Renal Ischemia/Reperfusion Injury: Functional Tissue Preservation by Anti-Activated β1 Integrin Therapy

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Renal ischemia/reperfusion injury (IRI) is an important cause of acute renal failure. Cellular and molecular responses of the kidney to IRI are complex and not fully understood (2–4). Several studies have examined the role of leukocytes and their surface adhesion molecules in the pathogenesis of postischemic renal damage. Leukocyte adhesion molecules seem to facilitate polymorphonuclear neutrophils recruitment during reperfusion, being implicated as mediators of renal IRI (5,6). Coexpression studies using mAb, antisense oligonucleotides, or gene “knockout” indicate that blockade of β2 integrins and/or intercellular adhesion molecule-1 attenuates IRI in some experimental models (7–13). Furthermore, it was proposed recently that T cells might also mediate IRI through the interaction with renal tubular epithelial cells (14).

Integrin activation and deactivation are not fully understood in vivo processes. It is conceivable that after IR, β1 integrins, which are basally localized in the stressed tubular cells, are deactivated. Conversely, as a new epithelium reforms, β1 integrins again become activated and return to an exclusively basal location. The state of integrin activation can be assessed by a group of mAb (HUTS) that selectively recognize β1 integrins in their active form (21). In fact, an activated epitope has been defined in the rat using the anti-human HUTS-21 mAb, which

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recognizes β1 integrins on rat lymphocytes after activation with divalent cations Mn²⁺ and Hg²⁺. This mAb confers protective effects in an in vivo autoimmune nephritis model (22).

We assessed herein the effects of HUTS-21 systemic administration, infused before the renal artery clamp in an IRI rat model. A single administration of this anti-activated β1 mAb resulted in the preservation of renal function and prevention of tissue damage after ischemic insult.

Materials and Methods

Animals

Male Sprague-Dawley rats (IFFA-Credo, Paris, France) that weighed 180 to 200 g were used throughout the experiments. Animals were treated according to the institutional guidelines that are in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Surgery and Experimental Protocol

Rats were anesthetized with an inhaled anesthesia mixture of isofluorane 2% (Abbott Laboratories Ltd., Queenborough, Kent, England) and oxygen 1 L/min and placed on a temperature-regulated table (39°C) to maintain body temperature. Renal ischemia was induced by clamping both renal pedicles during 45 min. Vehicle group (n = 36), received an intravenous injection of phosphate buffer (pH 7.4) 5 min before clamping. The HUTS-treated group (n = 36) received a single intravenous injection of 0.28 g/100 g body wt anti-activated β1 integrin HUTS-21 mAb (19) 5 min before clamping. The sham-operated group (n = 36) underwent the same surgical procedure, except that the clamp was not applied. Blood samples and kidneys were collected at different reperfusion times and processed for different studies.

Functional and Histopathologic Studies

Serum creatinine was determined using a modified Jaffé’s reaction, and blood urea nitrogen (BUN) was measured on the AEROSET System (Abbott Laboratories, Abbott Park, IL). Kidneys were processed for light microscopy examination according to standard procedures, and sections were stained with hematoxylin and eosin and periodic acid-Schiff. Histopathologic changes were analyzed for tubular epithelial cell necrosis, tubular dilation, proteinaceous casts, and medullary congestion as suggested by Racusen (23). The presence of interstitial leucocyte cell infiltrates was assessed by immunohistochemistry.

Immunohistochemistry Studies

Immunostaining was performed using an indirect immunoperoxidase technique. Snap-frozen renal tissues were staining with anti-α1 integrin (Chemicon, Temecula, CA) and HUTS-21 mAb. Paraffin-embedded renal sections were stained with anti-CD68 (ED1), anti-CD3 (1F4; Serotec, Oxford, UK), anti-focal adhesion kinase (FAK; C903), and anti-phosphorylated FAK (pFAK [Y397]; Santa Cruz Biotechnology, Santa Cruz, CA). Enumeration of interstitial infiltrating macrophages (CD68⁺) and T cells (CD3⁺) was determined by counting the total number of positive-labeled cells examined in 10 randomly chosen areas of interstitial infiltrates.

Western Blot Analysis

Pieces of snap-frozen kidneys were homogenized in lysis buffer that contained 100 mM buffer phosphate (pH 7.6), 150 mM NaCl, 10 mM EDTA, 0.5% Triton X-100, and a cocktail of protease and phosphatase inhibitors and then were incubated for 60 min on ice and centrifuged at 13,000 × g for 30 min at 4°C. Supernatants were collected, and protein concentration was quantified by Bradford colorimetric assay (24). Equal amount of proteins (30 μg) were separated by 10% SDS-PAGE and immunoblotted onto polyvinylidene difluoride membranes (Bio-Rad, Hercules, CA). β1 integrin expression was detected using anti-β1 antibody (M-106; Santa Cruz) and ECL Western blotting detection system (Amersham Pharmacia Biotech., Buckinghamshire, UK). Results were expressed as percentage of β1 integrin expression of sham-operated rat kidneys.

Quantitative Reverse Transcriptase–PCR

Total RNA was obtained from snap-frozen kidney tissue using the Ultraspec RNA isolation system (Biotec, Houston, TX). Two micrograms of DNasel-treated RNA were reverse-transcribed with MvUL reverse transcriptase (Roche, Indianapolis, IN). mRNA expression was quantified by real-time PCR following the manufacturer’s instructions (Lightcycler rapid thermal cycler; Roche) using the primers specific for exon sequences (5′→3′): ATG CCA TTC TGC GTC TGG ACC TGG C (β-actin, sense), AGC ATT TGC GGT GCA CGA TGG C (β-actin, antisense), CAC CTC TCA AGC AGA GCA CAG (IL-1β, sense), GGG TTC CAT GGT GAA TGC AAC (IL-1β, antisense), CCA GGA GAA AGT CAG CCT CCT (TNFα, sense), TCA TAC CAG GGC TGG AGC TCA (TNFα, antisense), CCC TTC CCA AGT TTC TGC CAG CAG (inducible nitric oxide synthase [iNOS], sense), GGG CTC CTC CAA GGT GTT GCC C (iNOS-antisense).

Isolation of Proximal Tubular Epithelial Cells

Proximal tubular epithelial cells (PTEC) were obtained from rat kidney cortices that were subjected to 45 min of ischemia and 3 h of reperfusion or sham operation, following the process described by Vinay et al. (25) with some modifications. Briefly, renal cortices were incubated in gassed Krebs-Henseleit saline (pH 7.4) that contained 0.1 mg/ml collagenase type I (Sigma, St. Louis, MO) at 37°C for 1 h and sieved throughout 0.250- to 0.035-mm mesh. Cell populations were sieved by gradient centrifugation in 50% Percoll (Sigma) solution (Kreb-Henseleit saline:Percoll, 1:1, gassed for 1 h on ice). PTEC that were obtained from the appropriate band were immediately used for cell adhesion experiments.

Cell Adhesion Assay

Cell adhesion assay was performed as described (26,27). PTEC (3 × 10⁴ per well), prepared as above, were incubated with 5 μg/ml HUTS-21 or irrelevant mAb (anti-N-cadherin [H-63; Santa Cruz]; anti-L-A [Serotec]) or without mAb in 1 μg/ml collagen IV (Sigma) or BSA-coated wells. Nonadherent cells were removed, and the percentage of attached cells was calculated by measuring absorbance at 540 nm after fixation and staining with 0.5% crystal violet in 20% methanol.

Statistical Analyses

Results are expressed as arithmetic means (± SD). Statistical comparisons between groups were performed by t test. The difference was considered to be significant at P < 0.05.

Results

IRI Diminishes Expression of Activated β1 Integrins on the Basal Cell Surface of PTEC

Previous studies suggest a role for β1 integrins in the tubular cell detachment and tubular lumen occlusion after IRI (17–20). Here, we analyzed the expression pattern of activated β1 integrins in a rat model of bilateral renal ischemia. As shown in Figure 1, activated β1 integrins (HUTS-21 mAb staining) local-
ize exclusively to the basal cell surface of proximal and distal tubules in the cortex and in the outer and inner medulla in normal kidneys (sham operated; Figure 1A, a). In contrast, much weaker immunostaining was observed after renal ischemia in the basal cellular layer of the same tubular structures (Figure 1A, b). After 24 h of reperfusion, activated \( \beta_1 \) integrin localized exclusively to basal cell surface of proximal and distal tubule in sham-operated rat kidney (a), decreasing the immunostaining after renal ischemia (0 h reperfusion; b) and 24 h of reperfusion (c). Immunodetection of \( \alpha_1 \) integrin in sham-operated rats (d), after renal ischemia (e) and 24 h of reperfusion (f), showed no changes. (B) Densitometric quantification (as a percentage of control, sham-operated) of \( \beta_1 \) expression under the different reperfusion times. Results represent the mean ± SD; \( n = 4 \). Representative Western blot analysis of \( \beta_1 \) integrin in total ischemic kidney lysates is also shown (bottom). Magnification, ×500 in A, immunoperoxidase (PO).

**Preischemic HUTS-21 Administration Confers Renal Protection**

Animals that underwent renal ischemia showed marked deterioration of renal functional parameters with significant increase in both creatinine and BUN levels, compared with basal levels from sham-operated and normal rats (Figure 2). Serum creatinine and BUN levels exhibited a steady increase from 0 h of reperfusion, reaching the maximal values after 24 h and declining thereafter to reach background levels after 7 d (data not shown). Preischemic administration of HUTS-21 produced a remarkable renal functional protection, as shown in the serum creatinine (0.40 ± 0.098 \textit{versus} 1.60 ± 0.324 mg/dl; \( P < 0.001 \)) and BUN levels (25 ± 11.0 \textit{versus} 120 ± 11.79 mg/dl; \( P < 0.001 \)) at 24 h of reperfusion (Figure 2). Administration of HUTS-21 to sham-operated and normal rats produced no changes in renal functional parameters (data not shown).

Parallel to the deterioration of functional parameters, histologic examination revealed patchy distributed renal lesions in the vehicle group (Figure 3). Renal lesions were evident as early as 1 h of reperfusion: Signs of sublethal injury (Figure 3a, arrows) and altered cell adhesion, such as tubular cell loss/
pauci-cellular epithelium with flattening as well as detached cells in tubular lumina, were observed. At 8 h of reperfusion, a massive PTEC detachment (Figure 3b, arrows) was observed. Renal lesions reached a peak between 12 and 24 h of reperfusion (Figure 3, c and d, respectively). At that time, more severe and lethal injury (tubular epithelial cell necrosis) was found, being most prominent in the outer medullary stripe, but also with patchy involvement of the cortical proximal segments. In addition, tubular dilation with proteinaceous and epithelial cell cast-filled lumen in the inner zone of the medulla (Figure 3, c and d, asterisk) and blood congestion in the outer zone of the inner stripe of the medulla were observed. The preischemic treatment with HUTS-21 showed complete absence of all renal lesions at 12 and 24 h of reperfusion (Figure 3, g and h, respectively), which paralleled the accelerated recovery of renal function.

An additional important aspect of IRI is the recruitment of circulating leukocytes into renal interstitium. Although polymorphonuclear neutrophil infiltration has been widely studied, less is known about the role of macrophages and T cells after IR insult. Here we studied the contribution of both cell populations to IRI (Table 1). Interstitial macrophages were first detected as early as 12 h of reperfusion, reaching the maximal number at 24 h (Figure 4a). In addition, a discrete number of T cells began to be detected at 24 h of reperfusion, reaching a prominent number at 72 h (Figure 4b). It is interesting that HUTS-21 administration showed a drastic reduction on both macrophages and T cells (Figure 4, c and d).

**HUTS-21 Administration Reduced Proinflammatory Cytokines and iNOS mRNA Levels in Renal IRI**

Because proinflammatory cytokines are keys for tissue inflammatory cell infiltration and its expression during IRI results in iNOS upregulation, we studied the effects of HUTS-21 administration on its local mRNA expression (Figure 5). Real-time quantitative RT-PCR analysis of renal tissue revealed similar kinetic profiles for TNF-α and IL-1β mRNA expression in ischemic kidneys (vehicle group), showing a maximum value at 12 h after ischemia, declining thereafter, and reaching basal levels at 3 d of reperfusion. By contrast, the analysis of this proinflammatory cytokine mRNA expression in the HUTS-treated group showed no changes, maintaining the basal values along reperfusion times.

Local expression of iNOS mRNA showed a delay compared with proinflammatory cytokines. HUTS-21 administration significantly prevented the increase in local iNOS mRNA expression.

**HUTS-21 Increases Adhesiveness of Ischemic PTEC**

To investigate the mechanisms of effects of HUTS-21 administration over IRI, we examined its effects on cell adhesion to ECM. PTEC from kidneys of IRI and control rats were incubated with HUTS-21, with irrelevant antibody, or without antibody, and PTEC adhesion to collagen IV was analyzed. Cell adhesion from ischemic or control PTEC on the BSA matrix in the presence or absence of all antibodies showed basal values (data not shown). As shown in Figure 6, adhesion of control PTEC was not further enhanced in the presence of HUTS-21. By contrast, adhesion of ischemic PTEC to collagen IV in the presence of HUTS-21 was threefold higher compared with ischemic PTEC, either in the absence or in the presence of control mAb.

**Ischemia-Induced FAK Dephosphorylation Is Prevented by HUTS-21 Administration**

FAK activity is regulated by phosphorylation after activation of integrins, including β1 integrins, among other factors. To investigate further in vivo β1 activation in the outcome of IRI, we evaluated the phosphorylation level of FAK (pFAK) during IR and upon administration of HUTS-21. Figure 7 shows pFAK levels in the tubular epithelium using a specific antibody that recognizes FAK phosphorylation at Y397 residue. Ischemia induced FAK dephosphorylation. During reperfusion (3 to 24 h), pFAK levels remained lower than the basal ones (sham-oper-
ated group). HUTS-21 administration preserved FAK phosphorylation from the early times of reperfusion, even at higher levels than basal pFAK. No changes in total FAK expression were detected by immunohistochemistry, neither upon IRI nor upon HUTS-21 administration (data not shown). These results suggest that IRI induces deactivation of β1 integrin that was restored after HUTS-21 administration.

**Discussion**

β1 integrins function mainly as cellular receptors for ECM proteins. The importance of β1 integrins in the architectural and functional maintenance of the tubular compartment of kidney tissue is known (28). β1 integrin–ECM interaction favors cell adhesiveness and epithelium integrity (15). Moreover, HUTS-21 administration preserved FAK phosphorylation from the early times of reperfusion, even at higher levels than basal pFAK. No changes in total FAK expression were detected by immunohistochemistry, neither upon IRI nor upon HUTS-21 administration (data not shown). These results suggest that IRI induces deactivation of β1 integrin that was restored after HUTS-21 administration.

**Table 1.** Macrophage and T cell infiltration in the kidney from rats that were subjected to renal ischemia followed by reperfusion

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Reperfusion Time (h)</th>
<th>Macrophages (× 10)</th>
<th>T Cells (× 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operated</td>
<td>3.75 ± 0.96</td>
<td>3.00 ± 0.82</td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>4.25 ± 0.96</td>
<td>1.75 ± 1.71</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.00 ± 1.63</td>
<td>3.00 ± 1.63</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3.25 ± 2.06</td>
<td>4.25 ± 1.26</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>52.50 ± 18.48</td>
<td>3.50 ± 2.08</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>204.75 ± 41.53</td>
<td>10.00 ± 2.58</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>117.50 ± 19.23</td>
<td>113.00 ± 13.93</td>
<td></td>
</tr>
<tr>
<td>HUTS treated</td>
<td>2.50 ± 2.08</td>
<td>2.75 ± 1.71</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.00 ± 1.41</td>
<td>3.25 ± 0.96</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4.50 ± 1.29</td>
<td>4.25 ± 2.22</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>5.50 ± 1.29a</td>
<td>3.75 ± 1.50</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>4.75 ± 2.36a</td>
<td>3.00 ± 1.41b</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>4.50 ± 2.38a</td>
<td>4.00 ± 1.82a</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.001, bP < 0.05 versus vehicle at the corresponding time.

**Figure 4.** Cellular infiltration into the postischemic kidney. Macrophages (CD68⁺ cells) and T lymphocytes (CD3⁺ cells) were detected in kidneys from the vehicle group (a and b, respectively), whereas no inflammatory cells were observed in HUTS-treated kidneys (c and d). Magnification, ×600 in a and b, PO; ×400, c and d, PO.

**Figure 5.** Proinflammatory cytokines and inducible nitric oxide synthase mRNA analysis. Real-time quantitative reverse transcriptase–PCR analysis of mRNA from sham-operated (△), vehicle (○), and HUTS-treated (□) kidneys. Results represent the mean ± SD, n = 4, and are normalized to β-actin expression measured in parallel in each sample. *P < 0.001; **P < 0.01; ***P < 0.05.
the α4β1 integrins are implicated in cell–cell interaction between circulating leukocytes and activated endothelium (29). Here, we report the protective effects of systemic HUTS-21 administration in a rat model of renal IRI. HUTS-21 recognizes an activation-dependent epitope on the human β1 integrin chain and cross-reacts with a similar activated epitope in the rat (21,22). We have focused our studies on both the PTEC detachment from the basal lamina and the adhesion and extravasation of circulating leukocytes into the renal interstitium.

Animals that underwent renal ischemia showed a marked deterioration of renal functional parameters with significant increase in both creatinine and BUN levels. Histopathologic renal lesions showed a massive PTEC detachment, with the renal lesions reaching a peak at 24 h of reperfusion. Preischemic HUTS-21 administration resulted in an accelerated improvement of renal function and complete absence of renal tissue lesions at 24 h of reperfusion.

Cellular adhesive properties are regulated by selective expression of the integrin repertoire and by the modulation of their binding properties. A distinctive feature of integrins is the ability to modulate the level of adhesiveness rapidly and reversibly (30). The process of activation is a direct consequence of integrin conformational changes resulting in an increased ligand binding affinity. Consequently, a potential strategy to maintain tubular cell integrity would be to avoid β1 integrin deactivation. It is interesting that binding of HUTS-21 to partially activated α4β1 integrins results in freezing active conformation of these receptors and enhancement of cell adhesion to α4β1 integrin ligands (21). Our experiments addressed the role played by activated α4β1 integrins in the course of renal IRI. Our data showed that during IRI, α4β1 integrins are deactivated as reflected in the loss of HUTS-21 staining on the basal cellular layer of stressed PTEC as well as FAK dephosphorylation. These results could not be explained on the basis of a loss of α4β1 integrin expression, because Western blot analysis showed no significant changes in its expression levels during IRI.

α4β1 integrins act as cell surface receptors for ECM proteins and extracellular signal transducers into the cell, including actin cytoskeleton reorganization. Thus, integrins clustered to the basolateral surface of adherent cells to form focal adhesion complex (FAC) (31). One important component of this adhesion complex is the p125 FAK that can be activated by autophosphorylation, and this activation is required for the FAC formation and function (revised in 32). Phosphorylated FAK can bind the cytoplasmic domain of α4β1 integrins and activate actin cytoskeleton assembly (33–35). Activation/deactivation of α4β1 integrins can be estimated indirectly by FAK phosphorylation, as assessed in this study. Previous reports have demonstrated that ischemia/hypoxia induced FAK dephosphorylation in vivo (36) and in vitro (37). Our results demonstrate that renal ischemia by itself induces FAK dephosphorylation, correlating with α4β1 integrin deactivation. Moreover, reperfusion induced slight β1 integrin activation but was not sufficient to increase FAK phosphorylation. However, HUTS-21 that recognizes partially activated β1 integrin “locked” the integrin in the active form, resulting in a strong FAK activation by phosphorylation. Administration of HUTS-21 could maintain the integrity and

Figure 6. Effect of HUTS-21 mAb on proximal tubular epithelial cells (PTEC) adhesion to collagen intravenously. PTEC from control (□) and ischemia/reperfusion injury (IRI; ■) kidneys were treated with HUTS-21 mAb or with irrelevant antibodies (anti–I-A, anti–N-cadherin). Adhesion of control PTEC to BSA in the absence of antibody was considered as basal adhesion, and the percentage of adhesion was calculated according to these values in each independent experiment. PTEC that were cultured in the absence of antibody served as reference. *P < 0.001.

Figure 7. Effect of HUTS-21 administration on expression of pFAK. Immunohistochemical staining of kidneys from sham-operated, vehicle, and HUTS-treated groups. Note the increased intensity of staining with anti-pFAK mAb in the HUTS-treated group in all reperfusion times compared with vehicle. (a) Negative control (b); sham operated (c and d); 0 h of reperfusion (e and f); 3 h of reperfusion (g and h); 24 h of reperfusion. Magnification, ×400 in a through g, PO; ×600 in h, PO.
functionality of FAC as determined by FAK phosphorylation, resulting in enhanced adhesiveness of PTEC.

The diminished immunostaining of HUTS-21, together with the decreased FAK phosphorylation in ischemic kidneys, suggests the functional disruption of FAC and, consequently, the loss of adhesion. PTEC require anchorage to the BM for normal function, which is mediated by integrins’ binding to ECM proteins, being collagen IV its major component. It was demonstrated previously (38) that β1 integrins’ binding to collagen IV and not to other ECM proteins elicits signal transduction events in injured PTEC that stimulate cell survival and are important for the recovery of physiologic functions. Our results revealed that ex vivo HUTS-21 was able to increase the adherence of ischemic PTEC to collagen IV, suggesting that in vivo HUTS-21 most probably increases the adhesiveness of β1 integrins to the basal lamina, thus contributing to prevent renal damage. It is conceivable that HUTS-21 administration increases the adhesion and focal contact between PTEC and basal lamina, thus diminishing cell detachment and casts formation.

Renal injury after ischemia seems to be a consequence of tissue hypoxia from renal blood flow deficiency but also from the process of reperfusion, leading to an active inflammatory response (4). Sublethal or even lethal injured proximal tubular and endothelial cells trigger this process through the release of proinflammatory mediators that will promote cellular infiltration. As compared with vehicle rats, HUTS-21 administration showed a drastic inhibitory effect on the recruitment of both macrophage and T cell leukocyte populations, as well as on the expression of proinflammatory cytokines, thus abrogating the inflammatory response. Although it is well demonstrated that leukocyte adhesion molecule blockade plays a tissue protective role in IRI in muscle and heart, it is still a matter of controversy in the kidney. CD11/CD18 and intercellular adhesion molecule-1 blockade are usually protective in experimental renal IRI (9,12). In contrast, induction of systemic neutropenia and selectin function blockade do not have a protective effect, suggesting a neutrophil-independent mechanism for renal protection (6). Although neutrophil infiltration has been widely studied (revised in 39), less is known about the role of macrophages and T cells after IR insult. Macrophages and CD4+ cell infiltration within a few days after IRI was described after warm ischemia (40) and after experimental cold ischemia (41), with limited information, however, as to its precise topographic localization and kinetics. Ysebaert et al. (42) reported that only at the later time of regeneration did a sequential infiltration of macrophages and T cells become prominent. Nevertheless, it has been reported (43,44) that the blockade of CD29-B7 co-stimulatory pathway, important for lymphocyte activation, significantly abrogates postischemic renal dysfunction in the rat. Rabb et al. (14) recently proposed that T cells might also mediate IRI through the interaction with PTEC. These observations concur with our findings regarding the early presence of mononuclear cells in the renal interstitium, supporting their important role in renal IRI.

In a previously reported study, we demonstrated the role of β1 activated integrins in the development and progression of an autoimmune model of nephritis (22). HUTS-21 administration was able to block circulating leukocyte extravasation, thus abrogating the development of interstitial nephritis. Although we do not know the exact mechanism/s responsible for this phenomenon, it is conceivable that the blockade of proinflammatory cytokine expression could contribute to the abrogation of the inflammatory response amplification and consequently to leukocyte cell infiltration. Although kidney interstitium infiltration by circulating leukocytes has been considered an important source of cytokines, evidence suggests that glomerular mesangial cells and tubular epithelial cells are additional major cellular sources of TNF-α (45). Moreover, the expression of proinflammatory cytokines during IRI gives rise to the upregulation of iNOS, leading to tissue injury (46–49). We found that HUTS-21 administration prevents expression of proinflammatory cytokines and iNOS from renal tissue, thus abrogating the inflammatory response.

We conclude that in vivo administration of HUTS-21 likely keeps β1 integrins in an activated state, as occurs in in vitro studies (50). Consequently, the sustained adhesion of PTEC to β1 integrin ligands on BM could attenuate tubular epithelial detachment into the lumen and tubular obstruction. The preservation of tubular epithelial integrity could explain the protective effect of HUTS-21, supporting renal function improvement, proinflammatory cytokine and iNOS suppression, and a decreased T cell and monocyte/macrophage extravasation into the renal interstitium.

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References


