Proceedings of

The sixth Asian colloquium in nephrology
The seminar on renal transplantation
The postgraduate course in nephrology

Edited by : Abu Bakar Suleiman
Zaki Morad

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Foreword

The Sixth Asian Colloquium in Nephrology was held in Kuala Lumpur, Malaysia, in November 1985. Previous colloquiua have been held in Singapore (1974), Bangkok (1976), Tokyo (1979), Hong Kong (1981) and Manila (1983).

Over 350 registrants from 22 countries attended the Colloquium and, for the first time, a Seminar on Renal Transplantation, a Postgraduate Course in Nephrology and a Seminar for Dialysis Nurses and Technicians were organized in conjunction with the Colloquium. These proceedings are based on the series of plenary and symposium lectures given during the Colloquium and the Seminar on Renal Transplantation, as well as lectures from the Postgraduate Course.

The programme was constructed with an emphasis on clinical nephrology and this stimulating collection of papers from a most distinguished panel of authors provides an excellent up-to-date review of the clinical aspects of nephrology.

Abu Bakar Suleiman
Zaki Morad
Kuala Lumpur, November 1985

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Current concepts in ischaemic acute renal failure

R.W. SCHRIER and J.I. SHAPIRO

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INTRODUCTION

Although the relative importance of vascular factors (such as decreases in renal blood flow or glomerular capillary permeability) and tubular factors (e.g. obstruction and back-leak) in the initiation and maintenance phases of ischaemic acute renal failure (IARF) have been investigated and debated for some time,[1] it is quite likely that the most relevant questions with respect to the eventual understanding and therapy of this disorder will be found at the cellular and subcellular levels. The role of cell organelles in response to ischaemia and reflow and the biochemical changes that accompany cellular injury and death are a major topic of research at many centres including the University of Colorado School of Medicine.

EFFECTS OF ISCHAEMIA ON RENAL METABOLISM

In understanding the consequences of ischaemia at a cellular level, an understanding of normal metabolic function is essential. The cells of the kidney, as do all cells, rely on the utilization of energy stored in the phosphate bonds of adenosine triphosphate (ATP). There is, however, heterogeneity in terms of substrate preference and glycolytic capability within the kidney itself. Medullary tissue has relatively high glycolytic activity and is less dependent than cortical tissue on oxidative metabolism.[2] Morphologic support for the importance of energy supply in ischaemic injury can be observed in the predominantly cortical site of cell necrosis (primarily proximal tubules) in animal[3] and human[4] cases of ischaemic acute renal failure.

Oxidative metabolism is dependent on the integrity of mitochondrial function as well as substrate availability. As will be discussed below, mitochondrial functions other than energy supply may ultimately lead to the organelle's failure to generate ATP. During ischaemia, ATP content as measured by standard biochemical techniques (freeze clamp and extraction) rapidly decreases. In one study by Hems et al.,[5] tissue ATP levels had decreased to 22% of control after 120 seconds of ischaemia. During long periods of ischaemia, tissue adenine nucleotide levels have been used to investigate the transition from reversible to irreversible cell injury. In two-hour warm ischaemia studies by Kahng et al.,[6] ATP levels fell quickly and remained low, adenosine diphosphate (ADP) levels fell more slowly and adenosine monophosphate levels, after an initial rise to 2.9 times control value, eventually fell to about 20% of control levels. Loss of the adenine nucleo-

tide pool with subsequent metabolism to purine breakdown products must create a substantial 'energy debt' with respect to the number of high-energy phosphate bonds that must be spent to resynthesize adenosine nucleotides from precursors. However, it is not clear from the tissue concentrations of the adenine nucleotides at which point cells have sustained a fatal insult rather than a reversible one.

A major problem with the standard biochemical techniques is that considerable portions of these nucleotide pools may be 'bound' and not 'biologically active'. In vivo ^{31}P nuclear magnetic resonance measurements may provide a different estimate of the unbound tissue nucleotide levels and perhaps shed new light on the metabolic consequences of ischaemia.[7] Studies by Siegel et al.[8] demonstrated that $MgCl_2$-ATP enhances recovery from IARF in a rat model; this recovery was associated with adequate nucleotide concentrations after ischaemic insult.

MITOCHONDRIAL FUNCTION DURING ISCHAEMIA AND REFLOW

Since kidney tissue, especially the renal cortex, is dependent on mitochondrial function for energy supply, our laboratory at the University of Colorado School of Medicine has centred considerable research interest on the function of mitochondria during ischaemia and reflow. The use of in vitro assessment of mitochondrial respiration has been a prominent approach in this work. Briefly, after mitochondria are isolated by differential centrifugation, mitochondrial respiration can be quantified in vitro by measurement of oxygen consumption. The rate of oxygen consumption per weight of mitochondria measured in the presence of substrate, i.e. succinate, State 4, ADP+succinate, State 3, or in the presence of an uncoupler of oxidative phosphorylation (FCCP), is a reproducible parameter that can be used to study mitochondrial function in models of ARF. The ratio of stimulated (State 3) to basal (State 4) respiration, termed the acceptor control ratio, has been particularly useful as an index of the integrity of mitochondrial function. These respiratory states are shown in Figure 1.

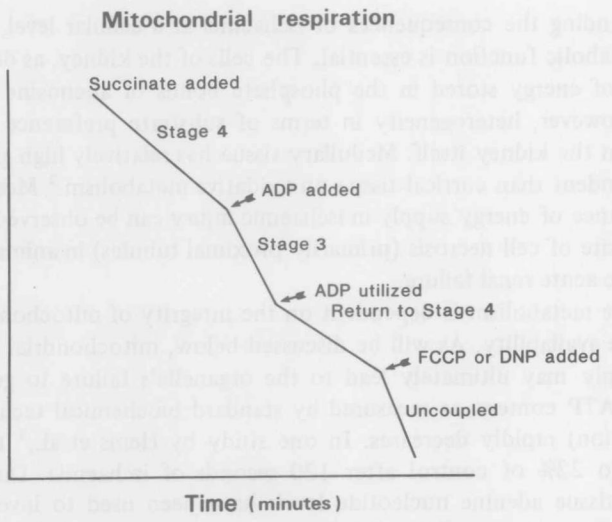

Fig. 1. Tracing showing oxygen (O_2) consumption of mitochondria in the presence of substrate (succinate) without ADP (State 4), with ADP added (State 3), with ADP depleted (State 4) and in the presence of an uncoupler of oxidative phosphorylation (dinitrophenol-DNP or FCCP) showing maximal oxygen consumption. ADP = adenosine diphosphate.

Two models of IARF have been used in our laboratories. A dog model involving 40-50 minutes of unilateral renal artery infusion of norepinephrine (NE model) and a rat model involving bilateral renal pedicle (artery and vein) clamping for 45 minutes (clamp model) have provided reproducible models of reversible ARF. In both of these models, mitochondrial respiratory dysfunction has been demonstrated after 24 hours of reflow.[9, 10] Detailed sequential studies in the clamp model in the rat during the 24 hours of reflow after the ischaemic insult have revealed a very interesting relationship between mitochondrial respiratory function and calcium content. After clamping is terminated, mitochondrial respiration, which is initially low, improves transiently but later decreases as reflow continues. This later deterioration corresponds to mitochondrial calcium accumulation which occurs progressively during reflow. A causative role for calcium accumulation in the mitochondrial dysfunction that occurs with reflow is supported not only by the close temporal association demonstrated using the clamp model[9] but also the protection against calcium accumulation and respiratory dysfunction which has been shown with calcium channel blockers in the norepinephrine model[10, 11] and the clamp model.[12] The question of why calcium is accumulated in mitochondria in spite of its noxious effect on mitochondrial respiratory function is perhaps best examined in the context of cytosolic calcium regulation and the role that calcium may play in cell injury and death (Fig. 2).

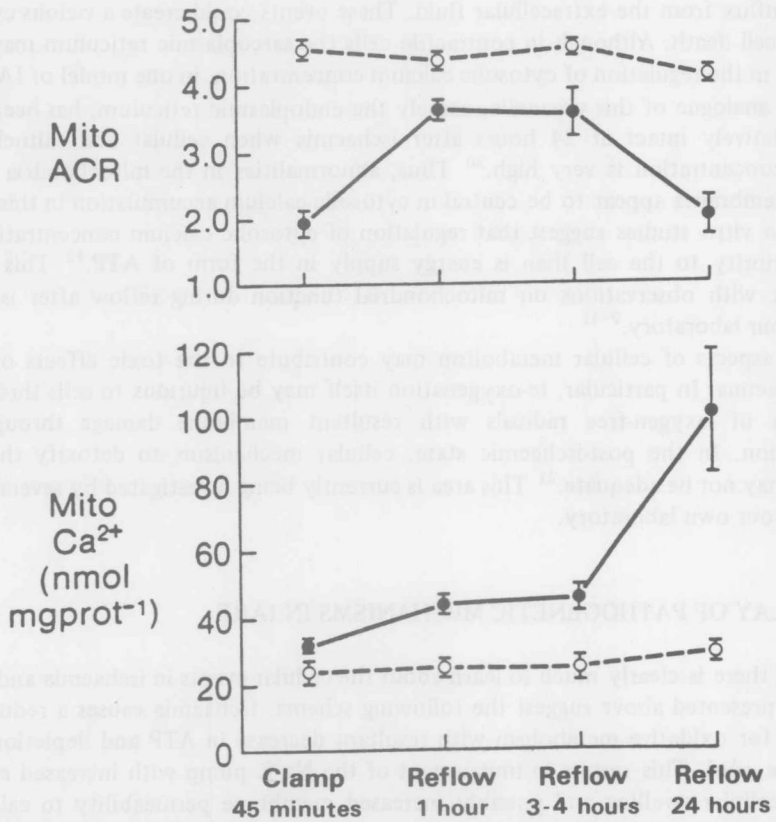

Fig. 2. Changes in mitochondrial (Mito) acceptor control ratio (ACR) and mitochondrial (Mito) calcium (Ca^{2+}) during renal pedicle clamping and at 1, 3-4 and 24 hours after reflow (closed circles) or in sham-operated rats (open circles). From Wilson et al. (1984).[9]

ROLE OF CALCIUM IN PATHOGENESIS OF ISCHAEMIC CELL INJURY

Much recent work has focused on the consequences of increased cytosolic calcium in both ischaemic and toxic models of cell injury.[13] Despite inherent difficulties in measuring cytosolic 'free' calcium, most investigators agree that cytosolic calcium is maintained at very low levels relative to the extracellular fluid. The subcellular organelles involved in this regulation appear to include the plasma membrane, endoplasmic reticulum and the mitochondria.[14] Interest in the relationship between cytosolic calcium and cell injury has been stimulated by several fascinating studies from J.L. Farber's laboratory which provided histologic and functional evidence of the toxicity of cellular calcium accumulation during reflow in an ischaemic rat liver model and also demonstrated a protective effect of chlorpromazine given before reflow. The effect of chlorpromazine may have been due to inhibition of cellular calcium entry, calmodulin and/or calcium-activated phospholipases.[13, 15] This work has been extended to renal ischaemia by several groups including our own by using calcium entry blockers.[11, 12] The mechanisms by which increased cellular calcium may mediate toxic effects may include activation of phospholipases,[16] which when activated cause breakdown of plasma membranes and release substrates for prostaglandin synthesis, inhibition of the Na/K pump in the plasma membrane,[17] and impairment of mitochondrial generation of ATP.[9, 18, 19] Each of these mechanisms may either directly or indirectly lead to sodium accumulation in the cytosol, cellular swelling and increased membrane permeability thus allowing increased calcium influx from the extracellular fluid. These events could create a vicious cycle and ultimate cell death. Although in contractile cells the sarcoplasmic reticulum may play a large role in the regulation of cytosolic calcium concentration, in one model of IARF, the epithelial analogue of this organelle, namely the endoplasmic reticulum, has been shown to be relatively intact at 24 hours after ischaemia when cellular and mitochondrial calcium concentration is very high.[20] Thus, abnormalities in the mitochondria and the plasma membranes appear to be central in cytosolic calcium accumulation in this setting. In fact, in vitro studies suggest that regulation of cytosolic calcium concentration is of higher priority to the cell than is energy supply in the form of ATP.[13] This is quite consistent with observations on mitochondrial function during reflow after ischaemic insult in our laboratory.[9-11]

Other aspects of cellular metabolism may contribute to the toxic effects of reflow after ischaemia. In particular, re-oxygenation itself may be injurious to cells through the formation of oxygen-free radicals with resultant membrane damage through lipid peroxidation. In the post-ischaemic state, cellular mechanisms to detoxify these free radicals may not be adequate.[21] This area is currently being investigated by several groups including our own laboratory.

INTERPLAY OF PATHOGENETIC MECHANISMS IN IARF

Although there is clearly much to learn about the cellular events in ischaemia and reflow, the data presented above suggest the following schema. Ischaemia causes a reduction in substrate for oxidative metabolism with resultant decrease in ATP and depletion of the nucleotide pool. This results in impairment of the Na/K pump with increased cytosolic sodium, cellular swelling and possibly increased membrane permeability to calcium as immediate consequences. With reflow, calcium enters the cell but is initially buffered via mitochondrial calcium uptake. If the ischaemic insult is mild enough, post-ischaemic mitochondrial calcium buffering does not preclude mitochondrial ATP resynthesis and regeneration of the adenine nucleotide pool. If, however, damage exceeds a certain

threshold, calcium uptake by the mitochondria impairs the ability of these organelles to provide sufficient energy resulting in continued calcium entry into the cytosol and resultant cell death. Oxygen radicals may contribute to membrane damage and 'leakiness' to calcium.

In view of the central role of calcium in ischaemic cell death (Fig. 3), it might be expected that calcium entry blockers might prevent mitochondrial calcium accumulation and protect mitochondrial respiratory function and overall renal function. Results from our laboratory have demonstrated renal functional and histological evidence of protection in the norepinephrine model[11] using treatment with verapamil or nifedipine. Treatment with these calcium channel blockers either before the ischaemic insult or at the time of reflow has a protective effect on renal function in the norepinephrine model in the dog (Fig. 4).

RELATIONSHIP OF CELLULAR MECHANISMS TO ORGAN PATHOPHYSIOLOGY

The cellular events described above may be integrated with theories of pathogenesis at the organ level. As seen in the schematic diagram (Fig. 5), increased cytosolic calcium, cell swelling and cell death may be incorporated into all of the currently prevailing theories of the pathogenesis of IARF.

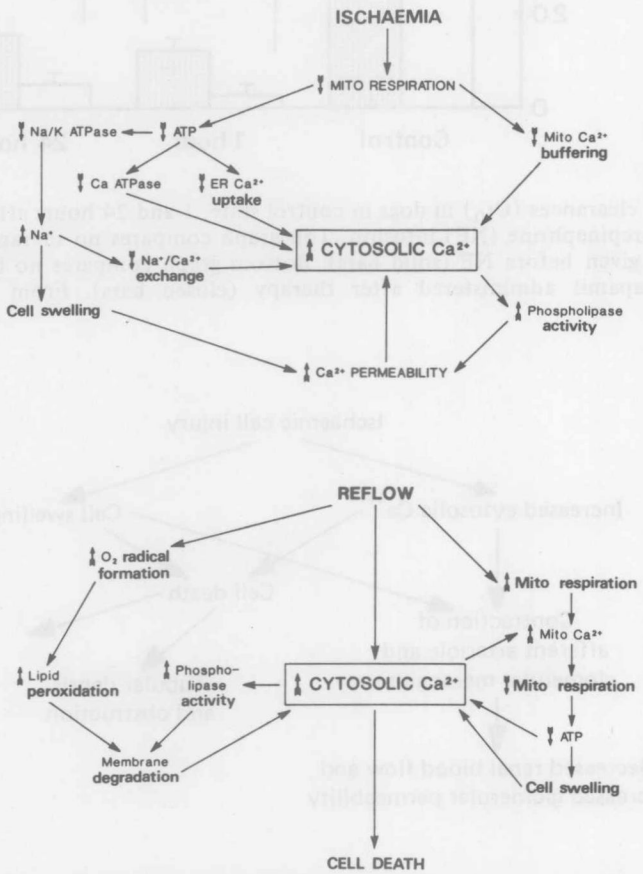

Fig. 3. Schematic of cellular events during ischaemic insult and reflow. ATP = adenosine triphosphate; ATPase = adenosine triphosphatase.

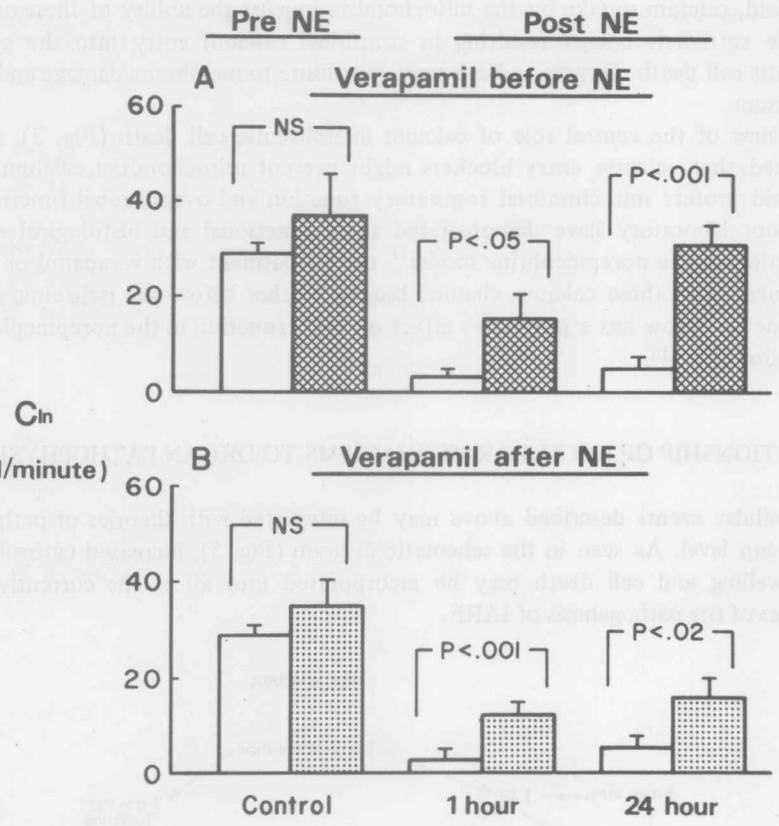

Fig. 4. Inulin clearances (C_{In}) in dogs in control state, 1 and 24 hours after 40 minutes of intrarenal norepinephrine (NE) infusion. Top graph compares no therapy (open bars) with verapamil given before NE (solid bars). Bottom graph compares no therapy (open bars) with verapamil administered after therapy (closed bars). From Burke et al. (In press.)[11]

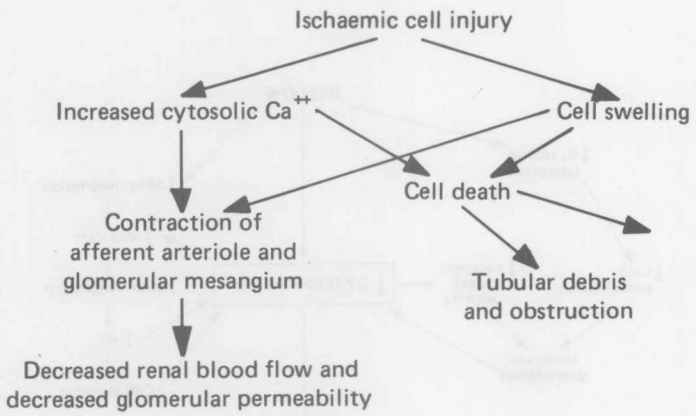

Fig. 5. Schematic representation depicting interplay of cellular events with classic tubular and vascular theories of the pathogenesis of ischaemic acute renal failure.

CONCLUSIONS

Investigation into the cellular mechanisms of IARF have focused our attention on the importance of oxidative metabolism and the central role that calcium accumulation may play in cellular injury. Studies from our laboratories have implicated the deleterious effects of mitochondrial calcium accumulation during reflow in different models of IARF.

Moreover, we have noted the beneficial effects of calcium entry blockers in modifying this phenomenon as well as preserving mitochondrial respiratory function and renal function in these animal models. The protective qualities of these agents not only serve to support the proposed role of calcium in the pathogenesis of ischaemic cell injury, but may ultimately prove to have important application in human IARF as well as other clinical settings of ischaemic cell injury resulting in organ dysfunction.

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Drug-induced acute renal failure

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INTRODUCTION

Acute renal failure (ARF) continues to be a major source of morbidity and mortality. In a recent prospective study, nearly 5% of patients admitted to a medical or surgical service at a university hospital developed an acute deterioration in renal function during their hospital stay. Drug-induced ARF constituted approximately 20% of these cases.[1]

Drugs may cause ARF by a variety of mechanisms. An alteration in renal haemodynamics is the mechansim by which several pharmacologic agents cause acute deteriorations in renal function. Non-steroidal anti-inflammatory agents[2] and angiotensin converting enzyme inhibitors[3] in the appropriate settings may cause renal failure by this mechanism.

Immunologic or allergic mechanisms may complicate the use of a variety of drugs. Classic examples include methicillin-[4] or sulphadiazine-[5] induced acute interstitial nephritis. Clinical features of rash, fever, eosinophiluria and eosinophilia while commonly noted in these classic cases are conspicuously uncommon in recent reports of interstitial nephritis associated with high-grade proteinuria attributed to fenoprofen, a non-steroidal anti-inflammatory agent.[6]

Direct toxicity is another mechanism by which drugs can cause ARF. Aminoglycoside antibiotics[7] and cis platinum[8] appear to be directly toxic to renal tubular epithelial cells. Some agents may act through multiple mechanisms or have toxicity that is poorly understood. The remainder of this discussion concentrates on aminoglycoside antibiotics and radiocontrast media which account for the majority of ARF observed on medical or general surgical services[1] and cyclosporin which is currently of great importance in the transplant population.[9]

AMINOGLYCOSIDE ANTIBIOTICS

Aminoglycoside antibiotics are the cornerstone of therapy for Gram-negative bacterial infections. With Gram-negative bacteria accounting for the majority of hospital-acquired infections, there is considerable use of these agents.[10]

Aminoglycosides generally cause a non-oliguric type of ARF that is related to the dose and duration of the drug.[11] Increases in serum creatinine generally occur after 8-10 days of therapy, although variation may occur.[12] Enzymuria is characteristic and often precedes changes in serum creatinine.[13] Potassium and magnesium wasting are well described.[14] Risk factors for the development of aminoglycoside nephrotoxicity include advanced age, renal insufficiency, hypovolaemia, potassium depletion, a recent previous course of aminoglycosides and concurrent use of other nephrotoxins.[11] A toxic synergism with cephalothin has been noted in clinical studies[15] but has not been supported by animal models.[16]

Aminoglycoside antibiotics accumulate in renal tissue. After a single dose in rats, the

serum half-life is only 30 minutes, whereas the half-life in renal tissue is 109 hours.[17] This long tissue half-life explains why a recent previous course of an aminoglycoside is a risk factor for aminoglycoside nephrotoxicity and how clinical evidence of nephrotoxicity may manifest itself after the course of aminoglycoside is completed.[11]

The mechanism by which aminoglycosides cause a decrease in glomerular filtration rate is poorly understood. One animal model using gentamicin causes a 30% decrease in single nephron glomerular filtration rate after seven days. The decrease in glomerular filtration rate is explained entirely by a decrease in glomerular permeability coefficient in this model.[18] A role for angiotensin II in mediating this decrease in glomerular permeability coefficient has been suggested by other workers who, by treating rats before and during the course of gentamicin with converting enzyme inhibitors, prevented the changes in glomerular haemodynamics and decreases in glomerular filtration rate.[19] However, more recent studies using potassium-depleted rats found a deleterious effect of captopril on gentamicin nephrotoxicity in these animals.[20] Therefore, where some studies explain the deleterious effects of aminoglycosides on alterations in glomerular haemodynamics mediated by angiotensin II, this explanation is probably incomplete. Moreover, although animal studies to date do not find a role for tubular back-leak and obstruction in aminoglycoside nephrotoxicity in animal models, this does not exclude their relevance to human aminoglycoside nephrotoxicity.

At the cellular level, gentamicin and presumably other aminoglycosides are actively taken up by renal proximal tubule cells.[11] Intraluminal calcium appears to compete for the gentamicin-binding sites and antagonize gentamicin uptake.[21] Amelioration of gentamicin nephrotoxicity by increases in dietary calcium has been shown in a rat model of nephrotoxicity.[21] Although intraluminal calcium appears to prevent gentamicin uptake and ameliorate nephrotoxicity, recent evidence from our laboratory suggests that changes in intracellular calcium may be involved in the pathogenesis of gentamicin toxicity. Burke et al.,[22] using calcium uptake kinetics with isolated proximal tubules, found that intracellular calcium compartment size increases in a rat model of gentamicin toxicity. This increase in intracellular calcium compartment size could be demonstrated after only one day of gentamicin therapy, before any histologic changes could be noted.[22] Whether these changes in intracellular calcium are causally related to the cellular toxicity of gentamicin is not known at this time. Support for this hypothesis, however, comes from Eliahou et al. who were able to demonstrate a protective effect of verapamil, a calcium channel antagonist, in a rat model of gentamicin toxicity.[23] Further experimental work on the complex interactions between calcium and gentamicin may eventually prove useful clinically.

At this time, clinical therapy of aminoglycoside nephrotoxicity involves identifying the patient at risk, careful attention to dosage and concurrent risk factors and the use of a less nephrotoxic drug when clinically indicated. Serum creatinine, potassium and magnesium should be followed in patients treated with these agents.

RADIOCONTRAST MEDIA

Radiocontrast media are used during a variety of radiologic procedures. Although the risk of ARF from these agents for the normal patient is quite small, the great number of contrast studies performed makes this a major cause of ARF.[1]

Clinically, radiocontrast media-induced ARF is usually oliguric, occurs within the first 24-48 hours after exposure, and is of moderate severity with most patients not requiring dialysis and completely recovering.[24] Radiocontrast-induced ARF is usually associated with a low urine sodium and a low fractional excretion of sodium.[25] Risk factors for the