GLomerulotubular function in cyclosporine-treated rats. A lithium clearance, occlusion time/transit time and micropuncture study

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Summary

Sprague-Dawley rats treated with cyclosporine (Cys) and appropriate controls were investigated with inulin, lithium and sodium clearances. It was found that Cys depressed glomerular filtration rate (GFR) and absolute proximal tubular reabsorption, while fractional proximal reabsorption was increased. As a normal proximal tubular reabsorptive capacity was found after volume expansion, and the intratubular pressure was normal, tubulotoxicity or obstruction of the tubular system by Cys was excluded. Increased fractional proximal reabsorption was also found with the occlusion time/transit time method. Intravenous Cys resulted in instantaneous renal functional changes qualitatively identical to those of prolonged Cys treatment. It was concluded that Cys nephrotoxicity is due to decreased ultrafiltration pressure, most probably due to a reversible spasm in the afferent glomerular arteriole.

Introduction

Since the earliest reports on the clinical effect of cyclosporine (Cys), a nephrotoxic side effect has been suspected. In man increases in serum creatinine and water, sodium and potassium retention are the main features of this adverse effect [1]. However, no specific morphological correlate of these functional effects has been accepted in man or animals. In the first years after the clinical introduction of Cys it was the general opinion that Cys was toxic to renal tubular cells [2], but this seems not to be true [3]. At present more attention is placed on Cys effect on the renin-angiotensin-aldosterone system, on the renal vasculature and on the glomerular permeability [3,4]. We hereby summarise our contributions to this new thesis concerning the nephrotoxicity of Cys.

Material and methods

The effect of Cys 0–12.5–25mg/kg/day over 13 days was studied in the conscious catheterised Sprague-Dawley rat [3]. Inulin, lithium and sodium clearances were measured. This allows the estimation of: glomerular filtration rate (GFR) =
inulin clearance ($C_{\text{in}}$), delivery of lithium, fluid ($\dot{V}_{\text{prox}}$) and sodium ($C_{\text{Na prox}}$) from the proximal tubule to the thin descending segment of the loop of Henle (lithium clearance, $C_{\text{Li}} = \dot{V}_{\text{prox}} = C_{\text{Na prox}}$), absolute proximal tubular lithium, sodium and fluid reabsorption ($C_{\text{Li}}-C_{\text{Li}}$), fractional proximal tubular reabsorption ($1-C_{\text{Li}}/C_{\text{in}}$), fractional lithium clearance ($C_{\text{Li}}/C_{\text{in}}$), absolute reabsorption of sodium in the nephron segment distal to the proximal tubules ($C_{\text{Li}}-C_{\text{Na}}$), and fractional reabsorption in the same segment ($1-C_{\text{Na}}/C_{\text{Li}}$) [5]. The effect of extracellular volume expansion with 2% of body weight (BW) saline was investigated in those rats treated with Cys over 13 days, as was the effect of a high sodium clearance obtained over 13 days with a high sodium content in the diet. In rats on the normal sodium diet the effect of Amiloride (MSD), a drug which is known to block conductive sodium channels in high resistance epithelium, such as those of the distal tubule and collecting duct, was investigated in order to see whether it would increase $C_{\text{Li}}$. In a separate group of anaesthetised Sprague-Dawley rats we measured tubular occlusion time (OT), lissamine green tubular transit time (TT), intratubular hydrostatic pressure (P) and $C_{\text{in}}$, $C_{\text{Li}}$ and $C_{\text{Na}}$. Estimates of fractional reabsorption in the convoluted part of the proximal tubules (PFR) from the clearance data (PFR$_{C_{\text{Li}}/C_{\text{in}}}$) and from the occlusion time/transit time (PFR$_{\text{OT/TT}}$) could then be compared using the formula [6]:

$$PFR = 1 - e^{-TT/OT} = 0.78 \times (1-C_{\text{Li}}/C_{\text{in}}).$$

The effect of intravenous Cys was investigated in rats with normal and high $C_{\text{Na}}$, in the conscious catheterised rat model. Plasma renin concentration (PRC) and serum Cys were investigated in all animals, and blood Cys in some.

**Results**

A typical example of the Cys modified renal function is shown in Table I. Cys reduces GFR and absolute proximal tubular reabsorption, while proximal tubular ouillary clearance increases.

<table>
<thead>
<tr>
<th>Cys</th>
<th>0</th>
<th>12.5</th>
<th>25</th>
<th>SPRD rats</th>
<th>µl/min/gKW</th>
<th>µl/min/gKW</th>
<th>µl/min/gKW</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{in}}$</td>
<td>1065±313*</td>
<td>734±324*</td>
<td>541±228*</td>
<td>µl/min/gKW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{Li}}$</td>
<td>187±47</td>
<td>125±115</td>
<td>59±47*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$1-C_{\text{Li}}/C_{\text{in}}$</td>
<td>82±3</td>
<td>88±9*</td>
<td>92±5*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{in}}-C_{\text{Li}}$</td>
<td>878±274</td>
<td>608±316*</td>
<td>490±196*</td>
<td>µl/min/gKW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{Na}}$</td>
<td>3.1±1.7</td>
<td>1.2±0.9</td>
<td>96.2±3.4</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$1-C_{\text{Na}}/C_{\text{Li}}$</td>
<td>98.3±1.1</td>
<td>97.1±3.4</td>
<td>96.2±3.4</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{\text{Li}}-C_{\text{Na}}$</td>
<td>183±46</td>
<td>123±114</td>
<td>49±47*</td>
<td>µl/min/gKW</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$C_{\text{in}}=\dot{GFR} \cdot C_{\text{Li}} = \dot{V}_{\text{prox}} = C_{\text{Na prox}} = $ delivery of lithium, fluid and sodium from the proximal tubule.

$1-C_{\text{Li}}/C_{\text{in}} = $ proximal fractional reabsorption. $C_{\text{in}}-C_{\text{Li}} = $ proximal absolute reabsorption.

$1-C_{\text{Na}}/C_{\text{Li}} = $ distal fractional reabsorption. $C_{\text{Li}}-C_{\text{Na}} = $ distal absolute reabsorption.

* = $p<0.05$, one-tailed two-sample t-test

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tubular fractional reabsorption (1-CLi/Cin) is increased. CLi (=\(\nu_{\text{prox}}=C_{\text{Na prox}}\)) is also decreased while absolute and fractional reabsorption in the nephron segment distal to the proximal tubule are both reduced. Table II shows that intravenous Cys (12.5mg/kg) results in alterations quantitatively identical to those of the longer Cys treatment. The following groups were treated with Cys 25mg/kg/day for 13 days (Table II). Extracellular volume expansion (2% BW saline) instantly increased GFR and absolute proximal tubular reabsorption (Cin-CLi) to normal values while the increased fractional proximal tubular reabsorption remained unaltered. High sodium content in the diet resulted in increased CNa and decreased fractional sodium reabsorption in the distal nephron segment (1-CNa/CLi) while CLi and glomerular filtration and proximal tubular reabsorption was identical to the corresponding values from groups with a moderate sodium content in the diet. Furthermore, Amiloride was unable to increase CLi (Table II).

<table>
<thead>
<tr>
<th></th>
<th>Cin</th>
<th>CLi</th>
<th>1-CLi/Cin</th>
<th>Cin-CLi</th>
<th>1-CNa/CLi</th>
<th>CLi-CNa</th>
<th>CNa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous Cys</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>NC</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Fortnight Cys</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>NC</td>
<td>↑</td>
</tr>
<tr>
<td>+ vol exp</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>NC</td>
<td>↑</td>
<td>NC</td>
<td>↑</td>
</tr>
<tr>
<td>+ high Na</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>↓</td>
<td>NC</td>
<td>↑</td>
</tr>
<tr>
<td>+ Amiloride</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
</tr>
</tbody>
</table>

Vol exp = 2% BW volume expansion. High Na = high sodium content in the diet.

In anaesthetised rats PFR estimated from OT/TT and CLi/Cin, respectively, was compared. PFROT/TT was 68 per cent, and PFRCLi/Cin was 69 per cent. Proximal tubular transit time (TT) was prolonged to a mean of 26 seconds (p<0.05), and the intratubular hydrostatic pressure was low in the normal range (11.6±1.4mmHg). PRC was approximately twice the normal value of Cys-treated groups, and did also increase immediately after intravenous Cys administration (not shown). Also, the increased PRC of the Cys-treated rats was found to be resistant to volume expansion (2% BW saline). Including all experiments conducted (>120 SPRD rats) we have been unable to show any significant correlation between s-Cys and renal function.

**Discussion**

Cys was found to reduce GFR in the Sprague-Dawley rat at dose ranges commonly used during human transplantation [1]. It has earlier been proposed that the nephrotoxic effect of Cys could be due primarily to proximal tubular damage [2]. GFR would then decrease as a consequence of depressed proximal tubular reabsorptive capacity and concomitant increase in intratubular pressure. This interpretation predicts a decreased proximal fractional reabsorption. But
as we have found a normal proximal tubular reabsorptive capacity (during volume expansion), a proximal tubular hydrostatic pressure low in the normal range, and an increased proximal fractional reabsorption [3] (the latter also shown by Tonnensen et al [7]), proximal tubular damage seems very unlikely. An interpretation of increased fractional but decreased absolute proximal reabsorption could have been a partial obstruction in the tubular system at a site distal to the proximal tubule in various numbers of nephrons, but the finding of a normal intratubular pressure also disproves this alternative. Two series of experiments were done to elucidate the possibility that \( C_{\text{Li}} \) should underestimate the outflow from the proximal tubule because of (pathological) distal lithium reabsorption. Firstly, a comparison was made between the fractional delivery from the end of the proximal convoluted segment (PFR) as measured directly by the OT/TT method (\( 1-e^{-\text{TT}/\text{OT}} \)), and as calculated from the formula \( \text{PFR} = 0.78 \times (1-C_{\text{Li}}/C_{\text{IN}}) \). The two estimates agreed quantitatively and thereby confirmed that no significant distal lithium reabsorption occurred. Secondly, Amiloride in a dose which in a separate control group was shown to reduce potassium clearance, and which has been shown to increase \( C_{\text{Li}} \) in rats with distal lithium reabsorption, was without effect on the \( C_{\text{Li}} \) of the Cys-treated rats.

Having excluded other possibilities, our data suggest the following pathophysiological mechanism and sequence of events: net ultrafiltration pressure is reduced by Cys, and due to inadequate reduction in the absolute rate of proximal reabsorption, proximal fractional reabsorption increases. As the functional pattern shows, many similarities with that seen during partial obstruction of the renal artery, we suggest that a vascular site of action, most likely on the glomerular afferent vessels, is responsible for the nephrotoxic effect of Cys.

The function of the distal nephron segment is also altered during Cys treatment (Table I). The finding that the fractional and the absolute reabsorption of sodium (\( 1-C_{\text{Na}}/C_{\text{Li}} \) and \( C_{\text{Li}}-C_{\text{Na}} \), respectively) in the nephron segment distal to the proximal tubule were reduced may suggest the following sequence of events: Cys reduces distal sodium (and water, data not shown) reabsorption resulting in natriuresis and azotemia, which then could reduce net ultrafiltration pressure. But as we and others [7] have found GFR to be reduced instantaneously after intravenous Cys, and even without natriuresis, this can also be excluded.

An interesting finding was that the proximal fractional reabsorption was markedly increased even when urine flow and electrolyte excretion was returned to normal by extracellular fluid expansion. This resistant change in glomerulotubular balance strongly suggests a drug-induced resetting of the tubulo-glomerular feedback mechanism.

The increased PRC in the Cys-treated animals could be explained by the decreased delivery of tubular fluid to the macula densa region, as \( C_{\text{Li}} \) was decreased. Increased PRC in rats has been shown earlier [3,4], and increased renin release from renal cortical slices in vitro has also been reported [8]. The finding that renal transplant patients do not always have higher PRC when treated with Cys than controls treated with other immunosuppression does not
disprove that Cys increases PRC [9]. Serum or blood Cys did not correlate significantly with any measure of renal function, and as it also can be difficult to show any such correlation in patients [1] this finding is not surprising.

In conclusion, as it can be excluded that Cys nephrotoxicity is due to decreased proximal tubular reabsorptive capacity, or to tubular obstruction, or to primary actions of Cys on the tubular epithelium of the nephron segment distal to the proximal tubule, it appears that Cys nephrotoxicity is due to decreased net ultrafiltration pressure, most probably secondary to an effect on the glomerular afferent vessels. The tubulo-glomerular feedback mechanism is resettled with a higher fractional proximal reabsorption, either because of a direct effect of Cys or as an insufficient adaption to the reduced GFR. Cys increases GFR, and the increase is resistant to volume expansion. Serum or blood Cys did not correlate with any measure of renal function. After increasing the ultrafiltration pressure by extracellular volume expansion, the GFR of the Cys-treated animals was returned to normal. It will be of great diagnostic and perhaps therapeutic importance to see if vasodilator therapy will also return renal function to normal, and whether the CTi method can be used to distinguish between renal transplant rejection and Cys nephrotoxicity.

Acknowledgments

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Open Discussion

MEES (Utrecht) How do you explain the increased renin secretion in your model?

DIEPERINK The increased renin we believe is explained by the decreased proximal delivery of sodium to the macula densa, but we cannot exclude a direct effect on the macula densa system.

MEES When you expanded these animals with saline did the renin come down to normal?
DIEPERINK It came down but not as much as we expected it would.

ALLISON (Glasgow) I was fascinated, but a little bit confused, so I apologise if I sound a little confused. First of all, why did you do individual measurements of a single nephron function?

DIEPERINK The micropuncture was in fact mainly for pressure measurements.

ALLISON You see I could not understand why you had to go to the bother of looking at lithium clearances in order to arrive at a measurement for proximal fractional reabsorption when one can simply measure late proximal F:P inulin concentrations to calculate proximal, absolute and fractional reabsorption. I think, therefore, one has to be very cautious about drawing conclusions about proximal fractional and absolute reabsorptions if you do not actually measure each individual nephron, because you will find that each individual nephron has very individual glomerulartubular balance.

DIEPERINK I somewhat disagree with you, because the advantages of the lithium clearance method is that it allows estimation, which is the mean for the whole nephron population of the animal.

ALLISON Nephrons are heterogeneous and not homogeneous, especially when you are looking at an animal that has been given a potentially nephrotoxic substance. I think to validate the method it would be very easy and simple if you were doing micropuncture to give the animal some inulin and measure the late proximal F:P inulin.

DIEPERINK I would draw your attention to the use of the occlusion time/transit time method.

CHAIRMAN I think Dr Allison's point is that when you look at proximal fractional reabsorption by the PFT inulin method you may find changes of 3–4 U:P inulin ratios. That is easy to detect and is far above the error of the method. When you look at the transit time/occlusion time method, which is a true estimate, but has such a wide scatter of data from tubule to tubule, you can just pick those results that you feel more comfortable with and come up with the numbers you want. In a way there is a different source of error and a different way of controlling the data with true measurement, such as the one that Dr Allison proposes, and the estimated way that you use, although mathematically correct.

ALLISON Yes, you are absolutely correct.

DIEPERINK No, I do not think I will go into more detail on that data, but I will mention one more advantage of the lithium clearance method: that is that you can use it in humans. We have found a lower lithium clearance in patients after cyclosporine treatment.
CHAIRMAN  I think you have worked on hydropenic rats?

DIEPERINK  No, I would not say that.

CHAIRMAN  I mean rats without extracellular fluid volume expansion.

DIEPERINK  We used several situations and most of the lithium clearance studies were from conscious curarised rats who have been curarised during anaesthesia and they are hydrated for the duration of the study.

CHAIRMAN  I think that the most probable explanation for the falling GFR in that condition is simply a decrease in renal plasma flow or better in glomerular plasma flow. It is well known that a single nephron GFR is plasma flow dependent. On the other hand you yourself have found a rise in afferent arterial resistance which can well account for a decrease in renal plasma flow.

CHAIRMAN  With hypertonic saline loading you increased or normalised the GFR and you normalise absolute reabsorption, but fractional reabsorption is still higher than normal. How is that possible?

DIEPERINK  We have tried to explain it by a specific alteration of the glomerular tubular balance against a higher fractional proximal tubular reabsorption.

CHAIRMAN  Yes, but what I am saying is that it is impossible mathematically to have a normal GFR and a normal absolute reabsorption and to have ratio between the two, which is the fractional reabsorption high.

DIEPERINK  They go back to the normal range, but when you see on the fraction of the two it is not normal.