J. Pharm. Pharmacol. 1997, 49: 1096–1101
Received May 16, 1997
Accepted July 30, 1997

Cold-storage of Rabbit Thoracic Aorta in University of Wisconsin Solution Reduces Endothelium-independent Vasodilation

J. V. D. GRYF-LOWCZOWSKI, D. SHERLOCK, J. SALISBURY*, I. S. BENJAMIN AND B. ALEXANDER

*Departments of Surgery and *Histopathology, King's College School of Medicine and Dentistry, Rayne Institute, 123 Coldharbour Lane, London SE5 9NU, UK*

Abstract

Optimum preservation conditions for storage of donor livers and blood vessels are essential for successful transplantation. The blood vessels are used as vascular conduits to facilitate anastomosis of the liver to the recipient's systemic vasculature. Failure of some transplants has been ascribed to thrombosis of these vascular conduits possibly because of alterations in vascular reactivity owing to inadequate storage techniques. To restrict data variability previously associated with studies using a heterogeneous sample of vessels from man, this study investigated changes in vascular reactivity in segments of rabbit thoracic aorta from male, age-matched, New Zealand White rabbits stored at 4°C in either University of Wisconsin solution (UW; Du Pont Pharmaceuticals, UK) or Krebs–Bülbring buffer (KB).

Percent vasodilation to acetylcholine remained significantly greater in UW than in KB at −log (M) concentrations of 7·0 (UW = 47·05 ± 4·26 compared with KB = 13·20 ± 7·20%; $P < 0·001$), 6·5 (UW = 66·82 ± 4·83 compared with KB = 26·60 ± 9·48%; $P < 0·01$), and 6·0 (UW = 83·68 ± 5·26 compared with KB = 31·20 ± 9·83%; $P < 0·001$). This was not significantly different to relaxation in unstored arteries and suggested improved endothelial function and structure, confirmed by electron microscopy. Percent vasodilation to sodium nitroprusside was significantly lower in UW than in unstored (D_0) arteries at −log (M) concentrations of 7·5 (D_0 = 28·27 ± 4·02 compared with UW = 15·21 ± 1·82%; $P < 0·01$), 7·3 (D_0 = 52·58 ± 5·05 compared with UW = 29·23 ± 1·94%; $P < 0·01$), 7·0 (D_0 = 69·70 ± 4·85 compared with UW = 49·72 ± 2·49%; $P < 0·05$), and 6·4 (D_0 = 93·16 ± 2·93 compared with UW = 71·29 ± 5·20%; $P < 0·05$). Percent vasodilation was also lower in UW- compared with KB-stored arteries at −log (M) sodium nitroprusside concentrations of 7·0 (UW = 49·72 ± 2·49 compared with KB = 64·11 ± 5·03%; $P < 0·05$) and 6·4 (UW = 71·29 ± 5·20 compared with KB = 96·91 ± 5·96; $P < 0·05$). Electron microscopy confirmed that this was not a result of degradation of smooth muscle structure. The nitric oxide synthase inhibitor L-N^G-nitro-L-arginine methyl ester (100 μM) did not significantly modulate sodium nitroprusside-induced vasodilation in unstored arteries, when endothelial function was maximum, or in UW-stored arteries, suggesting that the reduced responses in UW-stored arteries were not because of increased synthesis of nitric oxide. This reduced relaxation to sodium nitroprusside was therefore nitric oxide-independent and not a result of competition between sodium nitroprusside and endothelial 'nitric oxide donation' for cGMP.

In summary, cold-storage preservation with UW reduced endothelium-independent vascular relaxation by mechanisms other than competition with NO; this requires further evaluation.

Failures of donor-organ vascular reactivity induced by cold-preservation solutions critically prejudice the survival of transplanted organs (Clemens et al 1993). Segments of iliac arteries from man are harvested concurrently with livers destined for transplantation. The segments of artery are used to construct a conduit, when necessary, between the donor liver and the recipient's systemic circulation. Hepatic artery thrombosis occurs in 9–18% of transplants (Yanaga et al 1990) often on or around the 10th post-operative day (Marujo et al 1991) and leads to graft failure after transplantation. It has been suggested that the thrombosis might be a consequence of poor quality of the harvested vessels or inadequate storage techniques.

It is believed that optimum conditions are preserved to a greater extent by use of University of Wisconsin Solution (UW) rather than extracellular-type solutions such as Krebs' buffer, saline, eurocollins and crystalloid hyperkalaemic cardioplegic solution. The use of UW is established in liver transplantation (Todo et al 1989) and is increasingly advocated in heart (Wicomb et al 1993), lung (Hopkinson et al 1994) and small bowel (Schweizer et al 1994) transplantation. Reduced endothelium-independent coronary arterial relaxation to both sodium nitroprusside (Cartier et al 1993) and nitroglycerine (Wiklund et al 1994) has been reported after preservation of arteries with UW compared with extracellular-type solutions. This has been attributed to structural smooth muscle damage with the use of UW (Cartier et al 1993).

One recourse open to the surgeon after removal of the thrombosed artery is to re-anastomose a remaining segment of artery that has been stored at 4°C in UW. This study was designed to evaluate the vascular pharmacological changes that might occur in vessels stored under the conditions described, using rabbit thoracic aorta as the experimental model. The arteries were tested for endothelium-dependent and -independent vasodilation on harvesting and after storage for 8 days at 4°C, to reflect the time scale of current clinical practice of prolonged allograft cold-storage in UW. In addition, vascular reactivity was evaluated in a further group of arteries

Correspondence: B. Alexander, Department of Surgery, King's College School of Medicine and Dentistry, The Rayne Institute, 123 Coldharbour Lane, London SE5 9NU, UK.

stored in KB for 8 days at 4°C. Finally, scanning electron microscopy was used to compare the extent of retention of vascular morphology in the three groups of arteries.

Materials and Methods

Male New Zealand White rabbits, 2·2–2·9 kg, were killed by lethal injection of sodium pentobarbitone through a cannulated marginal ear vein (Schedule I killing). After thoraco-laparotomy the thoracic aorta was gently dissected out, avoiding vessel traction, and 5-mm rings were cut and placed under 2 g isometric tension upon fine-wire myographs, according to the technique of Mulvany & Halpern (1977) in oxygenated (95% O_2–5% CO_2) KB at 37°C. Similarly, harvested arteries were stored at 4°C in 20 mL sterile universal containers (Sterillin UK, Teddington, Middlesex, UK) containing either UW or KB and evaluated after 8 days cold-preservation. After 8 days preservation the vessels were placed upon the fine-wire myographs. All groups of vessels were left to equilibrate for 1 h before testing proceeded and, during this time, the baths were flushed with KB regularly every 15 min. In an additional group of freshly harvested arteries, the endothelium was removed, before testing. Endothelium was removed by gentle rotation of a pipe cleaner within the vessel lumen. The efficacy of endothelium removal by this technique was confirmed histologically (H&E staining) in randomly sampled arteries.

Drugs

UW was provided by Du Pont Pharmaceuticals UK. KB was freshly prepared in the laboratory for rabbit aortic ring KB-storage or organ bath perfusion. Its composition ((mM): NaCl 133, KCl 4·7, NaH_2PO_4 1·16, $NaHCO_3$ 20, $MgSO_4$ 0·60, glucose 7·8, $CaCl_2$ 2·52) was chosen to reflect extracellular-type preservation. Acetylcholine (chloride), sodium nitroprusside, noradrenaline and N^G-Nitro-L-arginine methyl ester (L-NAME) were purchased from Sigma (Poole, Dorset). Solutions (0·1 M) of acetylcholine, sodium nitroprusside and L-NAME were prepared in distilled water and used for further dilution. Noradrenaline was dissolved in 0·1 mM ascorbic acid to prevent oxidation.

Experimental protocol

Endothelium-dependent relaxation to acetylcholine and endothelium-independent relaxation to sodium nitroprusside were tested in rabbit aortic rings, under isometric conditions as described above, on harvesting and after 8 days cold-storage (4°C) in either UW or KB. Responses of a second group of rabbit aortic rings to sodium nitroprusside were again tested on harvesting and after 8 days storage in UW or KB in the presence of the nitric oxide synthase inhibitor L-NAME (100 μM). Cumulative concentration–response curves recording changes in isometric ring tension to increasing log concentrations of acetylcholine, sodium nitroprusside and sodium nitroprusside in the presence of L-NAME have been expressed as a percentage dilation from a submaximum preconstriction with a 2×10^6 (M) −log concentration of noradrenaline, determined from concentration–response curves constructed before each run.

Electron microscopy

For transmission electron microscopy, a separate group of specimen arteries was removed from either UW, or KB-storage after 8 days, minced, placed in fresh glutaraldehyde cacodylate solution for 1 h, then placed in cacodylate buffer. They were then post-fixed in 1% osmium tetroxide and embedded in epon. Sections (1 μm) were prepared and stained with toluidine blue. Sections with surface endothelial cells were identified, thin-sectioned, stained with uranyl acetate, and examined by a consultant pathologist with a Corinth AE1 electron microscope at magnification ranges of 2500–15 000.

Data analysis

Vasodilator responses were calculated as a percentage of the noradrenaline-induced increase in tone above that of resting tone. Results from concentration–response curves were expressed as the mean ± s.e.m. after confirmation that the data were normally distributed. Data were initially processed by the Newman–Keuls multiple comparisons test, using analysis of variance and, where differences were significant ($P < 0·05$), with post hoc Student's 2-tailed, unpaired t-test with the Bonferroni adjustment to exclude Type I_e errors (Ludbrook 1991).

Results

Noradrenaline induced concentration–dependent increases in vascular tone in all of the groups tested. The concentration of noradrenaline required to produce submaximum vessel contraction was established by construction of cumulative concentration–response curves at the start of every experiment. No significant differences were found between any of the groups tested and therefore all vessels were pre-constricted with a −log (M) concentration of 2×10^{-6} noradrenaline. This consistently reproduced an average of 71·3 ± 2·4% submaximum contraction. Acetylcholine induced concentration-dependent vasodilation in all the groups tested. The vasodilation was endothelium-dependent because it was abolished in freshly harvested, de-endothelialized preparations (Fig. 1). A trend

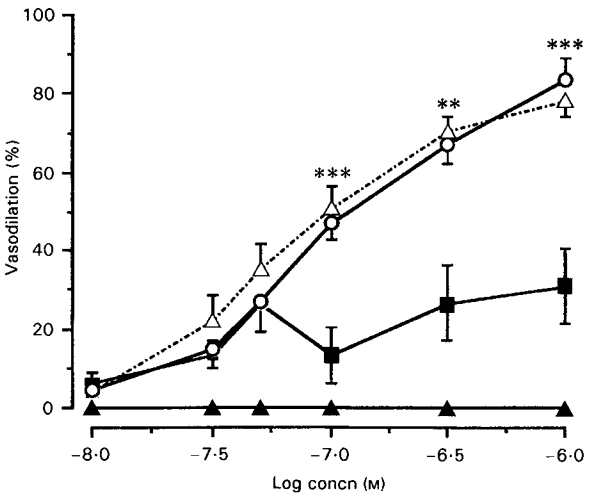

FIG. 1. Percent vasodilation to acetylcholine of pre-constricted sections of rabbit aorta on harvesting before (△) and after (▲) removal of endothelium at day 0 (i.e. on harvesting), and also after storage for eight days in UW (○) or KB (■). Vasodilation to acetylcholine was significantly greater in arteries stored in UW for 8 days than in those stored in KB, and was not significantly different from that measured for freshly-harvested arteries. $^{**}P < 0·01$, $^{***}P < 0·001$, significant differences between results from arteries stored in UW and in KB.

towards reduced retention of acetylcholine-induced vasodilation was observed in the KB group with increasing concentrations of acetylcholine when compared with the freshly-harvested and (day 8) UW groups. This became statistically significant at the three highest concentrations of acetylcholine tested when, after 8 days storage, the percentage vasodilation was significantly greater in UW- than in KB-stored arteries at $-\log$ (M) concentrations of 7·0 ($P < 0.001$), 6·5 ($P < 0.01$) and 6·0 ($P < 0.001$) (Fig. 1). Responses after UW-storage for 8 days were not significantly different from those of freshly-harvested arteries at all concentrations of acetylcholine tested.

Sodium nitroprusside induced concentration-dependent, endothelium-independent vasodilation in all groups tested (Fig. 2). Interestingly, a trend towards decreased vasodilation was observed in the (day 8) UW group compared with the KB group; this was significant at $-\log$ (M) sodium nitroprusside concentrations of 7·0 ($P < 0.05$) and 6·4 ($P < 0.05$). However, a significant attenuation of sodium nitroprusside-induced vasodilation was observed between UW-stored and D_0 (freshly-harvested) arteries at $-\log$ (M) sodium nitroprusside concentrations of 7·5 ($P < 0.05$), 7·3 ($P < 0.01$), 7·0 ($P < 0.01$) and 6·4 ($P < 0.01$). No significant differences were measured between the three groups at the highest concentration of sodium nitroprusside tested ($-\log$ M = 6·4). The action of the nitric oxide synthase inhibitor L-NAME (100 μM) upon sodium nitroprusside-induced vasodilation was then evaluated in a second group of experiments. Concentration-dependent vasodilation to sodium nitroprusside was readily demonstrated in freshly-harvested tissue for which sodium nitroprusside responses were unaltered by 100 μM L-NAME (Fig. 3A). The trend towards reduced sodium nitroprusside-induced vasodilation in UW-stored compared with D_0 arteries, shown in Fig. 2, persisted in the presence of L-NAME (Fig. 3B) and remained significant at the same $-\log$ (M) sodium nitroprusside concentrations of 7·5 ($P < 0.01$), 7·5 ($P < 0.01$), 7·0 ($P < 0.05$) and 6·4 ($P < 0.05$). Sodium nitroprusside-induced vasodilation remained statistically lower in UW-stored than in KB-stored arteries at $-\log$ (M) sodium nitroprusside concentrations of 7·0 ($P < 0.05$) and 6·4 ($P < 0.01$). Therefore inhibition of NO synthase did not reverse the previously observed reductions in sodium nitroprusside-induced vasodilation in UW stored arteries and, in addition, incubation of freshly-harvested arteries in L-NAME did not significantly alter this result.

Electron microscopy confirmed that the endothelial and smooth muscle layers of KB-stored tissue were severely damaged, possibly as a result of autolysis, after 8 days storage at 4°C (Fig. 4). In contrast, markedly superior endothelial and smooth muscle structural preservation was observed at both the cellular and sub-cellular level for UW-stored tissue. Mitochondria, endoplasmic reticulum and smooth muscle appeared unaltered, even after 8 days UW preservation at 4°C (Fig. 5).

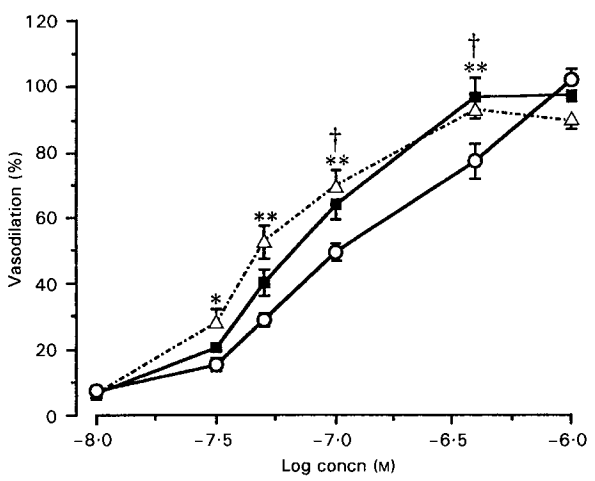

FIG. 2. Percent vasodilation to sodium nitroprusside of pre-constricted sections of rabbit thoracic aorta on harvesting (△) and after storage for eight days in UW (○) or KB (■). Vasodilation to sodium nitroprusside was significantly (*$P < 0.05$, **$P < 0.01$) less in arteries stored in UW for 8 days than in unstored arteries (△) and also significantly (†$P < 0.05$) less in arteries stored in KB for 8 days than in those stored in UW.

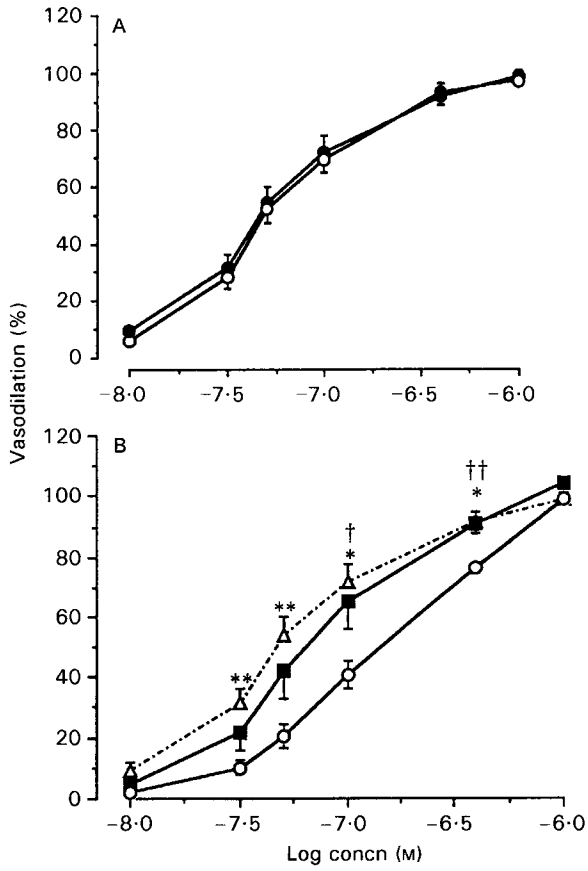

FIG. 3. A. Plots of response of freshly harvested arteries against sodium nitroprusside concentration with (●) and without (○) L-NAME. L-NAME did not significantly change sodium nitroprusside-induced vasodilation in freshly-harvested arteries. B. Plots of response, in the presence of L-NAME, of arteries stored for 8 days in UW (○), KB (■) and in freshly-harvested rings (△). In the presence of L-NAME sodium nitroprusside-induced vasodilation was significantly greater in arteries stored in KB for 8 days than in those stored in UW for $-\log$ (M) sodium nitroprusside concentration = 6·4 and 7·0. However sodium nitroprusside-induced vasodilation in freshly-harvested arteries remained elevated and unaltered above that for UW-stored arteries after incubation with L-NAME. †$P < 0.05$, ††$P < 0.01$, significant difference between results from arteries stored in UW and in KB. *$P < 0.05$, **$P < 0.01$, significant difference between results from freshly-harvested arteries and those stored in UW.

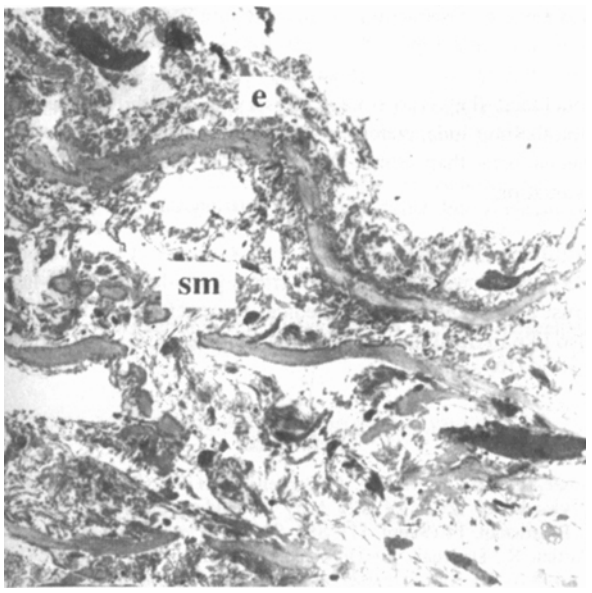

FIG. 4. Electron photomicrograph at ×2500 magnification of the intima and subjacent media of a section of rabbit thoracic aorta stored in KB. The endothelium (e) and smooth muscle (sm) layers are severely damaged.

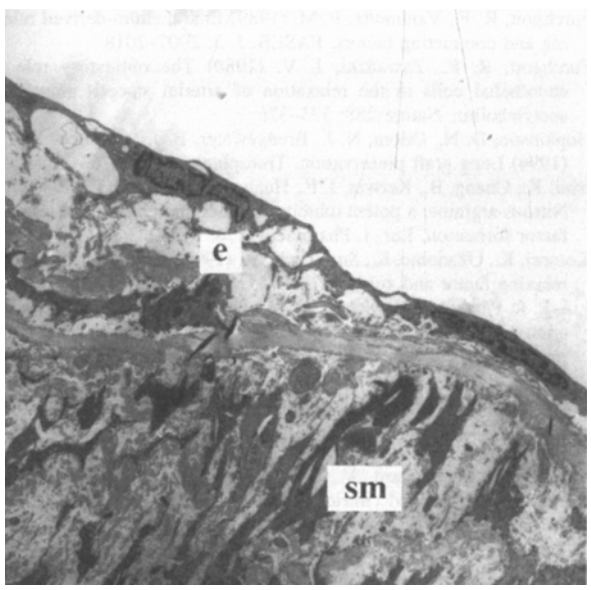

FIG. 5. Electron photomicrograph at ×2500 magnification of the intima and subjacent media of a section of rabbit thoracic aorta stored in UW. Normal endothelial (e) and smooth muscle (sm) morphology has been preserved.

Discussion

Rabbit thoracic aorta was used as the experimental model to limit data variability experienced in an earlier pilot study where vessels from man were used (data not presented). The heterogeneity of results might have been because of the use of donors of different sex or variations in size, age, anatomical location or the histopathology of the vessels used. Moreover, donors from different centres were given different drug regimens before donation and this factor alone might have confounded data interpretation. Previous attempts to identify degradative changes using tissue from man have proved inconclusive, possibly because of the aforementioned reasons (Komori et al 1991). Molecular studies have isolated defective expression of some compliment regulatory proteins but the relevance of this to the functional activity of stored blood vessels or organ microvasculature remains to be clarified (Scoazec et al 1994). Vessels of size comparable with those used during liver transplantation, but originating from aged-matched male New Zealand White rabbits were used during this investigation to obviate many of the above complications. The current study has provided new information about the degradative changes which occur in vascular material and possibly in organ microvasculature during cold-storage at 4°C. However, caution should be used when making direct extrapolations to man because major differences have been cited between vascular material from man and from animals (Lin et al 1991).

NO-dependent relaxation of the rabbit aorta to acetylcholine is believed to be entirely dependent upon an intact vascular endothelium (Furchgott & Zawadzki 1980). The current study attempted to evaluate endothelial function after storage in different preservation solutions by comparison of curves of concentration against response to acetylcholine in matched rabbit aortic rings. Endothelium-dependent responses to acetylcholine were unaltered after 8 days UW storage, were reduced after storage in KB and absent after de-endothelialization, suggesting that the use of UW improved endothelial preservation. The vascular pharmacological data were partially confirmed by the almost unaltered electron microscopic appearance of rabbit aortic endothelium after 8 days UW storage and reduced endothelial preservation after storage with KB.

Endothelium-independent vasodilation of UW-stored rings to sodium nitroprusside was reduced after 8 days storage although, interestingly, this was not a prominent finding in KB-stored rings. Indeed, the only statistically significant differences between KB- and UW-stored rings were measured at $-6{\cdot}4$ and $-7{\cdot}0$ log (M) sodium nitroprusside. Reduced endothelium-independent coronary artery relaxation to sodium nitroprusside has been reported after arterial preservation with UW and has been attributed to structural smooth muscle damage with the use of UW (Cartier et al 1993). Shelf-stored UW contains oxidized glutathione which is a powerful activator of the collagenolytic system and UW-storage has been shown to induce loss of cardiac collagen (Wolkowicz & Caulfield 1991). A smooth-muscle compartment damaged in this way might explain the smaller sodium nitroprusside responses obtained after UW-storage. The UW used in our experiments was also 'shelf stored' but electron microscopy confirmed that reduced responses to sodium nitroprusside after UW-storage compared with KB storage were, in fact, associated with markedly improved smooth muscle preservation in UW-stored compared with KB-stored rings. Therefore, in our experiments, reduced relaxation to sodium nitroprusside in UW-preserved donor vasculature did not appear to be because of structural damage as a result of this type of storage.

Contrasting endothelium-dependent and endothelium-independent relaxation after UW-preservation was surprising because both acetylcholine and sodium nitroprusside might have mediated vascular relaxation through cGMP-dependent phosphorylation and dephosphorylation of smooth muscle myosin light chains (Furchgott & Vanhoutte 1989). Therefore

the action of UW on preservation might have been expected to be non-selective for different vascular compartments. It has been reported that the endothelium-independent action of sodium nitroprusside on vascular smooth muscle involved increased production of cGMP owing to stimulation of soluble guanylate cyclase in the muscle wall (Walter et al 1983) probably via the generation of NO (Rappaport & Murad 1988). Endothelial NO release, however, was also reported to stimulate soluble guanylate cyclase to form cyclic GMP, and NO synthase inhibition caused a sodium nitroprusside-stimulated increase in rat lung fibroblast cGMP (Ishii et al 1990). The diminished sodium nitroprusside responses observed after UW-storage might be because an up-regulated release of basal NO induced a decrease in the available pool of cGMP for activation by sodium nitroprusside. It is, therefore, possible that greater responses to sodium nitroprusside in KB-stored rings were a result of reduced endothelial preservation owing to a comparatively reduced basal production of NO with this solution. This would be consistent both with the electron microscopy findings, which were suggestive of improved endothelial preservation with the use of UW, and with the pharmacological responses to acetylcholine of KB-stored rings.

It has been reported that the effects of nitrovasodilators such as sodium nitroprusside on isolated, unstored, arteries were inhibited by nitric oxide (Pohl & Busse 1987) but were enhanced both by removal of the endothelium (Shirasaki & Su 1985) and by nitric oxide synthase inhibitors (Moncada et al 1991; Ralevic et al 1991). It was suggested that competition for cGMP between endothelial NO and NO donation by sodium nitroprusside explained this observation (Pohl & Busse 1987; Busse et al 1989; Ralevic et al 1991). Therefore inadequate or poor endothelial preservation and reduced NO release in cold-stored vessels could have been observed as increased relaxation to sodium nitroprusside. Alternatively, reduced relaxation to sodium nitroprusside might reflect improved endothelial preservation and increased NO release after different types of storage. The concept that hypoxic or other endothelial damage might enhance localized sensitivity to nitrovasodilators might have important therapeutic implications, for instance in coronary artery disease (Luscher et al 1989). Therefore reduced sodium nitroprusside responses after UW cold-storage could be a result of improved endothelial preservation and increased release of NO and not, as has been suggested, because of structural damage as a result of the use of this preservation solution.

However, no statistically significant differences were found between sodium nitroprusside-induced responses in freshly-harvested rings before and after incubation with L-NAME when endothelial preservation was maximum. Moreover, the reduced sodium nitroprusside relaxation observed in UW rings, after 8 days storage, was also not significantly altered by L-NAME. This reduced relaxation to sodium nitroprusside was therefore endothelium-independent and was not a result of competition between sodium nitroprusside and endothelial 'NO donation' for cGMP. Similar changes were observed in coronary arteries after a much shorter (4 h) period of storage in UW solution (Cartier et al 1993) and did not appear to be related to structural degeneration.

It is suggested that donor organ microvascular reactivity, believed critical to whole-organ survival (Clemens et al 1993) was a result of reductions in endothelium-independent activity, similar to that reported in this study, possibly in a manner unrelated to structural degeneration, after UW-storage. It is concluded that cold-storage preservation with UW reduced endothelium-independent vascular relaxation by other mechanisms than competition with NO and requires further evaluation.

Acknowledgements

This project was supported by grants provided by The Mason Trust and King's College Medical Research Trust. We would also like to thank Du Pont Pharmaceuticals (UK, Ltd) for their gift of UW solution.

References

Busse, R., Pohl, U., Mulsch, A., Bassenge, E. (1989) Modulation of the vasodilator action of SIN 1 by the endothelium. J. Cardiovasc. Pharmacol. 14 (Suppl. 11): S81–S85

Cartier, R., Dagenais, F., Hollmann, C., Carrier, M., Pelletier, L. C. (1993) The role of preservation solutions in coronary endothelial damage during cold storage. Transplantation 56: 997–1000

Clemens, M. G., Chun, K., Miescher, E., Jones, D., Zhang, J. (1993) Leukocyte-dependent and -independent hepatic microvascular injury during reperfusion after warm ischaemia. In: Messmer, K., Menger, M. D. (eds) Liver Microcirculation and Hepatobiliary Function. Prog. Appl. Microcirc., Karger, Switzerland, 19: 139–151

Furchgott, R. F., Vanhoutte, P. M. (1989) Endothelium-derived relaxing and contracting factors. FASEB. J. 3: 2007–2018

Furchgott, R. F., Zawadzki, J. V. (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288: 373–376

Hopkinson, D. N., Odom, N. J., Bredgewater, B. J. M., Hooper, T. L. (1994) Lung graft preservation. Transplantation 58: 763–768

Ishii, K., Chang, B., Kerwin, J. F., Huang, Z. J., Murad, F. (1990) N^{ω}-Nitro-L-arginine: a potent inhibitor of endothelium-derived relaxing factor formation. Eur. J. Pharmacol. 176: 219–223

Komori, K., Okadome, K., Sugimachi, K. (1991). Endothelium-derived relaxing factor and vein grafts. Br. J. Surg. 78: 1027–1030

Lin, P. J., Pearson, P. J., Schaff, H. V. (1991). Endothelium-dependent contraction and relaxation of the human and canine internal mammary artery: studies on bypass graft vasospasm. Surgery 110: 127–135

Ludbrook, J. (1991) On making multiple comparisons in clinical and experimental pharmacology and physiology. Clin. Exp. Pharmacol. Physiol. 18: 379–392

Luscher, T. F., Richard, V., Yang, Z. (1989) Interaction between endothelium-derived nitric oxide and SIN-1 in human and porcine blood vessels. J. Cardiovasc. Pharmacol. 14 (Suppl 11): S76–S80

Marujo, W. C., Langnas, A. N., Wood, R. P., Statta, R. J., Li, S., Shaw, B. W. (1991) Vascular complications following orthotopic liver transplantation: outcome and the role of urgent revascularization. Transplant. Proc. 23: 1484–1486

Moncada, S., Rees, D., Schulz, R., Palmer, R. M. J. (1991) Development and mechanisms of a specific supersensitivity to nitrovasodilators after inhibition of vascular nitric oxide synthesis in-vivo. Proc. Natl Acad. Sci. USA. 88: 2166–2170

Mulvany, M. J., Halpern, W. (1977) Contractile properties of small resistance vessels in spontaneously hypertensive and normotensive rats. Circ. Res. 41: 19–26

Pohl, U., Busse, R. (1987) Endothelium-dependent relaxant factor inhibits effects of nitro compounds in isolated arteries. Am. J. Physiol. 252: H307–H313

Ralevic, V., Mathie, R. T., Alexander, B., Burnstock, G. (1991) N^{G}-Nitro-L-arginine methyl ester attenuates vasodilator responses to acetylcholine but enhances those to sodium nitroprusside. J. Pharm. Pharmacol. 43: 871–874

Rappaport, R. M., Murad, F. (1983) Agonist-induced endothelium-dependent relaxation in rat thoracic aorta might be mediated through cGMP. Circ. Res. 52: 352–357

Schweizer, E., Gassel, A. M., Deltz, E., Schroeder, P. (1994) A comparison of preservation solutions for small bowel transplantation in the rat. Transplantation 57: 1406–1408

Scoazec, J. Y., Delautier, D., Moreau, A., Durand, F., Degott, C., Benhamou, J. P., Belghiti, J., Feldmann, G. (1994). Expression of complement-regulatory proteins in normal and UW-preserved human liver. Gastroenterology 107: 505–516

Shirasaki, Y., Su, C. (1985) Endothelium removal augments vasodilation by sodium nitroprusside and sodium nitrite. Eur. J. Pharmacol. 114: 93–96

Todo, S., Nery, J., Yanaga, K., Podesta, L., Gordon, R. D., Starzl, T. E. (1989) Extended preservation of human liver grafts with UW solution. J. Am. Med. Assoc. 261: 711–714

Walter, U., Waldmann, R., Nieberding, M. (1988) Intracellular mechanism of action of vasodilators. Eur. Heart J. 9 (Suppl. H): 1–6

Wicomb, W. N., Levy, J. V., Holdfer, M., Collins, G. M. (1993) Functional integrity of vascular endothelium correlates with myocardial function in stored rabbit hearts. Transplant. Proc. 25: 1639–1641

Wiklund, L., Mantovani, V., Nilsson, B., Nilsson, F. N., Berggren, H. E. (1994) Temperature-dependent coronary reactivity after perfusion with University of Wisconsin solution in the isolated rat heart. Transplantation 57: 1314–1317

Wolkowicz, P. E., Caulfield, J. B. (1991) Cardioplegia with aged UW solution induces loss of cardiac collagen. Transplantation 51: 898–901

Yanaga, K., Lebreau, G., Wallis March, J., Gordon, R. D., Makowa, L., Tzakis, A. G., Todo, S., Stieber, A. C., Iwatsuki, S., Starzl, T. E. (1990) Hepatic artery reconstruction for hepatic artery thrombosis after orthotopic liver transplantation. Arch. Surg. 125: 628–631