THE ACTIVITY OF NA/K ATPASE IN EXPERIMENTAL NEPHROTIC SYNDROME

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Abstract

Background: Adriamycin nephropathy is a nonimmune-mediated rat model of proteinuric chronic glomerular disease, is induced by a single intravenous injection of doxorubicin hydrochloride into the tail vein. The development of edema and ascites in adriamycin induced nephrosis, due to sodium retention.

Objectives: to evaluate whether enhanced Na^{+}/K^{+}-ATPase activity in the kidneys is a general feature of experimental nephrotic syndromes and whether this enhanced activity is responsible for decreased urinary sodium excretion, and to study the role of zn^{+} supplement on ATPase activity and the probable mechanism of its increment.

Materials & Methods: 12 rats were involved in this work. Control nephrotic (adriamycin 15mg/100g BW) given IV in rat-tail vein. The other group; rats received zinc chloride 0.2 mg^{+}/Kg in addition to Adriamycin (same dose), after 2 week animals were scarificed. Blood sample were aspirated before and after administration of adriamycin. In all samples Na^{+}/K^{+}ATPase activity were estimated. Urine analysis (for proteinurea) was done using dipsticks. Kidneys were homogenized for estimation of Na^{+}/K^{+}ATPase activity in tissue from all groups. Lastly kidneys were dissected and examined by light microscope and Electron microscope.

Results: Histopathological sections of rat kidneys treated with ADR show nephrotic changes. The activity of Na^{+}/K^{+} ATPase was significantly increased in nephrotic rats (p<. 05), and was further increased in nephrotic rats receiving zinc chloride ($p<0.01$). Sections examined by E/M confirmed the diagnosis of nephrosis and revealed apoptotic changes mainly in tubular epithelial cells of nephrotic rats.

Discussion: The activity Na^{+}/K^{+}-ATPase was increased in nephrotic rats; this may be attributed to mobilization of some ion channels from basolateral to apical membrane, further increase in enzyme activity after zinc chloride supplements; since the enzyme is a metalloenzyme and require Mg^{+2} and Zn^{+2} for its full activity.

Conclusion: there was a marked increase in Na^{+}/K^{+}ATPase activity found in adriamycine nephropathy and the activity was further elevated upon zinc chloride supplement.

Key words: Na+/K+-ATPase, adriamycin , nephrotic syndrome

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Introduction

The kidneys clean the blood by filtering out excess water and salt and waste products from food. Healthy kidneys keep protein in the blood, which helps the blood absorb water from tissues. But kidneys with damaged filters may let protein leak into the urine. As a result, not enough protein is left in the blood to soak up the water. The water then moves from the blood into body tissues and causes swelling. The most common form of the nephrotic syndrome in children is called minimal change disease[1]. Adriamycin nephropathy (AN) is a widely used nonimmune-mediated rat model of proteinuric chronic glomerular disease and is usually induced by a single intravenous injection of doxorubicin hydrochloride (DX) into the tail vein[2,3,4]. Nephrotic syndrome characterized by: High levels of protein in the urine, Low levels of protein in the blood, In addition to proteinuria and podocyte foot process effacement, development of edema and ascites are permanent features in the majority of models and diseases[3,4]. Marked decreases in urinary sodium excretion are common clinical observations in human minimal change disease[5], and decreases in the fractional excretion of sodium are observed in animal models such as puromycin aminonucleoside nephrosis PAN[6], adriamycin nephrosis[7], immune glomerulonephritis resulting from rabbit anti-rat serum[8]. The collecting duct is the site of final Na reabsorption according to Na balance requirement, and this reabsorption is achieved by Na^{+}/K^{+} ATPase. Activation of local Na^{+}/K^{+} ATPase in cortical collecting duct could be involved in Na retention with or without

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generalized activation of tubular ATPases in nephrotic syndrome[9]. Na^{+}/K^{+}ATPase are P-type translocases consists of two transmembrane protein the larger of which, catalytic subunit; exchange extracellular k against intrracellular Na or proton, at expense of ATP hydrolysis. ATPases utilizes 80% of renal metabolic energy. Na^{+}/K^{+}ATPase is the major factor in primary renal Na retention[10].

Therefore, to evaluate whether enhanced Na^{+}/K^{+}ATPase activity in the kidneys is a general feature of experimental nephrotic syndromes and whether this enhanced activity is responsible for decreased urinary sodium excretion. Finally to study the role of zinc chloride in activation and/or restoring the activity of the enzymes. For this purpose, Na^{+}/K^{+}ATPase activity was measured in kidneys and blood, perhaps to explain the probable mechanisms responsible for this increment in its activity in adriamycin nephrosis in rats.

Materials & Method

12 rats were involved in this work. Rats were stratified into control group (4rats) and experimental group (ADR-induced nephropathy group; 8 rats). Rats (Rattus norvegicus albinus) weight (308±9) gram aged 3 months. The control group receives zinc chloride on 3 successive days.

The experimental group was then divided into:

Control nephrotic group (ADR-induced nephropathy; adriamycin 15mg/100g body weight) given iv in rat tail vein, then animals were scarified after 2 weeks.

Blood samples; blood sample aspirated before and after administration of adriamycin, third blood sample before scarifying the animal. In all samples estimation of Na^{+}-K^{+}ATPase activity was performed according to Reddy et al[11].

Second group; rats received zinc chloride 0.2 mg/Kg for 3 successive days, then 2 days rest ,then animal receive Adriamycin (15mg/100g body weight) in rat tail vein, after 2 week animals were scarified.

Urine analysis to check protein in urine; checked by dipstick (med-testcombi 3A Macherey-Naglel).

Kidneys were homogenized for estimation of Na^{+}-K^{+}ATPase activity in tissue from all groups.

Also kidneys were dissected and examined by light microscope after staining with (H and E) stain, and were processed for Electron microscope study according to the method of Reynold[12].

Results

Nephrotic syndrome was diagnosed on bases of: Edema; especially puffiness of the eyes and weight increment, proteinuria (>3g/ L), which was significantly higher level compared with control groups ($P < 0.05$).

Histopathological examination of the kidneys of rats treated with ADR revealed focal areas of mesangial proliferation and mild tubulointerstitial inflammation. These results similar to those found by Ozen (2001)[13] and were diagnostic of nephrotic syndrome.

The activity of Na^{+}/K^{+}ATPase was significantly increased in blood and tissue of nephrotic rats ($p<0.05$), and was further increased significantly in nephrotic rats receiving zinc chloride ($p<0.01$) as shown in table 1.

Table 1: Activity of Na^{+}/K^{+} ATPase in different groups of the study

	control/tissue	nephrotic/tissue	nephrotic/tissue/ receiving zinc chloride
Na/K-Atpase nmol/mg/min	97.6235 ± 10.4805	112.0758 ± 30.6908	206.9018 ± 37.63815
	Control/ blood	**nephrotic/blood**	**nephrotic/blood/ receiving zinc chloride**
Na/K-Atpase nmol/mg/min	38.34825 ± 5.627057	55.72275 ± 4.918427	121.6513 ± 9.651917

The sections of the kidneys were examined by electron microscope which confirmed our diagnosis of nephrosis and revealed apoptotic changes: In nephrotic rats tubular epithelial cells exhibit condensed and fragmented chromatin typical of apoptotic cell death while normal kidneys had 0.18±0.04 ($P<0.02$) apoptotic cell/HPF as compared with 2.76±0.2 ($P<0.001$) in edematous rats with end stage disease, apoptosis was mainly observed in tubules and only rare apoptotic cells observed in glomeruli from rat kidneys. The apoptotic changes in the kidneys of nephrotic rats are showing in figures 1 and 2.

Discussion

Polarized epithelial cells have characteristic cellular organization that include a surface plasma membrane organized into distinct apical and basolateral domains within these surface membrane domains are enzymes, transporters, hormone receptors and lipids are localized in a polarized fashion. The alterations in vectorial transport function were related to the redistribution of surface membrane phospholipids and domain specific apical and basolateral membrane enzymes. i.e. redistribution of Na^+/K^+ATPase from basolateral to apical membrane in renal proximal tubular cells, was associated with reduced Na reabsorption secondary to Na^+/K^+ATPase pumping Na+ back to the urinary lumen. Reestablishment of surface membrane Na^+/K^+ATPase was essential for restoration of normal cellular Na+ transport[6]. In PAN nephrosis Na reabsorption in outer medullary collecting duct does not altered while an increased synthesis and membrane expression of Na^+/K^+ATPase in cortical collecting duct occurs. Moreover, an altered normal trafficking of intracellular Na^+/K^+ATPase unit to the basolateral has been documented[9]. In vivo micro puncture experiments have shown that the collecting duct is the site of sodium retention in the kidney of nephrotic rats[14] and this phenomenon is independent on proteinuria or the level of vassopressin[15]. Na^+/K^+ATPase activity is significantly enhanced in the cortical collecting ducts (CCD) of rats with PAN nephrosis, during a period with a positive sodium balance and this is independent on endogenous inhibitors suggesting primary a paracrine or cellular mechanism[16].

While recent literature data suggest that a primary impairment in sodium excretion is the basic abnormality in the pathogenesis of edema formation in the nephrotic syndrome, the activity of the enzyme was measured in blood and tissue of adriamycin induced nephrosis[17].

ATPases are metalloenzymes; required magnesium for their maximum activity, the effect of zn^+ on ATPase activity was examined. A significant increase in enzyme activity was observed in both control normal and adriamycin induced nephrosis. The enzyme activity measured in blood samples and renal tissue show increment in the activity in blood and renal tissue in the same manner. Faurkov demonstrate the effect of trace metal cadmium on Cl-ions and the closely related metals zn^+ and Ni were also able to activate the Cl^- secretion. Moreover zn^+ and Ni^+ completely prevent the inhibition process of some inhibitors for transport[18]. However basolateral Na^+/K^+ATPase of CCD which causes Na retention if the activity is elevated may affect some how the apical membrane ATPase as it was found that basolateral Cadmium (or zn^+, Ni^+) causes calcium mobilization and activation of apical membrane sensitive channel[18], i.e. mobilization of some ions from basolateral to apical membrane and activate ATPases.

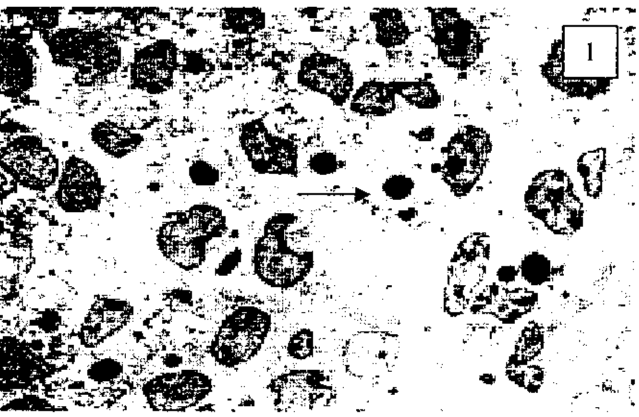
In this work apoptotic process was increased in end stage disease and was ameliorated by addition of zn^+ (this was proved in our previous work), since zinc can inhibit apoptotic process by different mechanisms[19].

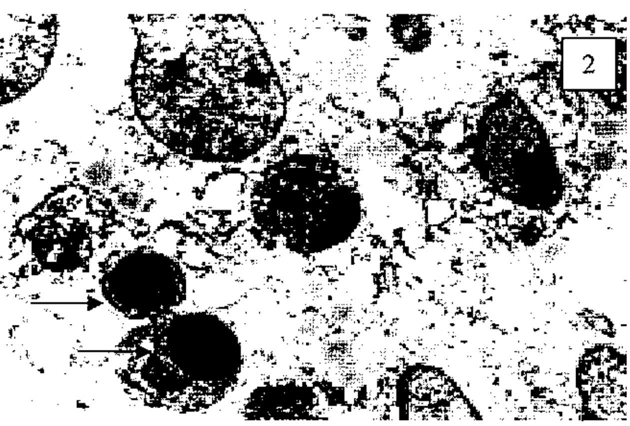
Zn^+ stimulate ATPase activity by inhibition of proteolysis, as zn^+ is well known caspase 3 inhibitor that regulate apoptotic process and hence protein proteolysis[20].

Marked increase in Na^+/K^+ATPase activity found (twofold) in nephrotic and further elevated upon zinc chloride supplement (another twofold), as nephrotic dysregulate distribution of transporter in CCD and elevated the number and size of basolateral ATPase to some extent that cause Na/water retention ,zinc will activate ATPase in both sites leading to another twofold increment. In puromycin aminonucleoside induced nephrosis (PAN), sodium retention originates in part from the collecting duct, and it is associated with increased Na^+/K^+ATPase activity lead to the formation of ascites and edema[9]. Cloning of four isoform of Na^+/K^+ and two of K^+/H^+ ATPases has provided a molecular bases to the heterogeneity of these ATPases beside its house keeping function, renal ATPases energizes most solute and water transport along whole nephrone. ATPases utilizes 80% of renal metabolic energy[10].

It was suggested that increase Na reabsorption associated with shift of Na^+/H^+ exchanger (NHE3) from an inactive pool to an active pool thus contributing to Na retention[21] i.e. dysregulation of Na^+/K^+ATPase in CCD is the major factor in primary renal Na retention in nephrotic syndrome. Water reabsorption and retention could be attributed to extensive down regulation of aquaporin and urea transporter expression this may represent an appropriate renal response to extra cellular volume expansion and may occur at expense of lecreased urinary concentrating and diluting :apacity[22].

Conclusion: The activity of Na^+/K^+ATPase is ncreased due probably to mobilization and activation of some ion channels from basolateral o apical membrane.





Figures 1 and 2: E/M of the kidneys showing nuclear fragmentation chromatin condensation in centrifugal pattern (arrow) on nuclear envelop: 1: x 1600, 2: x 5000

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