PRE-TRANSPLANT GRAFT RECONDITIONING WITH MESENCHYMAL STROMAL CELLS IN NON HEART BEATING DONOR EXPERIMENTAL MODEL

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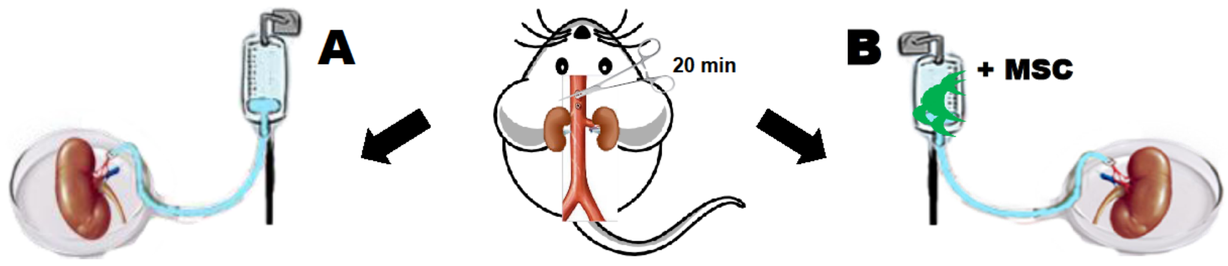
INTRODUCTION AND OBJECTIVE

Harvesting kidneys from non Heart Beating Donors (NHBD) is an effective but challenging way to face organ shortage. Infact, grafts from NHBD have a high risk for primary non- and delayed graft function, and worse long-term outcome (1). Viability of NHBD kidneys is improved by perfusing them with hypothermic machine perfusion (HMP), but the outcome of NHBD grafts remains worse in spite of HMP (2). Mesenchymal stromal cells (MSC) are multipotent cells that abate the immune and inflammatory response. MSC injection in a rat model of renal transplantation protects graft function and reduces tissue injury (3-4). We hypothesize that delivering MSC to the isolated kidney as part of HMP procedure can afford a full protection to NHBD graft by blocking at the earliest stage injury caused by ischemia/reperfusion and rejection. The aim of this study is to evaluate the effects of pretransplant graft reconditioning with MSC on tissue injury.

METHODS

Fisher rats (F) were used as kidney donors, Lewis rats (L) were used as MSC donors and Transgenic Sprague-Dawely rats expressing enhanced green fluorescence protein were used as MSC donors to track MSC. After 20 min of warm ischemia by abdominal aorta clamping, bilateral nephrectomy from F was performed and kidneys were perfused with Belzer UW solution (group A) or Belzer UW solution supplemented with 3 millions MSC (group B) for 4 h, at 4°C. 6 kidneys for each group were studied after 4 h from the beginning of the perfusion. Proliferating cell nuclear antigen (PCNA) expression was evaluated by immunochemistry. Renal damage was graded according to semiquantitative score in 15 non consecutive microscopic fields (X 20) (0-4) (0: 0%tubular casts /microscopic field; 4: >75% tubular casts / microscopic field). Tubular mitotic index was measured as tubules with mitotic cells/ total tubules number in 15 microscopic fields (X20). Malondialdehyde (MDA), marker of oxidative stress and lipid peroxidation (5), was measured in the collected perfusion fluid.



RESULTS

Histology showed no MSC capillary margination, not macro- or microvascular engorgement or thrombosis. We found MSC in interstitium, tubules, glomeruli. (Figure 1, panel A, B, C). Damage score was significantly lower in MSC perfused kidneys (A: 3.4±1; B 1.6±1.2, p< 0.0001) (Figure 2 - 3).
PCNA positive cells were increased significantly in group B compared with group A (A 27.9±12.1; B 35.0 ±15.7 , p< 0.0001) (Figure 4). Mitotic index was higher in group B than in group A (A:0.22± 0.14; B: 0.27 ±0.20, p< 0.005) (Figure 5). MDA levels in the collected fluid were significantly lower in group B compared with group A (p< 0,001) (Figure 6).

Figure 1

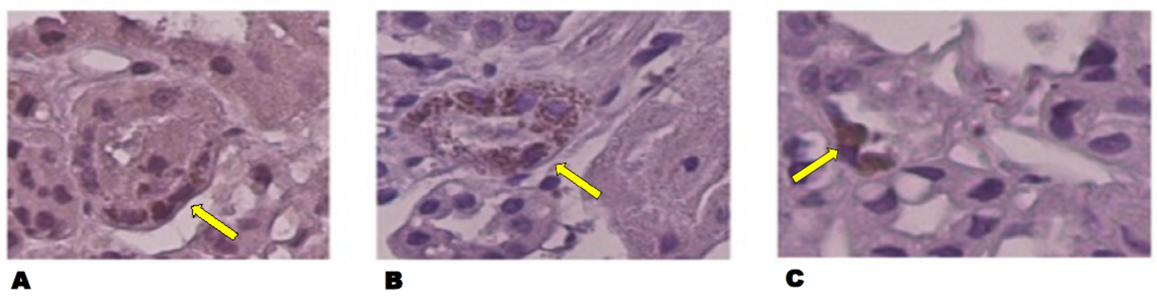


Figure 2

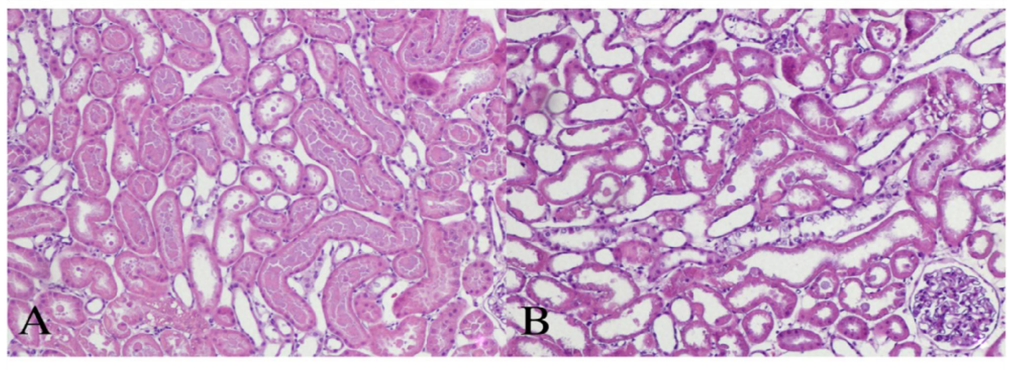


Figure 3

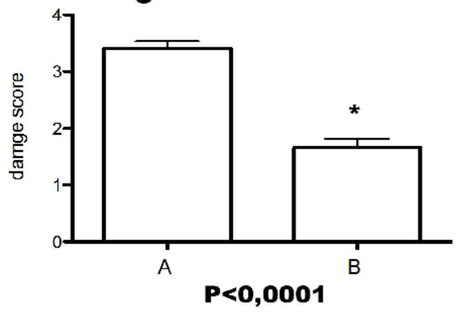


Figure 4

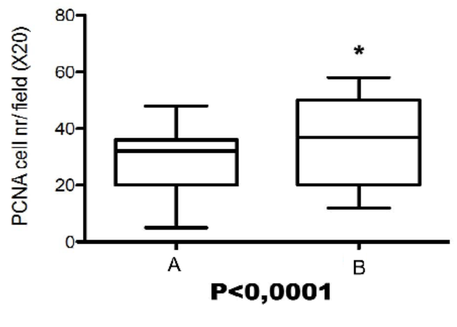


Figure 5

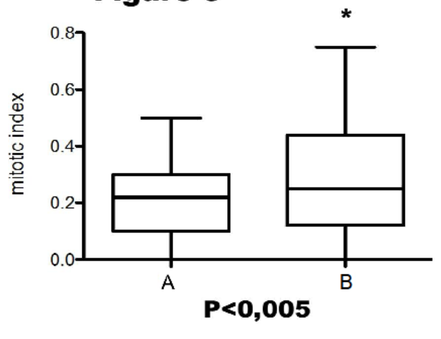
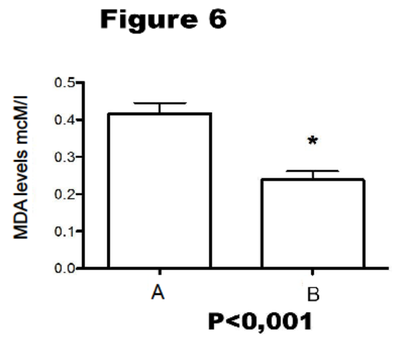


Figure 6



CONCLUSIONS

These results demonstrate that MSC infused in the kidney as part of the washing procedure afford early protection from ischemia injury. Confirmation in human transplant will simplify MSC handling and add further prevention by early blocking ischemic damage

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