Pathophysiologic Features and Prevention of Human and Experimental Acute Tubular Necrosis

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Abstract. Acute renal failure (ARF) remains a common and potentially devastating disorder that affects as many as 5% of all hospitalized patients, with a higher prevalence in patients in critical care units. The focus of this article is on categorizing recent pathophysiologic and clinically relevant developments in the field. The vascular and tubular factors in the pathogenesis of ARF, together with the potential mechanisms of recovery and repair of the injured kidney, are discussed. A number of experimental and clinical interventions to prevent ARF are summarized. Although the clinical treatment of these patients is still largely supportive and many recent clinical trials showed rather negative results, it is hoped that basic research will provide therapeutic tools to improve the grim prognosis of this disease in the future.

Acute renal failure (ARF) is defined as rapid deterioration of renal function associated with the accumulation of nitrogenous wastes in the body. Clinically, three major classes of ARF are distinguished, i.e., pre- and postrenal ARF and ARF attributable to renal or intrinsic causes. Intrinsic ARF that is not the result of primary vascular, glomerular, or interstitial disorders (approximately 15% of all cases) has been referred to as acute tubular necrosis (ATN).

ATN is caused by ischemic (50%) or nephrotoxic (35%) injury to the kidney. However, in 50% of the cases of hospital-acquired ARF, the cause is multifactorial.

The incidence of ATN is particularly high among patients admitted to an intensive care unit (ICU). Unfortunately, neither this incidence nor the morbidity and mortality rates associated with ATN have decreased, despite ongoing improvement in the supportive care of these patients and the advent of intermittent and continuous renal replacement therapy (1–4). One of the reasons for this lack of improvement is a change in the severity of the underlying diseases causing ARF.

The spectrum of ATN in the ICU, compared with that observed in other settings, is indeed different, with more patients developing ATN predominantly as part of a multiple organ dysfunction syndrome; isolated ARF is the usual presentation in non-ICU settings (5).

The pathophysiologic abnormalities in ARF include intrarenal hemodynamic changes and ischemic and toxic injuries to tubular cells (Figure 1). The interplay of these abnormalities forms the basis for the acute decrease in GFR, which is the result of intrarenal vasoconstriction, with a decrease in glomerular filtration pressure, tubular obstruction, transtubular back-leakage of the filtrate, and interstitial inflammation.

Experimental work has indicated important contributions of both the vascular and tubular factors, but clinical studies have failed to quantitate their relative importance.

In this article, the pathophysiologic features of ischemic ARF are briefly reviewed, with emphasis on more recent hemodynamic and molecular biologic findings. Where possible, we indicate the clinical implications of the experimental results, and we try to summarize the most recent experimental and clinical efforts to prevent and/or treat ATN.

It should be realized that most of the mechanisms that are described have been derived from experimental models of ARF, which, although extremely useful, still show major limitations in their extrapolation into clinical disease states (6).

A variety of agents have been tested for their abilities to prevent or attenuate injury or hasten recovery in ischemic and toxic ATN. Some of these agents have quite often been effective in altering the course of experimental models of ATN; however, none of them has consistently been shown to be of benefit in clinical ATN.

Table 1 summarizes the therapeutic approaches that have most frequently been used in several animal models of ATN. Some of these drugs have undergone clinical trials or are in one of several stages of clinical development but are not yet widely used.

We (7,8) and others (9,10) have extensively discussed these issues.

The classification of the drugs discussed in this review is based on their principal actions on the different pathophysiologic mechanisms. This classification is largely arbitrary, because many drugs simultaneously act by several mechanisms.

In the first part of this review, substances acting mainly on the hemodynamic factors are discussed. The second part focuses on drugs acting mainly on the tubular factors.

Vascular Aspects of ARF

Intrarenal Vasoconstriction

Research on the vascular aspects of animal models of ATN has focused on the role of intrarenal vasoconstriction or en-
hanced intrinsic vascular tone, impairment of renal autoregulation (making the kidney more vulnerable to recurrent ischemic injury), and more-selective reduction in blood flow in the outer medulla.

Many studies have demonstrated increased sensitivity of the renal vasculature to vasoconstrictor stimuli, including angiotensin II, endothelin (ET), and serotonin, and increased sensitivity to renal nerve stimulation (11). Chief among these vasoconstrictors is ET. ET, a 21-amino acid peptide, is the most potent vasoconstrictor discovered to date. The ET-1 gene is upregulated during ischemia and reperfusion (12), and monoclonal antibodies to ET or ET receptor antagonists improve both renal function and renal histologic features in the setting of renal ischemia-reperfusion injury (13,14), as well as in a number of nephrotoxic conditions, such as rhabdomyolysis and the administration of cyclosporine or radiocontrast agents (15).

Our laboratory explored the role of serotonin in postischemic kidneys. Serotonin-induced vasoconstriction of the renal vascular bed was augmented after ischemia, and this constriction was blocked by ketanserin, a serotonin S2 receptor blocker (16).

The potential role of the renin-angiotensin system in ARF is still unresolved and was recently reviewed elsewhere (17).

**Impaired Autoregulation of Renal Blood Flow and GFR**

It is well known that, in clinical ARF, fresh histologic lesions in the kidney may be identified in renal biopsies, even up to several weeks after the onset of ARF. One possible explanation for this finding is decreased renal blood flow autoregulation, allowing small decreases in BP to provoke recurrent ischemic damage.

This loss of autoregulation has been directly demonstrated in animal models by us and others (18,19). Although some beneficial effects of calcium entry blockers on this hemodynamic alteration were observed by Conger (18), serotonin blockers resulted in almost complete restoration of renal blood flow autoregulation in a postsischemic model of ATN and in cyclosporine nephrotoxicity (16,19). These results suggest that serotonin plays a pivotal role in the suppression of renal blood flow autoregulation, by a serotonergic S2 receptor-mediated vasoconstrictor effect, in postischemic kidneys.

It is also well known that the outer medullary region exhibits high vulnerability to toxic substances and/or to hypoperfusion (20,21). In particular, the tubules in the outer stripe of the outer medulla receive a very sparse blood supply. It is therefore easy to imagine that medullary congestion and hypoxia not only would promptly result in activation of the tubuloglomerular feedback mechanism, and thus a decrease in GFR, but also would directly provoke damage in this area (22).

**Anti-ET Substances.** Several studies have demonstrated remarkable protection in experimental ischemic ATN in animal models with either the nonpeptide ET receptor antagonist SB209670 or the selective ET receptor antagonist BQ-123 (13,14,23).

A multicenter trial has studied the effect of intravenously administered SB209670, a mixed ET A and ET B receptor antagonist, in the prevention of radiocontrast agent-induced nephrotoxicity in patients with preexisting chronic renal failure (serum creatinine concentration, >2 mg/dl) (24). All patients received intravenous hydration, with 0.45% saline solution, before and after contrast agent administration. The anti-ET infusion was initiated at least 30 min before the administration of the radiocontrast agent. An exacerbation of radiocontrast agent-induced nephrotoxicity was observed, inasmuch as the mean increase in serum creatinine concentrations measured 48 h after angiography was greater for the patients who received the experimental drug, compared with the placebo-treated group. This negative effect of the ET receptor antagonist was apparent in both diabetic and nondiabetic patients. In
addition, adverse effects, especially hypotension, were more common in the SB209670-treated group.

**Low-Dose Dopamine Treatment.** For many years, it was accepted that the renal actions of dopamine were mediated by two different dopamine receptors, termed D1 and D2. However, recent findings have indicated that this two-receptor hypothesis should be modified; to date, two D1-like and three D2-like receptors have been identified by molecular cloning (25–27).

When infused in so-called renal doses (0.5 to 2 μg/kg body wt per min), dopamine primarily activates both of these dopaminergic receptors. At these low doses, dopamine increases renal plasma flow, GFR, and sodium excretion. At higher doses (2 to 5 μg/kg body wt per min), dopamine also binds to β-adrenergic receptors; at doses of >5 μg/kg body wt per min, α-adrenergic receptors become activated. However, there is overlap in receptor activation at each of these doses, as well as intraindividual variation in binding activities. It is therefore not always easy to determine the extent to which a particular effect is purely dopaminergic in origin or is attributable to combined dopaminergic and adrenergic activation. Some of the renal improvement, if any, produced by dopamine can thus be explained by its inotropic effects, such as increases in cardiac output and BP. These inotropic effects of dopamine and dobutamine have recently been summarized (28). In addition to its hemodynamic effects, there is now good evidence that dopamine inhibits tubular Na⁺/K⁺-ATPase activity.

Low doses of dopamine have been used and are still used to increase urine output, in an attempt to prevent ATN, among oliguric, critically ill patients. However, as noted in the extensive and excellent reviews by Denton et al. (27) and Burton and Tomson (29), the ability of dopamine to achieve these goals is poorly documented and largely anecdotal.

Juste et al. (30) recently investigated the appropriateness of the use of a low-dose renal dopamine regimen for critically ill, adult patients, by studying steady state dopamine clearances. Very large interindividual variations in plasma clearances were observed, and the authors concluded that the concept of a selectively renovascular, low-dose dopamine infusion is invalid for critically ill patients.

In previous, largely uncontrolled, studies, it was shown that patients who remain oliguric despite high doses of loop diuretics or hypertonic mannitol might begin to experience diuresis when dopamine is added (31–33).

Low doses of dopamine are able to reverse the acute changes in glomerular function caused by orally administered cyclosporine in healthy subjects and to improve renal function for patients with cancer who are treated with recombinant interleukin-2 (34). This form of immunotherapy is often associated with acute prerenal failure, with oliguria, hypotension, weight gain, and a vascular leak syndrome. One study reported dopamine protection against radiocontrast agent-induced nephrotoxicity in patients with preexisting renal impairment, but few diabetic patients were included (35). Among diabetic patients, a much higher incidence, rather than a lower incidence, of

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Note: iNOS, inducible nitric oxide synthase; PAF, platelet-activating factor; RGD peptides, peptides containing the arginine-glycine-aspartic acid motif; mAb, monoclonal antibodies; ICAM-1, intercellular adhesion molecule-1; α-MSH, α-melanocyte-stimulating hormone; ANP, atrial natriuretic peptide; ET, endothelin; IGF, insulin-like growth factor.
contrast agent-induced nephrotoxicity with dopamine was previously observed (36).

A prospective, randomized, controlled, single-blind trial was recently conducted, in which 98 participants were randomized to undergo forced diuresis with intravenously administered crystalloid solution, furosemide, mannitol, and low-dose dopamine treatments versus intravenously administered crystalloid solution and matching placebo treatments (37). That study suggested that forced diuresis with intravenously administered crystalloid solution, furosemide, and mannitol (if hemodynamic conditions permit), beginning at the start of angiography, provides a modest benefit against contrast agent-induced nephropathy, if a high urine flow rate can be achieved.

In a double-blind, randomized, controlled trial, the effectiveness of dopamine or furosemide in the prevention of renal impairment after cardiac surgery was recently evaluated (38). A total of 126 patients with preoperatively normal renal function who underwent elective cardiac surgery received a continuous infusion of either renal-dose dopamine (2 \( \mu g/\text{kg per min} \)), furosemide (0.5 \( \mu g/\text{kg per min} \)), or isotonic sodium chloride solution (as placebo), starting at the beginning of surgery and continuing for 48 h or until discharge from the ICU, whichever came first. The increase in plasma creatinine concentrations was twice as great in the furosemide-treated group, compared with the groups treated with dopamine or placebo (\( P < 0.01 \)). Acute renal injury (defined as an increase in serum creatinine concentrations of >0.5 mg/dl) occurred more frequently among the patients treated with furosemide (six of 41 patients), compared with the two other groups. Also, creatinine clearance values were lower for the patients treated with the loop diuretic. It was further demonstrated that the continuous infusion of dopamine for renal protection was ineffective and was not superior to the administration of isotonic saline solution in preventing postoperative dysfunction after cardiac surgery.

In summary, there is presently no clear experimental or clinical support, from well controlled studies, for a renal protective effect of dopamine. Furthermore, there are potential risks associated with even low-dose regimens. These include the induction of tachycardia, cardiac arrhythmias, myocardial ischemia, and possibly intestinal ischemia (attributable to pre-capillary vasoconstriction), which might promote bacterial translocation from the intestinal lumen into the systemic circulation (27).

A less well known effect of dopamine on hormone release from the adenohypophysis in critically ill patients has been carefully explored by Van den Berghe et al. (39). In a series of elegant clinical investigations (summarized in reference 39), those authors described the inhibitory effect of dopamine on the secretion of virtually all anterior pituitary-dependent hormones, except for cortisol. This pattern of hypopituitarism induced by chronic severe illness and exogenous dopamine administration is reminiscent of the hormonal profiles observed in experimental models of chronic stress, suggesting that endogenous dopamine may play a deleterious role in the endocrine and metabolic responses to critical illness.

**Atrial Natriuretic Peptide and Urodilatin.** In this article, the generic term atrial natriuretic peptide (ANP) is used to refer to the circulating peptide and its synthetic analogs. The synthesis, release, metabolism, and renal effects of ANP have been reviewed (40,41).

At the glomerular level, ANP seems to dilate afferent arterioles but constrict efferent arterioles. Therefore, the increase in GFR is quite selective and may occur independently of an increase in renal blood flow. Of further importance, ANP has been reported to inhibit almost all tested vasoconstrictors that affect glomerular blood flow. ANP increases the tubular urinary flow rate and decreases calcium influx into cells (41), which is relevant for the potential action of ANP in ATN.

Several natriuretic peptides, including urodilatin, that are likely to be produced by the kidney and to exhibit an equal or even greater natriuretic potency, compared with human ANP, have been discovered. In most of the animal studies in which administration of ANP afforded protection against ischemic or toxic renal injury, the drug was administered either directly into the renal artery or intravenously in high doses; the latter often result in marked systemic hypotension (42,43).

Nonhypotensive doses administered intravenously to rats did not improve postischemic renal function. High doses of intravenously administered ANP, in conjunction with dopamine to maintain a normal BP, administered either before or after the induction of renal ischemia were effective in ameliorating the decrease in GFR in animal studies (44,45).

Interestingly, combinations of urodilatin and dopamine (46) or of nonhypotensive doses of ANP with mannitol (47) were able to improve or almost completely restore the GFR in experimental ischemic ATN.

A first controlled clinical study in patients with established ATN showed that a combination of either intravenously or intrarenally infused human ANP with furosemide or mannitol significantly increased creatinine clearance by 8 h of ANP treatment (48). This effect persisted for 24 h after discontinuation of ANP administration. Although there was a reduced need for dialysis in the ANP-treated group, the mortality rates for the treated and control groups were not significantly different. A larger and more rigidly controlled clinical trial with a 24-h infusion of ANP, compared with placebo, was then performed in patients with established ischemic or nephrototoxic ATN (49).

Primary efficacy parameters were the incidence of dialysis (by day 14) and dialysis-free survival rates (at day 21) for the two treatment groups. Although ANP failed to produce a beneficial effect on either parameter for the general study population, a decrease in the incidence of dialysis and an increase in the dialysis-free survival rate were observed for the subgroup of oliguric patients with ARF.

The efficacy of intravenously administered ANP in preventing radiocontrast agent-induced nephropathy was carefully investigated in a prospective, randomized, double-blind, placebo-controlled trial in patients with stable chronic renal failure, with or without diabetes mellitus (50). There were no statistical differences in the incidences of radiocontrast agent-induced nephropathy. Although the patients with diabetes mellitus ex-
hhibited a significantly higher incidence of radiocontrast agent-induced nephropathy, there was no effect of ANP in the diabetic or nondiabetic groups. A previous study in which ANP, dopamine, and mannitol were compared with saline solution alone indicated that all three drugs actually increased the risk for contrast agent-induced nephropathy among diabetic patients (36). ANP was superior to saline solution alone among nondiabetic patients, for whom mannitol and dopamine proved equally beneficial.

The effect of urodilatin on the peak serum creatinine concentration and the course of serum creatinine levels in critically ill patients with ARF after major abdominal surgery and the necessity for renal replacement treatment were recently investigated (51). Although there was a tendency for lower peak serum creatinine values in the urodilatin-treated group, the difference did not reach statistical significance; in addition, the total numbers of hemodialysis episodes attributable to oliguria/anuria and/or hyperkalemia were the same for the two groups. An excellent summary of all studies performed with urodilatin in ARF was recently published (52).

Theophylline. Adenosine is a potent renal vasoconstrictor and has been thought to play a role in the initiation phase of ATN. Ischemic preconditioning and adenosine pretreatment protected renal function and improved renal morphologic features in rats subjected to renal ischemia (53). A1 adenosine receptor activation mimicked and A1 adenosine receptor antagonism blocked adenosine-induced protection. In contrast, A3 adenosine receptor activation before renal ischemia worsened renal ischemia-reperfusion injury, whereas A3 adenosine receptor antagonism protected renal function. These data suggest that A1 adenosine receptor agonists and A3 adenosine receptor antagonists may have clinically beneficial effects when renal ischemia is unavoidable.

In a prospective, double-blind, placebo-controlled study (54,55), the effects of oral administration of theophylline, an adenosine receptor antagonist, on renal hemodynamic changes and tubular injury induced by radiocontrast medium were investigated in 80 well hydrated patients with preexisting chronic renal insufficiency (serum creatinine concentration, >1.5 mg/dl).

The results indicated that the GFR is preserved by hydration alone in these patients, without additional benefit from the use of theophylline. In another study in patients with preexisting chronic renal failure, all patients were hydrated with 0.45% saline solution and received a nonionic contrast agent. It appeared that neither dopamine nor aminophylline reduced the incidence of contrast agent-induced nephropathy, compared with saline hydration alone (56).

Prostaglandin E1. Very recently, three different doses of prostaglandin E1 (10, 20, or 40 ng/kg body wt per min) or placebo were administered to patients with renal impairment who underwent radiocontrast examinations. The mean elevation of the serum creatinine concentration after radiocontrast agent administration was markedly higher for the placebo-treated group, compared with the prostaglandin-treated patients (57). However, no clinically relevant changes in the creatinine clearance were observed for the four groups examined.

Role of Infiltrating Leukocytes

Anti-Intercellular Adhesion Molecule-1 (ICAM-1) Antibodies and ICAM-1 Antisense Oligonucleotides. Renal ischemia-reperfusion injury is also associated with increases in infiltrating neutrophils in the kidney (58), and the role of leukocyte adhesion molecules has been extensively studied (59,60). The mechanism by which infiltrating leukocytes can cause damage is still incompletely understood but, after adherence and chemotaxis, leukocytes can release reactive oxygen species and enzymes that can directly injure cells. In addition, upregulation of adhesion molecules such as ICAM-1 on endothelial cells after ischemia and reperfusion may promote the adhesion of neutrophils to these cells and cause damage to the tissue. The administration of monoclonal antibodies against ICAM-1 protected rats against ischemic ARF (61), whereas ICAM-1-deficient mice are protected against renal ischemia (62). In addition, an ICAM-1 antisense oligodeoxynucleotide with lipofectin protected the kidney against ischemic renal failure, and this functional protection was associated with amelioration of the ischemia-induced infiltration of granulocytes and macrophages (63).

These findings were later confirmed in an autotransplantation model in rats (64). Recently, the effect of a monoclonal anti-CD54 (anti-ICAM-1) antibody was evaluated in a nephrotoxic model of ARF induced by cisplatin in rats. Striking protection of renal function and an increase in the survival rate were observed for animals treated with the antibody, compared with control animals (65).

Platelet-Activating Factor (PAF) Antagonists. The potential role of PAF in the pathogenesis of ARF was recently reviewed (66). An oral PAF antagonist (Ro-24-4736) was administered to rats before or after interruption of blood flow to both kidneys for 30 min (67). In animals treated with the PAF antagonist before ischemia, renal function was less impaired and histologic abnormalities in the posts ischemic kidneys were less pronounced, compared with vehicle-treated animals. The PAF antagonist was also protective when administered 30 min, but not 2 h, after the ischemic insult. The concomitant use of an anti-ICAM-1 monoclonal antibody did not confer additional protection, compared with that observed with the oral PAF antagonist alone. These data suggest that PAF contributes to the pathophysiologic process of renal ischemic injury, perhaps by its effects on leukocyte-endothelial cell interactions. A PAF antagonist also seems to be protective against clinical posttransplant ARF (68,69) and cyclosporine nephrotoxicity (70).

Several lines of evidence now indicate a role for neutrophil activation, secondary to the release of inflammatory mediators, in the induction and/or maintenance of renal ischemia-reperfusion injury (71). For example, the effects of nitric oxide (NO) on neutrophils have recently been delineated. Distinctions must be made between constitutively produced NO [via endothelial NO synthase (NOS)] and NO produced by the upregulated isoforms of NOS [inducible NOS (iNOS)]. NO effects on neutrophils include decreases in neutrophil chemotaxis, decreases in NADPH oxidase activity, and downregulation of both neutrophil and endothelial cell adhesion molecule expres-
sion. The role of NO in ARF is, however, dependent on the model used to produce renal injury (see below). NO donors worsen injury in hypoxic isolated tubules, whereas NO inhibition worsens in vivo models of sepsis. In addition to neutrophil activation, there is evidence that infiltrating mononuclear leukocytes play an important role in injury regeneration (72).

α-Melanocyte-Stimulating Hormone (α-MSH). α-MSH is an anti-inflammatory cytokine that inhibits both neutrophil and NO pathways. α-MSH inhibits neutrophil migration and infiltration mediated by a number of mechanisms, which have recently been summarized (73). Administration of α-MSH, even initiated 6 h after injury, inhibited renal injury in both mice and rats (74). However, this hormone also decreases renal injury when neutrophil effects are minimal or absent, as in ICAM-1 knockout mice, indicating that α-MSH also inhibits neutrophil-independent pathways of renal injury. In addition, α-MSH inhibited cytokine-stimulated NO production in a cell line derived from proximal tubules (75).

Tubular Factors

A number of metabolic responses of tubular cells occur after renal ischemia or nephrotoxicity. These responses include depletion of cell ATP, cell swelling, increases in intracellular free calcium concentrations, activation of phospholipases and consequent alteration of the composition of the lipid bilayers of the plasma membrane and subcellular organelles, protease activation, and oxidant injury attributable to the formation of reactive oxygen species. These mechanisms have recently been summarized (11). Only some aspects of these alterations are discussed here, insofar as they are related to drug prevention and/or therapy.

Loss of Cell Polarity

In normal kidneys, sodium is vectorially transported from the proximal tubular lumen across the apical membrane microvilli into the tubular epithelial cells and then across the basolateral membrane into the interstitium and the peritubular interstitium. The sodium influx across the apical membrane into the polarized proximal tubular cells is passive, via the $H^+/Na^+$-exchanger and various sodium cotransporters. The $Na^+$ gradient is maintained by active transport via the $Na^+/K^+$-ATPase at the basolateral membrane of proximal tubular cells. During hypoxia, the tubular cells lose their polar differentiation; the $Na^+/K^+$-ATPase, together with other cytoskeletal proteins (see below), dissociates from its basolateral location, redistributes into the cytoplasm, and may move into the apical membrane (76). Similar alterations have been demonstrated in human forms of ARF, such as delayed graft function after transplantation (77). This loss of cell polarity impairs vectorial sodium transport and decreases tubular sodium reabsorption. This may explain the impaired sodium reabsorption and increased fractional excretion of sodium in established human ARF.

The structural polarity is exemplified by discrete structures that interact with the actin cytoskeleton, such as the apical microvilli, surface membrane protein complexes, junctional complexes (including tight junctions and adherens junctions), and sites of cell-matrix contact (76). In ARF, marked structural changes in renal epithelial cells occur rapidly, and the onset of these changes is correlated with structural alterations in the actin cytoskeleton (76). These alterations disrupt the ability of the renal epithelium to maintain polarity and thus maintain normal renal function. A detailed description of these changes is beyond the scope of this article and can be found in recent reviews (76,78).

It has also been demonstrated, in tubular cells exposed to oxidative stress, that detachment of viable tubular cells is caused by the movement of integrins from a predominantly basolateral location to the apical membrane (79). Integrins are heterodimeric glycoproteins that recognize the most universal tripeptide sequence, arginine-glycine-aspartic acid (RGD), which is present in a variety of matrix proteins, including Tamm-Horsfall protein. Depletion of integrins expressed on the basal membrane leads to loss of anchorage to the basement membrane and cell desquamation (79). Expression of integrin receptors on the apical membrane may lead to interactions, e.g., adhesion of desquamated cells to the cells remaining in situ, thus initiating the process of tubular obstruction (80–82). These effects result in the formation of tubular casts consisting of cells, blebs, and Tamm-Horsfall protein. Some of the detached tubular cells are excreted in the urine of patients with ARF; a substantial percentage of these cells are viable and can be cultured (83).

These integrin-based interactions can be blocked by synthetic RGD peptides. Infusion of these peptides prevented the elevation of intratubular pressure that is characteristic of ischemic tubular necrosis, indicating that they at least partly prevented tubular obstruction (82,84).

Calcium and Cysteine Proteases

Numerous studies have demonstrated a central role for calcium in the pathophysiologic processes of hypoxia- and toxicant-induced renal injury (for review, see reference 85). Hypoxia in isolated proximal tubules was associated with a significant increase in free intracellular calcium concentrations that preceded evidence of membrane damage, as assessed by nuclear staining with propidium iodide (86). Low extracellular calcium concentrations prevented the increase in free intracellular calcium concentrations that is normally induced by hypoxia, indicating that the increase in intracellular calcium levels is primarily attributable to net calcium entry (i.e., calcium influx > efflux) from the extracellular compartment into the cells (86).

Calpain, a major calcium-dependent cytosolic cysteine protease, becomes activated in the presence of calcium. The activated calpain degrades cytoskeletal proteins involved in the interaction of the cell cytoskeleton with the plasma membrane (see above) and, for example, the complex of Na$^+/K^+$-ATPase, ankyrin, and spectrin, which impairs the anchoring of Na$^+/K^+$-ATPase at the basolateral membrane, is disassembled. A major role for calpain as a mediator of hypoxic injury to rat proximal tubules has been suggested (87,88).

A new inhibitor of calpain (PD 150606) with no effect on
cathepsins was protective against hypoxic injury in isolated rat proximal tubules (88).

The caspases, which are a recently discovered group of cysteine proteases, play a major role in apoptosis and activation of proinflammatory cytokines. The expression and activity of caspases are increased during ischemia and reperfusion, whereas caspase inhibition protects against hypoxic injury (89,90).

In view of the calcium-related pathophysiologic mechanisms described above, it is not surprising that calcium entry blockers have been used to prevent or at least attenuate the clinical course of ATN. The majority of animal studies suggest that calcium antagonists afford protection against experimental ATN, although all available data are not fully in accord (91,92). This protection is related to improvement of the GFR by preferential preglomerular vasodilation, to the promotion of solute diuresis, or to direct cytoprotective effects on renal cells (91).

A number of studies (reviewed in reference 93) have demonstrated that oral administration of a calcium antagonist protects against the decrease in GFR that may occur after the administration of hyperosmolar radiocontrast media. Many of the studies that demonstrated protection included a limited number of patients or exhibited other flaws in the experimental protocol. A number of investigators demonstrated that the prophylactic administration of calcium channel blockers to recipients who received renal grafts flushed with these drugs (e.g., diltiazem) protected the grafts against posttransplant ATN (94–96).

When the recipient received the calcium antagonist immediately after transplantation but the graft was not perfused with diltiazem, the incidence of delayed graft function was not significantly different from control values. Interestingly, fewer rejection episodes and higher trough plasma levels of cyclosporine were noted for the diltiazem-treated patients (97). Other transplant groups reported similar results (95,98).

It thus seems that combined treatment of both the graft and the recipient with calcium antagonists may decrease the incidence of post-replant transplant ATN, in contrast to donor or recipient treatment alone. A criticism that can be made against the studies that demonstrate such a dramatic decrease in the incidence of post-transplant ATN with calcium antagonists is that a similar low incidence of ATN can be obtained without calcium antagonists, with an adequate hydration policy and the perioperative administration of a moderate dose of mannitol. An additional criticism may be that diltiazem, verapamil, and nicardipine, but not nifedipine, interfere with the metabolism of cyclosporine through the cytochrome P450 system (97,98). This interference may lead to increased formation of certain cyclosporine metabolites with additional immunosuppressive properties, explaining the lower incidence of acute rejection episodes in certain studies.

However, a critical meta-analysis of all studies performed with calcium entry blockers for the prevention of posttransplant ATN concluded that all studies that demonstrated protective effects of these agents were uncontrolled open studies, whereas the studies in which no effect could be demonstrated were blinded, placebo-controlled investigations (99). These findings suggest at least that the issue of renal protection with calcium entry blockers in the prevention of posttransplant ATN is not settled.

Role of Solute Diuresis

Arguments for the Use of Mannitol and Loop Diuretics.

There are some theoretical arguments for the use of mannitol and/or loop diuretics for either the prevention or treatment of ARF. Both mannitol and loop diuretics can induce diuresis, potentially washing out obstructing cellular debris and casts. Mannitol may preserve mitochondrial function by osmotically minimizing the degree of postsischemic swelling and by scavenging free radicals. Loop diuretics decrease active transport in the thick ascending loop of Henle, and the ensuing decrease in energy requirements may protect cells under ischemic conditions. In addition, loop diuretics may act as renal vasodilators in particular circumstances.

A number of recent comprehensive reviews on the use of mannitol and loop diuretics and their potential beneficial effects in the prevention and/or attenuation of both experimental and clinical ATN are available (100–105).

Mannitol. A recent review thoroughly discussed the potential beneficial effects of mannitol on kidney function during cardiac surgery but also emphasized the positive and negative effects of mannitol on the brain, lungs, heart, gastrointestinal tract, and red blood cells (105). The prophylactic use of mannitol has been advocated for certain groups of patients considered to be at high risk for ATN, such as those undergoing vascular (aortic aneurysm) surgery, cardiac surgery, or renal transplantation or those developing obstructive jaundice or rhabdomyolysis. Although it is based on anecdotal and poorly documented studies performed in the early 1960s, the prophylactic use of mannitol, together with an adequate hydration policy, is now standard practice in most vascular and cardiac surgical units. Many of these studies (summarized in references 100–105) indicate that mannitol increases urine flow but does not reduce the incidence of ATN. For surgery to treat obstructive jaundice, the results are conflicting. Earlier studies found that mannitol reduced the incidence of ATN, but a prospective evaluation found no additional beneficial effect of mannitol, compared with that of adequate hydration (106). More convincing are the results obtained with the preventive administration of mannitol just before clamp release during renal transplantation surgery. In a randomized study investigating moderate hydration with or without mannitol administration, the incidence of posttransplant ATN was significantly lower in the mannitol-treated groups, for both cyclosporine- and azathioprine-treated patients (107). There have been no formal studies on the prophylactic use of mannitol alone to treat clinical rhabdomyolysis. However, forced alkaline diuresis and mannitol administration are generally accepted as being very important for the prevention of ATN after severe crush injuries (108,109).

Mannitol has been evaluated for the prevention of radiocontrast agent-induced acute nephropathy. In most recommended hydration protocols, such as that for the prevention of radio-
contrast agent-induced ATN, mannitol (500 ml of a 20% solution) is included with furosemide and hypotonic saline solution. It is virtually impossible to appreciate the individual role of each component in such protocols. Some earlier studies found that mannitol was successful in preventing renal function deterioration among patients with mild to moderate chronic renal failure who were undergoing intravenous pyelography. Those studies, however, used historic controls, making the true contribution of mannitol difficult to assess. In contrast to current opinions, another investigation found that mannitol increased the incidence of acute contrast agent-induced nephropathy among diabetic patients but reduced this incidence among nondiabetic patients (36). Because results similar to those obtained with mannitol were obtained with two other renal vasodilators, *i.e.*, dopamine and ANP, that study suggests that the preventive administration of a renal vasodilator before radiographic examination probably cannot be recommended for diabetic patients. However, a well controlled study did not observe additional benefits, compared with those of adequate hydration, with mannitol treatment before and after radiocontrast agent administration to patients with chronic renal failure (110).

The potentially negative effects of mannitol include volume depletion and hypernatremia produced by too-strong osmotic diuretic effects. However, volume expansion, hyponatremia, hyperkalemia, and metabolic acidosis may develop when the hypertonic mannitol is retained. Excessively high plasma concentrations of mannitol (>1050 mg/dl) may cause ARF (111).

**Loop Diuretics.** Clinical experience with loop diuretics (mainly furosemide) has closely paralleled that with mannitol (112–114). Furosemide has been advocated as part of prophylactic regimens for ATN, although to date there is no convincing evidence for its efficacy. Two controlled randomized studies, involving 66 and 58 patients with established ATN (115,116), revealed that high doses of furosemide could induce high urine output and convert oliguria into nonoliguria for a substantial number of patients but failed to reduce the need for dialysis and, importantly, did not reduce the mortality rates. It is noteworthy, however, that in some of those studies a favorable diuretic response was observed when a diuretic drug (furosemide or ethacrynic acid) was administered within the first 24 h after the onset of oliguria. All studies except one (117) demonstrated that loop diuretics do not lower mortality rates among patients with ATN. Some studies suggest that the continuous infusion of furosemide is more efficacious than bolus injections (118).

It should be remembered that loop diuretic treatment in the setting of ATN is not without risk. As indicated in experimental studies, furosemide may promote the aggregation of Tamm-Horsfall protein in the lumina of the tubules, which is thought to cause intratubular obstruction (119).

Large doses of furosemide or ethacrynic acid may cause deafness, which may sometimes become permanent. Coadministration of aminoglycosides increases the risk of ototoxicity. However, the incidence of permanent hearing loss is rather low; hearing loss was observed primarily when high drug doses were administered in bolus injections. At administration rates of <4 mg/min, the risk is low (120). Finally, because furosemide may increase the risk of ATN attributable to aminoglycosides and cephalosporin antibiotics, it has no place in the prevention of these forms of ATN (121).

A recent prospective, placebo-controlled, double-blind study examined the role of loop diuretics (furosemide and torasemide) in the treatment of oliguric patients with ATN, all of whom were also treated with low doses of dopamine (122). No significant difference in any major outcome parameter (renal recovery, requirement for dialysis, or death) was observed after 21 d.

**NO as a Mediator of Tubular Cell Injury**

NO is formed by a family of NOS, including the constitutively expressed isofoms neuronal and endothelial NOS and iNOS. The role of NO in leukocyte infiltration in ischemic kidneys is described above. Several lines of evidence, obtained in both *in vitro* and *in vivo* experiments, indicate that NO is also an important direct mediator of ischemic tubular injury. The evidence can be summarized as follows (123,124): (1) hypoxia stimulates NO production in proximal tubules; (2) hypoxic rat and mouse proximal tubules are protected against cell membrane damage by the nonspecific NOS inhibitor N(G)-nitro-l-arginine methyl ester; (3) proximal tubules of iNOS knockout mice are resistant to hypoxic injury; (4) iNOS is enriched in the outer medulla during ischemia in rats and mice; (5) prevention of iNOS induction by α-MSH during ischemia-reperfusion is associated with functional protection; and (6) *in vivo* targeting of iNOS with oligonucleotides specifically directed against iNOS mRNA prevents both the induction of iNOS and the cytotoxic effects of NO produced via iNOS in the course of ischemic ARF (125).

In contrast to the protective effects of the nonselective NOS inhibitor N(G)-nitro-l-arginine methyl ester in isolated proximal tubules, this agent produces deterioration of renal function in the whole kidney (125), suggesting that *in vivo* the vascular effects of NO must also be considered.

NO plays a role in cell adhesion, not only in renal tubular epithelial cell-matrix attachment (126) but also in leukocyte-endothelial cell interactions (66,127). NO, through the action of its metabolite peroxynitrite (ONOO -), impairs renal tubular epithelial cell-matrix attachment (126). This NO/peroxynitrite-mediated impairment of adhesion may contribute to tubular obstruction and delayed recovery of the tubular epithelium during ARF. It is well known that NO and calpain may interfere with components of integrin receptors and, in cultured renal epithelial cells, inhibition of both calpain and NOS prevents cell detachment from the extracellular matrix (128).

**Necrosis and Apoptosis**

It is now well established that renal tubular cells that are lethally injured after an acute ischemic or nephrotoxic insult can die by necrosis or apoptosis (129). The mechanisms of apoptosis have recently been summarized (130). The two forms of cell death are distinct morphologically and biochemically. In experimental *in vivo* models of ATN, apoptosis occurs in two phases. The first phase occurs early, between 12
and 48 h after the acute ischemic or nephrotoxic insult. The second phase occurs many days later, during the recovery phase of ATN. Whereas the first phase of apoptosis contributes to tubular cell loss and tubular dysfunction, the apoptosis observed in the recovery phase contributes to the remodeling of injured tubules and facilitates their return to a normal structural and functional state. Therapeutic interventions that inhibit or promote tubular cell apoptosis may either minimize renal dysfunction or accelerate recovery after ATN. A detailed description of these therapeutic approaches is beyond the scope of this article but can be found elsewhere (93,129,131,132). All of the results reported to date were obtained in experimental models of ARF, and the clinical benefits of these treatments have not yet been demonstrated.

Recovery, Regeneration, and Repair

In normal adult kidneys, tubular cells are highly differentiated cells with very low rates of mitosis. It is remarkable that, during recovery after acute renal injury, the remaining intact cells in damaged areas of the nephrons dedifferentiate and undergo accelerated mitosis. This process requires resetting of the cell cycle clock and subsequent redifferentiation of cells, and it seems to be highly orchestrated by a large number of tightly regulated genes. These genes include those for transcription factors such as c-Fos, heat shock proteins, and others. A detailed description of these fascinating phenomena is beyond the scope of this review and can be found elsewhere (131,133–142).

After dedifferentiation and replication, the cells migrate to fill the epithelial defect, spread out, become attached to the tubular membrane, and reestablish their polarized differentiated structure.

Growth Factors. The expression and action of growth factor peptides during the recovery of functional and anatomic integrity of nephrons in ARF and the results of some experimental studies have led to clinical studies of growth factor treatment for patients with ARF (for review, see references 137 and 143). An important double-blind, placebo-controlled, multicenter study explored the effect of subcutaneously administered recombinant human insulin-like growth factor-1 (IGF-1) (100 μg/kg desirable body wt, twice per day for up to 14 d) on the enhancement of recovery of renal function in critically ill patients with ARF (144). Injections were initiated within 6 d after the onset of ARF. IGF-1 did not accelerate the recovery of renal function in patients with ARF and substantial comorbidity.

In another double-blind, placebo-controlled trial, it was demonstrated that postoperative administration of IGF-1 to patients who underwent surgery during which blood flow to the kidneys was interrupted was well tolerated and prevented the decrease in GFR that occurred in placebo-treated subjects (145). However, the incidence of ARF was too low to permit conclusions regarding a possible protective action of IGF-1 on the course of postoperative ARF.

Finally, although the administration of growth hormone can attenuate the catabolic responses to injury, surgery, and sepsis, recent studies showed that the administration of high doses of growth hormone to patients with critical illnesses was associated with increased morbidity and mortality rates (146).

Thyroxine. Because thyroxine has been shown to shorten the course of ARF in experimental models, a prospective, randomized, placebo-controlled, double-blind trial of thyroxine was performed in patients with ARF (147).

It was concluded that, in contrast to the beneficial effects observed in experimental ARF, thyroxine had no effect on the course of clinical ARF and could have a negative effect on outcomes through prolonged suppression of thyroid-stimulating hormone. Critically ill euthyroid patients should not undergo replacement treatment with thyroid hormone.

Osteopontin. Osteopontin is a highly acidic phosphoprotein that was first isolated from bone but is also produced in other tissues, including kidney tubular epithelium. Osteopontin can bind to different integrins through an RGD sequence and possibly also through non-RGD domains (148).

In ischemia, profound elevation of osteopontin expression along the entire nephron has been observed (149). Recently, however, different patterns of osteopontin upregulation after renal ischemia-reperfusion were found for the proximal and distal tubules. In the distal tubules, an early persistent increase in osteopontin staining, without morphologic injury, was observed; in the proximal tubules, however, the staining was delayed and was associated with morphologic regeneration (150). In addition, a reduced tolerance to ischemia was observed for mice with targeted disruption of the osteopontin gene (151). All of these findings suggest a strong renoprotective potential of osteopontin in acute renal ischemia.

Administration of recombinant human osteopontin to rats prevented the loss of kidney function associated with ischemic injury. This was associated with a decreased number of obstructed tubules, less inflammation because of decreased intra-renal accumulation of neutrophils, and a reduction in programmed cell death (152).

Conclusions

The clinical treatment of patients with ARF is still largely supportive, but basic research has provided many, albeit still unproved, approaches to future therapies. We need additional experimental models that better reflect the multifactorial causes of clinical ARF. It is becoming clear that single-drug therapy will probably never be effective, and multiple agents may be needed to improve outcomes for a disease in which different pathophysiologic processes are involved. In addition, drugs should be administered early in the course of the disease; therefore, early detection of ARF, especially in the setting of the multiorgan failure syndrome, is crucial. The optimal mode and amount of dialysis for these patients are equally important, although they were not discussed in this review. The nephrology community should not be discouraged by the negative results of recent clinical trials but should continually examine and reexamine the basic and clinical strategies, to improve the grim prognosis for this dreadful disease.
References


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