The Fanconi syndrome of cystinosis: insights into the pathophysiology

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Cystinosis is a lysosomal storage disease, and is one of the most common causes of the Fanconi syndrome. In vitro studies of the cystine-loaded tubule provided insights into the pathophysiology of the proximal tubular defect. Proximal tubules loaded with cystine have a generalized proximal tubule transport defect characteristic of the Fanconi syndrome. The decrease in proximal tubular transport with cystine loading is due to a decrease in active transport. In cystine-loaded tubules the ATP production is severely compromised. The cystine-loaded tubule has a lower intracellular phosphate concentration than that of control tubules. This low intracellular phosphate concentration in cystine-loaded tubules likely plays an important role in maintaining intracellular ATP level. Preservation of intracellular phosphate at control levels prevents the decrease in intracellular ATP and the proximal tubule respiratory dysfunction with cystine loading.

Key words: cystinosis, proximal tubule, Fanconi syndrome.

Introduction

Cystinosis in an autosomal recessive disease characterized by a high intracellular concentration of the amino acid cystine in various organs[1-3]. Cystine accumulates predominantly in lysosomes where the exodus of cystine from this compartment to the cystoplasm is defective[2-10].

The proximal tubule reabsorbs 70% of the filtered water and sodium chloride and 80% of the filtered bicarbonate. All of the filtered glucose and amino acids as well as most of the phosphate are reabsorbed by the proximal tubule.

Cystinosis is the most common inherited cause of the Fanconi syndrome[1]. Infants with cystinosis are typically asymptomatic for the first six months of life. They usually present with failure to thrive. Evaluation of serum electrolytes reveals a hyperchloremic metabolic acidosis, hypokalemia, and hypophosphatemia. The urine will have a hyperphosphaturia, positive anion gap, and glucosuria. The electrolyte disturbances seen in cystinosis are caused by cystine accumulation in proximal tubules. The metabolic acidosis and other electrolyte disturbances seen in pediatric patients with the Fanconi syndrome of cystinosis contribute to their failure to thrive and short stature.

Fanconi Syndrome: In Vitro Studies

There is no animal model for the Fanconi syndrome of cystinosis. Foreman et al.[11] were able to load proximal tubules with cystine using cystine dimethyl ester. Cystine dimethyl ester permeates across cell membranes and leads to the intracellular accumulation of cystine. Proximal tubule cells can be loaded to cystine concentrations comparable to those measured in patients with cystinosis[11,12]. The intracellular esterases cleave cystine dimethyl ester to liberate cystine. The in vitro studies have shown that much of the cystine in proximal tubules incubated with cystine dimethyl ester is found in lysosomes[13].

There are several potential mechanisms whereby cystine could produce a generalized dysfunction in proximal tubular transport. The driving force for apical membrane sodium transport is the low intracellular sodium concentration generated by the Na-K-ATPase on the basolateral membrane that is driven by ATP. Therefore, a defect in the generalized proximal tubule transport could be due to any of the processes mediating active transport. Bergeron et al.[14] suggested that the Fanconi syndrome could be explained by an alteration in passive transport. The glomerulus produces an

ultrafiltrate of plasma that is delivered to the proximal tubule. Within the first milimeter, the composition of the luminal fluid changes dramatically[15,16]. There is an active absorption of organic solutes and bicarbonate. This leaves the luminal fluid without glucose and amino acids, and with a lower concentration of phosphate and bicarbonate than the peritubular plasma. The Fanconi syndrome could be due to an increase in permeability of the proximal tubule allowing transported solutes to leak back in to the proximal tubule lumen[15].

A defect of transporters on the apical membrane could lead to an inhibition in active transport. Foreman et al.[17] prepared brush border membrane vesicles from proximal tubules that were incubated with cystine dimethyl ester. The uptake of the amino acid proline was no different in brush border membranes prepared from control or cystine-loaded tubules. Thus, cellular cystine loading did not affect the transporters on the apical membrane to result in the inhibition in transport. Brush border membrane vesicles from rats that received intraperitoneal injections of cystine dimethyl ester also had fewer glucose transporters on renal brush border membrane than control rats[17].

A generalized inhibiton in proximal tubule transport due to decrease in Na-K-ATPase activity was investigated by Coor et al.[18] and Foreman et al.[19] Neither was able to demonstrate that cystine loading inhibited the Na-K-ATPase directly.

Another possible hypothesis is that the reduction in proximal tubular transport in cystine-loaded tubules could result from a reduction in fuel (ATP) to the pump. Coor et al.[18] when a major reduction in intracellular ATP individually dissected proximal convoluted tubules were incubated with cystine dimethyl ester. Incubation of control tubules with exogenous 1 mM ATP did not significantly affect intracellular ATP levels and had no effect on transport in control tubules[18]. However, incubation of proximal tubules with cystine dimethyl ester resulted in a 89% reduction in proximal tubular transport, while in the presence of exogenous ATP there was only a 45% reduction in volume absorption[18]. Therefore, proximal tubule cystine loading resulted in a decrease in intracellular ATP, and repletion of ATP ameliorated the transport defect. A depletion in intracellular ATP content has also been demonstrated by Foreman et al.[19] utilizing suspensions of proximal tubules loaded with cystine.

Some researchers focused on the effect of proximal tubular cystine loading on cellular metabolism, especially the respiration component[20]. In control proximal tubules, addition of ouabain, an inhibitor of the Na-K-ATPase, resulted in a 50% decrease in oxygen consumption. Thus, under basal conditions, half of proximal tubule oxygen consumption is consumed to mediate proximal tubular transport. Oxygen consumption in cystine-loaded tubules was only 50% of control tubules, which is consistent with the previous studies that suggested a reduction in intracellular ATP. This effect has been shown by others[21]. Of interest was the fact that cystine-loaded tubules had an almost total inhibition in the oxygen consumption utilized for active transport[20]. Non-transport directed oxygen consumption remained intact. These studies suggest that when cystine loading injures a proximal tubular cell, maintenance of vital functions necessary for cellular survival are not affected.

Some studies have suggested that depletion in intra-cellular phosphate may play a role in the pathogenesis of the Fanconi syndrome[22]. Infusion of maleic acid results in a generalized proximal tubule transport dysfunction[22]. Al-Bander et al.[22] found that infusion of sodium phosphate attenuated the fall in glomerular filtration rate, the aminoaciduria, and bicarbonaturia in maleic acid treated dogs. These investigators suggested that the reduction in intracellular phosphate produced the defect in proximal tubule transport[22].

The proximal tubule is dependent on phosphate to maintain active transport[23,24]. Perfusion of proximal convoluted tubules with an ultrafiltrate-like solution without phosphate resulted in a total inhibition of active transport[23]. When proximal tubules were perfused, with an ultrafiltrate-like solution without glucose or with glucose and phlorizin, inhibitor of glucose transport, active transport was the same as that measured in the presence of phosphate. Similarly, the rate of proximal tubule oxygen consumption was impaired in the absence of phosphate in the incubation solution, only when glucose was present[23].

Glucose-induced inhibition in proximal tubule transport and oxygen consumption in the absence of phosphate are analogous to Crabtree effect[25], where there is a glucose-dependent decrease in intracellular phosphate due to the accumulation of phosphorylated glycolytic intermediates. In the presence of glucose, there is thus a critical depletion of free intracellular phosphate that compromises oxidative phosphorylation and produces depletion in ATP.

Bajaj et al.[26] incubated isolated proximal tubules with cystine dimethyl ester. Cystine-loaded tubules had a 40% reduction in intracellular phosphate. Following this in vitro study, as an in vivo experiment, rabbits were given an infusion of sodium phosphate, sodium sulfate, or sodium chloride prior to isolation of proximal tubules. Cellular cystine loading produced a reduction in both groups; however, the intracellular phosphate concentration in cystine-loaded proximal tubules that received a phosphate infusion was comparable to that of the proximal tubules from animals that received a sulfate infusion and were cystine-loaded. Thus, cystine-loaded tubules after phosphate infusion had an intracellular phosphate concentration comparable to control tubules. After cystine loading, there was a reduction in oxygen consumption in the sulfate and chloride group, but the tubules from animals that received a phosphate infusion had no reduction in cellular respiration after cystine loading.

Cellular cystine loading with cystine dimethyl ester produced a significant reduction in intracellular ATP in the chloride and sulfate group. Intracellular ATP was not reduced in cystine-loaded proximal tubules prepared from animals that received an infusion of phosphate. The same group of researchers also demonstrated that when cystine-loaded proximal tubules were perfused with solution containing 1 mM phosphate, there was a 75% reduction in tubule volume absorption, whereas the tubules perfused with 4 mM phosphate did not show any change in the transport. These data demonstrate that cystine loading results in a reduction in intracellular phosphate. Maintenance of intracellular phosphate preserves intracellular ATP and prevents the reduction in proximal tubular respiration and proximal tubular transport with cystine loading.

Most of the studies examining the pathogenesis of the Fanconi syndrome of cystinosis have utilized cystine to duplicate its pathogenesis in vivo. In addition, the Fanconi syndrome seen in other diseases may be generated by other mechanisms than those described above in the cystine-loaded tubules.

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