

## IMMUNOSUPPRESSANT AND ANTIOXIDANT THERAPIES IN THE PROPHYLAXIS OF INTESTINAL REPERFUSION INJURY

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A lot of evidence about the role of free radicals in the genesis of the reperfusion injury has been provided by the administration of antioxidants prior to ischemia. The aim of this study was to test the ability of these drugs to decrease the intestinal reperfusion injury once the ischemia has been induced, in order to parallel clinic situations.

**Material and methods:** Under nembutal anesthesia, ischemia was performed in female Sprague-Dawley rats (200 g) by clamping the superior mesenteric artery for 120 min. The different drugs were administered, diluted in 2 ml of saline, at a low perfusion rate through the femoral vein, 15 min prior to removing the clamps. Seven groups of 20 animals have been considered: (I) control: ischemia alone; (II) serum; (III) superoxide-dismutase (SOD, 7 mg/kg); (IV) vitamin E (20 mg/kg); (V) allopurinol (Allo, 50 mg/kg); (VI) folic acid (2.5 mg/kg); (VII) cyclosporin A (5 mg/kg, s.c.). The mortality rate (MR), the length of damaged intestine (LDI) and the mucosal damage index (MDI) were assessed.

**Results:** Mortality Rate: (I) 75.5 %; (II) 57.7 %; (III) 40 %; (IV) 55 %; (V) 25 %; (VI) 25 %; (VII) 60 %. Length of damaged intestine: (I) 30.9 %; (II) 46.5 %; (III) 24.7 %; (IV) 39.5 %; (V) 23.8 %; (VI) 20.3 %; (VII) 29 %. Mucosal damage index: (I) 12.54 %; (II) 9.76 %; (III) 8.4 %; (IV) 10.15 %; (V) 11.54 %; (VI) 8.54 %; (VII) 10.54 %. Serum treatment slightly decreased the MR and MDI, while frankly increasing the LDI ( $p < 0.01$ ). SOD, ALLO and folic acid significantly decreased the MR, the LDI and the MDI. Vitamin E did not modify the MR but it increased the LDI and the MDI. CsA did not improve the MR nor the MDI, but it prevented the increase of the LDI induced by serum ( $p < 0.005$ ).

**Conclusion:** The clinical trial of antioxidant drugs for the prevention of intestinal reperfusion injury may be justified.

## USE OF ISOLATED RAT INTESTINE IN ORDER TO TEST THE EFFICACY OF REPERFUSION SOLUTIONS

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Small bowel preservation implies two different problems: the damage produced by the ischemia, and the injuries derived from reperfusion. Oxygen-derived free radicals (OFR) produced during the early stages of reperfusion, and the subsequent activation of neutrophiles, play a decisive role in the primary function of the graft; and, perhaps, in the graft rejection. The aim of our experimental model is the reactivation of the cellular metabolism of the graft while casting aside the first minutes of venous drainage rich in OFR.

**Methods:** Male WAG rats weighing 250 g have been used. Under ether anaesthesia the whole small bowel is excised with an aortic patch and the whole portal vein. Both vessels and the luminal ends are cannulated. The graft is perfused with ice-cold Eurocollins, and harvested during six hours at 4 °C. Once the period of cold storage is over, the graft is reperfused during 40 min with a modified Ringer solution, enriched with oxygen ( $pO_2 = 300$  mmHg), at 37 °C. Sequential samples of the venous and luminal flushes are obtained for ionic gasometric measurements. The pressure in the superior mesenteric artery is monitored throughout this period.

**Results:** The experimental model makes it possible to perfuse the isolated small bowel with a flow similar to that found in the normal rat intestine (10-14 ml/min), maintaining the arterial pressure in a range of 100-120/80-110. In control animals, the perfusion with Ringer's lactate alone induces an intense edema which blocks the portal drainage after 10-15 min. Our modified-solution using Ringer's lactate with dexamethasone, sodium pentobarbital, norepinephrine, verapamil, glucose, insulin and the addition of SOD and/or folic acid, allows normothermic reperfusion, showing  $O_2$  extraction ( $pAO_2$ - $pVO_2/pAO_2$ ) (range 36-53 %),  $CO_2$  liberation ( $pACO_2$ - $pVCO_2 = -14$  mmHg) and pH diminution (range: 0.205-0.415).

**Conclusions:** This experimental model allows studies of intestinal graft preservation and reperfusion injury, avoiding the reimplantation of the organ. The number of experiments to be performed, the money and time to be expended, and the animal sufferings are all of them reduced. Only the drugs useful in vitro would proceed into «in vivo» experiences.