Pediatric renal allograft transplantation does not normalize the increased cortisol/cortisone ratios of chronic renal failure

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Abstract

Objective: The conversion of cortisol (F) to cortisone (E) is catalyzed by 11beta-hydroxysteroid dehydrogenase type 2 (11β-HSD2). Children suffering from chronic renal failure (CRF) have a decreased activity of 11β-HSD2 contributing to increased arterial blood pressure. The objective was to investigate whether a normal conversion of F to E is achieved after renal transplantation (TX) in children.

Methods: Fifteen children with CRF, 17 children with steroid-free immunosuppression after TX, and 18 healthy controls (CO) were enrolled. The activity of 11β-HSD2 in plasma was calculated using the ratio of F/E determined by tandem mass spectrometry, the ratio of tetrahydrocortisol (THF) + 5α-tetrahydrocortisone (5αTHF) in urine determined by gas chromatography/mass spectrometry, and the ratio of (THF + 5αTHF)/tetrahydrocortisone (THE) in urine determined by tandem mass spectrometry.

Results: The F/E ratio (mean±S.D./S.E.M.) was significantly higher in CRF (5.6±1.9/0.6, 7.12±3.1/0.9) than in CO (1.18±0.2/0.03, P < 0.0001) groups. The (THF + 5αTHF)/THE ratio in CRF (1.19±1.1/0.5) and TX (1.19±0.1/0.5) groups was significantly higher than in controls (0.21±0.05/0.18, P < 0.0001). Positive correlations between plasma and urinary ratios (P = 0.0004, R² = 0.73 in CRF, P = 0.0013, R² = 0.56 in TX, P < 0.0001, R² = 0.66 in CO) were found, whereas significant correlations between F/E or (THF + 5αTHF)/THE ratios and blood pressure, the number of antihypertensive drugs taken or creatinine clearance could not be found.

Conclusions: In all children with chronic renal failure plasma and urinary cortisol/cortisone ratios are elevated and do not return to normal levels after renal allograft transplantation. This suggests that renal transplantation does not normalize 11β-HSD2 activity.

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Introduction

The conversion of active cortisol (F) to inactive cortisone (E) is catalyzed by 11beta hydroxysteroid dehydrogenase type 2 (11β-HSD2) (1). In humans, 11β-HSD2 is active in the kidney and placenta (2–4). In the cortical collecting duct, where most of the catalyzation of F to E takes place, the metabolites of these glucocorticoids, tetrahydrocortisol (THF), 5α-tetrahydrocortisol (5αTHF) and tetrahydrocortisone (THE), are excreted in urine (5, 6). The isoenzyme 11β-HSD type 1 catalyzes the reverse action from E to F in adipose tissue, human lung and liver (4, 7). 11β-HSD2 dysregulation is involved in arterial hypertension in acquired disorders such as hypercortisolism and primary arterial hypertension (8, 9). Absolute 11β-HSD2 deficiency is the cause of apparent mineralocorticoid excess (AME), a rare autosomal recessive defect due to a mutation in 11β-HSD2 (10, 11). This syndrome is characterized by sodium retention and hypervolemia despite low plasma renin activity and reduced aldosterone levels. On a physiological base, the regulation of 11β-HSD activity is in a certain correlation with changes of arterial blood pressure during childhood (12, 13).

In chronic renal failure (CRF) 11β-HSD2 activity is reduced (14). Therefore, diminished cortisol inactivation is thought to contribute to arterial hypertension in CRF. Palermo and co-workers report a patient with AME who was cured by kidney transplantation. This case indicates that normalization of 11β-HSD2 activity and, consequently, cortisol metabolism after transplantation, is possible (15).

Arterial hypertension is very common in children after renal allograft transplantation (16, 17). The reasons are multifactorial, i.e. chronic allograft failure, immunosuppressive therapy and reno-vascular disorders (16, 18–20). Detection and therapy of arterial hypertension in these patients remains of great importance.

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importance in order to avoid severe damage to the transplanted kidney (12, 18, 21).

The possible contribution of reduced 11β-HSD2 activity to elevated blood pressure after renal transplantation has not been investigated. Therefore, the objective of the present study was to examine whether the plasma F/E ratio and urine (THF + 5αTHF)/THE ratio, indicating 11β-HSD 2 activity, are normalized in pediatric renal allograft recipients as compared with patients with chronic renal failure and a group of healthy children.

**Subjects and methods**

Fifty children were enrolled into the study. Fifteen patients (aged from 4.0 to 17.5 years) were suffering from chronic renal failure (CRF group), 17 patients (aged from 6.0 to 15.5 years) were pediatric renal allograft recipients (TX group) (time after TX from 0.5 to 10.0 years), and 18 children (aged from 6.0 to 16.5 years) served as normal controls (CO). This control group consisted of 10 female and 8 male individuals with normal arterial blood pressure and no relevant past medical history or medication. The patients’ characteristics are shown in Table 1. The antihypertensive drug therapy in patients from the CRF and TX groups consisted of one or more of the following: angiotensin converting enzyme (ACE)-blocking agents, beta-blocking agents, calcium channel-blocking agents and diuretics; in most of them a combination of different drugs was used. The immunosuppressive therapy was based on Ciclosporine A or Tacrolimus in combination with Mycophenolate-mofetil. No corticosteroids were applied at the time of examination. The therapeutical characteristics of the patients are shown in Table 2.

All clinical and auxological data were obtained during routine visits to the hospital between 0800 and 1000 h. Details are shown in Table 1.

Plasma cortisol and cortisone were determined simultaneously using liquid chromatography tandem mass spectrometry, with atmospheric pressure chemical ionization in the positive ion mode, according to a modified method of Vogeser (22). One hundred microliters of the samples and calibrators were deproteinized with methanol/zinc sulfate (50 g/l, 1/1 v/v). After centrifugation, the supernatants were applied to an online solid-phase extraction column with subsequent HPLC separation employing column switching (extraction column: Oasis HLB 2.1 × 20 mm, 15 μm (Waters, Milford, MA, USA)). The samples were washed with 5% methanol and eluted in back-flush with 2 mmol/l ammonium acetate/methanol (30:70, v/v) onto the analytical column (Chromolith RP 18e100 × 4.6 mm; Merck, Darmstadt, Germany) at a flow rate of 1 ml/min. Sample analysis was performed in the multiple-reaction monitoring mode with a dwell time of 150 ms per channel using the following transitions for quantification (figures in brackets represent qualifier transition): m/z 363.2/121.2 (363.2/309.4) for cortisol, m/z 361.1/162.9 (361.1/239.0) for cortisone, m/z 367.3/121.2 for cortisol (9, 11, 12, 12-D4) internal standard (cortisol-d4). The intra-assay coefficients of variation were 2.6%–4.0% for cortisone (concentration range 19–38 ng/ml) and 2.0%–5.6% for cortisol (concentration range 55–206 ng/ml).

Urinary steroid profiles were measured by gas chromatography/mass spectrometry using the extraction technique described by Schmidt et al. (23). Briefly, THE, THF and 5αTHF in urine (2 ml) were extracted on C18 SPE columns (Macherey-Nagel, Düren, Germany) and eluted in methanol (2 × 1.5 ml). The dried eluate was hydrolyzed with β-glucuronidase/arylsulfatase (20 μl, 3 h at 58°C; Roche, Penzberg, Germany) in sodium acetate buffer (0.1 mol/l, pH = 4.6). Following addition of the internal standards androstandioli, coprostanol and cortisol-d4, and further purification over amino extraction columns, methyloxime-trimethylsilyl ether derivatives were made.

**Table 1** Characteristics of patients suffering from chronic renal failure (CRF), pediatric renal allograft recipients (TX) and healthy normal controls. Patient number, age, gender, body mass index (BMI), diagnoses, systolic and diastolic arterial blood pressure SDS are listed and are expressed as means ± S.E.M. and range (age, BMI).

<table>
<thead>
<tr>
<th>Group CRF</th>
<th>Group TX</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years, range)</td>
<td>15.1 ± 4.1, 4.0–17.5</td>
<td>12.8 ± 4.8, 6.0–15.5</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>8/7</td>
<td>11/6</td>
</tr>
<tr>
<td>BMI (kg/m², range)</td>
<td>21.9 ± 5.2, 19.1–26.8</td>
<td>20.7 ± 4.3, 18.0–24.0</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Membrano-proliferative glomerulonephritis (1), paracellin deficiency (1), hemolytic uremic syndrome (3), renal dysplasia (2), nephrotic syndrome (end-stage renal failure) (8)</td>
<td>Nephrotic syndrome (6), hemolytic uremic syndrome (2), Prune-Belly syndrome (1), Bardet-Biedl syndrome (2), renal dysplasia (4), nephropathitis (1), cystinosis (1)</td>
</tr>
<tr>
<td>Systolic arterial blood pressure SDS</td>
<td>0.26 ± 0.33</td>
<td>0.25 ± 0.29</td>
</tr>
<tr>
<td>Diastolic arterial blood pressure SDS</td>
<td>0.06 ± 0.24</td>
<td>0.04 ± 0.28</td>
</tr>
</tbody>
</table>
Table 2 Therapeutical characteristics of patients suffering from chronic renal failure (CRF), pediatric renal allograft recipients (TX) and healthy normal controls. Time after allograft renal transplantation is listed and expressed as mean ± S.E.M. and range.

<table>
<thead>
<tr>
<th></th>
<th>Group CRF</th>
<th>Group TX</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of antihypertensive drugs</td>
<td>ACE-blocking agents (12), beta-blocking agents (10), calcium-channel-blocking agents (8), diuretics (3), no therapy (0)</td>
<td>ACE-blocking agents (13), beta-blocking agents (11), Ca-channel-blocking agents (8), diuretics (7), no therapy (2)</td>
<td>None</td>
</tr>
<tr>
<td>Number of antihypertensive drugs (number/patients)</td>
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<td>18</td>
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<td>0/2</td>
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<td>1/4</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2/6</td>
<td>2/6</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>3/3</td>
<td>3/4</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>4/3</td>
<td>4/1</td>
<td>None</td>
</tr>
<tr>
<td>Immunosuppressive drugs</td>
<td>No therapy (15)</td>
<td>Ciclosporin A (8), Tacrolimus (9), Mycophenolate-mofetil (17), Prednison (0), no therapy (0)</td>
<td>None</td>
</tr>
<tr>
<td>Time after renal allograft transplantation (years, range)</td>
<td>None</td>
<td>4.7 ± 2.7</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>0.5–10.0</td>
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using 2% methoxyamine hydrochloride in pyridine (50 μl, 1.5 h, 80 °C) and N-methyl-N-trimethylsilyltri-fluoracetamide (MSTFA)/1-trimethylsilylimidazole (TMCS)/trimethylchlorosilane (TMSIM) (1000/50/20, 27 h, 100 °C). These derivatives were then analyzed on a Shimadzu QP5050 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a mass selective detector. The gas chromatograph was fitted with a ZB-5 ms column (30 m × 0.25 mm, film thickness 0.25 μm; Phenomenex, Terrace, CA, USA). Helium was used as the carrier gas under the following conditions: interface temperature 300 °C, oven temperature initially 218 °C for 3 min increasing at 2 °C/min to 295 °C for 7.5 min. The following masses were selected for single ion monitoring (figures in brackets represent qualifier ions): m/z 398.5 (488.7, 578.7) THE, m/z 382.5 (652.7, 472.7, 562.7) THF and 5αTHF. The interassay coefficients of variation of quality control samples (n = 13) were 10% for THE (mean concentration 2.86 μg/ml), and 11% for THF (mean concentration 1.92 μg/ml) and for 5αTHF (mean concentration 2.11 μg/ml).

After centrifugation of blood samples, plasma and urine samples (collected over 24 h) were kept frozen for up to 8 weeks at 20 °C and were analyzed when all specimens had been obtained.

The activity of 11β-HSD2 in plasma was calculated using the precursor/product ratio of F/E. The activity of 11β-HSD2 in urine was calculated using the precursors/product ratio of (THF + 5αTHF)/THE. The creatinine clearance (CreatCl) was calculated according to the formula of Schwartz (24).

Values are shown as means ± S.D./S.E.M., steroids with range [min, max]. All groups of data passed normality tests and showed a Gaussian distribution. Data with Gaussian distribution were correlated by linear regression. Parametric data were compared by two-tailed t-test. In the case of multiple tests, data were compared using one-way ANOVA, and in the case of significance, a post-hoc t-test was used. P values were corrected according to Bonferroni. A P value < 0.05 was considered statistically significant (25, 26).

The study was approved by the local ethics committee (Friedrich-Alexander-University Erlangen-Nürnberg). Informed consent was obtained from parents and, as far as possible, also from the children.

Results

There was no significant difference between plasma cortisol values within the three groups, whereas plasma cortisone levels were significantly lower in the CRF (13.8 ± 4.7/1.2 [10.0, 19.2] ng/ml) and the TX groups (10.9 ± 6.2/1.6 [6.6, 16.8] ng/ml) compared with healthy controls (55.0 ± 28.7/6.7 [28.1, 72.0] ng/ml) (P < 0.0001). Plasma cortisone levels in the CRF and TX patients were not different. In the CRF group, we calculated an F/E ratio of 5.6 ± 1.97/0.55, while in the TX group 7.16 ± 3.09/0.86 was calculated. These values were significantly higher than in the control group (1.18 ± 0.15/0.03, P < 0.0001) (Fig. 1). Urinary THF was not different between the three groups (CRF: 90.6 ± 48.8/13.0 [52.5, 124.5] mg/24 h; TX: 43.8 ± 48.19/12.0 [15, 45.1] mg/24 h; CO: 59.8 ± 56.1/14.0 [15, 94] mg/24 h) and neither was urinary 5αTHF (CRF: 156.2 ± 37.3/55.2 [60.5, 165] mg/24 h; TX: 161.6 ± 42.6/57.2 [16.5, 194] mg/24 h). Urinary THE values were significantly lower in CRF (178.4 ± 16.9/29.6 [88.5, 251.5] mg/24 h) and TX patients (72.3 ± 47.9/12.4 [29.5, 105.5] mg/24 h) than in healthy subjects (69.3 ± 250/57.4 [456, 944] mg/24 h, P < 0.0001). THE values were not different between CRF and TX patients. The ratio of (THF + 5αTHF)/THE was not significantly different between CRF and TX patients.
aTHF)/THE was significantly higher in the CRF group (1.19±0.48/1.13) and TX group (1.19±0.5/0.13) than in the control group (0.21±0.18/0.05) (P < 0.0001), but the ratio was not different between CRF and TX patients (Fig. 2).

In two patients, urinary steroids before and after kidney transplantation were measured. In patient 1, urinary THF was 45.5 mg/24 h before and 47.5 mg/24 h after transplantation, 5αTHF was 97.2 mg/24 h before and 82.3 mg/24 h after transplantation, and THE was 85.3 mg/24 h before and 92.3 mg/24 h after transplantation; urinary (THF + 5αTHF)/THE ratios of 1.67 before and 1.41 after kidney transplantation were calculated. In patient 2, we found ratios of 1.32 before and 1.12 after kidney transplantation.

There were positive and highly significant correlations between plasma and urinary ratios in each group (P = 0.0004, R² = 0.73 in the CRF group, P = 0.0013, R² = 0.56 in the TX group, and P < 0.0001, R² = 0.66 in the CO group).

Neither systolic nor diastolic blood pressure standard deviation scores (SDS) (27) revealed any statistical differences when compared between the groups (Table 1). There was no significant correlation between blood pressure SDS and urinary (THF + 5αTHF)/THE or plasma F/E ratios (CRF group: P = 0.99, R² = 0.0000414; TX group: controls: P = 0.66, R² = 0.01; P = 0.69, R² = 0.01). There was no significant correlation between the number of antihypertensive drugs used and the (THF + 5αTHF)/THE ratio in the urine of CRF and TX patients (P = 0.56, R² = 0.027, P = 0.72, R² = 0.0087). In the CRF group, CreaCl was 19.8±5.8/1.5 ml/min/1.73 m², significantly lower than in the TX group (81.0±54.6/13.2 ml/min/1.73 m²), whereas CreaCl was normal in healthy controls (177.7±67.7/17.5 ml/min/1.73 m²). No significant correlations between CreaCl and the urinary (THF + 5αTHF)/THE or plasma F/E ratios were found in any group (CRF: P = 0.4, R² = 0.054; TX: P = 0.43, R² = 0.0042; CO: P = 0.62, R² = 0.015).

There was no significant correlation between time after renal allograft transplantation and the correlated ratios in plasma and urine in the TX group (P = 0.53, R² = 0.027).

Discussion

Our data show significantly higher plasma F/E ratios in children with chronic renal failure (CRF group) and those with pediatric renal allografts (TX group). The urinary precursor/product ratio calculated as the (THF + 5αTHF)/THE ratio was substantially higher in CRF patients and equally high in TX patients. Our data suggest reduced 11bHSD2 activity, both in children with end-stage renal failure and in patients after renal transplantation having steroid-free immunosuppressive therapy. For CRF children, these results are in line with those from other groups demonstrating reduced activity of 11b-HSD2 in adults with chronic renal failure (6, 28, 29).

There was no difference in the cortisol/cortisone ratios between patients suffering from chronic renal failure and the pediatric renal allograft recipients in this study. In our opinion, these data demonstrate that the allograft transplantation of a donor kidney,
an organ that is thought to be mostly responsible for the regulation of the cortisol/cortisone shuttle by renal 11β-HSD2 activity, does not normalize the 11β-HSD2 activity. There is only one case report of normal 11β-HSD2 activity after renal transplantation in a patient with AME (15). In this case, cortisol remained unconverted and was able to bind and activate the mineralocorticoid receptor leading to AME. The failure could, at least partially, be compensated by kidney transplantation. However, in contrast to our patients, this patient had a complete absence of 11β-HSD2 before renal transplantation. Although 11β-HSD2 appears to be reduced in CRF, its activity is much higher than in AME. Therefore, some restoration is to be expected after renal transplantation for AME.

The steroids data of two patients before and after renal transplantation showed that cortisol/cortisone ratios did not change considerably before and after renal transplantation. The number of examined patients in this study is relatively low. However, the number of pediatric transplant patients is also low: the total number of pediatric renal allograft transplantations in Germany is about 100/year.

Increased blood pressure, a frequent problem after renal allograft transplantation, is partially attributed to chronic allograft failure and immunosuppression (16, 18–20, 30). Our data show that the failure to restore 11β-HSD2 activity might also contribute to arterial hypertension. Furthermore, we have shown that neither urinary nor plasma F/E ratios are influenced by the level of chronic renal failure, as indicated by creatinine clearance, or by the age of the transplanted kidney. Therefore, we postulate that 11β-HSD2 cannot be restored to normal values by renal transplantation. The reduced activity of 11β-HSD2 leads to an increase in various metabolites inducing salt retention and leading to arterial hypertension (3, 5, 6, 9, 10, 13, 29, 31, 32). A very strict and aggressive antihypertensive drug therapy is necessary to avoid secondary complications of long-lasting arterial hypertension (12, 21, 33–35). In all our TX and CRF children there was the necessity to apply antihypertensive drugs using a variable combination of ACE-, beta-, calcium-channel-blocking and diuretic drugs. Systolic and diastolic blood pressure SDS values were normalized, not revealing any differences compared with healthy controls. This might be an explanation for the fact that we could not find any correlation between blood pressure values and plasma or urinary F/E ratios representing 11β-HSD2 activity. The number of

**Figure 2** (A) THF, (B) 5αTHF, (C) THE and (D) (5αTHF + THF)/THE ratio measured in urine using gas chromatography/mass spectrometry in patients suffering from chronic renal failure (group CRF), pediatric renal allograft recipients (group TX) and controls. Data are shown as means ± S.E.M. Significant differences between the study groups were evaluated using one-way ANOVA, P values were corrected according to Bonferroni.
antihypertensive drugs per patient did not correlate with the activity of 11β-HSD2. Possibly, the number of conflicting mechanisms does not allow for a simple relationship to be drawn between 11β-HSD2 and blood pressure. Different medications such as diuretic drugs (e.g. furosemide) probably modulate urinary 11β-HSD2 activity and this could be the subject of further investigations. Impaired liver function or cholestasis might modulate 11β-HSD2 activity. None of the children examined in our study showed hepatic insufficiency or cholestasis.

The measurement of cortisol and cortisone metabolites in urine is an estimate of the activity of human 11β-HSD in vivo (6, 36). The ratios reflect the activities of 11β-HSD2 and 11β-HSD type 1 enzymes in various organs such as kidney, colon, skin, salivary glands and lung. The positive correlation between plasma and urinary ratios in our study shows the suitability of either plasma or urine to estimate 11β-HSD activity. However, ratios of cortisol/cortisone are not an exact parameter for 11β-HSD activity, and therefore direct measurements of renal 11β-HSD2 expression might be helpful to determine the role of this enzyme more precisely.

Palermo and co-workers (15) suppressed endogenous cortisol secretion using methyl prednisolone, dexamethasone, cortisol or cortisone, and this procedure could provide a possible method to detect the ability of patients to metabolize cortisol. Unfortunately, in a pediatric cohort of patients such additional examinations for academic reasons only are difficult to countenance on ethical grounds.

In summary, all our patients presenting with chronic renal failure have increased cortisol/cortisone ratios potentially reflecting reduced 11β-HSD2 activity which could not be normalized by renal allograft transplantation. Normalization of systemic arterial blood pressure and clinically relevant changes in the creatinine clearance did not influence the cortisol/cortisone ratio. Reduced cortisol clearance by 11β-HSD2 might contribute to the development of arterial hypertension after renal allograft transplantation.

References