

Acute Dialysis Quality Initiative

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Workgroup 4

Animal Models of Acute Renal Failure

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Basic Concepts of ATN

Various nephron segments differ in their capacity to withstand hypoxic insults. Proximal tubules have little capacity for anaerobic glycolysis, and hence they are particularly sensitive to total cessation of renal blood flow and oxygen delivery, as occurs in the model of transient clamping of the renal artery (warm ischemia-reflow). By contrast, though thick ascending limbs (mTALs) have a greater capacity to generate ATP by glycolysis, hypoxic injury may be enhanced if reabsorptive activity persists. (1,2).

Renal parenchymal oxygenation is not homogenous: Physiologic medullary hypoxia exists because of limited oxygen supply (due to a unique regional microcirculation associated with a countercurrent oxygen exchange in the vasa recta) that marginally matches oxygen demand for high levels of tubular reabsorptive capacity. Ambient pO₂ normally declines at the cortico-medullary junction to 20-30 mm Hg. Not surprisingly, hypoxic tubular injury predominantly occurs among tubular segments in this region, namely mTALs and S3 (straight) segments of the proximal tubule in the outer medulla and medullary rays (2,3).

Various nephron segments are predominantly prone to different toxic insults, for instance cis-platinum principally damages S3 segments, while pigment nephropathy (induced by hemoglobin or myoglobin) involves S2 segments of the proximal convoluted tubule as well. Heterogenous distribution of toxin accumulation, related to selective sites of tubular reabsorption, may contribute to this variation.

The pathophysiology of renal tubular damage includes a clear-cut direct toxic effect (gentamicin, cis-platinum), hypoxic damage induced by altered medullary microcirculation and/or enhanced metabolic requirements (NSAIDs, radiocontrast, cyclosporine), or injury caused by free radical generation (heme pigments). These mechanisms often work in concert. For instance amphotericin directly injures tubular cell membranes, induces hypoxic tubular damage by profound renal vasoconstriction, and enhanced oxygen consumption to maintain cellular transmembrane electrolyte gradients.

Additional factors contributing to renal dysfunction include inflammatory processes which leads to compromised renal microvasculature, tubular obstruction, loss of tubular cell polarity and backleak of tubular fluids (4).

The ultimate indicator of renal dysfunction - declined GFR, may reflect primarily altered glomerular hemodynamics as occurs during hypovolemic shock or endotoxemia, or be secondary to a tubular injury with obstruction of the lumen and increased intratubular pressures as well as an activation of tubulo-glomerular feedback mechanisms.

Herein we critically review available animal models of acute renal failure (ARF) that are relevant to the clinical practice in the ICU. We will not discuss models of acute immune-mediated nephritis and most nephrotoxic models that utilize clinically less relevant agents such as heavy metals. The following text represents “consensus expert opinion” agreed upon by the four contributors, regarding the advantages and disadvantages of the discussed animal models. In addition, each participant will present his perspectives and individual comments related to unsettled issues. The reader is referred to four other important reviews addressing concepts and controversies regarding animal models of ARF (1,4-6).

General principles

We fully adopt the general principals, outlined by Piper and colleagues, in planing, conducting and critically evaluating studies utilizing animal models (7). We would like to especially emphasize the following principals:

1. Proper randomization of animals
2. Similar baseline characteristics of the experimental groups
3. Concurrent appropriate controls
4. Blinded assessment of outcome
5. Consideration and reporting of mortality
6. Numbers of animals studied should be appropriate to reproducibility of outcome measure in the particular model.

Additional basic principals agreed upon are:

1. Relevance to clinical situation should drive model use rather than let reproducibility of model itself drive the process.
2. Physiological parameters known to affect kidney function or susceptibility to injury should be controlled for, measured and reported (temperature, blood pressure, fluid status, type of anesthesia etc).

3. Better models depend upon better definition of human pathology. We should strive to extend our limited knowledge of human pathology.
4. Since clinical-pathological correlations are essential, it is important to consider carefully the appropriate preparation of tissue for valid pathological interpretation.
5. Fundamental requirements for a model should include morphology, hemodynamics and function.
6. It is important to look at outcome measures at multiple time points. One should strive to develop noninvasive techniques to monitor a dynamic outcome parameter (functional, hemodynamic, tissue oxygenation or metabolic status) in the same animal at multiple time points. Such technologies can later be adopted for the evaluation of human ARF.
7. We should strive to develop noninvasive biomarkers for renal parenchymal cell injury, to be tested in animal models and adopted for the evaluation of human ARF.
8. It is important to exploit models in vitro to gain additional insight into pathophysiological mechanisms as well as to validate observations in isolated cells and organs with confirmation in more complex models in vivo.
9. Models are generally done in healthy animals whereas human ATN is in setting of preexisting vascular alterations, multiorgan failure, multiple renal insults or an underlying chronic renal disease. Models should be created that explicitly address comorbidities that are believed to predispose to acute renal failure and outcome in humans.
10. Inter-species anatomical variations between model animals and humans may affect renal susceptibility in animal models of ARF.
11. In no model can we say with high level of conviction that we can predict that a protective effect in animals will translate to humans. This may or may not be a problem with the models themselves. It may at least to some extent be a problem with design of human studies in which the diagnosis is often delayed and the therapy is started well into the maintenance phase of ATN.
12. Experimental observations should be reproduced in other laboratories before they are generally accepted.
13. All animal models discussed below may be used for the evaluation of potential pharmacologic interventions, including vasodilators, immunomodulatory agents, free radical scavengers, and growth factors. Vasopressors may be studied exclusively in septic models. (see reservation by SN Heyman, individual remarks).

Individual Models

Below are additional consensus points regarding advantages and disadvantages of different animal models of ARF, generally classified under ischemic, toxic and septic etiologies.

A. Ischemic Models

Warm Ischemia - Reperfusion

In this most widely used model, conducted predominantly in rats (recently in mice as well), the renal vascular pedicle or renal artery alone is clamped for a variable length of time, with subsequent reperfusion. The renal temperature will decline, especially if the kidney is kept exposed, albeit not to the low range used for renal transplantation. Since hypothermia exerts a marked protective effect it is important to be very careful with temperature control. The principle preliminary pathophysiological insult is tissue hypoxia, with subsequent altered outer medullary microcirculation, inflammation and tubular injury, principally affecting the S3 segment. Reperfusion injury involves reactive oxygen species formation.

Advantages

1. Simple and reproducible (at ischemic periods > 45 minutes)
2. Graded response easily achieved
3. Response of the tubule recapitulates injury and repair process in humans
4. Medullary congestion may be comparable to human ATN
5. Inflammatory response comparable to human autopsy data
6. Good correlation between functional injury and pathology

Disadvantages

1. Clinically we do not often see ARF with pure ischemia alone
2. Complete cessation of renal perfusion is uncommon as a cause of human ARF
3. S3 damage may be much more severe than in humans (in fact in most experiments the ischemic period used ends with an almost complete necrosis of S3), whereas mTAL damage may be less prominent than in humans
4. Time of ischemia may be underestimated due to outer medullary
5. Congestion resulting in ischemic period extending beyond the point that the clamp is removed.

Cold Ischemia - Warm Reperfusion

In this model, the kidney is removed, flushed and kept at low temperature under conditions similar to cold preservation for transplantation. Kidneys are subsequently reimplanted following variable periods of cold preservation. This model has rarely been used in studies of experimental ATN, and the injury distribution and pattern may substantially differ from that of warm ischemia-reperfusion (8).

Advantages

1. Similar to what occurs with transplant ARF

Disadvantages

1. Inadequately studied
2. Difficult experimentally

Isolated perfused kidney

In this ex-vivo, whole organ model, the removed rat kidney is continuously perfused with oxygenated medium through the renal artery under controlled pressure and perfusate flow. "Urine" is collected, and glomerular filtration and tubular function are continuously monitored by the sequential determination of electrolytes and inulin in the perfusate and urine. Renal morphology can be assessed subsequently. Perfusion with oxygenated erythrocytes yields near-normal morphology and function, whereas perfusion without red cells (ie very low oxygen supply) results in progressive functional impairment with predominant mTAL hypoxic injury (9). (see reservations by W Lieberthal, individual remarks).

Advantages

1. As compared to isolated tubules and cell culture models, the renal anatomy and the overall vascular-glomerular-tubular anatomical associations are maintained.
2. There is an absolute control over all extrarenal factors.
3. Enables concomitant determination of hemodynamics, function and morphology
4. Experimental manipulation is easy

Disadvantages

1. The kidney is maximally vasodilated.
2. GFR is initially normal and therefore does not mimic ARF in humans
3. Factors that may participate in the pathophysiology of ARF, such as neuroendocrine mediators, leukocytes and various plasma factors are excluded

Contrast nephropathy

In vivo studies fail to reproduce contrast nephropathy in intact animals by the administration of radiocontrast alone. Rather, animals are predisposed to contrast-induced injury by additional insults including: salt depletion or angiotensin II infusion, concomitant administration of NSAIDs, inhibition of nitric-oxide synthase, induction of heart failure, hypercholesterolemia, diabetes, or reduction in renal mass. Frequently a number of these physiological perturbations are included with contrast administration. A predominant pathophysiological mechanism of renal failure following intravascular administration of radiocontrast is hypoxic outer medullary injury that results from altered renal microcirculation and enhanced metabolic demand for tubular reabsorption (10).

Advantages

1. All models involve predisposing factors which mimic risk factors for the clinical human syndrome.
2. Can be done in rats, rabbits, dogs
3. Clinical relevance, since contrast is a frequent cause of ARF in humans

Disadvantages

1. Complicated multi-component model
2. Variable response requires large numbers of animals
3. Agents that were effective in animals did not work in humans (eg furosemide with insufficient hydration [10]).

Other models of selective outer medullary hypoxic damage from combined insults

In these models, conducted predominantly in rats, various combinations of insults believed to adversely affect outer medullary oxygen balance are imposed, with some resemblance to the human predisposition for acute or progressive renal failure. In addition to radiocontrast (discussed separately above), these include the administration of NSAIDs, inhibition of nitric oxide synthesis, effective volume depletion (salt depletion, dehydration, heart failure), ureteral obstruction, or the administration of endotoxin, myoglobin, amphotericin, cyclosporine, chronic hypercalcemia etc. (3,10-13). Hypoxic outer medullary tubular injury plays the major role in the evolution of renal dysfunction in these animal models but other mechanisms may co-exist, such as enhanced cytotoxic effect due to increased intraluminal concentration of the nephrotoxin.

Advantages

1. Clinically relevant, reflecting both acute, subacute and chronic insults

2. In some models pathology resembles known human morphology
3. Adopts the principal of predisposing risk factors so relevant in human disease
4. Provide data regarding different types of cellular injury patterns (reversible injury, apoptosis and frank necrosis), gradients of damage, and acute and chronic inflammatory and reparatory responses.
5. Enables detection of changes in hemodynamics and tissue oxygenation during short term experiments

Disadvantages

1. Complicated models
2. Done almost exclusively in rats
3. In the acute models –uniformity is a problem with high level of variability
4. Papillary tip necrosis seen in some of these models does not mimic what is seen in humans in ARF
5. In less severe models there is poor correlation between function and extent of morphological damage. There is better correlation in more severe models.

Reccommendations: For experimental hypoxic ARF the use of ischemia reflow and IPK models will continue to provide basic insight regarding cellular response to hypoxic insults and potential therapeutic interventions, principally thanks to their simplicity, consistency and high reproducibility. However, the findings in these experiments, particularly regarding therapeutic interventions, should be subsequently tested on the more compound, yet perhaps more clinically relevant models of multiple insults. We should strive to create better complex models, no doubt. We also think that the cold ischemia-reflow model should be utilized more often, since it may have high clinical relevance to renal transplants.

B. Toxic Models

Gentamicin nephropathy

In this model, animals (usually rats) are given gentamicin parenterally at a dose generally ranging from 100-200 mg/kg body weight for 3-6 consecutive days (14,15). Direct tubular nephrotoxicity seems to be a predominant pathophysiologic process.

Advantages

1. Clinically highly relevant
2. A simple and reproducible model.

3. The presence of myeloid bodies in proximal tubular cells, a characteristic morphologic feature of gentamicin exposure in humans, is also seen in animal models of gentamicin toxicity.
4. Predisposing factors in the clinical practice (such as dehydration, bacteremia, ischemia and liver damage) can be reproduced in animals.
5. ARF in animals is reversible with a recovery phase comparable to the human syndrome.

Disadvantages

1. The dose required to get a reproducible model of ARF is far higher than that used in humans.
2. In humans gentamicin nephrotoxicity may appear with only minimal tubular changes on light microscopy, while the development of ARF in the rodent model requires extensive tubular necrosis.

Cisplatin nephropathy

ARF is induced in rats or mice by the injection generally of a single intraperitoneal dose (6-20mg/kg) (15-17). Direct tubular nephrotoxicity seems to be a predominant pathophysiologic process, with cisplatin being absorbed by and accumulates within cells in the proximal tubule (especially the S3 segment).

Advantages

1. A simple and reproducible model
2. ARF appears after a single administration, as may occur in humans
3. Pathology is comparable with the human disease: predominant S3 involvement.
4. Tubular dysfunction comparable to that seen in humans (glycosuria, hypomagnesemia, hypokalemia). Recovery phase also comparable
5. Dose appropriate to human use
6. Predisposing factors to ARF in humans, such as volume depletion is replicated in animals

Disadvantage

1. Declining clinical relevance: the use of cisplatin in humans has decreased due to availability of carboplatin, which is substantially less nephrotoxic.

Pigment nephropathy - Glycerol

In this model, used predominantly in rats, glycerol 50% is injected to hindlimb muscles (total dose 8-10 ml/kg), producing abrupt rhabdomyolysis, associated with rapidly progressive renal dysfunction. ARF is caused by complex pathophysiological processes, including dehydration, toxic proximal tubular cell

damage (induced in part by oxygen free radicals), vasoconstriction with subsequent hypoxic tubular damage, renal inflammatory process and tubular obstruction by casts (18,19).

Advantages

1. Simple and reproducible
2. Clinically relevant, resembling clinical rhabdomyolysis
3. Includes pathophysiological processes that occur in man, and provides all physiological features of muscle breakdown (third space fluid accumulation, pigment and purine load, systemic inflammatory response etc.)
4. Experimental maneuvers predisposing and protective in humans are also applicable in animals
5. Good model to study injury repair process of the proximal tubule

Disadvantage

1. Very severe model
2. Causes intravascular hemolysis as well as myoglobinemia

Pigment nephropathy - Myoglobin or hemoglobin infusion

In these models, used predominantly in rats, pigments are infused intravenously over an hour or so (19). Unlike the glycerol model, the pathophysiological processes do not include dehydration, spillage of other muscle contents, such as purines, and the induction of a systemic inflammatory response.

Advantages

1. Simple and reproducible model
2. Enables detection of changes in hemodynamics and tissue oxygenation during short term experiments

Disadvantages

1. Does not truly reproduce the pathophysiological aspects in humans with the exception of hemolysis.
2. Hypertension results from systemic vasoconstriction in contrast to what happens in the human crush syndrome
3. Induces a relatively mild renal damage

Recommendations: Studies in models of toxic nephropathies should shift to focus on newly recognized clinically relevant nephrotoxins, such as the new generations of anti-retroviral and immunosuppressive agents. An effort to produce renal failure by the incorporation of potential contributing risk factors in these models seems more relevant clinically than the use of extremely high doses of the nephrotoxins.

C. Sepsis models

Endotoxin infusion / injection

In this models, used in small and large animals, endotoxin (lipopolysaccharide, LPS) is administered by intravenous bolus injection or continuous infusion, with or without subsequent fluid resuscitation. Sometimes LPS is administered intraperitoneally. The primary pathophysiologic processes are probably altered renal hemodynamics (20), inflammation (21) with subsequent microvascular damage and obliteration and renal hypoxic parenchymal injury.

Advantages

1. Simple and inexpensive (when performed in small animals)
2. Suitable for study of new pharmacologic agents
3. Dose very well standardized

Disadvantages

1. Generalized vasoconstriction may occur in some animal models in the absence of fluid resuscitation, as opposed to generalized vasodilatation seen in humans.
2. Inadequate fluid replacement in most models.
3. ARF requires high doses, associated with high mortality. Consequently, these models have a short duration. On the other hand, with small doses renal dysfunction is transient without evident ATN.
4. Response variable to different sources of endotoxin, rate of administration, single or repeated administration and across species.
5. Comparisons difficult across laboratories
6. Renal morphology is poorly described

Bacterial infusion

In these models, used in small and large animals, an intravascular infusion of live bacteria is given. The principal pathophysiological processes involved are similar to those in the endotoxin models (22,23)

Advantages

1. Systemic hemodynamic response comparable to human sepsis
2. Insult can be standardized (bacterial dose)

Disadvantages

1. If animals survive, the model does not reproduce multi-organ dysfunction.
2. Expensive and cumbersome model in large animals
3. Very often standard supporting measures are lacking, including fluid resuscitation, antibiotics and mechanical ventilation.

Cecal ligation and perforation

The cecal ligation and perforation model was originally described in rats (23), but later successfully extended to mice and sheep.

Advantages

1. Simple and cheap
2. Utilized in small and large animals
3. In large animals septic shock develops with multiple organ dysfunction syndrome, including acute renal failure, acute lung injury and cardiac dysfunction, together with lactic acidosis.
4. Ongoing infection, mimicking the human situation.

Disadvantages

1. Variability of response within and across species (in part related to cecal distensibility, mode and size of cecal perforation, variable sealing of perforations etc.)
2. Does not always reproduce ATN seen with sepsis in humans
3. No standardized associated therapy (eg. antibiotics, fluids). Each center does it differently

Intraperitoneal infusion of bacteria

In this model bacteria are injected into the peritoneal cavity or implanted in a fibrin clot. Can be used in both small and large animals (24).

Advantages

1. Simple and cheap (when applied in small animals)
2. Rapid onset with disseminated bacterial administration into the peritoneum

3. Clot model reproduces aspects of sepsis in humans including: increased cardiac output, leukocytosis
4. Complete control over dose of bacteria
5. Model developed in large and small animals

Disadvantages

1. Disseminated bacterial peritonitis model is too severe in large animals
2. Does not produce ATN clinically or pathologically

Sepsis-related renal failure, often associated with multi-organ failure is a leading cause of ARF in critically ill patients. Yet, we are far from reaching a pathophysiologically adequate reproducible animal model that closely resembles the human syndrome. Many models fail to reproduce appropriate rate of multiorgan dysfunction and death. Others lead to overwhelming and dramatic hemodynamic deterioration, with ultimate death within 12 h, a time too short for the development of compensatory systems and a clinically relevant renal deterioration. Moreover, in most models fluid resuscitation, antibiotics and vasopressor therapy, the standard care in patients with septic shock, are not given. Finally, many studies fail to appropriately define clear endpoints such as death after 24 or 48 hours, or the occurrence of organ failure, precisely described in scoring systems. We believe that of all sepsis-ARF models, experimental ARF with LPS infusion may be the least appropriate. Large animal models with concomitant resuscitative maneuvers (fluids, antibiotics, hemodynamic and respiratory support) seems most appropriate, but considering complexity and expenses we should strive to develop comparable models in small animals.

Recommendations: 1. The magnitude of infection should be standardized. 2. The infection and host response should be easily reproducible in other labs. 3. Supportive care should be standardized and be comparable to the human standard care. 4. The insult and supportive care should allow enough time for the host response to occur. 5. Clear endpoints should be made.

Individual comments

Samuel N Heyman

I refer the reader to the long-standing debate, addressed in depth (1,5), regarding the predominant role of mTAL damage vs. S3 injury. On my opinion, the argument is to a lesser extent related to the type of nephron segment involved. In fact, these two sections are in close proximity to each other, compete on the limited regional oxygen supply (25), are compromised to the same extent by the altered medullary microvasculature that develops with the various types of experimental hypoxic ATN, and are both predominantly involved in human ATN (26,27). I believe that the main difference in opinions is conceptual: the warm ischemia-reflow model, with prolonged total cessation of renal blood flow may be an

excellent consistent probe to explore cell injury, but it does not represent what happens in human ATN, where renal blood flow never ceases and GFR decline is often gradual and incomplete. Since mTAL injury noted in the models of combined multiple insults is critically dependent on ongoing reabsorptive work, one should regard diminished GFR of whatever nature as a protective measure to prevent medullary oxygen imbalance and progressive tubular damage. This may contribute to the relative preservation of mTALs in the warm ischemia-reflow model, through total cessation of GFR. Indeed, we have recently found that pre-existing proximal tubular necrosis and diminished GFR (caused by ischemia-reflow or nephrotoxins) prevents downstream mTAL damage in the IPK model (28).

In this perspective, it is also conceivable that attempts to enhance GFR during ATN (strategies adopted from animal models of warm ischemia-reflow) might exacerbate mTAL injury. I have no idea what is clinically more important: a pharmacologically manipulated refined GFR or the amelioration of medullary oxygen balance to minimize progression of mTAL and S3 injury, at the price of diminished GFR. What can be said is that vasodilators that were found to improve GFR and to ameliorate damage in the warm ischemia-reflow model did not work in clinical trials, perhaps reflecting intensification of tubular hypoxic injury.

I am also concerned about the extent of ATN reproduced in our animal models. Mild and focal tubular injury (the hallmark of the human syndrome), induced by moderate insults often does not correlate with the extent of renal dysfunction (perhaps indicating a predominant impact of altered glomerular hemodynamics). Such models may also suffer from poor consistency and heterogeneous outcome, requiring large numbers of animals. By contrast, extensive damage induced by severe insults is highly consistent and more closely correlates with renal dysfunction. I think that our adherence to these convenient yet extreme models may underlie the failure to properly assess the true nature of the clinical syndrome and to develop effective protective measures that do work in humans.

An extreme insult is required to induce ATN in the normal animals, thanks to the ingenious complex control of renal parenchymal oxygenation (29). It is conceivable that these mechanisms are hampered in clinical conditions known to predispose to ATN. A recent example is the report indicating that experimental heart failure predisposes to outer medullary hypoxic injury (11). Thus, I am more than pleased that a consensus was reached regarding the implementation of clinically relevant predisposing insults in animal models of ATN. Since chronic renal disease (always associated with altered renal microcirculation) is probably the most important risk factor to ATN, I stress the critical need to develop animal models of acute on chronic renal failure (models other than unilateral or 4/5 nephrectomy).

I would like to respond to Dr. Lieberthal's criticism regarding the isolated kidney model, perfused without erythrocytes (see below). First, the extent of medullary thick limb necrosis in isolated kidneys perfused with a cell free medium is quite limited with the addition of amino-acids to the perfusate (30). Intracellular amino-acid depletion, induced by the ex-vivo settings markedly predisposes tubular cells to hypoxic injury (31) and this is probably attenuated by the addition of red cells. Secondly, This preparation preserves the

complex tubulo-interstitial glomerular construct that is unavailable in isolated tubules and cell culture preparations. The argument that the lack of significant oxygen carrying capacity in the perfusate is entirely non-physiological is correct, but this limitation provides the ability to study the vulnerability of the thick ascending limb to oxygen deprivation without the extensive reno-protective mechanisms present in vivo.

Finally, I would like to acknowledge the invaluable contribution of an appropriate renal tissue preparation and meticulous morphological evaluation provided by an experienced renal pathologist. We use in vivo perfusion fixation techniques that prevent tubular collapse and enable excellent assessment of tubular damage. This technique may be used in conjunction with conventional fixation in order to identify features of local inflammatory response, that may be removed by perfusion fixation.

Peter Rogiers

The clinical failure of several novel therapies in sepsis over the past decade may, in part, be attributed to the failure of the available animal models. Indeed, as it has been said before (22,24), the animal model of severe sepsis should be clinically relevant and therefore include hemodynamic measurements, provide aggressive fluid resuscitation and allow the development of multi-organ dysfunction. In most of the studies investigating hemofiltration endotoxin was used, either by intravenous bolus or by short infusion. Fluid resuscitation was minor or even absent, resulting in a hypodynamic septic state with low blood pressure and low cardiac output within a few hours. Most authors looked at global hemodynamic parameters like blood pressure and cardiac output and not at individual organ function. One should however question the relevance of these models. Indeed, in human septic shock we rather observe a hyperdynamic septic state with high cardiac output due to generous fluid administration, and low blood pressure due to low systemic vascular resistance needing vasopressor therapy. Recent reports about the beneficial effects of vasopressors and hemofiltration in an ovine peritonitis model (32,33) seem to be highly relevant to test the effects of hemofiltration in septic shock.

Wilfred Lieberthal

The renal artery occlusion model remains the most useful model currently available to study the mechanisms of ischemia-reperfusion injury to the kidney. I also believe that the glycerol model is the most suitable model for studying ARF due to rhabdomyolysis and is superior to myoglobin infusion for this purpose. Since the commonest cause of ARF in critically ill patients is sepsis, models of sepsis-induced ARF are most relevant to the aims of this survey. Our review indicates that many models that are widely used, such as LPS infusion, do not replicate the systemic events that occur in humans with sepsis. Also, small animals appear very resistant to sepsis. Sepsis induced by endogenous infection in larger animals, such as sheep, appear to be the best approach developed thus far for studying sepsis. However, even these models do not develop the ARF typical of sepsis in humans. Hopefully, ongoing work on the development of new models of sepsis, currently an active area of investigation, will yield better models in the near future.

As regards the isolated perfused kidney it is important for me to emphasize that there are two types of isolated perfused kidney models that have been studied, one perfused by a red cell-free perfusate (34) and another in which the perfusate is enriched with erythrocytes (35,36). These two models are very different from each other functionally and morphologically. In a perfusate that does not contain red cells, oxygen delivery is limited to the small amount of oxygen that can dissolve in the aqueous phase of the perfusate. In this system, the kidney is severely hypoxic. As a result the kidney is maximally vasodilated, cannot autoregulate perfusate flow and spontaneously develops severe necrosis of the mTAL segment. In this model, fractional sodium excretion is very high and the kidney cannot concentrate urine. By contrast, in the red cell-perfused kidney mTAL injury does not occur, renal vascular resistance is close to normal, and the kidney can autoregulate perfusate flow rate (36,37). Furthermore, in this system the fractional sodium excretion is much improved and the kidney concentrating capacity is maintained (36,38).

However, the most important difference between the two models is the morphologic pattern of injury that occurs in response to oxygen deprivation. Tubular injury in the red cell perfused kidney does not occur spontaneously as in the cell-free system, but can be induced by ischemia (temporarily stopping perfusate flow) (39) or by hypoxia (by reduction of oxygen tension of the red-cell containing perfusate)(35,40). Both ischemia and hypoxia in the red cell-perfused kidney are associated with proximal tubular injury while the mTAL remains relatively intact. It is also important to emphasize that injury to the mTAL does not develop in the hypoxic red cell-perfused kidney despite ongoing glomerular filtration and delivery of solute to the mTAL (35,40).

Furthermore, when isolated kidneys are perfused with red cell-free and red cell-containing perfusate at the same oxygen delivery rates, the proximal tubule remains the predominant site of injury in the presence of red cells and the mTAL segment the main site of necrosis in kidneys perfused without red cells (34,40). In my opinion, since the human kidney in vivo is never perfused without red cells, the extrapolations that have been made between morphology of the red cell-free perfused isolated kidney and ATN in humans are not valid. If the isolated kidney model is considered for the study of ARF for any reason, it should be the model that is perfused with red cells that should be used (35,36,40).

Joseph Bonventre

Fundamental to our understanding of various aspects of the pathophysiology of ARF is the use of animal models. Recognizing that they are not perfect, it is clear that they mimic many, albeit not all, of the features of human ARF. It is certainly true that ATN is rare in humans in the presence of ischemia alone. ATN is usually seen in the context of multiorgan failure, often on a background of vascular disease, the presence of anesthesia, sometimes in the presence of underlying chronic renal disease and frequently in the setting of sepsis. Because every aspect is not reproduced in the animal does not mean that we should ignore the value of the animal studies. It is in these models where specific features can be dissected. For example, using the clamp model of ischemia, we recently identified a protein (KIM-1) whose expression is upregulated

dramatically in the proximal tubule. This protein is also markedly upregulated in human ATN and may serve as an early marker for the disease (41).

Furthermore the lack of ability to demonstrate effectiveness of an agent in humans that had been effective in animals does not necessarily mean that the problem sits with the model. In fact the human clinical studies are often woefully under-powered, the agent is administered very late in the course of the ATN, and the population heterogeneity is not readily manageable in the context of the study. In order to alleviate the problem of late intervention it is critical to find better markers of early injury that are both sensitive and specific. With such markers we could intervene very early in the process and have a chance at finding an effective therapeutic agent.

With respect to comments previously raised discussing the isolated perfused kidney model, I believe that Dr. Lieberthal elegantly states when this model is useful and under what conditions.

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References

1. Bonventre JV, Brezis M, Siegel N, *et al.*, Edited by Lieberthal W and Nigam SK. Acute renal failure. I. Relative importance of proximal vs. distal tubular injury. *Am J Physiol* 1998, 275:F623-F632.
2. Brezis M, Rosen S. Hypoxia of the renal medulla: its implications for disease. *N Engl J Med* 1995, 332:647-655.
3. Heyman SN, Rosen S, Brezis M. The renal medulla: Life at the edge of anoxia. *Blood Purif* 1997, 15:232-242.
4. Molitoris BA, Weinberg JM, Manjeri A, *et al.*, Edited by Lieberthal W and Nigam SK. Acute renal failure. II. Experimental models of acute renal failure: imperfect but indispensable. *Am J Physiol* 2000, 278:F1-F12.
5. Rosen S, Heyman SN. Difficulties in understanding human "acute tubular necrosis": limited data and flawed animal models. *Kidney Int* 2001, 60:1220-1224.
6. Hammerman MR, Safirstein R, Harris RC, *et al.*, edited by Nigam SK and Lieberthal W. Acute renal failure. III. The role of growth factors in the process of renal regeneration and repair. *Am J Physiol* 2000, 279:F3-F11.
7. Piper RD, Cook DJ, Bone RC, *et al.* Introducing critical appraisal to studies of animal models investigating novel therapies in sepsis. *Crit Care Med* 1996, 24:2059-2070.
8. Harvig B, Engbert A, Ericsson JLE. Effects of cold ischemia on the preserved and transplanted rat kidney: structural changes of the loop of Henle, distal tubule and collecting duct. *Virchows Arch B Cell Pathol* 1980, 34:173-192.
9. Heyman SN, Brezis M, Rosen S. The isolated perfused rat kidney model in experimental renal injury. In "Clinical Nephrotoxins: Renal Injury from Drugs and Chemicals", Bennett WM, DeBroe ME, Porter GA and Verpooten GA (Eds), Kluwer Acad Pub, Dordrecht, 1998, pp 77-82.
10. Heyman SN, Reichman J, Brezis M. The pathophysiology of contrast nephropathy: a role for medullary hypoxia. *Invest Radiol* 1999, 34:685-691.
11. Goldfarb M, Abassi Z, Rosen S, *et al.* Chronic heart failure predisposes to hypoxic outer medullary tubular injury: studies in rats. *Kidney Int* 2001, 60:607-613.
12. Heyman SN, Rosen S, Darmon D, *et al.* Endotoxin-induced renal failure: II. a role for tubular hypoxic damage. *Exp Nephrol* 2000, 8:275-282.
13. Heyman SN, Rosen S, Epstein FH, *et al.* Loop diuretics reduce hypoxic damage to proximal tubules of the isolated perfused rat kidney. *Kidney Int* 1994, 45:981-985.

14. Spiegel DM, Shanley PF, Molitoris BA. Mild ischemia predisposes the S3 segment to gentamycin toxicity. *Kidney Int* 1990, 38:459-464.
15. Rosen S, Brezis M, Stillman I. The pathology of nephrotoxic injury: A reappraisal. *Miner Electrolyte Metab* 1994, 20:174-180.
16. Deng J, Kohda Y, Chiao H, *et al.* Interleukin-10 inhibits ischemic and cisplatin-induced acute renal injury. *Kidney Int* 2001, 60:2118-2128.
17. Shiraishi F, Curtis LM, Truong L, *et al.* Heme oxygenase-1 gene ablation or expression modulates cisplatin-induced renal tubular apoptosis. *Am J Physiol* 2000, 278:F726-F736.
18. Zager RA. Rhabdomyolysis and myohemoglobinuric acute renal failure. *Kidney Int* 1996, 49:314-326.
19. Heyman SN, Rosen S, Fuchs S, *et al.* Myoglobinuric acute renal failure in the rat: A role for medullary hypoperfusion, hypoxia and tubular obstruction. *J Am Soc Nephrol* 1996, 7:1066-1074.
20. Heyman SN, Goldfarb M, Darmon D, *et al.* Endotoxin-induced renal failure: I. a role for altered renal microcirculation. *Exp Nephrol* 2000, 8:266-274.
21. Shultz PJ, Raji L. Endogenously synthesized nitric oxide prevents endotoxin-induced glomerular thrombosis. *J Clin Invest* 1992, 90:1718-1725.
22. Deitch EA. Animal models of sepsis and septic shock: a review and lessons learned. *Shock* 1998, 9:1-11.
23. Wichterman KA, Baue AE, Chaudry IH. Sepsis and septic shock- a review of laboratory models and a proposal *J Surg Res* 1980, 29:189-201.
24. Fink MP, Heard SO. Laboratory models of sepsis and septic shock. *J Surg Res* 1990;49:186-199.
25. Heyman SN, Rosen S, Epstein FH, *et al.* Loop diuretics reduce hypoxic damage to proximal tubules of the isolated perfused rat kidney. *Kidney Int* 1994, 45:981-985.
26. Olsen TS, Hansen HE. Ultrastructure of medullary tubules in ischemic acute tubular necrosis and acute interstitial nephritis in man. *APMIS* 1990, 98:1139-1148.
27. Oliver J, McDowell M, Tracy A. The pathogenesis of acute renal failure associated with traumatic and toxic injury, renal ischemia, nephrotoxic damage and ischemic episode. *J Clin Invest* 1951, 30:1307-1351.
28. Heyman SN, Shina A, Brezis M, *et al.* Proximal tubular injury attenuates outer medullary hypoxic damage. Studies in the isolated perfused kidney. *Exp Nephrol* 2002, 10:259-266.

29. Brezis M, Heyman SN, Epstein F. Determinants of intrarenal oxygenation: 2. Hemodynamic effects. *Am J Physiol* 1994, 267:F1063-F1068.
30. Silva P, Rosen S, Spokes K, Epstein FH. Effect of glycine on medullary thick ascending limb injury in perfused kidneys. *Kidney Int* 1991, 39:653-658.
31. Weinberg JM, Nissim I, Roeser NF, *et al.* I. Relationships between intracellular amino acid levels and protection against injury to isolated proximal tubules. *Am J Physiol* 1991, 260:F410-F419.
32. Sun Q, Dimopoulos G, Nguyen N, *et al.* Low dose vasopressin in the treatment of septic shock in sheep. *Intensive Care Med* 2001, 27 Suppl 2:A113.
33. Rogiers P, Sun Q, Pauwels D, *et al.* Effects of hemofiltration with high cut-off membranine in ovine septic shock. *Intensive Care Med* 2001, 27 Suppl 2:A497.
34. Alcorn D, Emslie K, Ross B, Tange GRJ. Selective distal nephron damage and isolated perfused rat kidney. *Kidney Int* 1981, 19:638-647.
35. Endre ZH, Ratcliffe PJ, Tange JD, *et al.* Erythrocytes alter the pattern of renal hypoxic injury: predominance of proximal tubular injury with moderate hypoxia. *Clin Sci (London)* 1989, 76:19-29.
36. Lieberthal W, Stephens G, Wolf E, *et al.* Effect of erythrocytes on the function and morphology of the isolated perfused kidney. *Renal physiology* 1987, 20:14-24.
37. Lieberthal W, Wolf EF, Rennke HG, *et al.* Renal ischemia and reperfusion impair endothelium-dependent vascular relaxation. *Am J Physiol* 1989, 256:F894-F900.
38. Lieberthal W, Vasilevsky M, Valeri C, Levinsky N. Interactions between ADH and prostaglandins in the isolated erythrocyte-perfused kidney. *Am J Physiol* 1987, 252:F331-F337.
39. Lieberthal W, Rennke HG, Sandock KM, *et al.* Ischemia in the isolated erythrocyte-perfused rat kidney. Protective effect of hypothermia. *Renal Physiol Biochem* 1988, 11:60-69.
40. Endre ZE, Ratcliffe PJ. Patterns of ischemic renal cell injury. In: *Acute Renal Failure (First ed.)* edited by K Solez and L Racusen. Marcel Dekker Inc., New York, 1991, pp 173-181.
41. Han WK, Bailly V, Abichandani R, *et al.* Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int* 2002, 62:237-244.