Functional assessment of cryopreserved human aortas for pharmaceutical research

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Abstract

We evaluated the impact of standard cryopreservation on functional properties of human aortic homografts. From seven human donors, the thoracic descending aorta was obtained. Effects of cryopreservation on contractibility and endothelium function were tested. After cryopreservation no endothelium-dependent or endothelium-independent relaxation was found and the contractibility was strongly affected. Arteries showed no function and loss of endothelial integrity after cryopreservation and thawing.

Introduction

Cryopreserved human blood vessels may become important tools in both bypass surgery and peripheral vascular reconstruction in patients without sufficient autologous graft material and are actually interesting topics of pharmacological research (Stanke et al. 1998). Cryopreservation was carried out in the biologic refrigerator (CM25 Carburos Metalicos, SA, Madrid, Spain) with a programmed decrease of 1 °C min⁻¹ until −40 °C was reached and faster rates thereafter until −150 °C was reached.

However, evidence has come to light that after thawing of cryostored human arteries, the contractile force and endothelial function is reduced (Song et al. 1994, 1995; Müller-Schweinitzer et al. 1997, 1998; Stanke et al. 1998; Wusteman et al. 1995).

Accordingly, the purpose of the present study was to evaluate the effects of cryopreservation on the functional properties of the human thoracic descending aortic homograft, harvested as part of a donor program.

Material and methods

Tissue extraction

We used the thoracic descending aorta from seven multiple organ donors after permission in an aseptic procedure was obtained.

Tissue preparation

Samples were carefully harvested from seven multiple organ donors (mean age: 48 years; five men and six women). The tissues were immediately placed in saline solution at room temperature and transported to the laboratory. The arteries were cleaned...
of surrounding tissue. Each aorta was then cut into equal segments of approximately 3 cm length and were randomly assigned to each study group. The thickness of the wall is 2 mm and the diameter of the aorta is 1.5 cm.

Eighteen HAA rings were isolated and randomly assigned to one control group of unfrozen HFAs (4 rings) and one group of cryopreserved HFAs (14 rings).

The control group consisted of a segment of fresh unfrozen artery. The samples of ‘unfrozen arteries’ were used immediately for organ bath studies. Then, the arteries were frozen.

Cryopreservation, organ bath studies, histologic examination, drugs and data analysis

These analyses were carried out as previously has been described in the aforementioned article about human femoral arteries.

Uniquely, at the beginning of the experiments the rings were stretched to an initial tension of 2 g and allowed to relax and equilibrate for approximately 2 h in the bathing medium, which was changed every 15 min until a stable baseline tension of 2 g was achieved.

Results

Functional studies

The NA-induced contraction was significantly affected after cryopreservation (Table 1, Figure 1). An almost negligible response to ACH was demonstrated in aortic specimens directly after explantation, which further decreased after cryopreservation (Table 2, Figure 2).

Endothelium independent relaxation after addition of SNP was observed in fresh aortic rings and was significantly reduced after cryopreservation (Figure 3).

Morphologic examinations

Generally, cryopreservation of the arterial segments did not change the morphologic appearance of the medial layer in these tissues. However, there were considerable differences in the preservation of the endothelial lining. Anatomopathologic studies of the endothelium revealed marked endothelial denudation in rings from unfrozen human aortas and that had been frozen.

Discussion

Our study addressed the mechanical properties of the human aorta with and without cryopreservation. Tissues were taken from human donors and randomly divided into groups.

The present study showed that cryopreserved human aortic homografts have smooth muscle cellular contractibility (SMC contractibility) and endothelial cell function markedly attenuated (Song et al. 1995; Müller-Schweinitzer et al. 1998, 1994; Stanke et al. 1998; Wusteman et al. 1995). The effect of cryopreservation on SMC contractibility and endothelium cell function has remained a matter of discrepancy (Gournier et al. 1995; Stanke et al. 1998; Wagstaff et al. 1996). We found a general decrease in SMC contractibility and endothelium-dependent and independent vaso-dilatation after cryopreservation (Langerak et al. 2001; Song et al. 1995; Wusteman et al. 1995).

In accordance with our results, studies showed nearly abolished SMC contractibility in human internal mammary arteries (Nataf et al. 1995) and diminished contractile response of human coronary and internal mammary arteries after cryopreservation (Müller-Schweinitzer et al. 1997).

In addition, Ku et al. (1990, 1992) have shown that an identical cryopreservation procedure applied to canine and human coronary arteries

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<th>Unfrozen</th>
<th>Gas phase slow thawing</th>
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<tr>
<td>Efficacy (Emax)</td>
<td>Na</td>
<td>Na</td>
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<tr>
<td></td>
<td>0.78 ± 0.07</td>
<td>0.28 ± 0.33</td>
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<tr>
<td>Potency (pD2)</td>
<td>Na</td>
<td>Na</td>
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<td>3.60 ± 0.14</td>
<td>2.82 ± 0.83</td>
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Emax = maximal contraction expressed as gram isometric force developed by the rings. PD2 = –log EC50, negative logarithm of the molar concentration producing 50% of maximal response. Data are given as means ± SEM.

*Significant differences (p < 0.05) against values determined in unfrozen controls.