Functional assessment of cryopreserved human femoral arteries for pharmaceutical research

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Abstract

An established method for cryopreservation that might preserve the vascular and endothelial responses of human femoral arteries (HFAs) to be transplanted as allografts was studied. HFAs were harvested from multiorgan donors and stored at 4°C in saline solution before cryostorage. Thirty HFA rings were isolated and randomly assigned to one control group of unfrozen HFAs (eight rings) and one group of cryopreserved HFAs (22 rings).

Cryopreservation was performed in RPMI solution containing dimethylsulfoxide (DMSO) and the rate of cooling was −1°C/min until −40°C and faster rates until −150°C was reached. The contractile and relaxant responses of unfrozen and frozen/thawed arteries were assessed in organ bath by measurement of isometric force generated by the HFAs.

After thawing, the maximal contractile responses to the contracting agonist tested (noradrenaline) were in the range of 43% of the responses in unfrozen HFAs. The endothelium-independent responses to sodium nitroprusside were not altered whereas the endothelium-dependent relaxant responses to acetylcholine were weakly altered.

The cryopreservation method used provided a limited preservation of contractility of HFAs, a good preservation of the endothelium-independent relaxant responses, and a good preservation of endothelium-dependent relaxation. It is possible that further refinements of the cryopreservation protocol, such as a slower rate of cooling and a more controlled stepwise addition of DMSO, might allow better post-thaw functional recovery.

Introduction

The use of fresh or freeze-dried arterial homografts was abandoned long ago because of difficulties in tissue procurement, long-term degeneration, and the development of improved prosthetic commercially available grafts (Sheranian et al. 1959; Crawford et al. 1960; Key et al. 1960; Brock 1968; Barner et al. 1996).

Recently, improvements to the preservation techniques, such as the introduction of cryoprotectant agents and a controlled rate of freezing, have enabled the development of human blood vessel banks (Stanke et al. 1998). Cryopreservation was carried
out in the biologic refrigerator (CM25 Carburos Metalicos S.A., Madrid, Spain) with a programmed decrease of 1 °C/min until −40 °C was reached and faster rates thereafter until −150 °C was reached. So, cryopreservation has become an important tool for the storage of human vascular tissues.

However, it has been demonstrated that the post-thaw functional recovery of cryopreserved isolated blood vessels is generally associated with reduced contractile force and endothelial function (Song et al. 1994, 1995; Wusteman et al. 1995; Müller-Schweinitzer et al. 1997, 1998; Stanke et al. 1998).

Therefore, the aim of this study was to investigate the responses to various contracting and relaxing agents of both unfrozen human femoral arteries (HFAs) and cryopreserved HFAs.

Materials and methods

Tissue extraction

We used the HFAs from 11 multiple organ donors after permission in an aseptic procedure.

Tissue preparation

Samples were carefully harvested from 11 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 artery was then cut into equal segments of approximately 3 mm length.

Thirty HFA rings were isolated and randomly assigned to one control group of unfrozen HFAs (eight rings) and one group of cryopreserved HFAs (22 rings). The thickness of the wall is 1 mm and the diameter of the femoral is 4 mm.

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 as described in the following text.

Cryopreservation

First, the tissues were immediately placed in RPMI solution, pH 7.4, and the incubation in antibiotic solution was carried out (vancomycin 50 μg/ml, metronidazol 50 μg/ml, amikacin 50 μg/ml, amphotericin 5 μg/ml), for 20 h at 4 °C.

The samples of antibiotic solution were taken out. The samples were placed in a sterile GAMBRO DF 700 bag containing a solution composed of 100 ml RPMI + 10% v/v dimethysulfoxide (DMSO) and this solution was equilibrated during 30 min at 4 °C before cryopreservation.

To reduce cell damage due to osmotic changes, the cryoprotectant was removed by sequential washing and progressive dilution at 4 °C in saline solution (NaCl 0.9%). Cryoprotectant was performed by 1 min immersion of the artery in successive baths of diluted DMSO (8%, 6%, 4%, 2% NaCl 0.9%) at 4 °C.

Cryopreservation was carried out in the biologic refrigerator (CM25 Carburos Metalicos S.A., Madrid, Spain) with a programmed decrease of 1 °C/min until −40 °C was reached and faster rates thereafter until −150 °C was reached.

Samples were then transferred into the liquid nitrogen tanks for their storage in the gas phase.

Arterial segments were slowly thawed. Samples were first placed on dry ice for 10 min before being exposed for 6 min at room temperature and thawed within 4 min at 40 °C at a mean thawing rate of approximately 15 °C/min.

To minimize toxic effects of DMSO, the cryoprotectant was removed by sequential washing and progressive dilution at 4 °C in saline solution.

Organ bath studies

The arterial rings were mounted vertically between stainless steel hooks in organ baths for isometric recording of contraction and relaxation. The upper hook was connected to a force transducer. The signal was amplified and recorded on a computer. The organ bath medium was maintained at 37 °C, and pH was kept at pH 7.4 by bubbling the solution with O2/CO2 (95 : 5), since oxygenation of the solutions has been reported to improve tissue preservation. The tissue chambers contained 20 ml of Krebs–Henseleit (KH) solution of the following composition (mmol/l: NaCl 118, KCl 4.7, MgSO4 2.4, CaCl2 1.6, KH2PO4 1.2, NaHCO3 24, glucose 11.1). At the beginning of the experiment, the rings were stretched to an initial tension of 4 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 4 g.