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## Endothelial injury and dysfunction: Role in the extension phase of acute renal failure

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**Endothelial injury and dysfunction: Role in the extension phase of acute renal failure.** The pathophysiology of ischemic acute renal failure (ARF) involves a complex interplay between renal hemodynamics, tubular and endothelial cell injury, and inflammatory processes. A growing body of evidence supports the contribution of altered renal vascular function, especially at the microvascular level, in initiating and subsequently extending the initial tubular injury. The *extension phase* of ischemic ARF involves continued reduction in renal perfusion, ongoing hypoxia, and inflammatory processes that occur during reperfusion and contribute to continued tubular cell injury. Vascular endothelial cell injury and dysfunction play a vital part in this *extension phase*. With injury, the endothelial cell loses its ability to regulate vascular tone, perfusion, permeability and inflammation/adhesion. This loss of regulatory function has a detrimental impact upon renal function. Vascular congestion, edema formation, diminished blood flow, and infiltration of inflammatory cells have been documented in the corticomedullary junction of the kidney. However, linking their genesis to microvascular endothelial injury and dysfunction has been difficult. New diagnostic and therapeutic approaches to ischemic ARF must incorporate these findings to devise early recognition strategies and therapeutic approaches.

The cellular and molecular events involved in ischemic acute renal failure (ARF) have been identified and a much more complete understanding of how these events result in cellular and organ dysfunction has been established. Growing evidence indicates renal vascular endothelial injury and dysfunction play an important part in initiating and extending renal tubular epithelial injury and thus contribute to the ongoing pathogenesis of ischemic ARF. In fact, what is becoming rapidly apparent is that the lack of adequate renal cortical-medullary reperfusion may be more deleterious than the classical “reperfusion injury” secondary to oxygen and nitrogenous free radical formation. Therefore, the purpose of

this review is to summarize what is known about endothelial injury/dysfunction during and following ischemia in the kidney, and to place these data into a unifying hypothesis regarding immediate and delayed epithelial cell injury and organ dysfunction. Finally, how therapy of ARF should take into account the pathophysiology of endothelial dysfunction will be discussed.

### VASCULAR ENDOTHELIUM AS AN ORGAN

The concept of the endothelium as an organ has become widely appreciated [1] given the recent recognition of the consequences of endothelial injury and dysfunction in a range of disease states such as sepsis, hemolytic uremic syndrome/thrombotic thrombocytopenic purpura (HUS/TTP), diabetes, and hypertension. The endothelium regulates vascular permeability and modulates vasomotor, inflammatory, and hemostatic responses. Impairment of these vital endothelial cell functions during and following renal ischemia can contribute to the impairment of renal perfusion, continued renal hypoxia, and the subsequent epithelial cell injury and diminution in glomerular filtration rate (GFR) that are the hallmarks of ARF. Endothelial cells in different vascular beds have different characteristics [2]. Therefore, as the environment within the kidney changes dramatically from the outer cortex to the medullary region, endothelial cells may differ considerably within the kidney from region to region.

### EXTENSION PHASE OF ARF

The *extension phase* of ischemic ARF is ushered in by two major events: (1) continued hypoxia following the initial ischemic event and (2) an inflammatory response [3]. Both events are more pronounced in the corticomedullary junction (CMJ), or outer medullary region, of the kidney. Documentation of severely reduced blood flow, stasis, and accumulation of red blood cells and white

blood cells has been historically noted; however, the epithelial ramifications of these events have only recently been uncovered [4, 5]. It is during this phase that renal vascular endothelial cell damage likely plays a key role in the continued ischemia of the renal tubular epithelium, as well as, the inflammatory response observed with ischemic ARF. During this phase, S<sub>3</sub> proximal tubule and thick ascending limb cells, as well as endothelial cells, continue to undergo injury and death with both necrosis and apoptosis being present predominantly in the outer medulla [6]. In contrast, the proximal tubule cells in the outer cortex, where blood flow has returned to near normal levels, undergo cellular repair and improve morphologically during this phase. As cellular injury continues in the CMJ region during the *extension phase*, the GFR continues to fall. There is continued production and release of chemokines and cytokines that further enhance the inflammatory cascade.

### ISCHEMIC ARF—ALTERATIONS IN RENAL PERFUSION

A decrease in renal blood flow is of critical importance in initiating and extending the pathophysiology of ischemic ARF. Under physiologic conditions, the oxygen tension of the kidney decreases as one moves from the outer cortex to the inner medulla [7]. Regional alterations in renal blood flow persist after the initial ischemic event and play an important role in the *extension phase* of renal ischemic injury. During reperfusion a reduction in total renal blood flow of 40% to 50% of normal has been reported in both animal models of ischemic ARF and in human ischemic ARF [8]. Studies have demonstrated a persistent reduction in renal blood flow contributes significantly to the diminished GFR observed in human renal allografts following ischemic ARF [9]. These persistent perfusion deficits have been demonstrated to be of greater magnitude in the outer medulla than in the outer cortex or inner medulla in an animal model of ischemic ARF [10, 11].

Mechanisms involved in the alteration of renal perfusion following ischemic injury are incompletely understood. An imbalance between mediators of renal vasoconstriction and renal vasodilatation has been proposed to play a role in animal models of ischemic ARF. In support of this, antagonists to endogenous vasoconstrictors have been shown to ameliorate renal ischemic injury in animal models [12–14]. The role various vasoreactive mediators may play in controlling renal vascular tone following ischemic injury has been the subject of a recent review [15].

Congestion of the renal microcirculation, especially in the peritubular capillaries of the outer medullary region (vasa recta), contributes to deficits in renal perfusion. Accumulation of red blood cells and leukocytes in the

outer medulla has been demonstrated in animal models of ischemic ARF as well as in human ischemic ARF [10, 16–18]. This medullary congestion has been proposed to shunt blood flow away from the outer medulla resulting in continued hypoxia and cellular injury in this area. Experimental maneuvers to diminish trapping of red blood cells and leukocyte attachment in the renal microcirculation have been demonstrated to improve morphologic and functional aspects of renal injury in animal models of ischemic ARF [14, 19–22].

### ISCHEMIC ARF—MORPHOLOGY OF VASCULAR INJURY

Interestingly, no consistent morphologic changes of the renal vascular endothelium in ischemic ARF have been reported. In part this may be due to sampling bias in human ischemic ARF (i.e., predominantly cortical vs. medullary tissue on biopsy) and difficulty in visualizing the endothelium of potentially affected microvasculature in animal models. However, Sutton et al [23], using TIE2/GFP mice with selective endothelial cell expression of GFP, have shown actin cytoskeletal and junctional alterations occur in renal microvascular endothelial cells during ischemic injury in vivo.

Evidence of endothelial dysfunction and injury in other organ systems, as a result of ischemic injury, lends credence to the concept that endothelial dysfunction and injury play an important role in ischemic ARF. Separation of endothelial tight junctions, loss of endothelial attachment to the basement membrane, endothelial blebbing, and endothelial necrosis have been described in the cerebral and coronary vasculature following ischemic injury [24, 25]. In patients experiencing septic shock, a condition which shares many pathologic derangements with ischemic injury and is often a concomitant condition in human ischemic ARF [26], detached, circulating endothelial cells have been documented [27]. Potential functional consequences of these morphologic alterations include altered vascular reactivity, increased vascular permeability, increased leukocyte adherence and extravasation, and altered coagulation due to loss of normal endothelial function and/or barrier. Furthermore, circulating activated endothelial cells could potentially contribute to distant organ effects often attributed to leukocytes, such as pulmonary dysfunction following ischemic ARF [28].

### ISCHEMIC ARF—FUNCTIONAL ASPECTS OF ENDOTHELIAL INJURY

#### Alterations in endothelial permeability

Increased renal peritubular capillary permeability has been documented as a consequence of ischemic ARF in animal models [21–23]. Two general mechanisms can

account for increased endothelial permeability during ischemic injury: (1) increased paracellular permeability and/or (2) increased transcellular permeability [29]. Increased interstitial edema may contribute to further diminishing the compromised medullary blood flow by compressing peritubular capillaries [30]. Additionally, leakage of plasma from the vascular space through a leaky endothelium contributes to hemoconcentration that can lead to stasis and diminished perfusion in the CMJ as observed in other organs [31]. Hemoconcentration and stasis also increases the potential for endothelial-leukocyte interactions. Activated leukocytes can initiate an inflammatory cascade that leads to further endothelial cell injury and further dysfunction of the endothelial permeability barrier [32]. This may be particularly important in the medullary region as endothelial cells there, but not in the cortex, express surface markers important in lymphocyte activation [33].

#### Alterations in endothelial-leukocyte interactions

Endothelial-leukocyte interactions mediated through complementary adhesion molecules on endothelial cells and leukocytes play a key role in the local accumulation of leukocytes. Ischemic injury has been demonstrated to increase expression of P- and E-selectin on the surface of endothelial cells [34, 35]. Increased expression of intercellular adhesion molecule-1 (ICAM-1) by endothelial cells has also been demonstrated in vitro in response to oxidant injury [36]. The functional significance of these findings in ischemic ARF is underscored by evidence in animal models that inhibition of P- and E-selectin-mediated binding of leukocytes [20] and inhibition of ICAM-1-mediated binding of leukocytes [37] decreases renal injury. Endothelial cell injury and dysfunction may additionally contribute to the inflammatory response through loss of normal endothelial nitric oxide production [5, 38].

#### Alterations in coagulation

Fibrin deposition in the microvasculature following ischemic injury has been noted in a variety of organs systems, including the kidney [39]. Although the constitutive state of endothelial cells is an anticoagulant state, injury and activation of endothelial cells can induce a procoagulant response. Whether or not this occurs during prerenal azotemia is unknown. Furthermore, loss of nitric oxide production by injured endothelial cells may also contribute to an overall procoagulant state through loss of its inhibitory role on cytokine-induced expression of tissue factor [40]. While abnormalities in coagulation per se may have a deleterious role in ischemic ARF, recent studies have shed light on the relative importance the coagulation cascade plays as a mediator of inflammation in ischemic ARF [41, 42].

#### FUTURE THERAPIES AIMED AT THE EXTENSION PHASE

Recent advances made toward delineating the cellular mechanisms involved in ischemic ARF have not yet led to accepted therapeutic interventions that alter the natural course of ARF or improve the clinical outcome for ARF [43]. Reviews outlining potential therapeutic strategies for the treatment of ARF, and the barriers minimizing effective therapies, have been recently published [32, 44]. Human ARF is heterogeneous in its pathophysiology. Consequently, combined therapies targeting more than one pathophysiologic pathway may prove to be the most beneficial approach [32, 44]. It is also necessary to point out that differences may exist between animal models of ARF and what has been documented to occur during human ARF [45, 46]. However, a major problem is the lack of clinical biopsy data, especially in the CMJ area, during the early phases, initiation and extension, of human ARF. This has limited the ability to directly compare human and animal models of ischemic ARF.

Therapies directed at processes during the *extension phase*, including endothelial dysfunction/injury, and its myriad of pathophysiologic implications, should have a role in any therapeutic approach. Interrupting the amplification of this hypoxic and inflammatory cascade has important therapeutic implications. Therefore, early recognition of renal injury and prompt intervention remain important clinical challenges in this field [47].

#### CONCLUSION

A growing body of evidence lends support to the role endothelial dysfunction plays in overall renal injury during ischemic ARF. This may be especially important during the early events of ischemic ARF. Given that the endothelium is central to the myriad of biologic processes performed by the microvasculature, endothelial injury and dysfunction are crucial factors in the overall alteration of vascular function during both the *initiation* and the *extension phase*. Further investigation into the mechanisms of endothelial injury and dysfunction during the *extension phase* should provide further insight into the pathophysiology of ischemic ARF and reveal additional, as well as, novel therapeutic interventions.

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