency rate, lower incidence of atherosclerotic changes compared to saphenous vein grafts, and improved patient survival [1, 15, 24, 26, 37, 45]. The purpose of this study was to evaluate cryopreserved ITA as an alternative graft for aortocoronary bypass by assessing its patency, and studying its functional and biological characteristics following implantation.
**Materials and methods**

Internal thoracic artery pedicles were harvested from mongrel canines available from other projects. A central goal was to preserve internal endothelium and its function. Perfusion catheters were placed in the vessels, and were flushed and packaged with 1000 cc Plasma-Lyte A solution, with 4000 units of heparin and 120 mg papaverine available from other projects. Side branches were ligated and each ITA was measured for diameter and length. The arteries were then soaked for approximately 24 h in a low dose antibiotic solution at 4°C. The next day, the arteries were rinsed in RPMI at 4°C to remove residual antibiotic solution. The ITA graft material was returned to the study site and stored in vaporized liquid nitrogen at -179°C (in the Tissue Bank at the Deborah Research Institute) until the day of implantation, 6 days after harvesting. Twelve male and four female (n = 16) adult, mongrel dogs (mean weight 26.4 ± kg) were used in this study. Under general inhalation anesthesia (2% isoflurane), the carotid artery was exposed. Heparin was administered intravenously (100 mg/kg). The cryopreserved ITA (mean vessel diameter = 4 ± 1 mm) was prepared following the UCI protocol. Warm water baths (37-42°C) were employed to thaw the individual ITA grafts, after which the artery was removed from its protective pouch and placed in the first warm dilution solution to begin the process of cryoprotectant (DSMO) removal. The solution utilized for this step was RPMI with fetal calf serum. Two additional dilution baths (2 min each) removed all cryoprotectant. One last warm bath with RPMI remained prior to implantation.

The 16 cryopreserved ITAs were inserted as a bypass graft on the carotid artery of the unmatched canine recipient in an end-to-side fashion using continuous 6-0 Prolene suture above and below the site of the native carotid artery ligation. Following surgery, the animals were observed closely for complications. Analgesic medication, Nubain 1 mg/hk subcutaneously, was administered prior to removing the animal from the operating table, and as needed. The animals were boarded in the Deborah Research Institute vivarium for 3 months postoperatively, during which time they were examined on a daily basis.

**Histological and immunocytochemical evaluation**

Each specimen of harvested native ITA and cryopreserved ITA graft was fixed in 4% paraformaldehyde, processed, and embedded in paraffin for light microscopic evaluation. Each paraffin block was cut perpendicular to the long axis of the blood vessels, into seven micrometer sections. The slides were stained with hematoxylin and eosin. The morphological and structural components were examined histologically.

The presence of prostacyclin (PGI,) production was detected using immunocytochemistry techniques employing monoclonal antibodies against PGI,. The avidin-biotin-peroxidase complex method was employed with the monoclonal antibody to prostacyclin diluting 1:200 in phosphate buffered saline (PBS 0.01 M; pH 7.2). The intrinsic binding sites were blocked with normal goat serum, followed by primary antibody incubation overnight at room temperature. The specimens were then reacted with a secondary antibody (biotinylated goat anti-mouse) and streptavidin-biotin-peroxidase complex. The sections were lightly counterstained with hematoxylin and examined under a Nikon research light microscope.

**Results**

All animals survived the surgery and were evaluated as scheduled. The angiography performed 14 and 90 days postoperatively revealed no signs of visible atherosclerotic lesions, shrinking, stenosis or thrombus formation in open vessels (Fig. 1). The patency rates of the tested ITA grafts are presented in Table 1. No animal was sacrificed until the 90 day angiographic analysis. Recovery of occluded segments for analysis revealed technical error in two cases, with one early occlusion due to unknown causes. This vessel was not histologically evaluated.

Gross and histological evaluation of the cryopreserved ITA, performed 90 days after implantation, revealed that in general, ITA integrity was well preserved. The endothelial layer appeared to be intact without interruption. No thrombus formation was observed. The subendothelial layer also appeared to be normal. However, in the smooth muscle layer, there appeared to be a decrease in cellularity, mainly of smooth muscle cells. Collagen and connective bundles were well preserved and no cellular infiltrat...
Table 1 Angiographic patency rate of the cryopreserved internal thoracic artery at 14 (early) and 90 (late) days

<table>
<thead>
<tr>
<th>Dog</th>
<th>Vessel</th>
<th>Gender</th>
<th>Diameter</th>
<th>14 Days</th>
<th>90 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Graft</td>
<td>M</td>
<td>4 mm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Graft</td>
<td>M</td>
<td>4 mm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Graft</td>
<td>F</td>
<td>3 mm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Graft</td>
<td>M</td>
<td>4 mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Graft</td>
<td>M</td>
<td>4 mm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Graft</td>
<td>M</td>
<td>5 mm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Graft</td>
<td>M</td>
<td>4 mm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Graft</td>
<td>M</td>
<td>5 mm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Graft</td>
<td>M</td>
<td>3 mm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Graft</td>
<td>F</td>
<td>3 mm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Graft</td>
<td>F</td>
<td>5 mm</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Graft</td>
<td>M</td>
<td>4 mm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>Graft</td>
<td>F</td>
<td>5 mm</td>
<td>+</td>
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</tr>
<tr>
<td>14</td>
<td>Graft</td>
<td>M</td>
<td>5 mm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>Graft</td>
<td>M</td>
<td>4 mm</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>Graft</td>
<td>M</td>
<td>4 mm</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = open, - = closed

Fig. 1 Cineanglographic documentation of graft patency utilizing cryopreserved ITA as the conduit, at 90 days implantation.

Fig. 2 Cryopreserved ITA after implantation, stained with hematoxylin and eosin. Note that the integrity and the cellularity of the vessel were well preserved. L=Lumen, Bar=100 μM

Fig. 3 Immunocytochemical detections of prostacyclin in a cryopreserved graft 3 months after implantation. Photomicrograph shows portion of blood vessel wall and part of the lumen (L). The endothelial layer and the different muscle layers are intact. Although the smooth muscle cells are decreased in number, they contain prostacyclin immunoreactivity in their cytoplasm, indicating that the functional property was likely retained by those cells. The positivity is brown in color. Bar=100 μM

The vessel walls were of normal thickness and the lumen size was comparable to the control arteries. The adventitia was normal and no apparent adhesive material, proliferation or cellular infiltration was observed.

In the control ITA, prostacyclin immunoreactivity was detected in the endothelial cells and to a moderate degree in the cytoplasm of scattered smooth muscle cells. There was faint reaction in the connective tissue particularly in the adventitia, which might represent extracellular reactivity. Of note was that in the cryopreserved ITA, the activity of prostacyclin was similar to the control arteries, with faint staining in the endothelial cells and moderate staining in the smooth muscle layer (Fig. 3). However, as smooth muscle cells decreased in number, prostacyclin immunoreactivities were proportionately reduced. There appeared to be an increased reaction intensity in the extra-cellular connective tissue area in the adventitia of the cryopreserved ITA compared to control arteries. The controls for immunohistochemistry were negative, thereby confirming the specificity of the immunoreactions.
Functional data was obtained from 10 control ITAs and from 8 cryopreserved ITA grafts. Seven of 10 control ITA segments generated more than 0.1 g of force in response to 10 μM norepinephrine. The vessels which failed to respond to norepinephrine also failed to respond to KCl. The mean ± standard error for force generation by the 10 control ITA segments was 0.446 ± 0.119 g, (P<0.05). In contrast, only 4/8 ITA graft segments generated more than 0.1 g of force to norepinephrine. Those vessels not responding to norepinephrine also failed to respond to KCl.

**Discussion**

Autologous ITA and saphenous veins are the most commonly used coronary bypass substitutes. However, there are situations in which these grafts are not sufficient or suitable, and alternative autologous conduits may be necessary for complete myocardial revascularization. Although other autologous arterial conduits have been advocated, e.g. radial artery, gastroepiploic artery, inferior epigastric artery [16, 18, 27], late phase results are not yet available. For intra-abdominal harvest of arterial conduits, the abdominal cavity must be entered, the learning curve may be steep, and the graft might be difficult to harvest, if there has been previous abdominal surgery, or may not be satisfactory, if there is atherosclerosis of the abdominal aorta.

The search for alternative coronary grafts has intensified. Various materials have been assessed such as cryopreserved homologous saphenous veins [4, 5, 23, 29, 30], dialdehyde starch preserved bovine internal mammary artery [28, 35, 41, 44], arterialized cephalic veins [16], denatured venous homografts [22] and other synthetic bypass graft materials [13, 20, 33, 36]. Early phase results are mixed but, overall, the long-term results are discouraging. The purpose of the current study was to test cryopreserved ITA to determine if it possesses satisfactory clinical properties to permit its possible utilization as a coronary bypass conduit. Interest in this area was prompted by findings at this institution with the clinical utilization of cryopreserved saphenous vein [23].

Implantation in the canine carotid artery for evaluation can be justified as bypass of the carotid artery in small dogs is a reliable experimental model [7, 12, 35]. Cryopreserved ITA was chosen as a test graft material for several reasons. The relatively low susceptibility to atherosclerosis and the superior patency of ITA grafts make the ITA the conduit of choice for surgical revascularization [26]. Clinical studies suggest that in situ and free ITA grafts may be superior to saphenous vein grafts for long-term patency, and are associated with improved survival after coronary bypass grafting [25]. Studies by several groups indicate a mean patency rate of 96% for ITA grafts compared to 81% for saphenous vein grafts at 10 years [1, 15, 25]. Histological examination of ITA, compared to other autologous conduits, indicates that the ITA has important advantages for myocardial revascularization [39, 42]. The internal elastic lamina and elastic lamellae in the media may play a key role in the prevention of intimal thickening, as they form barriers to the invasion of smooth muscle cells from the media into the intima [38]. Also, the antithrombic and vasomotor properties of the arterial endothelium produce significant amounts of prostacyclin which may be one of the factors yielding a higher patency rate [12, 14].

Cryopreservation of isolated blood vessels at −70 to −195 °C in a medium containing DMSO offers the prospect of ready availability and virtually indefinite storage. Also, cryopreservation potentially maintains both biochemical and functional activities [4, 5]. Although deterioration in contractile force and overall endothelial cell function may occur [4, 5, 23], as evidenced by the immunocytochemical findings detecting a reduction in prostacyclin in the tested graft, the detection of PGI₂ in the cryopreserved ITA, at 3 months after implantation, is encouraging and indicates that the graft at least partially retained biochemical and structural integrity. The reduced cellularity found in the smooth muscle layer of the cryopreserved ITA could be due to an initial immune rejection of the graft; however, it did not appear to affect the overall integrity of the vessel. This change may have an influence on the functional characteristics and thrombogenicity of the vessel, predisposing to graft closure. However, in the 16 tested cryopreserved ITAs, two of the three early occlusions may have been due to a technical problem with constriction of the suture line causing stenosis close to the distal anastomosis. The angiographic evaluation of the 12 patent grafts revealed smooth flow without any stenosis or shrinking at 14 and 90 days postoperatively. The overall patency rates were 82% at 14 days and 75% at 90 days.

The intent of the functional studies was to evaluate the ability of cryopreserved, grafted ITA to contract in response to KCl and norepinephrine, and to relax in response to endothelium-dependent and -independent vasodilators. However, because so few of the grafted vessels contracted in response to KCl and norepinephrine, relaxation responses were not meaningful. It was apparent, however, that the grafted cryopreserved ITA segments lost some ability to contract in response to norepinephrine. It is uncertain whether this was due to loss of functional adrenergic receptors, altered signal transduction mechanisms (in the case of norepinephrine-induced contraction), or to loss of smooth muscle integrity. Vessels not responding to norepinephrine also failed to respond to the direct depolarizing effects of KCl, suggesting an impairment of fundamental contractile properties of the vascular smooth muscle. The implications of this loss of contractile activity, with respect to graft patency, are unknown at this time.

Internal thoracic artery, prepared by these techniques, appears to be a potentially suitable conduit when autolo-
gous conduits are not available. Accelerated atherosclerosis and neointimal proliferation seem to affect cryopreserved saphenous veins [4, 5, 23]; however, these processes appeared less apparent in cryopreserved ITA in this preliminary study. Prompted by data acquired utilizing this canine model, further long-term evaluation is needed prior to clinical evaluation of the utility of cadaveric, cryopreserved, homologous ITAs as coronary artery bypass conduits. The functional, metabolic and structural integrity of ITA needs to be studied over a longer period of implantation in the animal model prior to any human studies.

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References


**Discussion**

**Dr. R. Walpoh (Bern, Switzerland).** I wonder if you could comment some more on the three early closures, because that as at 14 days. Did you do angiographic controls after implantation to rule out surgical failures? But, on the other hand, that is the time when your endothelium will probably be removed and changed and that is the most thrombogenic time point, and that is why I am wondering whether you can differentiate between thrombosis of the graft or technical failure at this time?

**Dr. W. J. Boris:** Immediately after the initial operation, angiographic analysis was not performed. Therefore, we are not confident as to whether all three early graft failures represent technical mishap during graft implantation. However, it was noted at necropsy that, of those early grafts that had failed, there was evidence of technical error at the anastomotic site, with a twist in the graft in one and a narrowing at the anastomotic site in another.