

Graft cold preservation in a new solution over 2 weeks: Results on rat superior mesenteric artery (RSMA) and human internal mammary artery (HIMA)



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Background:

Vascular homo- or allografts are used for reconstructive cardiovascular surgery and transplantation. Besides cryoconservation and glutaraldehyde storage tissue cold storage is employed. The latter may gain considerably more importance in the future, if the limitations of tissue preservation due to less favourable storage solutions can be overcome. Of particular importance may be the preservation of endothelial function with respect to post-operative vessel occlusion and the immunological response.

Aim:

The present study addresses the question whether an optimized storage solution recently described (Wille et al., 2008) permits the extended storage of vessels with maintenance of endothelial structure and function. Therefore, we addressed endothelium-dependent vessel relaxation, protein expression and endothelial structure of organ arteries during extended storage protocols over 2-3 weeks.

Methods:

Human internal mammary arteries (HIMA) were harvested from patients undergoing coronary artery bypass surgery (Ethics Committee approval EK 307-12-2007). The clinical characteristics of these patients and their preoperative drug therapy are given in table 1. In addition, rat superior mesenteric arteries (RSMA) were used (permission 24D-9168.24-1-2006-23). The vessel segments were immediately placed in one of the following solutions (4°C):

- Physiological salt solution (PSS)
- Physiological saline (NaCl)
- Histidine-tryptophan-ketoglutarate solution (HTK)
- Potassium-chloride and N-acetylhistidine enriched solution (solution 8, Wille et al., 2008, table 2).

Vessel segments were subjected to function testing or biochemical and histological analyses within 4 hours after harvesting or stored under the respective condition for up to 3 weeks and then subjected to analysis. In further experiments the addition of iron chelators (LK 614 20 µM, desferal 100 µM) to solution 8 and HTK (table 2) was tested with respect to preservation of endothelial cell function.

Vessel rings were studied in a Mulvany myograph at a tension equivalent to an intraluminal pressure of 100 mm Hg. Constriction testing was done with potassium

Results:

When RSMA were studied within 2 h after isolation, they all showed proper tension development upon stimulation with high potassium solution (figure 2). At this time point there was no significant difference with respect to the storage solution. Similar results were obtained for HIMA vessels (not shown). However, RSMA stored in PSS more than 2 days did not develop any vessel tone, whereas vessels stored in solution 8 or HTK did develop tone even after 4 days. It should be noted, however, that failure rate to develop tone after 4 days was significantly higher after storage in HTK (p<0.05).

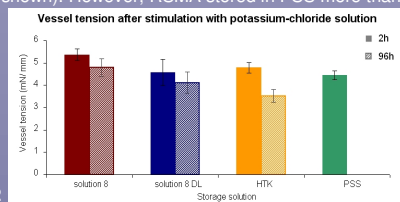


Fig. 2

The superior quality of vessels stored in new solution 8 augmented with iron chelators was also evident from data shown in figure 5. Vessels stored in traditional HTK exhibited impaired endothelium-dependent relaxation after 96 h storage, whereas full relaxation was seen only after storage in new solution 8.

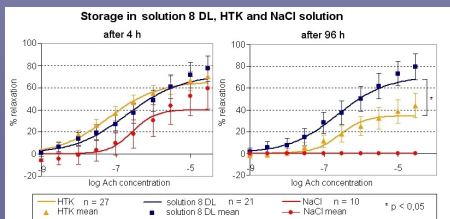
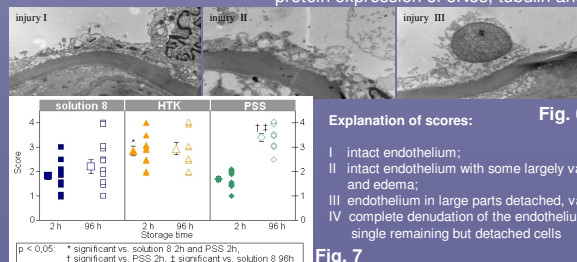


Fig. 5



Explanation of scores:
 I intact endothelium;
 II intact endothelium with some largely vacuoles and edema;
 III endothelium in large parts detached, vacuoles;
 IV complete denudation of the endothelium with single remaining but detached cells

Fig. 7

Table 1. Patient characteristics and drug therapies

Number of patients (males/females)	Age (mean ± SEM)	Hypertension (%)	Hypercholesterolemia (%)	Diabetes mellitus (%)	Obesity (%)	Current/past-smokers (%)	ACE-inhibitors (%)	beta-Blockers (%)	Calcium channel blockers (%)	Statins (%)	Coronary vasodilators (%)	Aspirin (%)
133 (102/31)	68.5 ± 0.77	96	71	47	50	14/20	71	82	23	78	99	85

Table 2. Composition of solutions

(mmol/L)	Solution 8	HTK
Cl ⁻	103.1	50
α-Ketoglutarate	2	1
Aspartate	5	1
H ₂ PO ₄	1	
Na ⁺	16	15
K ⁺	93	10
Mg ²⁺	8	4
Ca ²⁺	0.05	0.015
Histidine		198
N-Acetylhistidine	30	
Glycine	10	
Alanine	5	
Tryptophan	2	2
Sucrose	20	
Mannitol		30
Glucose	10	
pH	7.0	7.2
Osmolarity, mosm/L	305.2	310

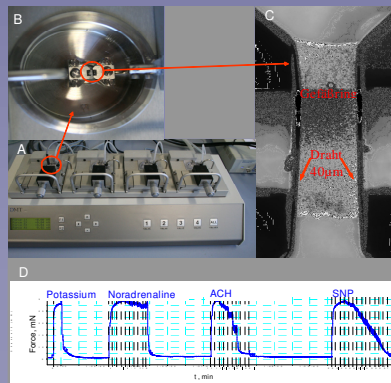


Fig. 1

enriched solution. Vessels constricted with noradrenaline (10 µM) were subjected to endothelium-dependent (acetylcholine (ACh) 10⁻⁹-10^{-4.5} M) and endothelium-independent relaxation (sodium nitroprusside (SNP) 10⁻⁹-10⁻⁴ M). Typical original recordings are displayed in figure 1D.

A fraction of RSMA vessels stored under the identical condition for 96 h were analyzed for structural changes using electron microscopy and protein expression using standard western blot techniques.

Responsiveness of HIMA toward norepinephrine was reduced and endothelium-dependent relaxation was absent after more than 8 h storage in either NaCl or PSS (Fig. 3). In contrast, if vessels were stored in solution 8 augmented with LK 614 and desferal endothelium-dependent relaxation was maintained for at least 96 h. Similar differences were found for the endothelium-independent stimulus with SNP (Figure 4).

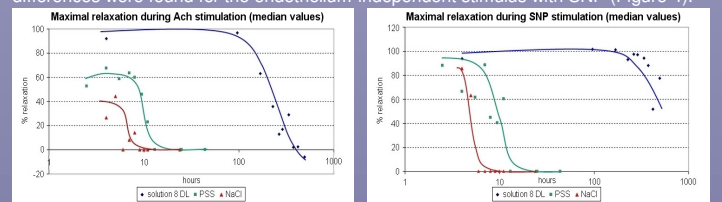


Fig. 3

Fig. 4

After 4 h storage in HTK a poor protection of endothelial cell structure of RSMA was objectified (figure 7). In contrast, cell structure was largely maintained even after 96 h storage of vessels in solution 8 augmented with LK 614 and desferal. Furthermore, 96 h cold vessel storage in solution 8 augmented with LK 614 and desferal fully protected protein expression of eNos, tubulin and β-actin.

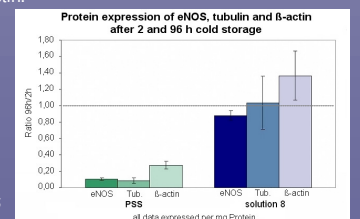


Fig. 8

Conclusions:

- Vessel cold storage in PSS or NaCl results in a rapid loss of endothelial cell and smooth muscle cell function.
- Cold storage in traditional HTK solution evokes structural injury of endothelial cells within 4 h followed by severe functional defects after 4 days storage.
- Functional loss can be greatly preserved for up to 14 days when stored in new potassium-chloride and N-acetylhistidine enriched solution 8.
- Addition of iron chelators LK 614 and desferal greatly augment the preservation of endothelial cell function after extended cold storage.