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Effects of a Pan Selectin Inhibitor on Renal Injury after Kidney Transplantation in Dogs

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Abstract : Selectins are differentially expressed carbohydrate binding proteins involved in the initiation of tissue inflammation by mediating the rolling and tethering of leukocytes on the vascular endothelium. This primary event in initiation of inflammation, as occurs during reperfusion injury, can be therapeutically targeted using selectin inhibitors, which generally block binding of sLex to E-, P-, and L-selectins. The objective of this study was to determine the role of selectins in renal ischemia/reperfusion injury after kidney transplantation. Canine kidneys were subjected to 60-min warm ischemia, flushed with UW solution, cold stored for 24 h, and autotransplanted into the nephrectomized donor. Renal autografts were monitored for 7 days by serum creatinine in the first study and by histology and myeloperoxidase activity after 4-hour reperfusion in the second study. In each study, one group of animals received TBC1269 (selectin inhibitor) and the other received saline vehicle. Serum creatinine rose quickly after transplantation and was not different between the groups. TBC1269 abolished a reperfusion-induced 2-fold increase in renal cortex neutrophil infiltration and improved histologic signs of ischemia after 4 hours of reperfusion. Selectin blockade does not improve the course of injury caused by warm renal ischemia. A minor benefit associated with the inhibition of early inflammation during reperfusion after kidney transplantation seems to occur.

Key words **:** Kidney Transplantation, Selectin, Delayed Graft Function, Myeloperoxidase, Dog

Introduction

Delayed graft function (DGF) of transplanted kidneys is a great problem and the incidence remains 21% of all kidneys in the last decade reported to UNOS[1]. Donors with prolonged exposure to warm ischemia, hypothermic storage, brain death, and other risk factors contributes to this injury. Furthermore, DGF consistently correlates with poor graft survival rate[1]. The infiltration and activation of neutrophils during normothermic reperfusion injury plays a significant role for lesion development in many organs and tissues[2,3]. Reperfusion of hypothermically preserved kidneys produce similar lesions and neutrophil infiltration is also implicated in the process. The loss of renal function at reperfusion is associated with ischemic changes at S-3 segment of proximal tubules, severe vascular congestion at the cortico-medullary junction, and infiltration of immune competent cells into the glomerulus and peritubular interstitium[4-7]. However, the role of leukocyte infiltration in delayed graft function induced by pre-exposure to warm ischemia before hypothermic preservation remains unknown.

The cellular inflammatory response that occur with reperfusion injury, begins by the rolling and tethering of inflammatory cells to the vascular endothelium followed by firm adhesive binding, and finally extravasation through the endothelial barrier into the tissue interstitium. The initial rolling behavior of these cells to the capillary endothelium results from the interaction of selectins with specific ligands. The selectins are a family of glycoproteins expressed on the cell surface of both the circulating inflammatory and endothelial cells[8]. The E-selectins are induced on endothelial cells by de-novo synthesis and P-selectins are induced on the surface of both platelets and endothelial cells by translocation of preformed P-selectin from intracellular vesicles (Weibel-Palade bodies) to the cell surface following inflammatory stimuli. By contrast, L-selectins are not induced but are constitutively expressed in high numbers on neutrophils and mononuclear cells. The selectins interact with sialyl Lewis-x (sLe^X) lectins on endothelial cells and neutrophils to slow down the passing of neutrophil and facilitate capture. These crucial interactions may be blocked by either antibodies specific for selectins or small molecule sLe^X mimetics that compete for the natural selectin binding ligands[9]. The experimental compound TBC1269 is a small molecule sLe^X mimetic that non-selectively inhibits the 3 classes of selectins from binding to their target ligands[9,10]. This agent was used to test the contribution of neutrophil infiltration in a model of renal DGF utilizing warm ischemia, hypothermic storage preservation, and transplantation reperfusion.

Materials and Methods

The present study was conducted on adult beagle dogs of both sexes weighing about 10 kg. The first series of experiments utilized long-term reperfusion after renal preservation (7 days) and the other series terminated reperfusion of the transplanted kidneys after 4 hours. Each series contained two

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groups of dogs. One group was treated with TBC1269 (Bimosiamose, Texas Biotechnology Corporation, Houston, TX) and the other group was kept as untreated control. Both groups were nephrectomies under general anesthesia using aseptic conditions. The left kidney was subjected to total ischemia by clamping the renal artery and vein for 60 minutes in situ condition. The kidney was then removed, flushed with 100-ml cold UW solution, and stored at 4°C for 24 hours. After storage, the kidneys were heterotopically autotransplanted into the original donors followed by immediate contralateral nephrectomy. The autografts were then allowed to reperfuse for the enxt 7 days with daily monitoring of renal function by measuring serum creatinine concentration. The untreated group (n = 4) was supplemented with normal saline and treated group with the selectin inhibitor TBC1269 and saline (n = 8). The TBC1269 agent was administered (25 mg/kg, I.V.) to the donor 15 minutes before warm ischemia and to the recipient 15 minutes before and 2 hour after reperfusion. This design allowed for the determination of the maximal effect from the inhibitor. The other series of experiments were conducted identical to the previous one except the kidneys were harvested after 4 hours of reperfusion. In these experiments, the renal tissue was processed for myeloperoxidase (MPO) activity and histological evaluation. Tissue MPO activity was used as an index of neutrophil content and was measured spectrophotometrically from tissue homogenates as described[11]. Histology samples were kept in formalin, embedded in paraffin, sectioned, stained with H&E, and examined by light microscopy in a blinded manner. Renal histology was graded from 0-4 using as primary criteria, the semi-quantitative extent of tubular necrosis and vascular congestion at the cortico-medullary junction.

Results

Renal function in the untreated animals confirmed a model of severe renal preservation injury where serum creatinine levels steadily rise over four days after transplantation and then begin to fall gradually (Fig 1). This degree of injury in this particular model is reproducible and represents maximal renal deficit tolerated without death of the animal. There were no statistical significant difference between the control and the treated group. Two-fold reperfusion-induced increase renal cortical MPO activity in the control after 4 hours of reperfusion. However, the increase of renal cortical MPO was not observed in TBC 1269 treated group (Fig 2).

The histological grade of injury of the kidneys of experimental animals was significantly less than those of untreated control after 4 hours of reperfusion ($p<0.05$, Fig. 3). The extent of tubular necrosis and the amount of subsequent engorgement of the remaining tubules with eosinophilic fluid and debris was remarkably less in the anti-selectin treated group with 4-hour reperfusion.

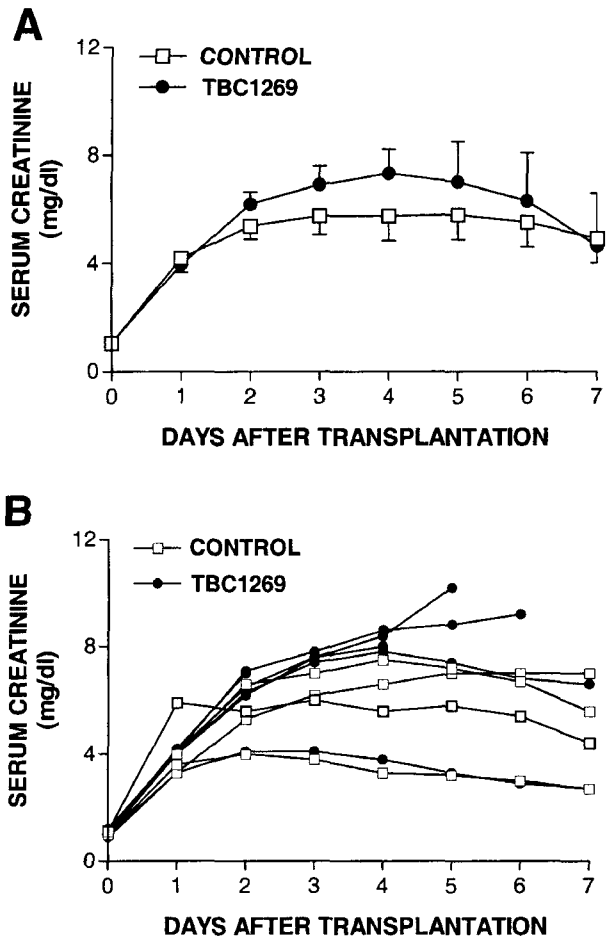

Fig 1. Serum creatinine concentrations after autotransplantation of kidneys subjected to 60 minutes of warm in-situ ischemia and 24 hours of cold storage in UW flush from dogs treated with the selectin antagonist TBC1269 and untreated controls. Panel A shows average creatinine values for all dogs and panel B shows the individual data from each experiment. Values are mean± standard deviation in panel A.

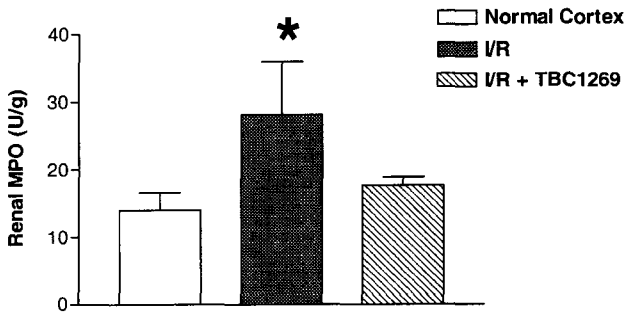

Fig 2. Renal cortical tissue myeloperoxidase (MPO) activity from normal tissue, tissue subjected to the preservation protocol and 4 hours of reperfusion (I/R) in untreated animals, and after reperfusion in TBC1269 treated animals. Values are mean± standard deviation, n = 6, *P< 0.05 by ANOVA.

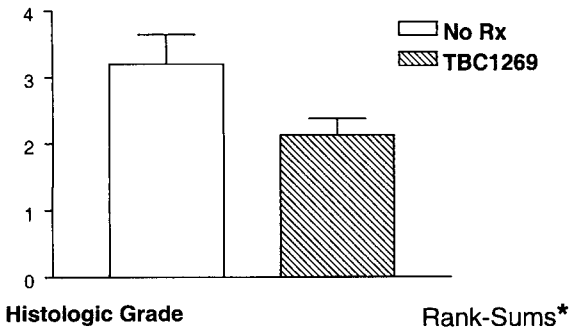

Fig 3. Histological grade of ischemic injury in kidneys after preservation and 4 hours of reperfusion from untreated and TBC1269 treated animals as determined by light microscopy. Values are mean±standard deviation, n = 6 for each group, *P < 0.05 by Rank-Sum test. The average grade for normal tissue was 0.67.

Discussion

Renal function in the treated group was not different from the control of the surviving animals. All of the animals were not survived, suggesting the inhibitor may have had a detrimental effect. A detrimental effect of the cold preserved grafts may be due to an unrecognized toxicity to the kidney that is probably independent of selectin inhibition. In short-term reperfusion experiments, the renal function of both groups was not measurable since the kidneys were anuric after 4 hours of reperfusion. The pharmacological effect of TBC1269 is selectin inhibition which subsequently inhibits neutrophil infiltration into the graft tissue during reperfusion. The data suggested that this occurred in control due to the two-fold reperfusion-induced increase of the MPO activity in the renal cortex with anti-selectin therapy after 4 hours of reperfusion (Fig 2). Thus it appears that the DGF (Fig 1) was not depend on neutrophil infiltration. However, tissue MPO levels were not measured before 4 hours of reperfusion where the functional data were obtained. So the early reperfusion of MPO data was not reflect the levels in the two groups at longer reperfusion times. Since MPO measures the presence of neutrophils and not their state of activation, a reduced neutrophil number in the tissue from treated animals does not rule out the enhancement of the existing cells. Similar activities of antibodies against P-selectins prevent the activation of circulating neutrophils in myocardial reperfusion injury were recorded [12].

Although it is tempting to extrapolate this result to the chronic reperfused series, histology was not assessed in this series and may not be similar. Similar results were conducted by using P-selectin neutralizing antibodies in rat kidneys subjected to 24 hours of cold storage[13]. It may be concluded that TBC1269 has a short term modest salutary effect on kidney within the first 4 hours of reperfusion in this study. Longer-term effects, however, may be achievable by alternate dosing regimes. It is predicted that renal preservation injury is neutrophil dependent in this experimental model and clinical settings.

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개의 신장이식에서 신장손상에 대한 Pan Selectin Inhibitor의 효과

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요 약 : Selectin은 조직 염증반응의 초기화에 관여하는 결합 단백질이며, 맥관내피에서 백혈구의 rolling 과 tethering을 매개한다. 따라서 selectin inhibitor를 이용하여, 이들 selectin의 수용체인 sLex 에대한 block을 유도함으로서 염증반응의 초기화를 억제할 수 있다는 가정하에, 본 연구에서는 장기이식 후 reperfusion에서 발생하는 손상에 대한 selectin inhibitor의 효과를 알아보았다. Beagle 견을 사용하여 신장이식을 실시하였다. 공여 신장은 60분의 warm ischemia를 유도한 후 UW solution으로 관류하고 24시간동안 냉장보관하여 자가이식하였으며, 반대쪽 신장은 적출하였다. 술 후 7일동안 혈청 creatinine치를 측정하였다. 2차실험으로, 12마리의 Beagle견을 사용하여 4시간의 reperfusion 후 조직학적 변화와 myeloperoxidase의 활성을 조사하였다. 각 실험의 대조군은 생리 식염수를, 비교군은 TBC1269 (selectin inhibitor)를 신장 적출 전과 신장이식 수술 후 reperfusion 직전에 각각 투여하였다. 혈청 creatinine은 신장이식후 급격히 증가 하였으나, 두군 사이에 유의적인 차이는 없었다. 신장피질의 백혈구 침윤은, 4시간 reperfusion후 생리식염수를 투여받은 군에서 2배의 유의적인 증가를 보였다. 그러나, TBC 1269로 처리한 군에서는 백혈구의 침윤이 유의적인 억제를 보였으며, 허혈에의한 조직학적 변화도 유의적으로 적었다. 개의 신장이식 수술에서 *Selectin*의 차단은 *warm renal ischemia*에서 기인된 손상을 개선하지는 못하나, 백혈구의 침윤을 억제하므로 delayed graft function과 관련된 술 후 염증반응의 초기화를 억제하는 효과가 있는 것으로 사료된다.

주요어 : 신장이식, selectin, delayed graft function, myeloperoxidase, 개