

Viable Vein Graft Preservation

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Homologous vein grafts are becoming increasingly useful in patients without suitable autologous saphenous veins for peripheral arterial bypass procedures [4, 8]. However, fresh veins are the only tissue that has been used successfully in homograft vascular reconstruction in terms of long-term patency [4]. The advantages of a reliable method of long-term storage of viable vein segments and the ultimate creation of a vein "bank" are therefore apparent.

Dimethylsulfoxide (DMSO) is a low molecular weight compound that diffuses readily across cell membranes [2] and has been shown to be an effective cryoprotectant avoiding cellular dehydration, a major cause of cell death during freezing [2]. The DMSO has been used for successful cryopreservation of other tissues [3, 6], but has not been utilized for vein graft preservation.

This study assesses a method for the long-term preservation of viable vein segments by freezing in liquid nitrogen with DMSO cryopreservation.

METHODS

Contraction Studies

Segments of jugular veins from healthy mongrel dogs of either sex were used throughout the study. *In vitro* contraction studies were carried out with 12 fresh veins incubated for 1 hr in either physiologic salt solution (PSS), 15% dimethylsulfoxide (DMSO), 20% DMSO, or 25% DMSO at room temperature. After incubation, 3-mm segments from each vein were suspended

from a strain gauge transducer in PSS in an organ bath at 37°C for 30 min. The transducer was connected to a 4-channel strip chart recorder. After 1-g of tension was applied to each vein segment, 5×10^{-6} g epinephrine was added to the organ bath, and the vein contraction measured and recorded.

Another group of 12 veins were excised and incubated in either PSS, 15% DMSO, 20% DMSO, or 25% DMSO. The vein segments were then placed in small sterile polyethylene bags¹ and rapid-frozen (5°C/sec) by immersion in liquid nitrogen. The bags were stored at -140°C for 24 hr and then rapid-thawed (5°C/sec) to 37°C. The veins were suspended from the transducer in an organ bath, and the vein smooth muscle contraction measured and recorded in response to 5×10^{-6} g epinephrine.

A third group of 12 fresh veins were tested for smooth muscle contraction in the organ bath immediately after removal from the dog.

Histochemistry

Sixteen vein segments were incubated for 1 hr in PSS, and another 16 veins were incubated in 15% DMSO. Eight veins from each solution were frozen in liquid nitrogen, using the above described techniques, and stored at -140°C for 1 wk. The remaining eight veins from each solution were stripped of all adventitial fat, and homogenized in a sucrose-saline solution for 1 min. The

¹Hemoflex Bags, Union Carbide Corporation.

following enzymes were then measured in the homogenate using standard laboratory procedures:² lactic dehydrogenase (LDH), acid phosphatase (acid phos.), alkaline phosphatase (alk. phos.), glutamic oxalo-transaminase (GOT), and glutamic pyruvate transaminase (GPT). The same enzymes were measured in the frozen vein groups after thawing to room temperature.

Histology

Segments of veins were examined by both light and electron microscopy after incubation in 15% DMSO or PSS for 1 hr and after freezing in liquid nitrogen for 1 wk. In addition, a control group of fresh veins was evaluated by both light and electron microscopy immediately after excision. Tissues examined by light microscopy were stained with hematoxylin and eosin, Verhoeff's elastic tissue stain and Masson's TriChrome. Transmission electron microscopy was completed utilizing standard procedures and staining with Uranyl acetate and lead citrate.

In Vivo Studies

In vivo evaluation was performed by implantation of autologous canine jugular vein grafts in the carotid artery. Before implantation, 3-mm segments of each vein were removed for contraction studies. In each group, the vein grafts were evaluated by surgical exploration 1 month after implantation. Patent vein grafts were biopsied and graft continuity restored by end-to-end anastomosis. Vein grafts found to be thrombosed at exploration were removed. All material removed was examined by light microscopy and transmission electron microscopy. The remaining grafts were evaluated 6 months postoperatively by arteriography.

The dogs in this series were divided into three groups. Group I (10 dogs) had 1 jugular vein frozen for 24 hr in PSS and the contralateral jugular vein frozen for 24 hr in 15% DMSO. After thawing, the veins were

implanted as autografts in the carotid arteries. Group II (8 dogs) had 1 jugular vein frozen in PSS for 28 days and the other jugular vein frozen in 15% DMSO for 28 days. These veins were likewise implanted as autografts in the carotid arteries; Group III (8 dogs) had 1 jugular vein frozen in 15% DMSO for 1 wk, thawed and implanted as an autograft in 1 carotid artery. The contralateral jugular vein was implanted immediately after removal into the other carotid artery.

RESULTS

Contraction Studies

The results of *in vitro* contraction studies are summarized in Table 1. Simple incubation of vein segments in 20% DMSO and 25% DMSO significantly ($p < .05$) reduced smooth muscle contraction in response to epinephrine when compared to fresh control veins and veins incubated in PSS for 1 hr. However, incubation in 15% DMSO did not significantly alter vein contractility. After freezing in liquid nitrogen for 24 hr, 15 and 20% DMSO preserved vein contraction best, while veins frozen in PSS did not contract.

Histochemistry

The results of tissue enzyme studies are summarized in Table 2. Incubation of veins in PSS for 1 hr did not alter any of the tissue enzymes measured when compared to controls (fresh tissue). However, freezing veins for 1 wk in PSS significantly reduced the levels of all enzymes tested ($p < .05$).

TABLE 1

Mean Contraction Values of Vein Segments in Response to 5×10^{-6} gm Epinephrine after Incubation and Freezing in Liquid Nitrogen

	Contraction after 1 hr Incubation (gm)	Contraction after 24 hr Freezing (gm)
Fresh	1.20 ± 0.19	—
PSS	1.16 ± 0.21	0.00
15% DMSO	0.87 ± 0.20	*0.57 ± 0.16
20% DMSO	*0.48 ± 0.12	*0.46 ± 0.11
25% DMSO	*0.24 ± 0.08	*0.18 ± 0.09

* $p < .05$ compared to fresh control.

²Sigma Prepared Kits, Sigma Corporation.

TABLE 2
Mean Enzyme Activities in Units/gm of Vein Tissue after 1-hr Incubation and after Freezing
in Liquid Nitrogen for 7 Days

	LDH	GOT	GPT	Alk. Phos.	Acid Phos.
Fresh	4072	73.3	11.4	6.0	1.2
PSS 1-hr incubation	3894	81.4	8.5	6.2	1.6
PSS 7 days freezing	*975	*12.4	*2.5	*2.0	*0.24
15% DMSO 1-hr incubation	9860	68.5	8.3	*0.58	*0.46
15% DMSO 7 days freezing	3316	62.6	7.8	*1.9	*0.65

* $p < .05$ compared to fresh tissue control.

Incubating veins in 15% DMSO for 1 hr and freezing veins for 1 wk in 15% DMSO significantly decreased tissue acid and alkaline phosphatase. However, normal levels of LDH, GOT, and GPT were maintained both after incubation in 15% DMSO and after freezing for 1 wk in 15% DMSO.

Histology

Examination of vein segments by light microscopy did not show any obvious differences among the different groups.

However, transmission electron microscopy revealed a number of changes in the ultrastructure of frozen veins. Vein segments frozen in PSS showed loss of muscle striations and the muscle bundles appeared granular compared to fresh (control) veins. Also, there was loss of detail in the collagen. The mitochondria were often normal in appearance, but there were some alterations noted in the endothelium. (Figs. 1, 2a,b)

Vein segments frozen with 15% DMSO appeared well preserved (Fig. 3). The endothelium appeared similar to fresh veins. Smooth muscle and collagen appeared to be unaltered. However, in some sections the mitochondria were swollen and the cristae distorted. In other sections, the mitochondria appeared unchanged when compared to fresh veins.

In Vivo Studies

The results of the *in vivo* studies are summarized in Table 3. Segments of veins removed for contraction studies before implantation were in agreement with the prior contraction studies, i.e., veins frozen in 15%

DMSO contracted in an essentially identical manner to fresh control vein segments, while veins frozen in PSS had a significantly smaller contraction response ($p < .05$). Vein segments frozen in DMSO were similar to fresh veins when used as autografts. Both had a low rate of thrombosis (23 and 25%, respectively). Veins frozen in PSS had a very high thrombosis rate (87.5%), which was significantly different from the thrombosis rates of both fresh and DMSO preserved veins ($p < .05$).

DISCUSSION

While homograft veins have been used for several years with reasonable long-term patency [4, 8], little attention has been directed toward practical methods of long-term storage of vein segments. Greater use of homologous vein grafts would no doubt save many limbs, but the lack of a reliable method of vein storage has limited its usefulness.

Dimethylsulfoxide (DMSO) is a low molecular weight compound that readily diffuses across cell membranes and has been shown to prevent intracellular dehydration during freezing [2]. The DMSO has been shown to successfully protect other tissues at temperatures of -70° to -190° C [3, 6]. Thus a method of storage of vein segments using DMSO at liquid nitrogen temperatures (-190° C) seemed worth evaluating.

In vitro vein contraction was utilized to determine the optimal concentration of DMSO that would protect the vessel smooth muscle. In addition, there was good correlation between smooth muscle contraction



FIG. 1. Transmission electron photomicrograph of fresh vein. Endothelium is superior. Note intact endothelial structures and well-defined smooth muscle and collagen bundles ($\times 12,000$; uranyl acetate and lead citrate stain.)



FIG. 2a. Transmission electron photomicrograph of vein segment after freezing and thawing in physiologic salt solution. Endothelium is superior and to the left and shows loss of detail. Collagen and smooth muscle do not appear well defined. In this section, the mitochondria appear distorted and swollen ($\times 25,000$; uranyl acetate and lead citrate stain.)



FIG. 2b. Transmission electron photomicrograph of vein segment after freezing and thawing in physiologic salt solution. The endothelium is superior and to the left and shows better definition of ultrastructure. Note loss of muscle striations and granularity and loss of detail in muscle bundles and collagen ($\times 20,600$; uranyl acetate and lead citrate stain).



FIG. 3. Transmission electron photomicrograph of vein segment after freezing and thawing in 15% DMSO. The endothelium is superior and to the right. Note generally well-preserved appearance similar to fresh vein (Fig. 1). There is preservation of smooth muscle striations with well-defined collagen and smooth muscle bundles. The endothelium appears unaltered and in this section the mitochondria are normal ($\times 12,500$; uranyl acetate and lead citrate stain).

TABLE 3
Mean Vein Contraction in Response to 5×10^{-6} gm Epinephrine and Autograft Patency

Group	Mean contraction (gm)	No. patent at 1 mo. exploration	No. patent at 6 mo. arteriography
Fresh	1.10 ± 0.20	6/8 (75%)	6/8 (75%)
15% DMSO frozen 1 day	0.74 ± 0.11	8/10 (80%)	8/10 (80%)
15% DMSO frozen 7 days	0.82 ± 0.15	7/8 (87.5%)	7/8 (87.5%)
15% DMSO frozen 28 days	0.91 ± 0.16	5/8 (62.5%)	5/8 (62.5%)
PSS frozen 1 day	*0.21 ± 0.06	2/10 (20%)	1/10 (10%)
PSS frozen 28 days	*0.27 ± 0.08	0/8 (0%)	0/8 (0%)

* $p < .05$ compared to fresh control.

in vitro and the usefulness of the vein as an arterial graft. The use of venous contraction as a measure of viability has not been reported previously.

The consistent thrombosis of veins that were frozen in PSS is of interest, and suggests that damage due to freezing in PSS is not present in veins frozen in DMSO. Light microscopy failed to demonstrate specific endothelial lesions in veins frozen without DMSO cryoprotection. However, transmission electron microscopy showed degenerative changes in vascular smooth muscle and in the organelles of the endothelial cells in PSS frozen veins. It is not clear, however, how these relatively non-specific changes are related to the rapid and consistent thrombosis of the PSS frozen veins. Scanning electron microscopy is presently being employed in this laboratory for the examination of these veins and may provide additional insight into subtle endothelial damage that may be present.

Histochemical evaluation of vein segment tissue enzymes showed statistically significant differences between fresh and PSS frozen tissues that were not present in DMSO frozen veins. Because the enzymes measured are basically contained in muscle, this is not surprising and correlates well with the smooth muscle contraction studies that show little viable smooth muscle in veins frozen in PSS.

The *in vivo* assessment of autologous vein grafts employed in this study has shown that veins frozen in 15% DMSO for up to 28 days function in an identical fashion to fresh vein autografts. Veins frozen in PSS are not

useful as arterial grafts, presumably due to irreversible smooth muscle and endothelial damage, resulting in rapid thrombosis of the graft.

Studies of the immunology of homologous venous tissue suggest that matching major blood groups might reduce rejection and improve patency of the graft [4, 5, 7]. Freeze-drying has been shown to alter the antigenicity of tissues [1] and this suggests the possibility that DMSO freezing might also decrease the antigenicity of vein homografts; studies with venous homografts are now underway in this laboratory to investigate this possibility.

It now appears feasible to consider removing saphenous veins from cadavers and by surgical stripping, and storing them in 15% DMSO in liquid nitrogen. These veins can be grouped according to ABO blood type, and matched in this respect to a prospective recipient. Prior studies with blood and other tissues suggest that storage in liquid nitrogen with adequate cryopreservatives can be carried out almost indefinitely [3]. The homologous vein graft is a satisfactory alternative to small diameter plastic prostheses, and can now be more readily available through storage in liquid nitrogen.

SUMMARY

In vitro and *in vivo* evaluation of veins preserved in DMSO has shown:

- (1) 15% DMSO is the optimal concentration for the cryopreservation of venous tissue in liquid nitrogen.

- (2) Veins preserved in PSS in liquid nitrogen are not useful as arterial grafts.
- (3) Veins preserved in 15% DMSO in liquid nitrogen are similar in function to fresh veins when used as arterial autografts.

Long-term storage of veins for use as arterial homografts in a vein "bank" appears feasible.

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