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CD47 Blockade Reduces Ischemia Reperfusion Injury and Improves Outcomes in a Rat Kidney Transplant Model

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Abstract

Background—Ischemia/reperfusion injury (IRI) significantly contributes to delayed graft function and inflammation leading to graft loss. IRI is exacerbated by the thrombospondin-1/CD47 system through inhibition of nitric oxide signaling. We postulate that CD47 blockade and prevention of nitric oxide inhibition reduces IRI in organ transplantation.

Methods—We used a syngeneic rat renal transplantation model of IRI with bilaterally nephrectomized recipients to evaluate the effect of a CD47 monoclonal antibody (CD47mAb) on IRI. Donor kidneys were flushed with CD47mAb OX101 or an isotype-matched control immunoglobulin and stored at 4°C in UW solution for 6 hours prior to transplantation.

Results—CD47mAb perfusion of donor kidneys resulted in marked improvement in post-transplant survival, lower levels of serum creatinine, BUN, phosphorus and magnesium and less histologic evidence of injury. In contrast, control groups did not survive more than 5 days, had increased biochemical indicators of renal injury and exhibited severe pathological injury with tubular atrophy and necrosis. Recipients of CD47mAb-treated kidneys showed decreased levels of plasma biomarkers of renal injury including cystatin C, osteopontin, TIMP1, β 2-microglobulin, VEGF-A and clusterin compared to the control group. Furthermore, laser Doppler assessment showed higher renal blood flow in the CD47mAb-treated kidneys.

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Conclusions—These results provide strong evidence for the use of CD47 antibody-mediated blockade to reduce IRI and improve organ preservation for renal transplantation.

Keywords

Antibody therapy; Nitric oxide; Ischemia reperfusion injury; Kidney transplant

INTRODUCTION

Kidney transplantation is a life-saving treatment for those with end stage renal disease. Although many improvements have been made in organ procurement, transplant techniques and pharmacologic immune suppression, ischemia-reperfusion injury (IRI) remains a major obstacle that contributes to primary graft non-function, delayed graft function and graft failure. Furthermore, organs from expanded criteria donors and donation after circulatory death (DCD) are even more susceptible to IRI (¹).

Components of IRI which contribute to adverse transplant outcomes include inflammation, reactive oxygen species production (ROS), necrosis, apoptosis, and thrombosis (^{2,3}). ROS production, an immediate response to ischemia/reperfusion, triggers or amplifies many downstream processes including vasoconstriction and thrombosis that further deprive the graft organ of oxygen and nutrients. Increased inflammation causes immediate tissue damage, sets the stage for graft versus host disease, and primes adaptive immunity to promote rejection. Together, these processes lead to apoptosis or necrosis of the graft organ with endothelial cells often being the primary target. This further compromises perfusion and amplifies the cycle of IRI leading to massive death of parenchymal cells (⁴).

Preclinical data indicate that augmenting nitric oxide (NO) signaling ameliorates many of the components of IRI damage (⁵). However, the NO signaling pathway is tonically inhibited by an endogenous ligand-receptor system in which the ligand thrombospondin-1 (TSP1) binds to its receptor CD47 and limits downstream effects of NO including guanylyl cyclase activation, production of cyclic guanosine monophosphate (cGMP) and protein kinase G activation. CD47 is ubiquitously expressed and TSP1 is secreted by cells throughout the vascular system in response to hypoxia, thrombosis and other stresses (⁶). When the vascular system is damaged and TSP1 is released, as in IRI, this endogenous braking system on NO signaling worsens inflammation, vasoconstriction, and cell death. Consistent with this model of IRI, the kidneys of CD47^{-/-} mice sustaining IRI from vascular occlusion exhibit reduced histologic injury, inflammation and cell death (⁷). Based on these findings, we postulate that inhibition of the TSP1/CD47 system with an anti-CD47 monoclonal antibody (CD47mAb) can similarly mitigate IRI and improve transplant outcomes. Here, we show that blocking CD47 by perfusion of the donor organ with CD47mAb at the time of organ procurement improves organ preservation, reduces IRI and improves transplant outcomes in a rat kidney transplant model.

RESULTS

Optimization of cold ischemic time for syngeneic rat kidney transplant survival

We evaluated the duration of cold ischemia time (CIT) on the transplant outcomes in the syngeneic (Lewis to Lewis) rat kidney transplant model to find the optimal time that would result in sufficient IR injury to allow observation of improvement with CD47mAb treatment. Rat donor kidneys were flushed with cold UW solution and placed in cold storage for 2, 4, 6, 18 or 24h before transplantation. Transplant outcomes were evaluated two days following transplantation. We determined that 2h CIT produced marked elevations in serum blood urea nitrogen (BUN) (115.6 ± 42.8 mg/dL, $n=5$ vs 16.4 ± 0.9 for sham operated controls, $n=7$, $p<0.05$) and creatinine (3.38 ± 0.06 mg/dL, $n=5$ vs 0.44 ± 0.06 for sham operated controls, $n=7$) levels, but only mild histological damage was observed 2 days post-transplantation (Fig. 1 A–C). 6h CIT produced greater elevations in the serum BUN (189.9 ± 22.2 mg/dL, $n=6$, $p<0.0001$) and creatinine (4.67 ± 0.73 mg/dL, $n=6$, $p<0.01$) and pronounced histological damage including acute tubular necrosis, proximal tubular dilatation and cytoplasmic blebbing (Fig. 1D), whereas 18h CIT resulted in diffuse acute tubular necrosis (Fig. 1E). We judged the severe damage observed with 18h CIT to be non-recoverable and therefore selected 6h CIT for CD47mAb treatment, as it resulted in substantial but potentially reversible injury. These results are consistent with the severity of rat kidney damage seen with moderate CITs⁽⁸⁾.

CD47mAb binding is localized to renal vasculature

To evaluate structural localization of CD47mAb binding within the kidney and the effectiveness of a post-procurement CD47mAb administration, we flushed the procured kidney with 5mL cold UW solution containing 50 μ g of CD47mAb. The kidney was subjected to 6h CIT, flushed with fresh saline to remove unbound antibody and then frozen in optimum cutting temperature (OCT) media. Frozen sections were stained with an anti-mouse labeled Polymer to localize the bound CD47mAb. With this immunohistochemical analysis, we observed that CD47mAb was evenly distributed on the endothelium of the kidney peritubular capillaries and was strongly localized to the glomerular capillaries (Fig. 1G) consistent with the localization of endogenous CD47 in the kidney (data not shown).

CD47 blockade improves rat kidney transplantation outcomes

To evaluate the effects of CD47 blockade with CD47mAb on kidney transplantation outcomes, we used a syngeneic rat model of kidney transplantation performed in bilaterally nephrectomized recipients so that survival was completely dependent upon graft function. Donor kidneys were flushed with 5mL of cold UW solution containing 50 μ g of the CD47mAb or an isotype matched IgG1 as a control. The kidneys were subjected to 6h CIT and then transplanted. CD47mAb treatment of donor kidneys significantly prolonged survival of recipient rats, as compared to the control IgG immunoglobulin (Fig. 2A, $p<0.01$). None of the recipients of control IgG-treated kidneys ($n=5$) survived for more than 5 days, while 80% of the recipients of the CD47mAb-treated kidneys ($n=5$) survived for 7 days, the duration of the study.

Consistent with improvements in overall survival post-transplant, serum biochemical indicators of renal injury were less pronounced with CD47mAb treatment. Two days post-transplantation, serum creatinine (6.12 ± 0.29 control IgG vs 0.50 ± 0.08 mg/dL sham, $p < 0.0001$), BUN (207.8 ± 6.1 vs 17.8 ± 0.5 mg/dL, $p < 0.0001$), phosphorus (16.62 ± 1.23 vs 5.30 ± 0.31 mg/dL, $p < 0.0001$) and magnesium (3.34 ± 0.33 vs 1.83 ± 0.09 mg/dL, $p < 0.01$) were markedly elevated in animals that received the IgG-treated kidneys compared to sham operated control animals ($n=5$, Fig. 2B). In contrast, these values were only moderately elevated in the recipients of the CD47mAb-treated kidneys (creatinine 1.08 ± 0.18 , BUN 74.2 ± 18.7 , phosphorus 5.90 ± 0.59 and magnesium 2.54 ± 0.12 , $n=5$, Fig. 2B). At 7 days post-transplant, serum creatinine, BUN, phosphorus and magnesium values returned to sham levels in the recipients of the CD47mAb-treated kidneys (creatinine 0.60 ± 0.09 , BUN 31.8 ± 2.1 , phosphorus 4.80 ± 0.23 and magnesium 1.98 ± 0.12 , $n=4$, Fig. 2B). No rats receiving IgG-treated kidneys survived for 5 days; therefore, the control IgG values are only available for day 2.

Histologic evidence of IRI protection with CD47 blockade

Because recipients of control, IgG-treated kidneys did not survive beyond 5 days post-transplant, we evaluated the transplanted kidneys at the two-day time point for histological evidence of IRI. CD47mAb-treated kidneys had significantly less injury (Fig. 3B) than kidneys receiving the control IgG antibody which exhibited severe pathological injury characterized by tubular atrophy, dilation, cytoplasmic blebbing, tubular cell shedding and coagulative necrosis (Fig. 3C). When these histologic sections were morphometrically analyzed and scored in a blinded manner for percent acute tubular injury (ATI) and necrosis (ATN) at day 2, the CD47mAb-treated kidneys ($n=5$) exhibited less injury with $29\% \pm 5$ versus $91\% \pm 8$ ATI/ATN in IgG-treated kidneys ($n=5$, Fig. 3D; $p < 0.0001$). Although none of the recipients receiving IgG-treated kidneys survived to 5 days, the reduction of injury persisted for 7 days in CD47mAb-treated kidneys ($32\% \pm 8$, $n=4$, Fig. 3D, $p < 0.0001$).

CD47 blockade mitigates biomarkers of renal injury after transplantation

To further evaluate the effects of CD47mAb treatment on rat kidney transplant outcomes, we determined plasma levels of proteins associated with renal injury using the rat kidney multianalyte panel developed by Myriad RBM. We tested plasma collected 2 and 7 days (for CD47mAb-treated kidney recipients) after transplantation with the proteomic Rat KidneyMAP to evaluate the presence of proteins associated with renal injury. We found that 6 proteins exhibited patterns consistent with the biochemical and histologic indicators showing decreased injury with CD47mAb treatment prior to transplantation: cystatin C, osteopontin, TIMP1, β 2-microglobulin, VEGF-A and clusterin (Fig. 4). The data for calbindin and glutathione S-transferase did not meet statistical significance but trended toward values also consistent with decreased injury with CD47mAb treatment.

Increased kidney blood flow with CD47 blockade

To evaluate differential degrees of apoptosis between CD47mAb and control IgG-treated kidneys, we performed TUNEL and activated caspase-3 staining but found no significant difference (data not shown). We also quantified mRNA levels of inflammatory markers

TNF- α , IL-6, IL-1 β and CCL2 in kidney tissue using quantitative PCR and did not find a difference between CD47mAb and IgG treated groups at day 2 (data not shown).

IRI can have dramatic effects on blood flow. Therefore, we evaluated the change in blood flow in the transplanted kidneys using laser-Doppler analysis. We performed renal transplantation as above following 6h CIT and used laser Doppler to measure blood flow immediately following reperfusion of the organ (0 h) and 1h after reperfusion. Twenty four hours after reperfusion, the animals were re-anesthetized and opened, and another blood flow measurement was obtained (Fig. 5A). At the time of reperfusion, the organs treated with CD47mAb had significantly increased flow compared to kidneys in the IgG control group (mean 274 ± 84 Perfusion Units (PU) vs 83 ± 60 PU, $n=4$ each group, $p<0.006$, Fig. 5B). At 24h, all kidneys treated with CD47mAb showed greater flow compared to their initial measurements at 0hr. The IgG control group had two kidneys that attained improved flow at 24hr whereas the other two kidneys had persistently low flow. The two kidneys with persistently low flow did not have vascular thrombosis on gross examination. Overall at 24h, the CD47mAb group had increased flow compared to the IgG control group (mean 634 ± 66 PU vs 295 ± 280 PU, $p<0.05$).

DISCUSSION

Using a syngeneic rat kidney transplant model, we have demonstrated that perfusion of kidneys prior to cold storage with a CD47mAb (1) improves overall function of the transplanted kidneys resulting in improved survival of recipients, (2) reduces serum biochemical and biomarker indicators of renal injury and (3) mitigates histologic evidence of renal injury. Accumulating evidence indicates that the mechanism by which this occurs is through augmentation of NO and cGMP signaling pathways. The CD47 receptor and its ligand TSP1 are expressed throughout the vascular system and limit NO signaling by inhibiting the NO/cGMP pathway at multiple points. An increase in NO/cGMP signaling with CD47 blockade or genetic knockouts has been demonstrated in both endothelial and smooth muscle cells and in platelets and leukocytes (^{7,9}). These *in vitro* findings have been mirrored in a number of *in vivo* studies in which lack of TSP1 or CD47 or CD47 blockade augments the downstream effects of NO including increased vasodilation and decreased necrosis, apoptosis, thrombosis and inflammation (¹⁰⁻¹³).

The rationale for CD47 blockade to ameliorate IRI in kidney transplantation follows a progression of studies that provides evidence for the role of CD47 in exacerbating IRI: In kidney and liver models of warm IRI, CD47 knockout mice were found to sustain less injury following periods of vascular occlusion followed by reperfusion (^{7,14}). In therapeutically relevant studies, CD47mAbs have been used to mitigate IRI in various models of tissue ischemia. Administration of the rat anti-mouse CD47mAb 301 or the mouse anti-rat CD47mAb OX101 improved survival of ischemic skin flaps and skin grafts (^{15,16}). The mouse anti-rat CD47mAb OX101 also reduced the severity of monocrotaline-induced pulmonary hypertension which results, in part, from IRI (⁹). In addition, administration of the CD47mAb 301 virtually eliminated cellular damage due to IRI in a model of *in situ* transient warm IRI in both mouse liver and kidney models (^{7,14}).

In this report, we demonstrate the effectiveness of a CD47mAb to reduce IRI following renal transplantation. We employed a preclinical, functional life-sustaining kidney transplantation model following IRI and the commercially available mouse anti-rat CD47mAb OX101. We chose the syngeneic rat transplant model (Lewis to Lewis rats) to eliminate the confounding effects of an immune response and the toxicities related to immunosuppression to prevent allograft rejection, thus allowing us to focus entirely on IRI effects. To assess the effects of IRI upon renal function, we performed standard serum electrolyte and biochemical tests with parallel morphometric and histologic examination. In addition, we determined plasma biomarkers of renal injury following renal transplantation. These biomarkers hold the potential for early detection of the onset of acute kidney injury (^{17,18}), and we have evaluated their use in the context of post-transplantation monitoring. We demonstrate that cystatin C, osteopontin, TIMP1, β 2-microglobulin, VEGF-A and clusterin indicate a clear pattern of renal injury with transplantation, and that a marked abrogation of these renal injury markers occurs with CD47mAb perfusion of transplanted kidneys. Interestingly, plasma KIM-1 and NGAL were higher in recipients receiving CD47mAb-treated kidneys, which has also been found in other transplant settings and which may be indicative of ongoing repair processes (¹⁹). This panel of biomarkers was developed to provide more sensitive indicators of kidney injury, particularly in the setting of drug induced kidney injury (Myriad RBM, Rat KidneyMAP White Paper). At the time points we monitored, the elevated levels of many of these biomarkers paralleled the elevations in serum creatinine and BUN.

We found the improved outcomes of renal transplantation with CD47mAb treatment were correlated with increased rates of blood flow to the graft after reperfusion. This effect is seen immediately at the time of organ reperfusion and persists at least to 24h afterwards (^{7,13,20}). Beyond the aggregate perfusion numbers, the changes in the blood flow of individual kidneys over the period of 24h differed between the treatment groups. All of the kidneys treated with CD47mAb in our experiment exhibited high perfusion at 24h post-reperfusion. Half of the kidneys in the IgG control group showed comparable organ perfusion at 24h to that of CD47mAb treated organs, but the other half had persistence of low perfusion. It is difficult to fully define the outcomes of these kidneys with persistently low perfusion because of the inability to perform hemodialysis to support these animals while awaiting return of renal function, as is done in the human transplant setting. However, these results suggest that CD47 blockade may be useful as a treatment to decrease the rates of post-transplant delayed graft function, which is associated with increased rates of rejection, poorer graft survival and increased health-care costs (^{21,22}).

In summary, we have established a proof of concept use of anti-CD47 mAb therapy to ameliorate the effects of IRI following kidney transplantation. Perfusion of procured rat kidneys with CD47mAbs prior to cold ischemia provides substantial protection against histological damage and improves markers of both kidney damage and function resulting in improved survival of the organ. The physiological impact of this improvement in functional parameters is seen in the enhanced survival of recipients which are completely dependent on the function of the transplanted graft. Most importantly, the marked improvement in survival and indicators of kidney function that we clearly demonstrated with CD47 blockade was obtained by treating only the donor kidney with the CD47mAb. Further studies will be

required to determine if treatment of the transplant recipient provides additional benefit. The syngenic transplant model of IRI that we employed for this proof of principle demonstration of CD47mAb protection benefits from the absence of the confounding effects of adaptive immunity. Allogenic renal transplant models using different forms of ischemia and immunosuppression are currently being pursued. Reducing IRI could not only improve the success rate for transplantation of standard criteria donor SCD organs, but may also allow greater use of extended criteria and donation after circulatory death organs, thereby increasing the number of utilized organs.

MATERIALS AND METHODS

Rat Kidney transplant IRI model and CD47mAb treatment

Animals experiments were approved by the Washington University Animal Studies Committee. Male Lewis rats (275–300g, Charles River Laboratories, Wilmington, MA) were housed in controlled environments. The donor animal was anesthetized with 2% isoflurane and the left kidney was mobilized. The aorta was clamped proximal and distal to the renal arteries and the kidneys were perfused through a 25-gauge needle with 5mL of University of Wisconsin (UW) solution containing either 50µg of an IgG1 control or CD47mAb, OX101 (Santa Cruz Biotechnology, Dallas, TX). The infrarenal inferior vena cava was transected distal to the renal veins. The left kidney was then placed into UW for 6h.

A syngeneic recipient was then anesthetized and a left nephrectomy was performed. The transplant was performed first with the arterial anastomosis using the end-in-end sleeve technique in which the recipient renal artery was telescoped into the donor renal artery and then fixed in place with sutures⁽²³⁾. The donor inferior vena cava was anastomosed end-to-end using a cuff technique. The donor renal vein was passed through and then everted through a cuff. The donor cuffed vein was then telescoped through the recipient renal vein and then fixed into place with sutures. The ureter was anastomosed to the bladder, and a right nephrectomy was performed. Surviving transplanted rats were sacrificed at 2 or 7 days following transplantation. Sham control operations were performed without nephrectomies.

Renal injury and function assays

Serum creatinine, BUN, phosphorus and magnesium were measured using a Beckman AU480 Chemistry Analyzer. Serum biomarkers of renal injury were determined using the Luminex Bead assay platform (Myriad RBM, Austin, TX) using the multianalyte panel (MAP) Rat KidneyMAP® v1.0.

Histology and immunohistochemistry

The kidneys were fixed in 10% buffered neutral formalin solution and stained with hematoxylin/eosin. Acute tubular injury was defined as tubular dilatation, epithelial flattening, cell sloughing or coagulative necrosis. This was scored as percent total kidney damage in a blinded manner by a renal pathologist (J.P.G.). Total kidney damage, given in 10% increments, was determined by visual estimation. For immunohistochemical analysis of CD47mAb binding, the kidney was flushed with the CD47mAb, stored for 6h on ice, and then frozen in OCT. Frozen sections were prepared and the bound CD47mAb to the donor

kidney was visualized using an anti-mouse HRP secondary antibody complex (Envision +System-HRP Labelled Polymer, Dako, CA).

Statistical analysis

Comparisons between groups were performed using one-way ANOVA; differences with $p < 0.05$ based on Tukey's multiple comparisons were considered significant. Survival analysis was performed using Kaplan-Meier analysis and the log-rank test. Analyses were performed using Prism (GraphPad Software, Dan Diego, CA).

Laser Doppler flow measurements

Renal blood flow was measured using the moorLDI2 laser Doppler imager (Moor Instruments, Devon, UK). After reperfusion of the transplanted kidney, blood flow was measured (0h). Temporary skin closure was performed for 1h, and another measurement was taken. The transplant was completed and the animal was allowed to recover. 24h after reperfusion, the animal was re-anesthetized and opened for the final measurement.

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Abbreviations

CD47mAb	CD47 monoclonal antibody
CIT	cold ischemia time
IRI	ischemia reperfusion injury
NO	nitric oxide
ROS	reactive oxygen species
TSP1	ligand thrombospondin-1

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FIGURE 1.

Biochemical and histological damage following increasing amounts of cold ischemic time assessed 2 days following transplantation (A–E). (A) Rat donor kidneys were procured and placed in cold storage for 2h (n=5), 4h (n=3), 6h (n=6), 18h (n=8) or 24h (n=5). Serum creatinine and BUN were measured 2 days post-transplant and found to be moderately elevated. CIT of 2h (B) and 4h (C) did not result in significant histological damage. However, 6h of CIT (D) resulted in focal acute tubular necrosis, proximal tubular dilation and cytoplasmic blebbing. 18h of CIT (E) caused diffuse acute tubular necrosis. Immunohistochemical localization of bound CD47mAb following administration by flush (F–H). Donor kidneys from Lewis rats were isolated and flushed with 5 mL cold UW solution containing 50 µg of the CD47mAb, stored in at 4°C for 6 hours, flushed with saline and frozen in OCT. Frozen sections were prepared and the bound CD47mAb was visualized using an anti-mouse HRP secondary antibody complex (Envision+ System- HRP Labeled Polymer). The bound CD47mAb was uniformly distributed on the endothelium throughout the kidney in interstitial arteries (F) and with strong staining in the glomerular capillaries as well as mesangium (G). No staining was detected in the untreated control tissue (H).

FIGURE 2.

Treatment of the kidney graft with CD47mAb improves post-transplant outcomes with 6h CIT. (A) A syngeneic Lewis rat kidney transplant model with bilaterally nephrectomized recipients was used. Donor kidneys were flushed with 5 mL of UW solution containing 50 μ g of CD47mAb (n=5) or isotype matched IgG control (n=5) antibody. No control-treated kidney recipients survived for more than 5 days, whereas 80% of CD47mAb-treated kidney recipients survived for 7 days ($p < 0.01$). (B) Serum markers of kidney function are reduced by treatment of donor kidneys with CD47mAb treatment. Serum samples were obtained from sham operated rats (n=5) and recipients that received IgG or CD47mAb treated kidneys. Samples were obtained at 2 days from recipients of IgG- (n=5) and CD47mAb- (n=5) treated kidneys, and at 7 days from recipients of CD47mAb-treated kidneys (n=4). Creatinine, BUN, phosphorus and magnesium were measured using an autoanalyzer. Two days following transplantation, serum creatinine, BUN, phosphorus and magnesium were markedly elevated in animals that received the IgG-treated kidneys, but were only moderately elevated in the recipients of the CD47mAb-treated kidneys. At 7 days post-transplant, values in the recipients of the CD47mAb-treated kidneys had returned to sham levels. Since no rats receiving control IgG-treated kidneys survived for 7 days, the IgG control values are only available for day 2.

FIGURE 3.

Donor kidneys with CD47mAb treatment exhibit histologic evidence of IRI protection. (A) The kidneys of rats undergoing sham operations had minimal evidence of renal injury. (B) CD47mAb-treated donor kidneys (n=5) showed less histologic injury when examined 2 days post-transplant than kidneys receiving control IgG antibody (n=5) (C). (D) Histologic sections were scored in a blinded manner for acute tubular injury (ATI) and acute tubular necrosis (ATN). CD47mAb-treated kidneys had $30\% \pm 5$ ATI/ATN versus IgG control-treated kidneys with $90\% \pm 8$ ATI/ATN ($p < 0.0001$).

FIGURE 4.

Biomarkers of renal injury are diminished with CD47mAb treatment versus control post-transplantation. Two days following transplantation, plasma collected from kidney recipients was evaluated for renal injury protein biomarker levels. Recipients receiving CD47mAb-treated kidneys had diminished levels of cystatin C, osteopontin, TIMP1, β2-microglobulin, VEGF-A and clusterin compared to those receiving control IgG-treated kidneys. Calbindin and GST-α did not reach statistical significance but trended towards lower values in the CD47mAb treated kidneys.

FIGURE 5.

CD47mAb treatment increases perfusion of transplanted kidney. Transplanted kidneys were imaged with a laser Doppler immediately after reperfusion (0hr) and 1hr after reperfusion. The animals were awoken and allowed to recover overnight. The next day, the animals were reanesthetized, and imaging was performed at 24hr after reperfusion. (A) Representative color Doppler and grayscale photographic images of the kidneys are shown at 0hr and 24hr for CD47mAb and control IgG treated animals. (B) The individual flux measurements from all four animals in each group are shown. At 0hr, the CD47mAb treated grafts (n=4) had higher flux than those treated with IgG (n=4) (** $p<0.006$). At 24hr, all CD47mAb treated animals had higher flux than their 0hr baseline values. This was true for only two of the IgG treated kidneys (* $p<0.05$).