Acute-on-Chronic Renal Failure in the Rat: Functional Compensation and Hypoxia Tolerance

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Key Words

Kidney failure, acute · Kidney failure, chronic · Ischemia-reperfusion · Hypoxia · Medulla · Radiologic contrast media · Hypoxia-inducible factor · Endothelin-B receptors · Pimonidazole

Abstract

Background: We hypothesized that chronic renal parenchymal disease may predispose to acute renal failure (ARF), facilitating the induction of hypoxic medullary tubular injury. **Methods:** To induce chronic renal parenchymal injury, rats underwent sham operation (control) or bilateral 50-min clamping of the renal artery [ischemia-reperfusion (IR)]. One or 3 months later, both groups were subjected to an ARF protocol, consisting of radiocontrast and the inhibition of prostaglandin and nitric oxide synthesis. Renal function and morphology were determined 24 h later. **Results:** Chronic tubulointerstitial changes (fibrosis, atrophy and hypertrophy) in the IR group correlated with baseline tubular function, but glomerular function was preserved. Functional deterioration after the ARF protocol was only marginally more

pronounced in the IR group, and the degree of medullary acute tubular necrosis (ATN) was unaffected by prior IR. The extent of both tubular necrosis and chronic tubulointerstitial changes independently predicted the acute decline in renal function. Immunostaining of IR kidneys disclosed critically low medullary pO₂ (determined by pimonidazole adducts), regional hypoxic cell response (hypoxia-inducible factors) and upregulation of endothelin-B receptors. **Conclusions:** Compensatory changes result in normal plasma creatinine 1 and 3 months after IR, despite diminished tubular function. Preexisting renal disease only marginally predisposes to ARF, and the extent of ATN is not significantly enhanced. These findings illustrate the complex interaction between chronic and acute renal injury and dysfunction and parallel the difficulty of their assessment in the clinical practice. Adaptive cellular responses to chronic hypoxia in conjunction with parenchymal loss and decreased oxygen demand might alleviate acute hypoxic injury.

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Background

Chronic kidney disease is a prominent clinical risk factor for the development of acute nephrotoxic or hypoxic renal failure (ARF) [1–9]. It is believed that reduction of renal mass predisposes remnant nephrons to larger doses of nephrotoxins. In addition, nephrosclerotic changes lead to altered renal microcirculation that may compromise renal parenchymal oxygenation. Remnant nephrons may suffer hypoxic insults as a result of diminished blood supply, combined with augmented oxygen demand for transport activity. This may be especially true at the outer medulla that normally functions at low ambient pO_2 [10].

Despite its clinical relevance, animal studies of acute injury superimposed on kidneys with chronic changes are generally unavailable [11], with the exception of subtotal renal ablation prior to the acute insult [12]. However, this kind of model involves tissue loss without the diffuse parenchymal distortion that characterizes usual chronic renal injury. Recently, Basile et al. [13] have reported that following renal ischemia-reflow tubular morphology quickly recovers, whereas prominent vascular depletion develops within the outer medulla. Using pimonidazole, a marker of critical tissue hypoxia, these investigators illustrated the accentuation of regional hypoxia [13] that was alleviated by *L*-arginine [14]. They have further suggested that peritubular medullary vascular depletion and aggravation of chronic regional hypoxia might participate in a subsequent gradual progression of renal failure [13]. In that perspective, we hypothesized that predisposition to ARF in patients with chronic renal failure might reflect a comparable scenario: the development of acute tubular necrosis (ATN), caused by compromised outer medullary oxygen balance, the consequence of an abnormal renal microcirculation, coupled with increased oxygen demand for hypertrophic remnant nephron elements. Our objectives were (a) to develop an animal model of acute-on-chronic renal injury; (b) to assess if chronic renal damage indeed predisposes to hypoxic outer medullary tubular injury, and (c) to explore the association between the acute and chronic morphological changes and renal dysfunction. The lack of an obvious predisposition to ATN by chronic tubulointerstitial disease demonstrated in these studies initiated a search for evidence for the induction of adaptive renoprotective systems.

Methods

Male Sprague Dawley rats (200–225 g) were used for all experiments, fed on regular chaw and having free excess to water. Experiments were conducted in accord with the NIH Guide for the Care and Use of Laboratory Animals. Chemicals were purchased from Sigma. In order to establish chronic tubulointerstitial disease, the ischemia-reflow model was chosen, characterized at 1 month by prominent outer medullary peritubular vascular depletion and mild tubulointerstitial changes [13]. A multi-insult model was used for hypoxic ATN [15], consisting of radiocontrast, administered following the inhibition of nitric oxide and prostaglandin synthesis, well-characterized systems important in maintaining medullary oxygen balance [10, 16].

Experimental Design

Fifty-eight animals were anesthetized with ketamine (100 mg/kg) and randomized to sham operation [control (CTR)] or bilateral 50-min clamping of the renal artery [ischemia-reperfusion (IR)]. One or 3 months later, following a 24-hour dehydration period, first urine samples were obtained in metabolic cages for the determination of maximal urinary concentration, and water supply was resumed. Subsequently, following a baseline 24-hour collection period (day 0), the rats were anesthetized with ketamine. The femoral artery and vein were cannulated for drug administration, and baseline blood samples were obtained. The ATN protocol consisted of parenteral indomethacin (10 mg/kg, i.v.), followed at 15 min and at 30 min by *N* ω -nitro-*L*-arginine methyl ester (10 mg/kg, i.v.) and meglumine iohalamate (Conray 60%, 6 ml/kg, intra-arterial). The rats were allowed to recover in the metabolic cages for an additional 24-hour period, and then were anesthetized with pentobarbital (60 mg/kg). A second set of urine and blood samples were obtained (day 1) and the kidneys were perfusion fixed *in vivo* with glutaraldehyde for morphologic evaluation through the abdominal aorta.

Blood and urine samples were analyzed for creatinine, urea, Na and K, using the Kodak dry reagent system, and the corresponding creatinine clearance, tubular sodium reabsorption (TRNa) and fractional potassium excretion (FEK) were calculated. Urinary osmolality following dehydration was determined with a freezing-point osmometer (Micro-Osmette 5004, Precision Systems, Natick, Mass., USA).

Renal Morphology

Kidney slices were postfixed in buffered 2% OsO_4 , dehydrated, and embedded in an Araldite-EM bed 812 mixture. Large sections were cut perpendicular to the renal capsule, containing cortex and medulla. Methylene-blue-stained sections (1 μ m) were analyzed in a blinded fashion for morphologic alterations.

Chronic tubulointerstitial changes (interstitial fibrosis, tubular atrophy and hypertrophy) were assessed semi-quantitatively on a scale from 0 to 2: 0 = unremarkable; 1 = zonal maintenance with focal fibrosis in the outer medulla and cortex (usually restricted to medullary rays); 2 = zones blurred with extensive fibrosis and tubular atrophy, both in the cortex (medullary rays and labyrinth) and outer medulla.

Acute changes were assessed as previously detailed [15, 17]. Tubular necrosis was determined separately for S3 proximal tubules in the outer stripe and for medullary thick ascending limbs (mTALs) in the outer, mid and inner zones (A, B and C zones, respectively) of the inner stripe of the outer medulla. The extent of

Table 1. Functional parameters

Group	IR 1 month (n = 18)	CTR 1 month (n = 16)	Unpaired t test	IR 3 months (n = 16)	CTR 3 months (n = 8)	Unpaired t test
Weight gain, g	40 ± 4	47 ± 4	NS	75 ± 2	80 ± 4	NS
Max. urinary osmolality, mosm						
Prebaseline	2,519 ± 115	3,505 ± 198	0.001	2,455 ± 69	3,143 ± 168	0.02
Urine volume, ml/h						
Baseline	0.68 ± 0.10	0.41 ± 0.08	0.05	0.39 ± 0.05	0.30 ± 0.05	NS
Day 1	0.56 ± 0.08	0.71 ± 0.08*	NS	0.46 ± 0.09	0.52 ± 0.12	NS
Plasma creatinine, µm/l						
Baseline	53 ± 2	53 ± 2	NS	54 ± 5	51 ± 3	NS
Day 1	116 ± 14*	96 ± 11*	NS	119 ± 20*	87 ± 8*	NS
Plasma urea, mm/l						
Baseline	8.5 ± 0.6	7.1 ± 0.3	0.04	7.0 ± 0.3	7.6 ± 0.3	NS
Day 1	33.2 ± 2.8*	30.0 ± 2.5*	NS	29.0 ± 3.3*	30.0 ± 4.6*	NS
ClCr, ml/min/100 g						
Baseline	0.39 ± 0.03	0.35 ± 0.03	NS	0.37 ± 0.04	0.43 ± 0.06	NS
Day 1	0.14 ± 0.02*	0.23 ± 0.04*	0.05	0.19 ± 0.03*	0.20 ± 0.03*	NS
TRNa, %						
Baseline	99.52 ± 0.05	99.67 ± 0.05	0.03	99.73 ± 0.04	99.74 ± 0.06	NS
Day 1	98.85 ± 0.60	99.09 ± 0.27	NS	99.41 ± 0.22	99.56 ± 0.09	NS
FEK, %						
Baseline	12 ± 2	8 ± 1	NS	17 ± 3	13 ± 3	NS
Day 1	35 ± 8*	28 ± 5*	NS	30 ± 6*	27 ± 4*	NS

Maximal urinary concentration was determined in 6–14 rats only in each group; unpaired t test for between-group comparison; * p < 0.05 vs. baseline, paired t test. ClCr = Creatinine clearance.

damage was expressed as the percentage of necrotic tubules out of the total tubules counted. The degree of papillary necrosis was semi-quantitatively assessed using a scale from 0 to 2, adopted from a detailed analysis previously performed [17].

Assessment of Chronic Adaptive Responses

In an additional complementary study, 6 rats were studied 1 month after IR, and compared with 3 CTR animals. In these studies, the rats were not subjected to the ATN protocol. The hypoxia marker pimonidazole (HypoxyProbe, Pharmacia International, Belmont, Mass., USA) was injected (60 mg/kg, i.v.) and an hour later the kidneys were perfusion fixed with 3% paraformaldehyde through the abdominal aorta, stored in ice-cooled PBS, and processed for paraffin embedding. Two-micrometer paraffin sections were processed for routine histology or immunohistochemistry. Hematoxylin and eosin staining was performed according to standard procedures. Counterstaining with Richardson's reagent served for a better discrimination of interstitial compartments.

Immunohistochemistry was performed with the following primary antibodies as previously reported [18]: mouse-anti-human hypoxia-inducible factor (HIF)-1α (α 67, Novus Biologicals, Littleton, Colo., USA, 1:10,000), rabbit-anti-mouse HIF-2α (PM9, gift from Patrick Maxwell, Wellcome Trust Centre for Human Genet-

ics, Oxford, UK, 1:10,000), rabbit-anti-rat heme oxygenase-1 (Stressgen, Victoria, Canada, 1:60,000), mouse-anti-pimonidazole (Hypoxyprobe, 1:1,000), and anti-endothelin-B (anti-ET_B) primary monoclonal antibodies (Alomone Labs, Jerusalem, Israel, 1:200).

Statistics

Data were analyzed using Crunch 4.0 statistical software (Oakland, Calif., USA) and are presented as mean ± SEM. Functional and structural parameters were compared between the groups, using two-way analysis of variance and nonpaired t test, respectively. The potential impact of structural changes upon functional parameters was evaluated using simple correlation and multiple regression analysis.

Results

Kidney Function

By 1 and 3 months after the initial perturbation, glomerular function recovered, baseline (before ARF protocol) plasma urea, creatinine and creatinine clearance in

Fig. 1. The extent of chronic and acute tubular changes in rats subjected to ARF protocol 1 or 3 months after bilateral IR procedure or sham operation (CTR). The extent of both chronic tubulointerstitial changes and acute papillary necrosis are assessed semi-quantitatively, using a 0–2 score (1+ = fibrosis with intact zonation, 2+ = fibrosis without intact zonation; 1+ = 50% and 2+ = 100% papillary tip necrosis). The degree of outer medullary tubular necrosis is separately assessed for S3 tubular segments in the outer stripe, and for mTALs in the superficial (A), mid (B) and deepest (C) zones of the inner stripe. Tubular necrosis is expressed as the percentage of injured tubules out of all tubules examined. * $p < 0.05$; ** $p < 0.0001$ vs. corresponding CTR.

the IR group were comparable to those of the control sham-operated animals (table 1). By contrast, tubular function in the IR group remained compromised: at 1 month, baseline urine volume was larger, and maximal urinary concentration and TRNa were decreased as compared to the CTR group. Plasma urea was also slightly, though significantly higher, probably reflecting altered renal water preservation. At 3 months, urine volumes were smaller in both groups (presumably related to a different batch, disparate yearly season and additional different environmental settings). Yet, volumes tended again to be higher in the IR group (table 1).

Induction of the ARF protocol resulted in ARF. Within 24 h, plasma creatinine was doubled in the CTR groups with creatinine clearance falling 1/3–1/2 at both time points (table 1). A fourfold rise was noted in plasma urea. Functional deterioration in the IR groups was only marginally more pronounced, reaching a statistically significant difference from CTR only for the decline in creatinine clearance at the 1-month time point.

Renal Morphology

Morphologic evaluation of perfusion-fixed kidneys disclosed variable *chronic* tubulointerstitial changes in the IR groups, including interstitial expansion and fibro-

sis, with tubular atrophy and hypertrophy of remnant nephrons. These changes, reaching 0.8 ± 0.2 and 0.6 ± 0.1 on the 0–2 score range used at the 1- and 3-month time points, respectively, did not progress over time (fig. 1 and 2).

As previously detailed [15, 17, 19, 20], *acute* morphologic changes associated with the ARF protocol consist of outer medullary congestion and tubular hypoxic damage (manifested as loss of brush border in S3 segments, mitochondrial swelling, cytoplasmic volume loss and nuclear pyknosis in mTALs, and finally cell membrane disruption in both tubular segments). In CTR animals, tubular necrosis was focal and restricted to the outer medulla, involving at the 1-month time period some 10% of S3 segments in the outer stripe, and 17% of mTALs in the mid zone of the inner stripe (fig. 1 and 3). As previously pointed out [19, 20], outer medullary injury pattern showed a gradient of damage, minimal around vascular bundles, and increasing towards the mid-interbundle zone, away from vasa recta. Papillary tip necrosis was noted as well (fig. 4), averaging 0.6 ± 0.2 on the 0–2 scale (fig. 1).

The extent of tubular necrosis in the outer medulla was comparable in the IR and CTR groups for both S3 segments and mTALs at both time points (fig. 1). By contrast, papillary necrosis was markedly attenuated in the IR

Fig. 2. Morphologic findings 30 days after IR: degree of chronic changes. Displayed in both pictures are low-power views of the cortex and adjacent outer stripe of the outer medulla (left) and complete outer medulla (right). **A** Fibrotic changes are present, but zonation is preserved. **B** Fibrotic changes are present and zonation is obscured (magnification $\times 30$).

group at the 1-month time point, averaging 0.1 ± 0.1 only (fig. 1).

Morphology-Functional Correlations

As shown in table 2, chronic tubulointerstitial changes, absent in CTR animals, closely correlate with baseline tubular function, namely urine volume, urea, TRNa, and fractional potassium excretion, but not at all with baseline creatinine or creatinine clearance.

Table 3 illustrates that both chronic tubulointerstitial changes and ATN (predominantly involving TALs) cor-

related with the decline in kidney function. Multiple regression analysis (table 4) reveals that the extent of both chronic tubulointerstitial changes and acute mTAL necrosis independently predict the degree of the evolving renal dysfunction: interestingly, however, while chronic changes predominantly foretell the decline in glomerular function (fall in creatinine clearance), ATN, particularly involving mTALs, predicts tubular dysfunction (manifested by the fall in TRNa and the rising FEK and plasma urea).

Fig. 3. Morphologic findings 30 days after IR: acute-on-chronic changes in the inner stripe of the outer medulla. Necrosis is present in mTALs (*) both in sham-operated (**A**) and IR animals (**B**) given the ARF protocol. Nephron elements show hypertrophic changes in the IR animal. The tubular necrotic changes are adjacent to collecting ducts and are located in the central interbundle zones (magnification $\times 400$).

Table 3. Structural-functional correlations 24 h after ARF protocol

	Chronic changes	S3 tubular necrosis	mTAL necrosis	Papillary necrosis
Urea	NS	NS	0.51 (0.0002)	NS
Creatinine	0.40 (0.005)	0.39 (0.006)	0.52 (0.0001)	NS
Creatinine clearance	-0.42 (0.03)	-0.27 (0.06) NS	-0.35 (0.02)	NS
TRNa	-0.40 (0.05)	0.42 (0.003)	-0.46 (0.001)	NS
FEK	0.52 (0.0001)	NS	0.51 (0.0002)	NS

The two experimental groups (IR and CTR) at the two time points (1 and 3 months) are included in the analysis (Pearson correlation and two-tailed p value). Functional parameters are those obtained after the induction of ARF.

Immunostaining Studies

As shown in figure 5, pimonidazole adducts, absent in CTR kidneys, were detected in all kidneys 30 days after IR, principally in the outer stripe of the outer medulla and in the papilla, indicating critically low regional pO_2 . Hypoxic stress response, represented by HIF-1 α accumulation, also absent in CTR kidneys, has been noted in all IR kidneys predominantly in the tubular and interstitial cells in the papilla. Cortical tissue immunostaining for both markers of hypoxia and HIF was negative. HIF-2 α immunostaining was invariably negative, and heme oxygenase-1, an HIF-mediated target gene, was rarely and inconsistently detected (not shown).

Table 2. Correlation of chronic tubulointerstitial changes and baseline kidney function (before ARF protocol)

Variable	Data of the 1-month period only	Data of the 1- and 3-month periods combined
Urine volume	0.53/0.004	0.33/0.02
Plasma urea	0.66/0.0004	0.52/0.0001
Plasma creatinine	0.1/NS	0.26/NS
ClCr	0.38/0.05	-0.18/NS
TRNa	-0.56/0.003	-0.33/0.02
FEK	0.63/0.0004	0.43/0.002

Figures indicate Pearson correlation/two-tailed p values. The 2 experimental groups, IR and CTR, are included in the analysis. Functional parameters are those obtained at baseline, before the induction of ARF. ClCr = Creatinine clearance.

Fig. 4. Morphologic findings: papillary necrosis 30 days after IR. In the sham-operated (CTR) animals, infarctive necrosis of the papillary tip was present (inset, $\times 30$). At higher power, collecting ducts (*) and capillaries (C, example) have lost their lining cells. The endothelium has been replaced by aggregated platelets (magnification $\times 360$).

Table 4. The contribution of structural damage to the acute decline in kidney function 24 h after the ARF protocol (multiple regression analysis)

Dependent/predicting variables	Chronic changes	S3 necrosis	mTAL necrosis	Papillary necrosis
Urea day 1				
IR groups	NS	NS	5.2/0.03	NS
All groups	NS	NS	7.4/0.009	NS
Cr day 1				
IR groups	3.8/0.06 (NS)	6.1/0.02	NS	NS
All groups	11.0/0.0001	NS	3.8/0.05	3.8/0.06 (NS)
ClCr day 1				
IR groups	10.7/0.003	NS	NS	NS
All groups	10.8/0.002	NS	NS	NS
TRNa day 1				
IR groups	4.3/0.05	NS	12.2/0.002	NS
All groups	12.5/0.001	NS	16.1/0.0002	NS
FEK day 1				
IR groups	17.8/0.0004	NS	8.4/0.009	NS
All groups	22.2/0.0001	NS	19.9/0.0001	NS

Analysis is carried out for the pooled data of 1 and 3 months, either for the IR groups alone or for IR and CTR groups combined. All four morphologic variables were included in the regression analysis to determine their individual contribution to renal dysfunction (F-to-remove/p value). Functional parameters are those obtained after the induction of ARF. ClCr = Creatinine clearance.

Fig. 5. Renal morphology and immunostaining of rats 30 days after IR or sham operation (CTR). Rats were injected with pimonidazole (Hypoxy-probe) 1 hour prior to sacrifice. Two IR kidneys (with moderate vs. advanced fibrosis) are compared with CTR. Both pimonidazole adducts in the outer stripe and papilla and papillary and HIF-1 α immunostaining are more pronounced in the kidney with moderate fibrosis. S3 = Straight segment of the proximal tubule; VB = vascular bundle (vasa recta). **A-F, J-L** Magnification $\times 100$. **G-I, M-O** Magnification $\times 200$.

As illustrated in figure 6, ET_B immunostaining, practically absent in the outer medulla in control kidneys, was detected in vasa recta stroma and inner stripe interstitial tissues in all kidneys subjected to IR, its extent and intensity in close correlation with the degree of fibrosis.

Discussion

Chronic renal failure is the most important risk factor for acute renal dysfunction following renal hypoxic and toxic insults [1–9]. We hypothesized that outer medullary microvascular depletion and hypoxemia in chronic renal disease predisposes to tubular hypoxic damage at this region during acute insults. We examined this hypothesis experimentally by applying acute hypoxic medullary insult in animals previously subjected to IR injury, a procedure known to compromise medullary microvasculature [13]. Herein we report that unexpectedly, renal dysfunction following the ARF protocol was only marginally more pronounced in animals subjected to IR, as compared with sham-operated animals. Moreover, the extent of ATN in the outer medulla was not significantly increased. As shown by our complementary studies, these negative results could reflect hypoxia-induced upregulation of stress response genes that might confer tissue protection against acute hypoxic or toxic insults.

Indeed, in concordance with reports by Basile et al. [13, 14], we observed pimonidazole adducts in the outer and inner medulla 1 month after IR, indicating severe regional hypoxia. Furthermore, we found that HIF-1 α , a member of the ubiquitous master regulators of hypoxic adaptation [21, 22], accumulates in the hypoxic regions identified by pimonidazole (fig. 5). This continuously synthesized molecule undergoes oxygen-dependent proteolysis by prolyl hydroxylase. Under hypoxic conditions, HIF-1 α degradation ceases, leading to its accumulation and binding with a β -subunit. The formed heterodimer activates numerous genes involved in red blood cell production, iron metabolism, vascular tone and architecture, energy metabolism, cell proliferation, differ-

entiation and viability [21, 22]. It is assumed that this unique mechanism of hypoxia-induced activation of stress response genes, such as heme oxygenase or erythropoietin, provides protection against repeated insults [23–26]. The marked attenuation of acute papillary damage in the IR group, as compared to CTR animals at the 1-month time point (fig. 1), supports this hypothesis, as chronic ongoing expression of HIF-1 α has been principally noted at that region.

Upregulation of ET_B receptors, noted in animals subjected to IR (fig. 6), is another plausible mechanism designed to protect against acute hypoxic stress. This endothelin receptor subtype exerts NO-mediated local vasodilation, and its important role in maintaining medullary blood flow has been well documented [27]. We have previously noted upregulation of ET_B receptors in vasa recta and medullary collecting ducts in rats with experimental heart failure [28], and in vasa recta and peritubular capillaries in streptozotocin-induced diabetic rats, in association with enhanced medullary hypoxia [29]. Interestingly, the marked upregulation of ET_B in kidneys subjected to IR is predominantly in the expanded interstitial tissues between the vasa recta and in the interbundle zone. Recent findings indicate that the particular distribution of ET_B upregulation may be induced by hypoxia, possibly mediated through HIF [30]. Regardless its cause, ET_B upregulation with subsequent nitrovasodilation may attenuate the effect of vasoconstrictive stimuli in the animals with heart failure, enhance oxygen supply for increased tubular transport activity in the diabetic kidney, and improve regional blood flow in the anatomically compromised medullary microcirculation.

Interestingly, as shown in figure 5, medullary immunostaining of both pimonidazole and HIF-1 α is maximal in kidneys with mild-to-moderate interstitial fibrosis, whereas in the presence of advanced tubulointerstitial changes their staining intensity is lower. Though these preliminary findings in a small number of animals require confirmation by a larger-scale study, they possibly indicate that diminished tubular mass at the later advanced stage is associated with decreased regional oxygen consumption, with attenuation of ambient hypoxia. In this perspective, some of the local renal stress responses after IR may be downregulated and waned over time, and may even be absent by day 30, as clearly shown by Forbes et al. [31], in parallel with the declining HIF signal. Indeed, heme oxygenase-1, a major HIF-mediated gene, was only minimally and inconsistently expressed in our IR kidneys at 1 month, as well as in THY-1-induced chronic tubulointerstitial disease [Rosenberger, unpublished data].

Fig. 6. ET_B receptor immunostaining in IR kidneys. **A** Intense ET_B immunostaining is noted in the outer stripe interstitial tissue in the presence of extensive fibrosis, calcifications and tubular atrophy. **B** Vasa recta stroma and inner stripe interstitial tissue are immunopositive in kidney with moderate interstitial fibrosis. Magnification $\times 360$.

This contrasts the abrupt HIF-mediated upregulation of heme oxygenase-1 within 8 h after acute hypoxic stress [18]. The cause of the decline in chronic hypoxic stress response remains speculative, but conceivably chronic hypoxia elevates the HIF activation threshold. This could be due to upregulation of some HIF prolyl hydroxylase, the key enzymes of HIF- α degradation and at the same time an HIF target gene. The lack of susceptibility to ATN is extended to the 3-month time point, perhaps related to ongoing diminished chronic medullary hypoxemia, associated with decreased regional tubular mass and reabsorptive activity.

Assessment of acute deterioration in kidney function is a compelling challenge in the clinical practice, especially in the presence of preexisting renal disease. Diagnosis of ATN in such patients is based predominantly upon clinical judgment, underscoring the overt limitation of currently available diagnostic tools. Our report provides functional-structural correlations at baseline and following the induction of ATN, which may give some insight regarding patients' assessment in the clinical practice.

First, chronic tubulointerstitial disease is evidently underdiagnosed by conventional evaluation, restricted to the assessment of the glomerular filtration rate (GFR). Rats fully regained filtration capacity by 1 month after IR. By contrast, larger baseline urinary volumes, higher plasma urea and lower maximal urinary osmolality indicate altered concentrating capacity, as already noticed by Basile et al. [13]. Table 2 illustrates the close correlation of these parameters with the extent of chronic morphologic findings.

Secondly, the acute decline in kidney function following the ATN protocol not only closely correlated with the degree of tubular necrosis (in particular mTALs, as has already been shown before [18]), but also with the amount of chronic tubulointerstitial changes (table 3). Moreover, multiple regression analysis revealed that both the extent of mTAL necrosis and chronic tubulointerstitial changes independently predicted the degree of evolving renal dysfunction (table 4). Noticeably, the acute decline in GFR was predicted by the chronic tubulointerstitial changes, rather than ATN, while the later injury principally predicted the development of acute tubular dysfunction. Placing these findings into patient context, one would conclude that acute hypoxic renal injury is not necessarily augmented by a substrate of chronic renal disease, and that both acute and chronic changes present are independent variables for the degree of clinical renal failure. In other words, in patients with preexisting renal disease, it

is currently impossible to predict the extent of acute tubular structural damage in the setup of ARF, based on clinical assessment. Moreover, the predilection of patients with chronic renal failure to develop ARF, for instance following radiologic contrast agents, does not necessarily imply a more extensive acute tubular damage. Declining GFR under these settings is related to a greater extent to the underlying chronic renal parenchymal disease.

The preserved baseline GFR and these regression analysis findings suggest that adaptive processes may take place at the glomerular level in rats with chronic renal disease to maintain GFR, such as a reset of glomerular hemodynamics or of the filtration coefficient. Turning off of these adaptive processes may explain the predisposition of preexisting renal disease to the acute decline in kidney function.

In summary, though chronic tubulointerstitial injury aggravates medullary hypoxia, it does not predispose to hypoxic ATN. HIF-mediated medullary adaptive responses and upregulation of regional ET_B receptors might participate in this unexpected tolerance to acute hypoxic stress. Stable chronic tubulointerstitial disease may be well compensated and thus underdiagnosed by standard functional studies of glomerular filtration. Functional deterioration related to ATN in these settings may reflect both the disintegration of adaptive compensatory mechanisms and factual acute tubular injury. New methods and technologies are, therefore, critically required to correctly assess the true extent of tubular damage in ATN in order to cleverly choose appropriate therapeutic strategies.

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