

## UREMIC CARDIOMYOPATHY: ROLE OF CIRCULATING DIGITALIS LIKE SUBSTANCES

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### 1. ABSTRACT

Patients with chronic renal failure develop a cardiomyopathy characterized by marked diastolic dysfunction and left ventricular hypertrophy. Interestingly, they also have substantial increases in the circulating concentrations of digitalis like substances. Digitalis like substances produce reactive oxygen species as part of the signal cascade induced by binding to the sodium pump and patients, and this signal cascade appears to induce hypertrophy of cardiac myocytes grown in culture. Also, patients with chronic renal failure develop an oxidant stress state without a known mechanism. From these data, we propose that it is these digitalis like substances which cause cardiomyopathy of renal failure as well as the systemic oxidant stress state.

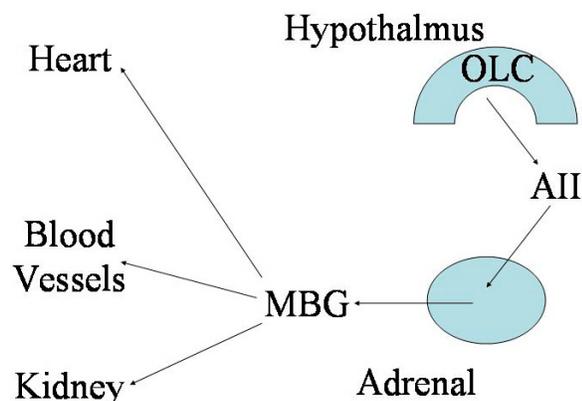
### 2. INTRODUCTION

The existence of circulating inhibitors of the Na/K-ATPase or digitalis like substances (DLS) which accumulate in chronic renal failure was proposed more than 4 decades ago. This topic is extensively reviewed elsewhere in this symposium. Support for this concept

includes the observation that the plasma or serum of patients with chronic renal failure inhibit in vitro assays of the Na/K-ATPase, that some digitalis assays are positive in patients with chronic renal failure not treated with digitalis, and finally the demonstration that patients with chronic renal failure have elevated levels of cardiac steroids structurally related to digitalis with immunological assays and, quite recently, with more definitive analytic techniques.

Although the mechanism for the elevation of these DLS in chronic renal failure may include a combination of decreased renal elimination and increased production, it is very clear that one would anticipate the development of such elevations if they were, as had been postulated, natriuretic hormones which increased sodium excretion under conditions where sodium loading occurred and/or renal elimination of sodium was impaired by loss of renal function. To this end, increases in the circulating concentrations of bufodienalydes have been consistently noted with both salt loading and renal insufficiency. In contrast, the changes in circulating concentrations of

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**Figure 1.** Schematic illustrating how OLC and MBG interact and cause end-organ effects.

ouabain have been rather inconsistent, and it has been speculated that ouabain functions more as a neurohormone. This has also been extensively reviewed elsewhere in this symposium.

Currently, the clinical treatment of patients with chronic renal failure is complicated by the almost universal development of a “uremic cardiomyopathy” which is characterized by diastolic dysfunction and progressive left ventricular hypertrophy. In this review we will examine the possibility that signaling through the Na/K-ATPase by these circulating DLS may contribute to the development of this uremic cardiomyopathy.

### 3. DLS IN CHRONIC RENAL FAILURE

In the late 70s and early 80s, it became clear that patients with chronic renal failure often had “false positive” digoxin levels (i.e., digoxin was detected when the patient wasn't taking this medication) with some antibodies (1, 2). In addition to playing a role in sodium balance, Bricker and others proposed that effects of DLS on other tissues explained aspects of the uremic syndrome which complicates chronic renal failure. The term “trade off hypothesis” was coined to explain this phenomenon (3).

The evidence for a circulating inhibitor of Na/K-ATPase in chronic renal failure is extensive. Many, if not most, of the studies in this area have used red blood cell Na/K-ATPase activity as a model to examine pump activity in uremic subjects. With very few exceptions, decreases in red blood cell sodium pump activity have been consistently found. The findings of reduced pump activity have also been observed in white blood cells, adipocytes, transporting epithelia, and muscle cells. In large part, most of the inhibition of the sodium pump can be attributed to a dialyzable (e.g., relatively small molecular weight substance) substance whose “concentration” in uremic plasma also tracks with extracellular fluid volume (4-9).

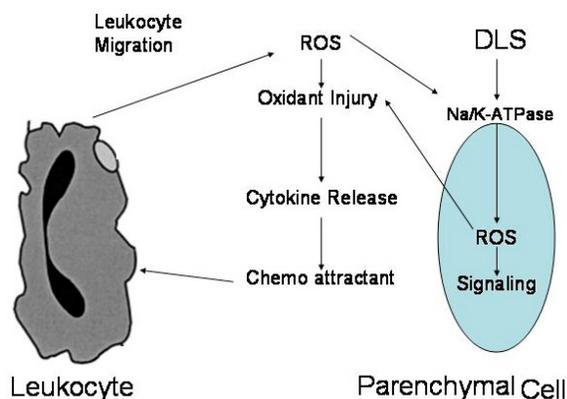
Our understanding of this issue has evolved considerably. It now appears that ouabain, the prototypical digitalis glycoside (or something structurally quite similar), is essentially a neurohormone, and that MBG (or something

structurally quite similar) is the circulating DLS which has a substantial effect on the alpha-1 isoform of the Na/K-ATPase (especially in rodents), MBG concentrations in the plasma increase in a variety of experimental and clinical settings associated with volume expansion and hypertension (10-13). Recently, it has been demonstrated that synthesis of this substance occurs in mammalian adrenal cells (14). Interestingly, modulation of the response of Na/K-ATPase to MBG appears to result from PKC inhibition (15). This concept is illustrated in Figure 1.

Of the circulating DLS that have been identified and characterized, ouabain has perhaps been the best. Ouabain is a cardiac steroid derived from plant tissue, and it is ouabain that is probably the DLS of first choice for study in laboratory preparations. As discussed above, a compound that is immunologically quite similar to plant derived ouabain can be detected in a number of mammalian tissues. Recent studies have isolated such an OLC from the hypothalamus of cattle, and identified this ouabain molecule to be an optical isomer of ouabain derived from plants. Artifacts determined during the isolation procedure require that further work remains for unequivocal determination of the chemical structure of OLC (16). In contrast, MBG and bufalin have also been detected in body fluids with Ab based assays, but the chemical identification of these substances have been a bit less rigorous. Studies from the laboratory of Dr. Bagrov have identified that his Ab assay recognizes a chemical with the retention time on an HPLC column and same mass determined with mass spectroscopy as amphibian derived MBG (17). Recent studies from the laboratory of Takahashi have unequivocally demonstrated MBG and telocinobugin, a closely related compound in human serum using mass-spectroscopy and NMR spectroscopy. These workers also found that the concentrations of these compounds were markedly increased in patients with chronic renal failure (18).

Na/K-ATPase as an energy transducing ion pump has been studied extensively since its discovery in 1957. Although early findings suggested that the enzyme also played a role in regulation of gene expression and cell growth (19-23), only in recent years have the mechanisms by which this plasma membrane enzyme communicates with the nucleus and other intracellular organelles been investigated (24-38). This research, performed mostly with neonatal rat cardiac myocytes, shows that in addition to pumping ions, the Na/K-ATPase interacts with neighboring membrane proteins and organized cytosolic cascades of signaling complexes to send messages to various intracellular organelles. The signaling pathways elicited by the interaction of ouabain with the enzyme are independent of changes in intracellular ion concentrations and contractility include activation of Src and Ras, transactivation of the EGFR, and increased production of ROS (24, 26, 27, 29, 33, 39). This topic is extensively reviewed elsewhere in this review, so we will not focus on it in this manuscript. Suffice it to say that the knowledge that ROS generation is critical to the genomic effects of signaling through the Na/K-ATPase suggests the possibilities that this ROS contributes in some meaningful

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**Figure 2.** Schematic demonstrating the potential relationship between oxidant stress and inflammation in patients with renal failure and elevated circulating levels of DLS.

way to the oxidant stress state associated with renal failure and that interference with ROS might be an effective strategy for preventing the cardiac disease seen with renal failure (vida infra).

That said, it is very clear that the development of a hypertrophic phenotype in neonatal cardiac myocytes, cell growth in both neonatal and adult cardiac myocytes, as well as some changes in ion concentrations stimulated by DLS can be blocked by a variety of anti-oxidant chemicals including a poorly characterized, but extremely potent, aqueous extract of green tea. We have tested the importance of such oxidant signaling in the pathogenesis of uremic cardiomyopathy and found green tea extract to markedly attenuate the development in an animal model of this condition (40).

### 4. OXIDANT STRESS IN CHRONIC RENAL FAILURE

Our group and others first proposed that oxidant stress contributed to the progression of chronic renal failure in the mid 1980s. The concept which we proposed was that oxygen consumption by the chronic renal failure kidney could not be explained by the amount of tubular sodium transport performed in the setting of a reduced glomerular filtration rate. This observation was followed by the somewhat surprising observation that rather than oxidant stress being limited to the diseased kidney, there appeared to be systemic oxidant stress in patients with chronic renal failure. Although the treatment modality used to treat the renal failure was initially suspected as the source of the oxidant stress, more recent studies clearly indicate that oxidant stress appears to complicate the chronic renal failure itself. Patients with chronic renal failure consistently demonstrate elevations in circulating levels of oxidized proteins and byproducts of lipid peroxidation. This oxidant stress has been implicated in the pathogenesis of uremic cardiovascular disease on several levels (41).

There has been a tremendous amount of speculation regarding why there is oxidant stress in chronic

renal failure. As mentioned above, the modality itself has been implicated. Specifically, interactions between the hemodialysis membrane and circulating proteins and/or white blood cells has been postulated to produce oxidant stress. However, the similar degrees of protein and lipid oxidation products observed in patients with chronic renal failure not yet treated with hemodialysis (or other modalities) and the lack of increase of oxidant stress in those treated for some time with dialysis strongly suggest it is the chronic renal failure which causes the oxidant stress (42). At the time that this symposium is being reported, the mechanism for this systemic oxidant stress is not known. However, a tight link with inflammation suggests that circulating white blood cells, perhaps themselves activated by uremic toxins, contribute to this oxidation stress (43, 44). This schematic is shown in Figure 2.

### 5. CLINICAL UREMIC CARDIOMYOPATHY

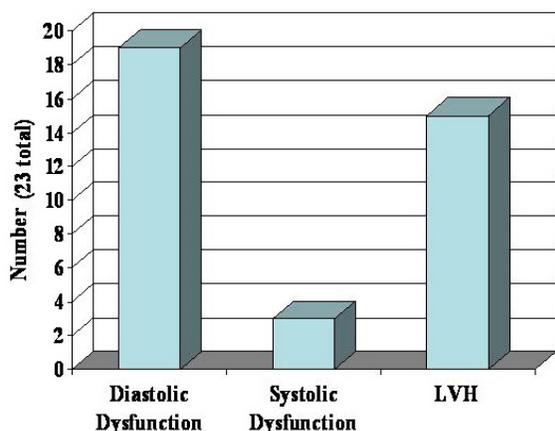
The current treatment of patients with chronic renal failure is complicated by the tremendous cardiovascular mortality associated with it. Recent studies demonstrate that mortality rates in ESRD patients remain extremely high in the United States. More than 50% of this mortality can be attributed to cardiac causes (45, 46). Conversely, it is not as if the high mortality seen with HD and peritoneal dialysis (PD) patients is simply due to complications of the therapies. Recent data strongly suggest that pre-ESRD patients have similar cardiac mortality rates as patients with ESRD (46-49).

A number of studies utilizing echocardiography have demonstrated that both left ventricular hypertrophy (LVH) and diastolic dysfunction (as assessed by left ventricular, atrial and pulmonary venous doppler flow studies) are extremely common in end stage renal disease (ESRD) patients treated with HD (50), as well as patients incident to ESRD (51-53). In Figure 3, we show data from our center which indicate that most patients incident to HD treatment have demonstrable diastolic dysfunction and LVH, whereas systolic dysfunction is relatively rare. In general, most studies have demonstrated that there is a very strong association between diastolic dysfunction and LVH which are both common in this population, whereas systolic dysfunction is much less often demonstrable (54). Parfrey and colleagues have demonstrated that the development of LVH in HD patients predicts a high mortality rate (54). Others point out a correlation between the degree of LVH as assessed by left ventricular mass index (LVMI) and the occurrence of ventricular arrhythmias in hemodialysis patients (55-57). In short, ESRD patients treated with HD have a high prevalence of diastolic dysfunction and LVH, and the clinical implications of these cardiac abnormalities, especially in this population, are grave.

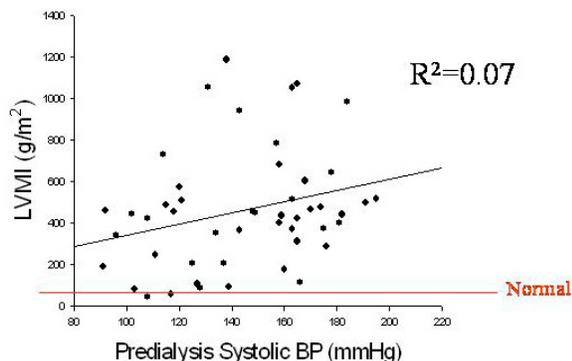
### 6. PATHOGENESIS OF UREMIC CARDIOMYOPATHY

Several pathogenetic factors known, or believed to contribute to, cardiomyopathy are present in patients with chronic renal failure to varying degrees. Specifically,

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**Figure 3.** Prevalence of diastolic dysfunction (determined by the E/A wave ratios), systolic dysfunction (determined by fractional shortening) and LVH (determined by calculation of LVMI) in 23 incident ESRD patients studied with echocardiography at the Medical College of Ohio Hospitals.



**Figure 4.** Correlation between LVMI determined with echocardiography and systolic blood pressure in 71 patients followed at the Medical College of Ohio Hospitals.

anemia, hypertension and parathyroid hormone have been implicated as potential pathogenetic factors.

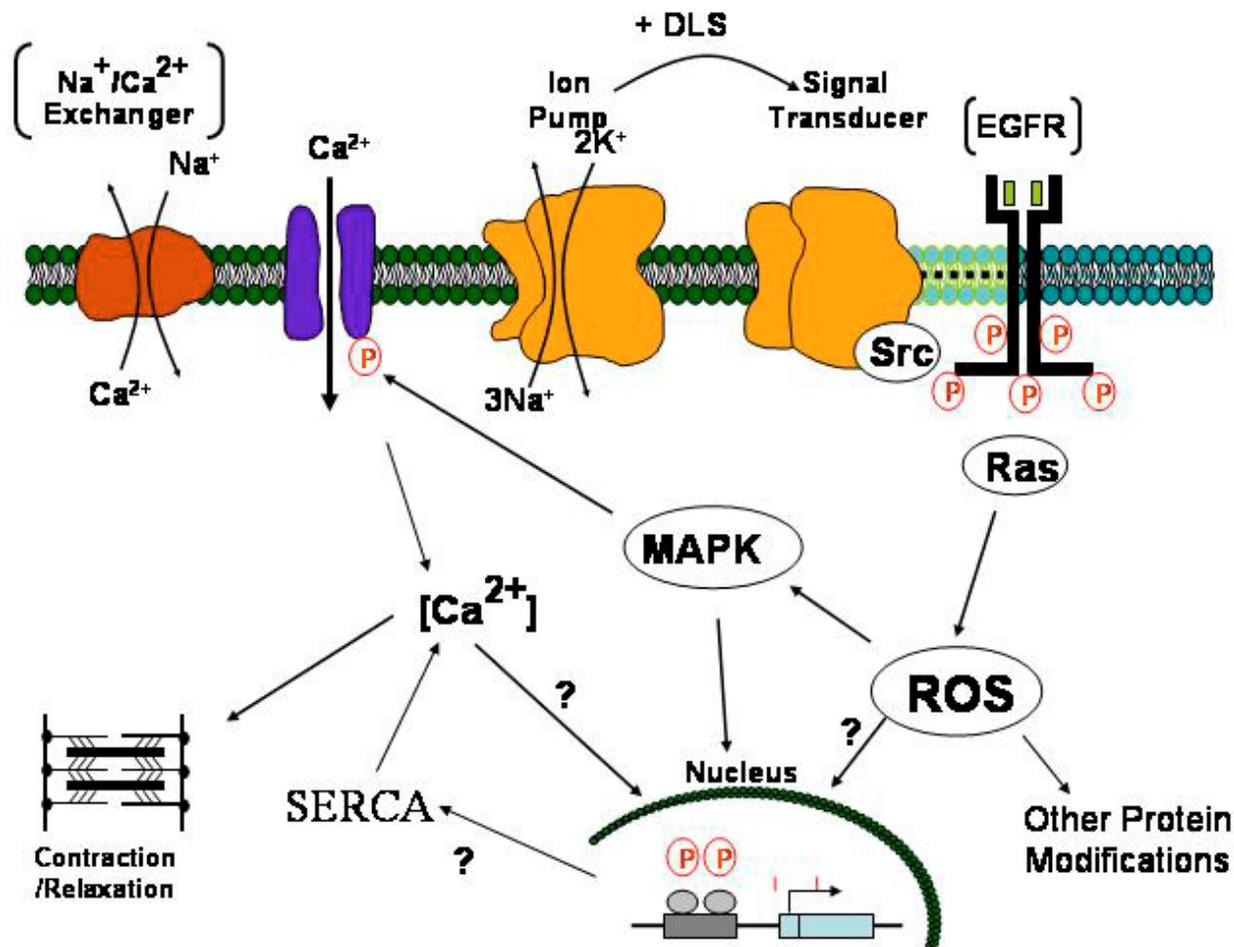
Anemia commonly complicates chronic renal failure, and is essentially the rule in ESRD patients treated with intermittent HD. Probably the best data to support a pathophysiological role for anemia in the LVH of ESRD patients is the observation that treatment with recombinant erythropoietin may actually cause regression in LVH to some degree. In some studies, a 10-20% reduction in LVMI has been observed with sustained increases in hematocrit (58-60). In contrast, the type of hypertrophy observed in ESRD patients is usually of the concentric type (50); this is not the hypertrophy pattern that one would expect if anemia were the dominant factor (see below). Also, multiple regression analyses have generally found only a relatively small correlation between hemoglobin (or hematocrit) and LVH (61-63). Probably because of worsened BP control, Minagawa and colleagues found that erythropoietin therapy actually worsened LVH (64).

Hypertension is the physiological factor in ESRD patients which has been best linked to LVH. A number of publications demonstrate significant correlations between the magnitude of LVH on echocardiogram and either the predialysis blood pressure, 24 hour ambulatory blood pressure, or number of antihypertensive medications (54, 65-67). Certainly hypertension is a treatable factor which must be addressed aggressively in patients with ESRD. However, we stress that while hypertension certainly contributes to the LVH seen in ESRD, blood pressure alone cannot explain the frequency and severity of LVH in ESRD patients (62, 68-71). This is illustrated by the extremely poor correlation between the LVMI and predialysis systolic blood pressure that we observe in our HD population (Figure 4).

Hyperparathyroidism causes abnormalities in cardiac energy metabolism, function and growth in experimental models (72, 73). On a molecular basis, it is possible (although not studied to date) that PTH stimulated Na/K-ATPase endocytosis (74) might actually amplify DLS signaling through the Na/K-ATPase. Hyperparathyroidism has also been associated with LVH in several clinical studies of patients with renal failure. Sato and colleagues found considerable regression of LVH in 24 patients with ESRD following parathyroidectomy (75). Covic, et al, also reported improvements in LVH after parathyroidectomy (76). However, it is clear that significant hyperparathyroidism is not a necessary condition for the development of LVH in ESRD patients (77, 78).

In addition to the aforementioned pathogenic factors, the renin angiotensin system (79-81), as well as lipid abnormalities (82), have been suggested to play a role in the pathogenesis of LVH in ESRD patients treated with HD. In addition, smaller studies have identified other factors ranging from aluminum intoxication to activation of the sympathetic nervous system (83, 84).

In experimental chronic renal failure induced by partial nephrectomy, we have observed that left ventricular hypertrophy develops quite early and that impaired myocyte relaxation accompanies the cardiac enlargement (85). In fact, the partial nephrectomy model of experimental renal failure induces a cardiomyopathy quite similar to that seen in clinical renal failure replete with diastolic dysfunction. In fact, the impaired myocyte relaxation appears to be associated with a marked downregulation of SERCA2a mRNA, protein and activity (85). SERCA2a is the dominant isoform of the sarcoplasmic reticulum calcium ATPase and is responsible for the rapid reduction in cytosolic calcium following systole (86, 87). We have found excellent correlations between the reduction in SERCA2a expression and SERCA enzymatic activity, as well as calcium renormalization following electrical stimulation. We have also found marked abnormalities in cardiac myocyte calcium concentrations during both systole and diastole. It is unclear at present whether the abnormalities in SERCA2a expression explain all of the changes in calcium cycling or active relaxation (85). Interestingly, we have observed that



**Figure 5.** Schematic illustrating the role of the Na/K-ATPase as a signal transducer based on work detailed in (8, 24, 25, 27-34, 39, 85, 88-92).

cardiotonic steroids (e.g., ouabain, MBG) acutely impair cardiac relaxation in normal rat cardiac myocytes. We have even seen this acute effect from the serum isolated from patients with chronic renal failure (8).

In the partial nephrectomy model of experimental uremic cardiomyopathy, we have found that administration of a decaffeinated green tea extract markedly attenuates the development of the cardiac hypertrophy. This same green tea extract also attenuates the cellular hypertrophy induced by ouabain or marinobufagenin in an isolated cardiac myocyte preparation in concert with the blockade of increased ROS produced by these agents in this system (40).

**7. SPECULATIVE ROLE FOR DLS IN UREMIC CARDIOMYOPATHY**

We propose that DLS signaling through the plasmalemmal Na/K-ATPase is the proximate cause of uremic cardiomyopathy and the oxidant stress state seen in chronic renal failure. A schematic of this potential pathway is shown in Figure 5. The evidence supporting this hypothesis can be summarized as follows; DLS

concentrations increase in renal failure, DLS stimulation results in ROS production, ROS production may participate in a positive feedback system potentiating the effects of circulating DLS and blockade of DLS through antioxidants ameliorates both the effects of DLS at a cellular level, as well as the development of uremic cardiomyopathy in an experimental model.

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