Moving Points in Nephrology

Renal Parenchymal Hypoxia, Hypoxia Adaptation, and the Pathogenesis of Radiocontrast Nephropathy

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Background and objectives: Renal parenchymal Po_2 declines after the administration of iodinated radiocontrast agents, reaching critically low levels of approximately 10 mmHg in medullary structures.

Design, setting, participants, & measurements: In this review, the causes of renal parenchymal hypoxia and its potential role in the pathogenesis of contrast nephropathy are appraised.

Results: Commonly associated predisposing factors are associated with a propensity to enhance renal hypoxia. Indeed, animal models of radiocontrast nephropathy require the induction of such predisposing factors, mimicking clinical scenarios that lead to contrast nephropathy in high-risk individuals. In these models, in association with medullary hypoxic damage, a transient local cellular hypoxia response is noted, initiated at least in part by hypoxia-inducible factors. Some predisposing conditions that are distinguished by chronically aggravated medullary hypoxia, such as tubulointerstitial disease and diabetes, are characterized by *a priori* upregulation of hypoxia-inducible factors, which seems to confer tolerance against radiocontrast-related hypoxic tubular damage. Renal dysfunction under such circumstances likely reflects to some extent altered intrarenal hemodynamics, rather than acute tubular injury.

Conclusions: Real-time, noninvasive novel methods may help to differentiate between evolving tubular damage and altered hemodynamics and in the design of appropriate preventive interventions.

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The pathophysiology of radiocontrast-induced nephropathy (CIN) likely consists of combined hypoxic and toxic renal tubular damage, associated with renal endothelial dysfunction and altered intrarenal microcirculation. A primary alteration of renal hemodynamics without tubular damage has been suggested, reflected by a decline in glomerular filtration with preserved tubular sodium reabsorption (1). Direct tubular toxicity, mediated principally by oxygen free radicals has also been proposed, principally based on studies *in vitro* (2–4). *In vivo*, administration of radiocontrast agents resulted in proximal tubular vacuolar changes and brush border simplification (flattening/loss of microvilli) (5,6). Nevertheless, these tubular anatomic findings seem to represent exposure to radiocontrast rather than true indicators of CIN and lack correlation with kidney dysfunction (7). Moreover, electron microscopy, at least in animal models, revealed that the "vacuolar" changes were in fact basolateral membrane outpouchings with otherwise intact cellular structures (8). A more likely pathogenic mechanism for radiocontrast-induced kidney dysfunction is hypoxic renal medullary injury, based on clear-cut evidence for aggravation of physiologic medullary hypoxia after radiocontrast administration.

Physiologic Medullary Hypoxia, Aggravated by Radiocontrast Agents

As reviewed elsewhere (9), the mammalian renal medulla functions normally at ambient Po_2 as low as 30 mmHg, reflecting limited regional oxygen supply, hardly matching high local oxygen consumption for tubular reabsorption. Compound mechanisms, including prostaglandins, nitric oxide (NO), and adenosine, continuously adjust medullary tubular transport activity to the limited available oxygen supply, acting by both enhancement of regional blood flow and downregulation of distal tubular transport, particularly in medullary thick ascending limbs (mTAL). Their overall effect helps in maintaining medullary oxygen sufficiency, whereas acute tubular necrosis (ATN) often reflects defects in one or more of these protective mechanisms. Reduced cortical blood flow and GFR and enhanced proximal tubular reabsorption, as happens with prerenal azotemia, also serve to maintain medullary oxygen balance by decreasing distal tubular reabsorptive activity and oxygen consumption (9).

The administration of radiocontrast agents markedly affects

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renal parenchymal oxygenation (Figure 1). Using oxygen microelectrodes, Brezis and colleagues (7) found that after the injection of the high-osmolar ionic agent sodium iothalamate, cortical Po_2 declined from 40 to 25 mmHg. In the already hypoxic outer medulla, Po_2 fell from 26 mmHg at baseline to mean levels as low as 9 mmHg. Liss *et al.* (10) reported a comparable decline of medullary Po_2 from approximately 30 to 15 mmHg with the administration of ionic and nonionic low-osmolar radiocontrast agents, as well as iso-osmolar dyes. The intensification of medullary hypoxia by contrast agents has also been documented noninvasively in rats (11) and humans (12), using blood oxygen–level-dependent magnetic resonance imaging (MRI), detecting increased unsaturated hemoglobin concentration within the renal medulla over extended periods of time.

Radiocontrast administration induces a host of systemic effects that may compromise renal tissue oxygenation, including pulmonary ventilation-perfusion mismatch, reduced cardiac output and renal perfusion pressure, altered rheologic properties of the blood, and increased oxygen-hemoglobin association (12). However, the greater part of the decline in renal parenchymal oxygenation can be attributed to altered intrarenal balance of oxygen supply and demand, summarized in Figure 2 and as discussed in the sections that follow.

Radiocontrast Agents Increase Renal Oxygen Demand

Radiocontrast injection leads to an abrupt but transient increase in renal plasma flow, glomerular filtration, and urinary output (13). This response, which resembles the effect of mannitol, is mediated at least in part by an increase in plasma volume and the release of natriuretic peptides (14–16). Natriuresis and diuresis may also be related to dye-induced endothelin release (14), mediated through ET_B endothelin receptors (17). All of these factors, in addition to the substantial osmotic load provided by hyperosmolar radiocontrast agents, enhance solute delivery to the distal nephron and lead to increased oxygen consumption caused by enhanced tubular sodium reabsorption. The decline in outer medullary Po_2, despite enhanced regional blood flow (outlined next), only emphasizes the important role for increased reabsorptive activity in the consequent regional hypoxia. Indeed, as shown in Figure 1, the inhibition of transport activity with the loop diuretic furosemide abruptly reverses radiocontrast-induced medullary hypoxemia (7,18). Furosemide-related attenuation of medullary hypoxemia takes place despite the profound regional vasoconstriction induced by this agent (19), again emphasizing the crucial role of regulated tubular transport activity in the determination of medullary oxygenation.

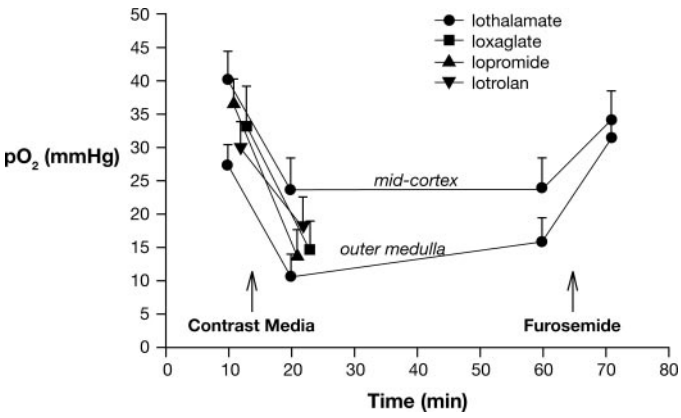

Figure 1. Renal parenchymal oxygenation after the administration of radiocontrast agents in rats. Outer medullary Po_2 markedly declines after administration of the high-osmolar agent iothalamate, the low-osmolar ionic (ioxaglate), and the nonionic (iopromide) dyes, as well as the iso-osmolar agent iotrolan. Note the protracted effect of the dye, the reversal of medullary hypoxia by furosemide, and the comparable, although less pronounced, effect of iothalamate on cortical oxygenation. All measurements represent parenchymal Po_2 determined in the outer medulla. For iothalamate, cortical measurements are shown as well (7,10).

Radiocontrast-Induced Decrease in Renal Oxygen Supply

The decline in renal parenchymal oxygenation may also reflect dye-induced altered renal regional microcirculation. Indeed, after a brief transient increase in renal blood flow after the administration of radiocontrast, a prolonged decline of 10 to 25% below baseline is noted (20). The fall in medullary oxygenation cannot be attributed to this modest decline in total renal blood flow *per se*, because the latter predominantly reflects changes in cortical flow, whereas medullary flow, representing only 10% of renal blood flow, is usually preserved (21). As outlined already, under such circumstances, medullary oxygenation is expected to improve as a result of diminished GFR and distal tubular transport (21).

The possibility that dye-related medullary hypoxia is a reflection of altered medullary microvasculature has been explored by direct determination of the local microcirculation. Nygren *et al.* (22) and Liss *et al.* (23), using laser Doppler probes, found that ionic high-osmolar as well as nonionic low-osmolar radiologic contrast media markedly reduced inner medullary papillary blood flow. They also documented near cessation of red blood cell movement in papillary blood vessels, associated with red cell aggregation within papillary vasa recta, using video microscopy (23).

This group of researchers (24) noted a similar decline in outer medullary regional blood flow, using both laser Doppler needle probes and hydrogen washout techniques; however, at very high volumes of injected radiocontrast, outer medullary regional microcirculation markedly increased (25–27), as long as NO or prostaglandin synthesis remained intact (25). Thus, so far, the impact of radiocontrast on outer medullary perfusion remains a matter of debate, with the different patterns of response possibly related to the type, volume, and route of administration of the dye.

Altogether, these findings indicate that dye-induced accentuation of inner medullary hypoxia is principally mediated by a decline in regional microcirculatory blood flow and oxygen supply. By contrast, intensification of outer medullary hypoxia

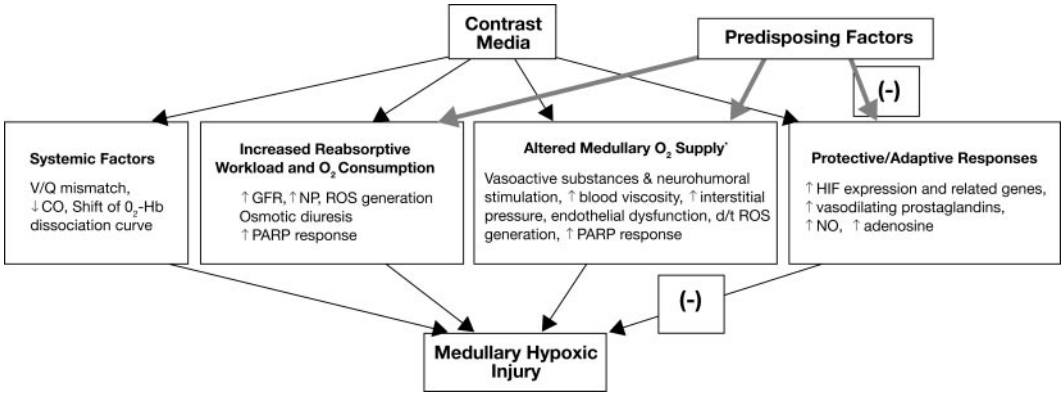

Figure 2. Intrarenal mechanisms that lead to radiocontrast-associated decline in medullary oxygenation. Some factors intensify medullary hypoxia through enhanced tubular transport and oxygen consumption, whereas others alter medullary regional blood flow. The latter group consists of both vasoactive mediators and mechanical factors (increased blood and urine viscosity and interstitial pressure). Concomitant activation of HIF-mediated hypoxia adaptation takes place, conferring hypoxia tolerance. Furthermore, the release of nitric oxide and vasodilating prostaglandins, as well as the accumulation of adenosine, help to maintain medullary oxygenation, both by the enhancement of medullary blood flow and by the downregulation of tubular transport. Factors that predispose to radiocontrast nephropathy are characterized by altered protective mechanisms, by enhanced tubular transport, or by distorted regional oxygen supply. *Happens in the inner medulla; might occur in outer medulla in the presence of altered protective mechanisms. CO, cardiac output; ET, endothelin; GFR, glomerular filtration rate; Hb, hemoglobin; HIF, hypoxia-inducible factors; NO, nitric oxide; NP, natriuretic peptides; PARP, poly-(ADP-ribose) polymerase; ROS, reactive oxygen species.

may represent reduced regional microcirculatory blood flow, as well as enhanced oxygen consumption, that may not be fully compensated even during increased regional oxygen delivery. The cause for the disparate and dosage-related papillary (inner medullary) and outer medullary microcirculatory response to dye is unknown, but it may reflect structural and functional differences in regional vasculature, diverse distribution of vasoactive mediators or their receptors, corticomedullary redistribution of blood flow at very high dosages of the dye used in some of these experiments, or artificial effects related to the technical procedures used.

Numerous neurohumoral mediators may contribute to the changes in renal microcirculation caused by radiocontrast injection. Plasma levels of atrial natriuretic peptide (14) and, seemingly, of other related molecules rapidly rise within 5 min after the injection of the contrast material, in parallel with the abrupt transient rise in renal blood flow and diuresis. Intrarenal NO synthase activity and NO concentration are also altered after contrast administration (26), and plasma endothelin increases (14). Other prominent mediators that are believed to participate in the renal hemodynamic response to dye are adenosine (13,28), prostaglandins (29), and vasopressin (30). Indeed, the renal hemodynamic pattern of cortical vasoconstriction, coupled with preserved or even augmented medullary blood flow, resembles the renal microvascular response to the administration of some of these mediators, such as adenosine, endothelin, or angiotensin II (31–33). The disparate distribution of receptor subtypes of these mediators in the renal cortex and medulla may be responsible for the different regional hemodynamic responses. Potential additional participants in the renal hemodynamic response to dye are serotonin, bradykinin, leukotrienes, histamine, catecholamines, and the sympathetic nervous system.

Mechanical factors may also adversely affect the renal microcirculation after dye administration. Radiocontrast-induced increased blood viscosity (34), in part related to red blood cell aggregation (35), may markedly alter flow within the low-pressure complex medullary microcirculation. Noteworthy, the achievement of iso-osmolality of third-generation dyes comes at the price of a substantially increased viscosity (34,36). In addition, the early enhanced diuresis after contrast administration is associated with increased renal parenchymal mass (swelling), presumably as a result of tubular luminal expansion and increased interstitial volume (37). Tubular intraluminal urine viscosity may increase as well, particularly at the distal nephron, after the reabsorption of most of the filtered fluids. It is conceivable that these changes might augment renal interstitial pressure, leading to compression of the vasa recta and peritubular capillaries, with subsequent compromised regional oxygenation.

In summary, radiocontrast dyes markedly affect the renal microcirculation in a way that particularly compromises medullary oxygen supply. The individual importance of the various vasomotor mechanisms and physical factors involved remains speculative.

Hypoxic-Toxic Interactions in CIN

Evidence for direct tubular damage as an important mechanism of dye nephrotoxicity relies mainly on cell culture and isolated tubular segment studies, providing biochemical evidence for tubular membrane oxidative damage (2,4). More important, dye-induced critical medullary hypoxia may lead to the forma-

tion of reactive oxygen species (ROS) with subsequent membrane injury and DNA damage. Indeed, studies *in vitro* underscore a role for ROS in radiocontrast nephropathy (2–4). Initial hypoxic stress caused by radiocontrast dyes may thus trigger the formation of ROS. Furthermore, oxygen free radicals increase tubular transport activity and oxygen consumption (38), induce endothelial dysfunction, and interfere with the generation of hypoxia adaptive response (39). Thus, a vicious circle of hypoxia, free radical formation, and further hypoxic injury may be activated after radiocontrast exposure. Activation of high-energy-consuming reparative processes such as poly-(ADP-ribose) polymerase (PARP) may further aggravate intracellular energy store depletion and subsequent endothelial dysfunction, further augmenting regional hypoxic injury (40).

Risk Factors for CIN Predispose to Medullary Oxygen Insufficiency

The incidence of CIN among patients without known risk factors is considered to be negligible, underscoring the importance of those multiple mechanisms, outlined previously, that maintain medullary oxygen sufficiency by adjusting local transport activity to the limited available oxygen supply (9). In rats, the inhibition of prostaglandin or NO synthesis was found to blunt the increase in outer medullary blood flow induced by radiocontrast and to aggravate regional hypoxia (7,25). Similarly, as reviewed elsewhere (41), altered protective mechanisms bring about the susceptibility to develop CIN in high-risk patients. Indeed, as demonstrated in Figure 2 and in Table 1, many known predisposing risk factors, such as preexisting renal dysfunction, diabetes, and congestive heart failure, are characterized by compromised medullary oxygen sufficiency. The last is likely related to defective nitrovasodilation or prostaglandin synthesis, to increased reabsorptive workload or enhanced systemic vasoconstrictive stimuli, or to structural changes of the renal microcirculation (41).

For example, diabetes may lead to enhanced distal tubular reabsorption as a result of increased GFR, osmotic diuresis, and tubular hypertrophy. At the same time, nitrovasodilation is characteristically altered. Not surprising, basal outer medullary Po_2 is significantly reduced in diabetic animals (42), associated with enhanced expression of hypoxia-inducible factors (HIF) (43) (see next section). Altered nitrovasodilation is also present in the aged, among those with hypertension, and in patients with hypercholesterolemia or atherosclerosis. Certainly, it is widely known that the administration of nonsteroidal anti-inflammatory drugs, which block prostaglandin synthesis, may also predispose to CIN. Preexisting renal disease is associated with hypertrophy of remnant nephrons and with structurally altered medullary microcirculation. Anemia predisposes to CIN (44), plausibly as a result of reduced oxygen delivery, whereas heart failure, cirrhosis, nephrotic syndrome, and dehydration all are risk factors characterized by effective volume depletion and increased neurohumoral vasoconstrictive stimuli that might compromise medullary oxygenation.

Table 1. Factors that predispose to contrast nephropathy[a]

Defective protective mechanisms
altered nitrovasodilation: diabetes, hypertension, aging, hyperlipidemia, atherosclerosis
altered renal prostaglandin synthesis: aging, NSAID
Increased reabsorptive workload
diabetes, chronic renal disease
Enhanced systemic vasoconstrictive stimuli
volume depletion, cirrhosis, nephrosis
low CO, acute MI
Structural changes of the renal microvasculature
chronic renal disease
Reduced oxygen delivery
anemia, low CO, volume depletion

[a]MI, myocardial infarction; NSAID, nonsteroidal anti-inflammatory drugs.

Experimental Models of CIN: The Concept of Medullary Hypoxic Damage

In rat kidneys, perfused *ex vivo* with cell-free oxygenated medium, contrast agents hasten the usual decline in kidney function and extend hypoxic tubular damage, which primarily affect mTAL and S3 segments in the outer medulla (8). *In vivo*, resembling healthy humans, experimental laboratory animals that are subjected to contrast media do not develop CIN. As detailed next, the dye-associated decline in medullary Po_2 invokes adaptive cellular hypoxic stress response, initiated by posttranscriptional medullary accumulation of HIF, with preservation of renal integrity and function (45); however, as detailed elsewhere (46), the introduction of other insults that parallel predisposing clinical conditions leads to tubular damage in experimental models of CIN. Resembling CIN in clinical practice, these clinically relevant conditions that are found to predispose to experimental CIN are characterized by systemic vasoconstrictive stimuli, enhanced oxygen requirements, altered protective mechanisms, generation of endothelial dysfunction, and enhancement of interstitial hydraulic pressure (46). With dye administration under these preconditions, apoptotic and necrotic damage rapidly develop (7,8,25,45,47,48), predominantly affecting mTAL and, to a lesser extent, S_3 segments in the outer medulla and medullary rays. Papillary tip necrosis may develop as well (47). Furthermore, in such experimental models, uncontrolled activation of energy-consuming reparative systems, such as PARP, or the formation of ROS may lead to endothelial dysfunction, which may further aggravate regional oxygen insufficiency.

Indeed, the severity of renal dysfunction is proportional to the number of the applied co-perturbations, resembling clinical observations. Renal dysfunction correlates with the extent of ATN, identified in the more severe protocols, but less so with the more moderate models that cause limited tubular damage, as probably happens in general in the human scenario of "ATN," in which the degree of glomerular filtration dysfunction does not correlate with the extent of focal tubular injury, underscoring a potential role for ensuing altered glomerular hemodynamics as the basis for the decline in kidney function

(46). In such circumstances, novel technologies such as sodium MRI for the early detection of evolving altered medullary countercurrent mechanism (49) or the use of potentially noninvasive real-time molecular imaging of necrotic/apoptotic injury (50) might help to differentiate between evolving true ATN and renal dysfunction reflecting altered glomerular hemodynamics.

Hypoxia Stress Response and Hypoxia Adaptation

Accentuated renal parenchymal hypoxia leads to hypoxia stress response and adaptation, at least in part regulated by HIF. HIF are heterodimers of a constitutive β subunit and one of at least two alternative α subunits. HIF-α is regulated by oxygen-dependent proteolysis by so-called HIF prolyl hydroxylases (51). These key enzymes, regulating HIF-α degradation, are oxygen dependent and can be considered cellular oxygen sensors because their activity varies in the range of physiologic and pathologic oxygen tensions. Whereas under normal tissue oxygenation α subunits are constantly degradated, proteolysis is inhibited when cellular oxygen content declines. Consequently, α subunits accumulate, bind with β subunits, translocate into the nucleus, and activate a host of target genes that govern a wide spectrum of biologic processes (*e.g.*, metabolism, vascular tone, erythropoiesis, angiogenesis, cell cycle, scavenging of free radicals, inflammation, immune response, tumor growth). Upregulation of HIF-mediated erythropoietin or heme oxygenase-1 (HO-1) have proved to be cell protective in experimental renal injury; however, the role of the HIF system *in vivo* and its possible impact on renal disease are still not completely understood. So far, we know that renal HIF-α subtype expression is cell specific, with the 1α isoform expressed in tubular cells, whereas HIF-2α appears in vascular endothelial and interstitial cells. We have also learned that tubular segments vary in their ability to mount an HIF response (predominantly medullary collecting ducts and, to a much lesser extent, mTAL), and that whereas moderate decline in ambient Po_2 triggers HIF, critically low Po_2 turns off hypoxia adaptation, presumably favoring p53-mediated activation of programmed cell death (52,53).

The administration of radiocontrast causes accumulation of HIF isoforms in the outer and inner medulla (but not in the cortex), with spatial distribution pattern identical to that of severe hypoxia, as defined by pimonidazole adducts (45). Radiocontrast-induced HIF expression is further intensified in animals with concomitant inhibition of prostaglandin and NO synthesis. HIF immunostaining is maximal 2 h after the administration of the radiocontrast and disappears during the subsequent 2 to 6 h, when HIF-dependent proteins, such as HO-1, begin to appear (45). It is interesting that furosemide intensifies HIF response, presumably by the attenuation of hypoxic stress into a "window of opportunity" of moderate cellular hypoxia that favors maximal HIF expression over p53 upregulation (45,52). Thus, *acute* hypoxia, induced by radiocontrast, causes HIF accumulation, presumably reflecting sublethal cellular stress and hypoxia adaptation.

Chronic medullary hypoxia, noted in chronic tubulointerstitial disease (54) and in experimental diabetes (42,43), leads to persistent HIF accumulation, presumably associated with protracted adaptive gene response. In such models, despite a more prominent reduction of GFR after the injection of the radiocontrast, the extent of ATN was not intensified as compared with control animals that were subjected to similar radiocontrast-mediated hypoxic insults (43,54). Conceivably, chronic HIF-mediated hypoxia adaptation provides acute hypoxia tolerance. Importantly, in an acute-on-chronic renal failure model of CIN, declining GFR was mostly predicted by the extent of chronic tubulointerstitial disease, whereas medullary hypoxic tubular injury could be correlated with evolving tubular dysfunction, such as defects in urine concentrating capacity and sodium transport, assays not routinely used in clinical practice (54).

In the perspective of these unexpected findings of resistance to radiocontrast-induced hypoxic tubular cell damage, particularly in the two most established predisposing conditions to CIN, we propose the following hypothesis (Figure 3): In intact individuals without hypoxia adaptation, acute hypoxia produced by contrast agents may lead to ATN and renal dysfunction (right arm). By contrast, under conditions characterized by chronic hypoxia and hypoxia adaptation, renal dysfunction after radiocontrast may predominantly reflect a transient, reversible injury or disintegration of adaptive changes in glomerular hemodynamics that maintain GFR (left arm). This may explain the low sodium excretion occasionally noted in such patients. Conceivably, these two situations coexist in some patients, and plausibly the degree of acute hypoxia is the major determinant regarding the potential to develop tubular injury. Unfortunately, we cannot say which arm is activated in a particular patient with declining kidney function after radiocontrast. We clearly need noninvasive, real-time technologies, such as the sodium MRI, molecular imaging technologies, or reliable urinary biomarkers of tubular cell injury, to differentiate between evolving true tubular hypoxic damage and altered renal hemodynamics. Such a distinction is essential for tailoring timely and appropriate interventions.

Protective and Adaptive Responses to Preserve Medullary Oxygenation

As reviewed elsewhere (41), proper hydration is clearly renoprotective, perhaps through attenuation of renal vasoconstrictive stimuli (with improved renal oxygen supply), downregulation of tubular transport, and reduced concentration of tubular intraluminal dye concentration. Importantly, this last effect might reduce blood and urine viscosity, with subsequent improved microcirculation. Additional protective effects of urine alkalization, either by the use of bicarbonate infusion or the administration of acetazolamide, might be related to improved renal oxygenation through the attenuation of free radical formation. Neutralizing ROS remains a plausible interventional approach (55), because it may improve medullary oxygenation and the ability to mount appropriate HIF response (43). It is obviously important to avoid concomitant use of other nephrotoxins, especially those with potential deleterious effects on renal oxygenation, such as nonsteroidal anti-inflammatory agents (41).

Currently, we basically interpret declines in GFR as a marker

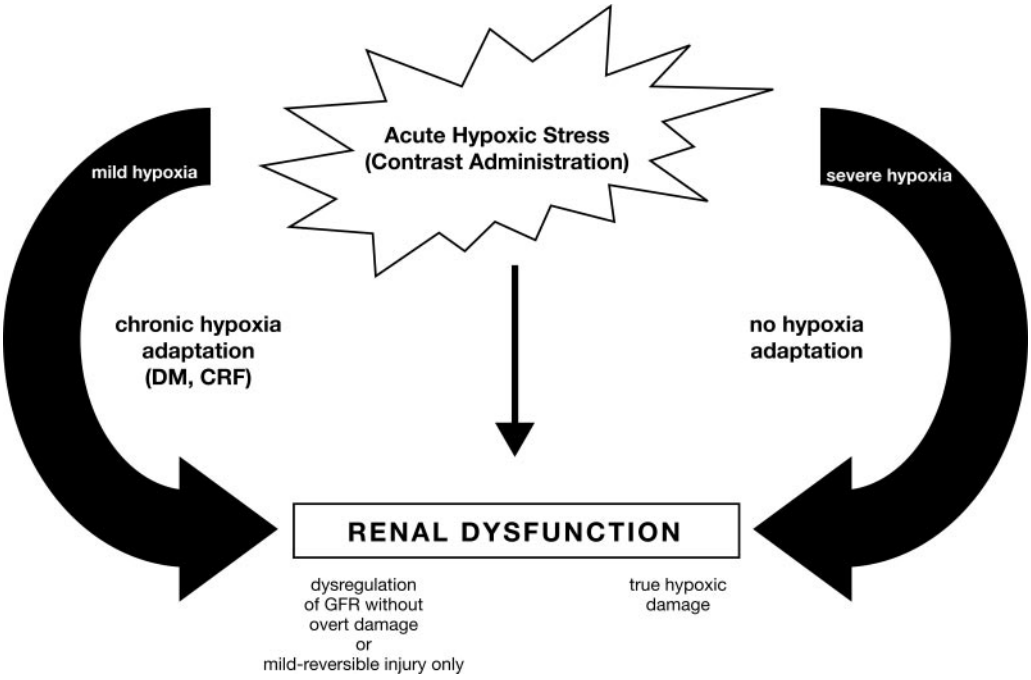

Figure 3. Hypothesis: The combined effects of acute hypoxia and HIF-mediated chronic hypoxia adaptation in the pathogenesis of radiocontrast-induced renal dysfunction. In patients without preexisting renal hypoxic stress and HIF-mediated hypoxia adaptation (right arm), acute radiocontrast-induced hypoxia may result in hypoxic tubular damage and renal dysfunction. By contrast, HIF-mediated adaptive processes occur in clinical conditions characterized by chronic hypoxia, such as diabetes or chronic tubulointerstitial disease (left arm). Conceivably, under such settings, acute dye-induced hypoxic tubular injury is prevented or attenuated, and renal dysfunction reflects dysregulation of compensatory mechanisms that preserve glomerular filtration (54). The extent and severity of acute tubular damage also largely depends on the severity of the dye-induced acute hypoxic stress (in part related to the presence or absence of predisposing factors). The more pronounced renal dysfunction (reduced GFR) consistently noted in patients with preexisting renal failure or diabetes might represent dysregulated adaptive mechanisms designed to maintain GFR, rather than a more pronounced true tubular damage.

of CIN, whereas in fact such changes might reflect activation of protective mechanisms designed to prevent medullary hypoxic injury. This has led to what might be considered conceptually illogical therapeutic interventions, designed primarily to enhance GFR, which might intensify medullary hypoxia. Indeed, dopamine and atrial natriuretic peptide prophylaxis both have failed to prevent CIN and in patients with diabetes might have increased the risk for renal dysfunction (56,57). Conceivably, these agents augment medullary oxygen debt by increasing GFR and consequent tubular reabsorptive workload, overcoming adenosine-mediated adaptive downregulated GFR (28). Mannitol prophylaxis has failed as well (58), plausibly because it intensifies medullary hypoxia as a result of osmotic diuresis and enhanced GFR, leading to increased distal tubular reabsorption and oxygen consumption (18,19). This might be the reason for the clinical failure, so far, of other new strategies, such as fenoldopam, implemented to augment GFR after radiocontrast administration. Improving GFR by the nonselective inhibition of adenosine receptors with theophylline might also adversely affect medullary oxygenation, both by increasing GFR (by blocking A-1 adenosine receptors) and by reducing medullary blood flow (A-2 adenosine receptor blockade) (59).

Probably, for similar reasons, the use of nonselective endothelin receptor blockers has failed and, in fact, was found to affect adversely the clinical outcome (60). In the perspective of the important role of endothelin ET-B receptors in maintaining medullary blood flow (32), the use of selective ET-A receptor antagonists seems more reasonable (61). Indeed, such treatment was reported to attenuate radiocontrast-induced medullary hypoxia (62) and renal dysfunction (63) in experimental settings.

Amelioration of altered medullary nitrovasodilation is another consideration in the prevention of CIN. In fact, L-arginine improves radiocontrast-induced altered renal hemodynamics and renal dysfunction in hypercholesterolemic rats (64). Recent experimental data suggest that the renoprotective effect of acetylcysteine may be related to improved nitrovasodilation and medullary oxygenation (65), in addition to the effect of scavenging free radicals. Inhibition of PARP activation in response to hypoxic stress may improve cellular energy depletion, attenuate endothelial dysfunction, and protect against medullary hypoxic injury (66). In the same manner, reduction of ROS generation or their scavenging might improve medullary oxygenation (38) and HIF upregulation (43) and may be beneficial in the attenuation of CIN. Zager *et al.* (4) found no effect of antioxidant or pro-oxidant interventions on lipid peroxidation in isolated proximal tubules, incubated with contrast medium. Nevertheless, Goodman *et al.* (67) recently reported that the

induction of HO-1 attenuated experimental radiocontrast nephropathy, underscoring a potential role for antioxidants in the prevention of contrast nephropathy.

The loop diuretic furosemide, inhibiting reabsorptive activity (19), was found to reverse medullary hypoxia that was induced by radiocontrast (7) and to prevent hypoxic outer medullary damage in a rat model of experimental CIN (68). Liss *et al.* (69) reported that pretreatment with furosemide did not prevent the dye-induced decline in medullary Po_2, but because baseline oxygen tension was higher, the decline in ambient Po_2 did not reach the critically low levels noted without furosemide. In clinical studies, however, furosemide treatment yielded a harmful outcome: Whereas kidney function was maintained with fluid regimen in patients who had preexisting renal dysfunction and underwent contrast studies, it significantly deteriorated with the addition of furosemide (58). This unexpected observation does not necessarily weaken the case for hypoxic insult, because it may be related, at least in part, to ensuing negative fluid balance and prerenal azotemia (70). In addition, as shown with blood oxygen–level-dependent MRI, loop diuretics may not be effective in ameliorating dye-induced medullary hypoxia among aged patients (71). Thus, as suggested from the Prevention of Radiocontrast Induced Nephropathy Clinical Evaluation (PRINCE) trial (72), studies with furosemide should be repeated with a more adequate fluid replacement to compensate for ensuing negative water balance.

Because increased reabsorptive workload and interstitial hydraulic pressure possibly contribute to dye-induced altered medullary microcirculation and oxygenation, we believe that vascular modulation by a selective medullary vasodilator, for instance an ET-B agonist, may be insufficient to prevent CIN. Therefore, a more global strategy may be tried, combining the inhibition of tubular transport together with fluid replacement and measures to restore medullary microcirculation.

Taken together, experimental and clinical data regarding therapeutic interventions only partially support the hypoxia theory outlined in this review. Nevertheless, substantial data call for a prudent preventive approach that takes into account maintaining renal parenchymal oxygenation during radiocontrast studies.

Conclusions

Medullary hypoxia plays a critical role in CIN, resulting from altered renal microcirculation and enhanced oxygen consumption for tubular reabsorption (Figure 2). The importance of intact homeostatic mechanisms in maintaining medullary oxygen balance and tubular integrity is illustrated in experimental models of CIN, mimicking the clinical situation of predisposing risk factors. Radiocontrast-induced renal medullary hypoxia invokes a spectrum of cellular regional response, ranging from hypoxia adaptation and reversible damage through apoptotic to necrotic cell death, depending on certain predisposing conditions and the extent of hypoxic stress. Chronic hypoxia and hypoxia adaptation may provide tolerance to dye-induced hypoxic tubular injury. Under such circumstances, evolving renal dysfunction may predominantly reflect altered renal hemodynamics rather than true tubular damage. New technologies that may noninvasively detect in real time the ensuing critical renal parenchymal hypoxia, tubular dysfunction, and evolving tubular cell injury may enable us to assess the relative impact of these components that lead to CIN and allow us to tailor appropriate therapeutic interventions.

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