180 The Morphology of Dog Arterial Grafts Preserved in UW-Solution as Studied by Scanning Electron Microscopy and Light Microscopy. Preliminary Results of Allograft Implantation

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Introduction. Cold preserved cadaveric arteries might be used as grafts for reconstructive purposes. The aim of this study was to morphologically assess the luminal surface and vessel wall of dog arterial grafts preserved in the University of Wisconsin preservation solution (UW). The morphological effects were compared with arteries stored in phosphate buffered saline, pH 7.4 (PBS).

Material and Methods. Carotid arteries were harvested from dogs and placed in either UW or PBS. The blood vessels were stored at 4 °C for 1–15 days and were examined by light microscopy (LM) and scanning electron microscopy (SEM). In addition, a preliminary implantation study was performed in dogs: 2.5 cm segments were used to substitute an equal segment of carotid artery in allograft studies. No immunosuppressive agents or anticoagulants were administered. During the observation period the grafts were assessed by Doppler ultrasound and after 28 days of implantation by arteriography, LM and SEM.

Results. The UW preserved arteries (n = 10) showed an intact endothelial lining up to 12 days of storage, with morphologically normal nuclei protruding in the lumen and distinct cell margins. In the PBS group (n = 10), deterioration of the endothelial layer was apparent after 1 day and after 3 days, the endothelial layer was completely lost. The smooth muscle layer was also better preserved in the UW stored grafts. Allograft arterial implants (n = 3) stored up to 14 days in the UW solution were all patent after 28 days.

Conclusion. The endothelial layer and vessel wall of arteries are well preserved in UW up to 12 days of storage. Preliminary results of allograft implantation studies showed that grafts preserved up to 14 days remained patent for at least one month.

181 Use of Isolated Rat Intestine in Order to Test the Efficacy of Preservation Solutions

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Organ preservation implies two main problems: the anoxic damage and the injuries derived from reperfusion. The second problem is specially important when working with the gut. Oxygen-derived free radicals (OFR) produced in the very first minutes of reperfusion and the activation of neutrophiles due to OFR play a decisive role in the small bowel reperfusion syndrome. The aim of our experiment was to work out a technique capable of 'reactivating' cellular metabolism of the graft in the absence of neutrophiles while casting aside the venous drainage reach in OFR. The preliminary results are reported.

Methods. Male WAG rats weighing 250 g have been used. Under ether anesthesia the whole small bowel is excised with an aortic patch and the whole portal vein. Both vessels and the proximal and distal ends of the graft are cannulated. The intestinal lumen is washed with ice-cold Ringer while the graft is perfused with Eurocollins. The organ is harvested in ice-cold Ringer. The ischemic damage is assessed histologically. Once the period of cold storage is over, the graft is perfused with a modified Ringer solution enriched with oxygen ($pO_2 = 300$ mm Hg) at 37 °C for 60 min. Throughout this period, the pressure in the superior mesenteric artery is registered, and sequential samples of the venous and luminal flush are obtained for biochemical analysis.

Results. This technique makes it possible to perfuse the isolated organ with an intermittent pressure which mimics the normal arterial pulsation (175/100 mm Hg). In control animals, perfused with a non-modified Ringer solution, an intense edema is produced after 15–20 min which blocks the venous drainage. The modified-Ringer solution (dexamethasone, sodium pentobarbital, norepinephrine, verapamil, glucose and insulin) allows normothermic reperfusion of the graft for more than 60 min. The analysis of the perfusate and the venous drainage show O_2 consumption and CO_2 production by the isolated organ. The metabolic activity of the intestine is also demonstrated by the decrease in glu-

cose concentration observed in the venous flush. To assess the damage induced during the normothermic reperfusion the histopathology and PA, GOT, CK and LDH are studied.

Conclusions. Our experimental model makes it possible to study intestinal graft preservation avoiding the reimplantation of the organ, which allows a greater number of experiments to be performed, and reduces time, money and animal suffering. Only those drugs or techniques which prove useful in vitro would require in vivo experiences (organ transplantation).

182 Glutaminase Activity as Parameter of Small Bowel Preservation Injury

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Successful small bowel transplantation has been performed with increasing frequency in humans. Since the quality of organ preservation may significantly influence the early postoperative outcome, small bowel preservation is gaining increased interest. Parameters that reflect early postoperative graft function are currently not established. We evaluated the mitochondrial enzyme glutaminase for this purpose.

The heterotopic model of small bowel transplantation was used, and grafts were preserved with saline or a modified PBS (phosphate buffered sucrose) solution and transplanted after various preservation times. Glutaminase activity was determined in mucosa biopsies obtained from the proximal part of the graft 20 min after reperfusion and when animals were sacrified. Histology referred as reference of the extent of preservation injury at the same time points.

In the first study glutaminase activity 20 min after reperfusion correlated significantly with duration of preservation (saline: $R^2=32.8\%$, $p\leq0.01$, and PBS: $R^2=52.3\%$; $p\leq0.0001$) and the histologic injury. Survival for grafts preserved for 1 h with either solution was 100%, for 6 h with saline 67% and with PBS 100%, but decreased to 0% after 12 h of preservation with either solution. Glutaminase activity also correlated with postoperative graft and recipient survival for grafts preserved in PBS: $R^2=49.6\%$; $p\leq0.001$ with a sensitivity of 92% and a specificity of 100%. The cut-off for surviving and non surviving graft recipients was established at an activity of 0.9 μmol glutamate/h/mg protein. In a second study, where grafts were preserved for 1, 6, 9 and 12 h in saline, leading to 100, 83, 50 and 0% graft survival, respectively, glutaminase activity correlated again with duration of preservation ($R^2=55.3\%$; $p\leq0.0001$) and the histologic graft injury. Using the previously established cut-off, glutaminase activity predicted graft survival ($R^2=27.9\%$; $p\leq0.01$) with a sensitivity of 71% and a specificity of 100%.

Conclusion. Glutaminase activity determined 20 min after reperfusion is a reliable parameter, which is easily to use in a clinical setting and correlates with the extent of preservation injury and predicts postoperative graft survival after small bowel transplantation.

183 Does Adrenergic Activity in the Mesentery Reappear after Small Bowel Transplantation?

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Small bowel transplantation inevitably leads to disconnection of autonomic nerves within the gut as well as within the mesentery. Aim of the study was to investigate whether 1) adrenergic activity disappears in the graft mesentery, and 2) if so, does it reappear?

The entire jejunoileum was transplanted heterotopically in an isogeneic rat model (Lewis/Lewis), $n=14$. The graft artery and mesenteric vein were anastomosed to the infrarenal aorta and vena cava, respectively. The proximal end of the graft was closed and the distal and anastomosed in an E/S fashion to the ileum of the recipient bowel. Two animals were sacrificed with an overdose of anesthesics at days 7, 14, 21, 28, 35, 42, 49. The mesentery of the graft as well as the recipient mesentery were analysed histochemically by fluorescence microscopy. After the first and second week, grafts were found to be completely depleted of noradrenaline ($n=4$). At the end of the third week, fluorescence became detectable along graft mesenteric arteries ($n=2$) and showed normal intensity from the end of week four until week seven ($n=8$).

From these findings it is concluded that adrenergic fibres within the mesentery of rat small intestine are extrinsic and are depleted of noradrenalin following transplantation. Noradrenergic activity does regenerate in this model within three weeks despite the fact that the mesenteric artery is not implanted at its original site.