British Journal of Plastic Surgery (1993), **46**, 288–291

BRITISH JOURNAL OF PLASTIC SURGERY

Amelioration of secondary ischaemic injury by perfusion with University of Wisconsin (UW) solution in rat skin flaps

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SUMMARY. **This study was designed to observe the effect of perfusion with University of Wisconsin (UW) preservation solution on skin flap survival following secondary ischaemia caused by venous obstruction in rats. An epigastric flap model was used.**

Saline-perfused flaps exhibited no significant improvement in survival compared to untreated animals (NS). Skin flaps perfused with UW solution, however, had a significant increase in survival to 40% (8/20) ($p < 0.01$) when perfused before the onset of primary ischaemia and 30% (6/20) ($p < 0.05$) when given before the onset of secondary ischaemia. These results show that UW solution improves skin flap survival, presumably through preservation of the microvasculature.

The use of perfusion solutions to enhance ischaemia tolerance of skin flaps has produced mixed results. Several early studies failed to show a benefit.[1–3] On the other hand, Rosen *et al.*[4] reported that flaps perfused with a neutral and osmotically balanced solution of salts and amino acids had a marked increase in ischaemia tolerance and better functioning microcirculation. The University of Wisconsin (UW) solution is an empirically derived, physiologically balanced preservation medium originally developed for pancreas preservation.[5] UW solution has shown great usefulness in liver[6] and cardiac[7] preservation. Turk and coworkers recently reported that perfusion of a skin flap with UW solution produced a three-fold increase in survival after primary arteriovenous (AV) ischaemia.[8]

Primary ischaemia is the first insult a tissue suffers before the restoration of blood flow, as in a free tissue transfer. If blood flow is disrupted postoperatively, for example by a vascular thrombosis or obstruction, the tissue encounters a secondary ischaemic insult. Skin has been shown to be less tolerant to secondary AV ischaemia than to primary AV ischaemia in porcine[9] and rat skin flap models.[10] Oxygen-derived free radicals have been implicated in ischaemia/reperfusion injury in prolonged primary ischaemia.[11] The survival rates of flaps subjected to secondary ischaemia have been shown to be improved by treat-

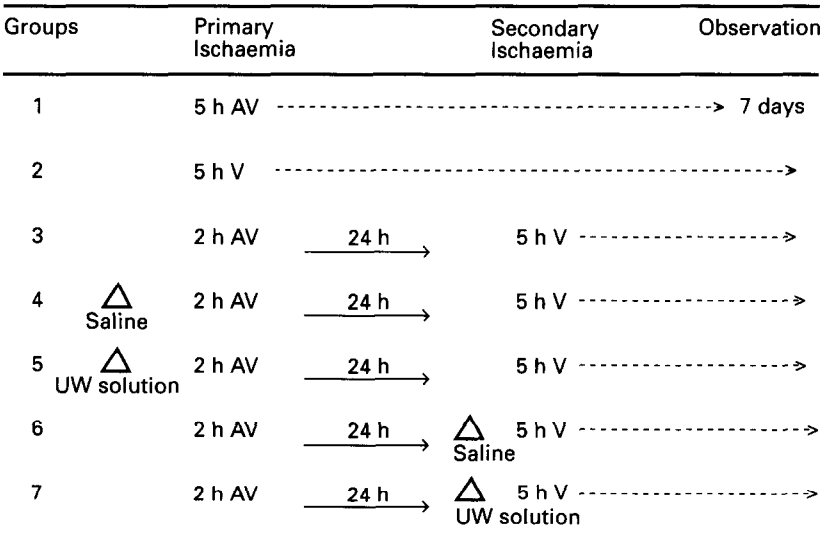

Fig. 1

Figure 1—Experimental protocol. AVO, arteriovenous occlusion; VO, venous occlusion; △, perfusion.

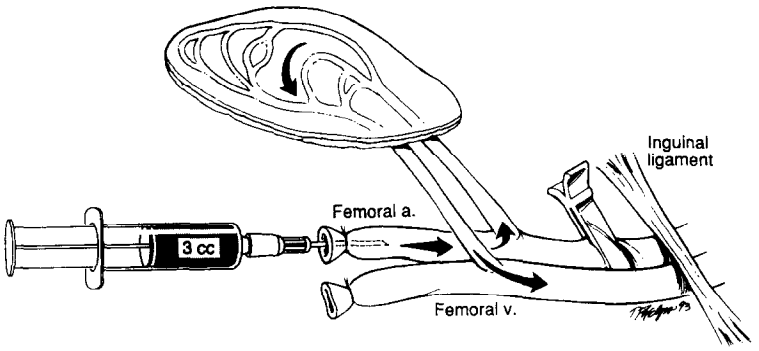

Fig. 2

Figure 2—Illustration of the operative procedure and irrigation method.

ments with a thromboxane synthetase inhibitor,[12] deferoxamine, an iron chelator,[13] or non-glucocorticoid 21-aminosteroid, an inhibitor of lipid peroxidation,[14] suggesting oxygen free radicals as important mediators in secondary ischaemic injury.[15,16]

The current study investigated whether perfusion of a skin flap with UW solution, which has proved to protect the microvasculature against an ischaemic insult in other organs, enhances the tolerance of skin to secondary venous ischaemia.

Materials and methods

One hundred and forty male Sprague-Dawley rats, 270–310 g, were used in the experiment. A 3 × 6 cm abdominal skin flap, based on the epigastric vessels, was elevated with sodium pentobarbital (40 mg/kg) anaesthesia as previously described.[17] The microvascular clamps used were "V" series (ST-B-IV) for vessels 0.4–1.0 mm in size (Accurate Surgical Scientific Instruments, Westbury, NY, USA). In a pilot study, the functional status of vascular clamps was tested by intravenous injection of fluorescein dye; only proven clamps which did not leak the dye were used during the experiments. The muscular branch and the distal branches (saphenous and popliteal) of the femoral vessels were cauterised and divided. The experimental design ensures that blood flow of the flap occurs solely via the superficial epigastric/femoral vessels. Primary ischaemia was produced by arteriovenous occlusion for 2 h: a microvascular clamp was placed on the femoral artery and vein between the origins of the deep (profunda) branch and the epigastric vessels.[10] Twenty-four hours after primary ischaemia, venous drainage was occluded by placing a microvascular clamp on the femoral vein *alone* for 5 h. The flaps were sutured in their donor sites and observed daily to assess survival for 7 days after secondary ischaemia.

Experimental animals were divided into 7 groups (Fig. 1). The first three groups were controls: group 1 (n = 20), primary AV ischaemia was produced by 5 h of arteriovenous occlusion; group 2 (n = 20), primary venous ischaemia was produced by occluding the vein *alone* for 5 h, and group 3 (n = 20), was 5 h of secondary venous obstruction which received no treatment.

Treatment groups (4–7) (n = 80): Animals were subjected to 5 h of secondary venous obstruction. UW solution (Via Span®) was obtained from Dupont Pharmaceutical Co. (Wilmington, DE, USA) and its composition is presented in Table 1.[18] Three ml of saline or UW solution were perfused via a catheter placed in the distal femoral artery, to the skin flap over a 5 min period and allowed to flush out blood from the flap into the femoral vein (Fig. 2) at either the onset of primary AV ischaemia (groups 4 and 5) or at the onset of secondary venous ischaemia (groups 6 and 7).

In all groups except group 5, survival was an all-or-none phenomenon; several flaps in group 5 showing a preponderance of necrosis, with small areas of viability, were considered necrotic. Fisher's exact tests were used for comparison of flap survival rates between groups.

Results

The survival rates are summarised in Table 2. In our previous report, 3 and 5 h of secondary ischaemia inflicted by venous occlusion 24 h after 1.5 h of primary ischaemia produced 54% and 0%, respectively.[12] In the present study, group 1 (5 h AV, primary ischaemia) had the best survival rate (100%) ($p < 0.05$, at least). Primary venous obstruction (group 2) resulted in survival of 50% compared to group 1 ($p < 0.001$) and untreated secondary venous obstruction (group 3) showed no flap survival ($p < 0.001$).

Saline-perfused groups (4 and 6) showed no statistically significant differences in flap survival com-

Table 1 Composition of UW solution

Component	*Concentration*
K Lactobionate	100 mM
NaH_2PO_4	25 mM
$MgSO_4$	5 mM
Glutathione	3 mM
Raffinose	30 mM
Allopurinol	1 mM
Insulin	100 U/L
Bactrim	0.5 ml/L
Hydroxyethyl starch	5 g%
Na^+	30 mM
K^+	120 mM
Osmolarity	320–330 mOsm/L

mM = millimole; U/L = units/litre; g% = grams percent; mOsm/L = milliosmols/litre.

Table 2 Flap survival rates after perfusion with UW solution

Type of ischaemia, treatment	*Number of flaps* (*n*)	*Necrosis*	*Survival*	*Survival rate* (%)	*P Value**
Controls					
Group 1					
Primary AV ischaemia, untreated	(20)	0	20	100%	
Group 2					
Primary venous ischaemia, untreated	(20)	10	10	50%	< 0.001
Group 3					
Secondary venous ischaemia, untreated	(20)	0	0	0%	—
Perfusion at the time of primary ischaemia					
Group 4					
Secondary venous ischaemia, saline	(20)	18	2	10%	NS
Group 5					
Secondary venous ischaemia, UW solution	(20)	12	8	40%	< 0.01
Perfusion at the time of secondary ischaemia					
Group 6					
Secondary venous ischaemia, saline	(20)	17	3	16%	NS
Group 7					
Secondary venous ischaemia, UW solution	(20)	14	6	30%	< 0.05

* Two-tailed P value by Fisher's exact test vs the untreated 2 ischaemia flaps (group 2). NS = not significant.
Summary of all groups. AV = arteriovenous 1 = primary; 2 = secondary.

pared to untreated controls (group 3). Saline-perfused flaps exhibited a 10% survival when saline was used at the time of primary AV ischaemia and a 16% survival when used at the time of the secondary venous ischaemic insult.

Skin flaps perfused with UW solution, on the other hand, demonstrated a significant increase in survival to 40% ($p < 0.01$) when given immediately before the onset of primary AV ischaemia and 30% ($p < 0.05$) when administered at the onset of secondary venous ischaemia.

Discussion

Free tissue transfers have a success rate of approximately 90% in most clinical microsurgical centres.[19,20] Failure of free flaps is secondary to postoperative thrombosis causing vascular obstruction.[21] The early postoperative recognition of such a vascular compromise has been noted as an important determinant to correct and improve the success rate.[22] In the present study, it has been observed that rat skin flaps perfused with UW solution either at the time of primary AV ischaemia or at the time of secondary venous ischaemia demonstrate an increased survival rate. The results suggest that perfusion of a skin flap with UW solution during free tissue preparation or after postoperative detection of a vascular compromise may allow a greater tolerance to subsequent vascular obstruction, if it does occur.

In a rodent model, we first demonstrated that secondary ischaemia caused by complete pedicle interruption (AV ischaemia) was less well tolerated than primary total ischaemia.[10] Harashina and coworkers[23] first showed that acute venous obstruction was more damaging to experimental flaps than AV ischaemia. This observation has been previously confirmed[24] and found true in this study in which all flaps subjected to 5 h of primary AV ischaemia survived, while 50% of those subjected to primary venous obstruction survived. Secondary venous obstruction was even less tolerated, with no untreated skin flaps surviving 5 h of venous obstruction.

We arbitrarily chose in our initial study, as did Kerrigan, the length of the primary ischaemic insult and the interval between the primary and secondary ischaemic insult. We explored in subsequent papers[25,26] the effects of varying these parameters. It was observed that as little as 5 minutes of primary ischaemia adversely affected skin flaps subjected to subsequent AV secondary ischaemia.[25] In that study there was also a control for the second anaesthetic, which showed that the decreased survival of AV secondary ischaemic flaps compared to AV primary ischaemic flaps was not due to an anaesthetic effect.

Rosen *et al.*[4] have demonstrated that perfusion of a skin flap with a particular perfusion solution was beneficial for survival. Flaps perfused with saline solution in the present study did not show increased survival: this result is consistent with the report of Chait and others.[2] Simply washing out stagnant blood with a saline or other preservation solution does not guarantee an increased ischaemic tolerance. Among the components of UW solution (Table 1), lactobionate, raffinose and glutathione are found to be essential to liver preservation in rabbits.[18] Lactobionate and raffinose are oligosaccharides which mini-

mise permeability changes in vascular cell membranes. Glutathione may act as an antioxidant. Allopurinol, a specific inhibitor of xanthine oxidase, has therapeutic value for preventing ischaemia/reperfusion injury in rat skin.[27]

Microvascular failure is manifested by increased permeability and no-reflow phenomenon in experimental skin flaps.[28] The importance of the microvasculature for flap survival has been demonstrated.[29,30] The present study did not intend to characterise each component of UW solution for its effect on skin tolerance to ischaemia. All components of UW solution may be beneficial to the microvasculature of ischaemic skin flaps. The ideal modification of UW solution for skin may also necessitate the inclusion of a vasodilator to overcome spasm, which can also be a problem at the time of reperfusion.[31]

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Paper received 26 May 1992.
Accepted 12 March 1992, after revision.