Uremic Cardiomyopathy and Insulin Resistance: A Critical Role for Akt?

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ABSTRACT

Uremic cardiomyopathy is a classic complication of chronic renal failure whose cause is unclear and treatment remains disappointing. Insulin resistance is an independent predictor of cardiovascular mortality in chronic renal failure. Underlying insulin resistance are defects in insulin signaling through the protein kinase, Akt. Akt acts as a nodal point in the control of both the metabolic and pleiotropic effects of insulin. Imbalance among these effects leads to cardiac hypertrophy, fibrosis, and apoptosis; less angiogenesis; metabolic remodeling; and altered calcium cycling, all key features of uremic cardiomyopathy. Here we consider the role of Akt in the development of uremic cardiomyopathy, drawing parallels from models of hypertrophic cardiac disease.

its characterization in the uremic heart remains incomplete.

**AKT SIGNALING PATHWAY**

Akt, known previously as protein kinase B, is a serine/threonine protein kinase with homology to protein kinase A and protein kinase C. Three isoforms of Akt exist in mammals (Akt 1 through 3), although in the heart, Akt1 and Akt2 predominate.42 In resting cells, Akt resides in the cytosol, and stimulation of either PI3K/PIPK1 or insulin or IGF-1, or PI3K/PIPK2, through G-protein–coupled receptors, leads to generation of phosphatidylinositol-3,4,5-trisphosphate and recruitment of Akt to the plasma membrane (Figure 2).43 There, Akt is phosphorylated at two regulatory sites by phosphoinositide-dependent kinases 1 and 2, respectively.

Phosphorylation of both regulatory sites is required for full activation of Akt,44 which has a number of intracellular targets (Figure 2). The consequences of Akt activation vary greatly depending on the route of activation and its duration and the specific isoform affected. For example, in normal health, insulin-PI3Kα-Akt signaling induces physiologic hypertrophy, yet insulin-stimulated Akt activity also augments pathologic hypertrophy in the context of pressure overload45; Akt activation through G-protein–coupled receptors and PI3Kγ results in pathologic hypertrophy.46–48 Furthermore, whereas Akt1 is the dominant isoform implicated in regulation of postnatal cardiac growth,49 Akt2 plays the dominant role in coronary angiogenesis, glucose metabolism, and cell survival.50 It is this variation that makes Akt such a vital signaling component.

**UREMIC CARDIOMYOPATHY**

The cardinal features of uremic cardiac disease are LVH, reduced capillary density, fibrosis, and ventricular remodeling. Of these, LVH is predominant, increasing in prevalence from 26% of patients with stage 3 CKD to 75% of hemodialysis patients.51 Hypertrophy is a powerful independent predictor of survival in CKD52,53 and regression of LVH is associated with reduced cardiovascular risk and improved survival.54 Ventricular Hypertrophy Cardiac hypertrophy is an adaptive response to a variety of physiologic and pathologic stresses; however, hypertrophied hearts are more susceptible to in-
The phosphorylation of Akt.45
of hyperinsulinemia and increased phosphorylation, which directly correlates with the degree of pathologic hypertrophy, the degree of pressure overload accentuates thermore, high carbohydrate feeding/H9253 Akt through GPCR-PI3K pathway directly,63 long-term treatment pertrophic stimuli do not activate this axis.49,59 – 68Although pathologic hypertrophy when Akt1 overexpression was induced in adult mice for 2 to 6 weeks.73 The transition to pathologic hypertrophy occurs in association with a decrease in capillary density, a feature typical of uremic cardiomyopathy. In contrast, nuclear-targeted overexpression of Akt1 does not lead to pathologic hypertrophy; instead, hearts display increased numbers of cardiomyocytes, enhanced contractility, and protection from ischemia-reperfusion injury (IRI).74,75
Thus, the insulin-Akt1 pathway is involved in both physiologic and pathologic cardiac hypertrophy. Both the route and context of activation (presence of concurrent stimuli for pathologic hypertrophy) are important in determining which will dominate, as are the duration and subcellular localization of Akt activity. In the human heart, Akt activity increases as hypertrophy deteriorates into heart failure,76 although it is not clear whether this is a causal relationship. Experimental studies showed perturbations in both total and phosphorylated Akt in the uremic rat51,52 but did not determine the differential expression or activation of Akt1 versus Akt2; however, uremia exposes the heart to long-term increase of serum insulin concentrations in the context of increased afterload, a situation that worsens pathologic cardiac hypertrophy in association with increased total Akt phosphorylation.45

**Angiogenesis**

Physiologic growth of cardiomyocytes is accompanied by increased angiogenesis maintaining capillary density. In experimental and clinical studies of CKD, capillary growth failed to keep pace with myocyte hypertrophy,77,78 resulting in decreased density. This effect is not seen in experimental essential hypertension, suggesting it is specific to uremic cardiomyopathy.

Coronary angiogenesis is under the dual control of vascular endothelial growth factor (VEGF) and angiopoietin-2 (Ang-2), both of which are upregulated during short-term cardiac Akt1 overexpression associated with physiologic hypertrophy.73 In contrast, after chronic overexpression of Akt1 and consequent pathologic hypertrophy, VEGF and Ang-2 are downregulated in association with reduced capillary density.73 Furthermore, short-term overexpression of Akt1 produces pathologic hypertrophy when VEGF is inhibited simultaneously,73 and inhibition of VEGF during pressure overload accelerates the transition to heart failure.79

Thus, during chronic experimental Akt1 activation, the stimulus for myocyte growth is maintained, but the stimulus for capillary growth declines, resulting in a drop in myocardial capillary density that contributes to the transition to heart failure. A similar state likely occurs within the uremic heart.73,78

**Fibrosis**

Cardiac fibrosis in uremia has been recognized since the 1940s and was found in ex-
perimental and postmortem studies of CKD. It is of the reactive type, a consequence of endothelial-to-mesenchymal transition followed by activation and proliferation of new interstitial fibroblasts. Compared with control hearts with a similar degree of hypertension and LVH, the uremic cardiac interstitium demonstrates increased expression of proinflammatory mediators, such as PDGF, with correspondingly increased fibrosis, suggesting renal disease enhances myocardial fibrosis. This interstitial fibrosis contributes to ventricular stiffness and diastolic dysfunction and cardiac dysrhythmias and may further compromise molecular exchange between cardiomyocytes and capillary bed.

Studies investigating the mechanisms underlying this fibrosis are scarce; however, experimental work implicates signaling through mammalian target of rapamycin (mTOR), a downstream target of Akt. Although there was no evidence of increased phosphorylation of Akt in these studies, neither specific phosphorylation of Akt1 and Akt2 nor Akt activity was determined. Furthermore, the development of insulin resistance was not determined; therefore, although the delineation requires further clarification, fibrosis in CKD is mediated in part by activation of a profibrotic intracellular signaling mechanism involving mTOR. Furthermore, in a nonuremic model, chronic hyperinsulinemia produced pathologic hypertrophy and fibrosis by activation of a complex network of intracellular pathways, including Akt, whereas the use of peroxisome proliferator–activated receptor γ (PPAR-γ) agonists in models of salt-sensitive hypertension decreased cardiac hypertrophy and fibrosis in association with reduced Akt phosphorylation. Furthermore, as mentioned already, chronic overexpression of Akt1 can produce cardiac fibrosis associated with LVH.

Thus, although cardiac fibrosis is a feature of the uremic heart, knowledge of the underlying mechanisms is still sparse. There is direct evidence that downstream targets of Akt are involved, and evidence from nonuremic models confirms that perturbations in Akt signaling induce cardiac fibrosis. The potential role of Akt in this process deserves further clarification.

**Apoptosis**

Outcomes after acute myocardial infarction in CKD remain poor, despite optimal conventional therapy, and experimental studies showed the increased susceptibility to IRI of uremic hearts. Previous work on IRI demonstrated that cell death occurs through necrosis and apoptosis and that inhibiting apoptosis during reperfusion significantly improves outcomes. Cardiomyocyte apoptosis also plays a causal role in the development of uremic nonuremic heart failure, whereas inhibiting apoptosis reduces cardiac dysfunction in heart failure.

Maintaining cardiac function requires timely de novo production of ATP; however, the production of ATP is reduced within the uremic heart, as evidenced by a decreased phosphocreatine-ATP ratio. In addition to uremic heart, this restriction has multiple causes, including decreased oxygen and substrate supply as a result of impaired capillary–myocyte exchange, metabolic remodeling, and alterations in creatine kinase. Compromised ATP synthesis also results in a loss of mitochondrial membrane potential and functional deterioration, resulting in a further decline in ATP production and contributing to the inability of the uremic heart to adapt to hemodynamic alterations. A situation similar to that in the failing human heart, Mitochondrial damage causes the release of cytochrome c triggering apoptosis and cell death, jeopardizing the survival of remaining cardiomyocytes. The importance of apoptotic cell death in the transition from compensated hypertrophy to heart failure is paramount. Akt stimulation is a potent antiapoptotic signal, and evidence demonstrates that upregulation of the PI3K-Akt pathway, by either administration of insulin/IGF-1 or genetic manipulation, reduces apoptosis and improves functional recovery in the face of IRI. Akt activation does not protect against cell loss from IRI and is in fact detrimental, potentially as a result of Akt-mediated inhibition of P13K, again highlighting important differences between acute and chronic activation of the Akt pathway.

**Remodeling**

**Metabolic**

Under normal conditions, the adult heart displays a preference for oxidation of fatty acids, with 60 to 90% of ATP production resulting from this route and the remaining 10 to 40% from glucose and lactate and a small fraction from ketones; however, LVH is associated with downregulation of fatty acid oxidation and upregulation of glucose oxidation. One explanation for this is a switch to “oxygen efficient” fuels during times of metabolic stress. Although this may be initially compensatory, it may contribute to cardiac injury by lipotoxicity or loss of metabolic flexibility. Certainly, in models of pressure overload hypertrophy, although cardiac basal glucose uptake is increased, insulin-stimulated uptake is impaired. The mechanism for this involves reduced GLUT4 translocation, rather than changes in GLUT4 or GLUT1 expression. Within the uremic heart, expression of GLUT4 and GLUT1 transporters are also unchanged, although there is also some evidence for a defect in GLUT4 translocation in the early stages of experimental uremic cardiomyopathy; the situation in more advance uremic cardiomyopathy is unknown.

Akt2 regulates glucose influx into cardiomyocytes by increasing translocation of GLUT4 while concurrently decreasing fatty acid oxidation through downregulation of transcription factors, such as PPAR-α and PPAR-γ coactivator-1, which are involved in regulation of fatty acid oxidation. The net effect of acute Akt2 stimulation is increased glucose and decreased fatty acid oxidation, a beneficial effect during hypoxic conditions; however,
once again, the consequences of acute and chronic Akt activation differ, with chronic stimulation increasing basal but significantly blunting insulin-stimulated glucose uptake as a result of decreased GLUT4 expression in insulin-sensitive intracellular vesicles,\(^\text{122}\) a picture similar to that seen in hypertrophy with uremia. Reduced myocardial insulin sensitivity could lead to a decreased ability to increase ATP generation in times of need or alter substrate use to match supply, leaving it susceptible to energy depletion. Furthermore, chronic increases in glucose uptake enhance glucose metabolism through non–ATP-generating pathways, including the oxidative pentose phosphate and hexosamine biosynthetic pathways, both of which contribute to myocardial fibrosis and cell death.\(^\text{113}\)

Calcium Cycling

Uremia is associated with depressed cardiac function at the level of the myocyte,\(^\text{123–125}\) independent of gross alterations in cardiac structure, or \(\beta\)-adrenoreceptor desensitization.\(^\text{123,124}\) Although the underlying mechanism is not fully understood, two groups independently identified prolongation of the calcium transient,\(^\text{123,125}\) without a change in amplitude or rate of sarcoplasmic reticulum calcium release.\(^\text{125}\) Recovery of the calcium transient is dependent on the sarcoplasmic calcium-ATPase (SERCA2a) and the sarclemma sodium–calcium exchange. In clinical and experimental studies, the transition from compensated hypertrophy to heart failure was associated with downregulation of SERCA2a expression,\(^\text{126,127}\) which was also seen in experimental uremia.\(^\text{123}\) Consequently, the uremic cardiomyocyte is desensitized to calcium, requiring greater diastolic and systolic intracellular calcium concentrations to maintain contraction.\(^\text{123}\) The delayed recovery of the calcium transient and increased diastolic intracellular calcium may translate into clinical diastolic dysfunction.

The insulin-Akt signaling pathway impinges on calcium cycling at several stages, with the net effect of chronic activation of Akt increasing the size of the calcium transient and thus myocyte contraction. Akt acts on L-type calcium channels,\(^\text{128,129}\) enhancing calcium influx, and sarcoplasmic reticulum calcium reuptake, increasing expression of SERCA2a\(^\text{130}\) and phosphorylation of phospholamban.\(^\text{79}\) Whether these components are direct substrates for Akt is not known; however, the reduced expression of SERCA2a seen in uremia might be predicted by a defect in Akt signaling related to uremic insulin resistance.

**CONCLUSIONS**

Insulin resistance is an established integral component of the uremic syndrome and suggests disruption in the balance of insulin’s pleiotropic and metabolic actions. This imbalance may be more important than any individual defect and is reflected by differential alterations in the intracellular signaling pathways of insulin. In particular, the Akt pathway displays a discrete defect in Akt2 activity alone in other insulin-resistant states. As this Akt2 defect produces a compensatory hyperinsulinemia, Akt1 signaling may actually be upregulated, exacerbating the imbalance between Akt1 and Akt2 activity. This differential may explain many of the phenotypic features of the uremic heart. In particular, decreased activity of Akt2 predicts impaired insulin-stimulated glucose uptake, increased susceptibility to IRI, and a reduction in SERCA2a with its consequent effects on diastolic function. In turn, the preserved or chronically enhanced Akt1 effects produce cardiac hypertrophy associated with fibrosis and reduced capillary density. The overall effect generates a convincing mimic for uremic cardiomyopathy.

Although other factors and intracellular mechanisms are undoubtedly also of critical importance in the development of uremic cardiac disease, insulin resistance and alterations in Akt signaling warrant further investigation, particularly because therapies to manipulate this system are already in clinical practice and could be rapidly applied to the treatment of uremic cardiomyopathy. Already there is experimental evidence for thioglitazones increasing Akt activity and ameliorating IRI in ischemia/reperfusion models\(^\text{131,132}\) and hypertrophy in pressure overload,\(^\text{84}\) whereas rapamycin, targeting mTOR downstream of Akt, reduces cardiac hypertrophy and fibrosis in uremic mice.\(^\text{80}\) With proper clinical study, targeting future therapies at these underlying cellular mechanisms of uremic cardiomyopathy may finally start to reduce the burden of uremic cardiomyopathy in the CKD population.

**ACKNOWLEDGMENTS**

This work was supported by a clinical research fellowship from the Hull York Medical School (Kingston-upon-Hull, UK).

**DISCLOSURES**

None.

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