Epigenetic control of 11 beta-hydroxysteroid dehydrogenase 2 gene promoter is related to human hypertension

Simonetta Friso a,∗,1, Francesca Pizzolo a,1, Sang-Woon Choi b, Patrizia Guarini a, Annalisa Castagna a, Viviana Ravagnani c, Antonio Carletto c, Patrizia Pattini a, Roberto Corrocher a, Oliviero Olivieri a

a Department of Clinical and Experimental Medicine, University of Verona School of Medicine, Verona, Italy
b Vitamins and Carcinogenesis Laboratory, Jean Meyer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA
c Department of Clinical and Experimental Medicine, Unit of Rheumatology, University of Verona School of Medicine, Verona, Italy

Received 20 September 2007; received in revised form 16 November 2007; accepted 21 November 2007
Available online 7 February 2008

Abstract

Background: Lower activity of 11 beta-hydroxysteroid dehydrogenase 2 (11beta-HSD2) classically induces hypertension by leading to an altered tetrahydrocortisol- versus tetrahydrocortisone-metabolites (THFs/THE) shuttle. Recent cell culture and animal studies suggest a role for promoter methylation, a major epigenetic feature of DNA, in regulation of HSD11B2 expression. Little is known, however, of human HSD11B2 epigenetic control and its relationship with the onset of hypertension.

Objective: To explore the possible relevance of HSD11B2 promoter methylation, by examining human peripheral blood mononuclear cell (PBMC) DNA and urinary THFs/THE ratio as a biochemical indicator of 11beta-HSD2 activity, in blood pressure control.

Methods: Twenty-five essential hypertensives and 32 subjects on prednisone therapy were analyzed, the latter to investigate 11beta-HSD2 function in the development of hypertension.

Results: Elevated HSD11B2 promoter methylation was associated with hypertension developing in glucocorticoid-treated patients in parallel with a higher urinary THFs/THE ratio. Essential hypertensives with elevated urinary THFs/THE ratio also showed higher HSD11B2 promoter methylation.

Conclusions: These results show a clear link between the epigenetic regulation through repression of HSD11B2 in PBMC DNA and hypertension.

Keywords: HSD11B2; 11Beta-HSD2; Hypertension; Epigenetics; DNA methylation; Cortisol to cortisone metabolites ratio; THFs/THE ratio; Gene expression; Peripheral blood mononuclear cells

1. Introduction

Recent evidence suggests a role for the enzyme 11 beta-hydroxysteroid dehydrogenase type 2 (11beta-HSD2) (EC 1.1.1.146) in essential hypertension (EH) [1-3].

The enzyme catalyzes the dehydrogenation of 11beta-hydroxylglucocorticoids to 11keto-steroids with NAD⁺ as a cosubstrate [4,5], including conversion of cortisol to its inactive metabolite, cortisone. Loss of 11beta-HSD2 activity leads to activation of mineralocorticoid receptors by cortisol causing a renal sodium retention [6] and increased blood pressure [7,8]. HSD11B2 mRNA expression is inversely related with measured urinary THFs/THE ratio [9], evidence that the THFs/THE ratio reflects 11beta-HSD2 activity [4].

Polymorphic sites within the HSD11B2 gene have been associated with a slightly reduced enzyme function [10]. However, there is no clear evidence for a functional genetic
defect responsible for an impaired enzyme activity and raised blood pressure in essential hypertension. Epigenetic modulation of the HSD11B2 gene has been recently demonstrated in both a rodent model and cultured human cell lines [11]. CpG islands within the promoter region and first exon of the gene were found to be heavily methylated, associated with reduced gene expression [11]. Whether promoter methylation of HSD11B2 is related in vivo to blood pressure control in EH patients is yet to be established.

The main purpose of the present study was to evaluate the potential relationship between HSD11B2 gene promoter methylation, the cortisol to cortisone metabolite ratio as a biomarker for 11beta-HSD2 enzyme function and the development of hypertension in a human study. Because glucocorticoid therapy may lead to development of hypertension, we chose to study a group of patients on prednisone therapy, as a model for understanding a potential role for 11beta-HSD2 in the onset of hypertension, to explore a possible link between HSD11B2 promoter methylation and high urinary THFs/THE ratio.

2. Subjects and methods

2.1. Subjects

2.1.1. Glucocorticoid-treated patients

Thirty-two patients were selected from the Rheumatology Unit of the University of Verona School of Medicine with rheumatoid arthritis and undergoing chronic glucocorticoid treatment. Inclusion criteria were normotension (\(<140/90\) mmHg) at baseline before treatment was prescribed. All patients were on oral prednisone at a dosage range 5–15 mg/day for at least 3 months before urinary cortisol to cortisone metabolite assay in a 24 h urine collection. Patients were regularly followed-up, and at the time of blood and urine sampling were in remission from rheumatoid arthritis disease.

2.1.2. Essential hypertensive patients

Twenty-five patients from the Hypertension Unit of the University of Verona School of Medicine over the last 2 years were evaluated by routine biochemical and urinary metabolite assays. All patients were on a sodium-controlled diet (NaCl 110–120 mmol/day) for 3 days before blood and urine sampling, and were taking no anti-hypertensive drugs other than verapamil and/or alpha-blockers over the previous 4 weeks [12]. Patients with any history of glucocorticoid treatment or who consumed liquorice were excluded. The study was approved by our Institutional review board, and all patients provided informed consent prior to inclusion in the study.

2.2. Biochemical analyses and urinary steroid metabolites

Venous blood was collected into EDTA-containing Vacutainer tubes after overnight fasting for routine analyses including plasma creatinine, serum electrolytes and cortisol levels as previously described [12]. Urinary tetrahydrocortisol (THF), 5α-tetrahydrocortisol (αTHF) and tetrahydrocortisone (THE) were analyzed by gas chromatography/mass spectrometry [13].

2.3. Molecular analyses

2.3.1. Bisulfite treatment

DNA from PBMC was collected on the same day as urine for steroid analysis and stored at \(-80^\circ\)C before extraction with a Wizard® Genomic DNA Purification Kit (Madison, WI, USA). Bisulfite treatment was performed by the CpG Genome™ DNA modification kit (Chemicon International, Inc. Temecula, CA, USA). DNA was immediately used for PCR reaction or stored at \(-80^\circ\)C until use.

2.3.2. HSD11B2 gene promoter methylation

Bisulfite-treated DNA from PBMC was amplified by PCR as previously described [14]. The HSD11B2 gene promoter region contains two CpG islands (GenBank accession no. U27317) which we considered in designing the specific sets of primers to detect CpG sites as either methylated or unmethylated. PCR was performed using hot start Taq polymerase (AB Analitica, Padova, Italy). Optimal conditions were established for PCR using both unmethylated and methylated primer sets using the same protocol (primers and conditions available upon request). To verify the efficiency of the newly designed primers, both unmethylated and methylated primer sets were tested with bisulfite-treated fully unmethylated and fully methylated DNA (CpG Genome™ Universal Unmethylated or Methylated DNA, respectively by Chemicon International, Inc. Temecula, CA, USA) which were both run during each PCR reaction. Amplification products were resolved by gel electrophoresis and stained with ethidium bromide. The index of methylation was obtained by calculating the band density (intensity/mm²) measured under UV light by a Gel Doc XR 170-8170 (Bio-Rad, Laboratories, Hercules, CA, USA) and expressed as the percent density of [methylated-band/(methylated-band + unmethylated-band)].

2.4. Statistical analysis

Statistical analysis was performed with the SPSS Statistical software program for Windows, version 13.0 (SPSS, Inc., Chicago, IL, USA). Distribution of continuous variables is expressed as mean ± SD. Logarithmic transformation was performed on all skewed variables to normalize their distribution. Such data are then presented as geometric means (antilogarithms of the transformed means) with estimation of 95% confidence intervals (CIs). Analysis of comparisons between groups was undertaken by Student’s unpaired t-test. Pearson’s \(\chi^2\) test was used for qualitative variables. Comparison of proportions was carried out by cross-tabulation and Pearson’s \(\chi^2\) test. Values of \(P<0.05\) were considered statistically significant.
Table 1
Clinical and biochemical features of glucocorticoid-treated and essential hypertensive patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Glucocorticoid-treated patients (n = 32)</th>
<th>Essential hypertensive patients (n = 25)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>54.56 ± 12.6</td>
<td>51.92 ± 14.3</td>
<td>NS</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>4/28</td>
<td>14/11</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>131.41 ± 15.2</td>
<td>170.20 ± 22.1</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82.34 ± 10.1</td>
<td>103.30 ± 14.5</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>S-Na⁺ (mmol/L)</td>
<td>142.32 ± 2.1</td>
<td>141.76 ± 2.1</td>
<td>NS</td>
</tr>
<tr>
<td>S-K⁺ (mmol/L)</td>
<td>3.80 ± 0.3</td>
<td>3.97 ± 0.4</td>
<td>NS</td>
</tr>
<tr>
<td>U-Na⁺ (mmol/24 h)</td>
<td>150.00 ± 57.7</td>
<td>190.80 ± 50.1</td>
<td>NS</td>
</tr>
<tr>
<td>U-K⁺ (mmol/24 h)</td>
<td>58.60 ± 17</td>
<td>46.90 ± 25.5</td>
<td>NS</td>
</tr>
<tr>
<td>S-Creatinine (µmol/L)</td>
<td>68.00 ± 18.7</td>
<td>80.90 ± 20.0</td>
<td>&lt;0.05†</td>
</tr>
<tr>
<td>S-Cortisol (µg/dL)</td>
<td>9.95 ± 7.2</td>
<td>19.53 ± 6.4</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>P- ACTH (pg/mL)</td>
<td>15.46 ± 12.6</td>
<td>38.36 ± 27.9</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>P- Aldosterone (pg/mL)</td>
<td>214.6 ± 186.6</td>
<td>223.0 ± 79.8</td>
<td>NS</td>
</tr>
<tr>
<td>P- Renin (pg/mL)</td>
<td>22.72 ± 57.7</td>
<td>11.74 ± 13.8</td>
<td>NS</td>
</tr>
<tr>
<td>ARR (pg mL⁻¹/pg mL⁻¹)</td>
<td>29.00 ± 46.6</td>
<td>33.10 ± 26.1</td>
<td>NS</td>
</tr>
<tr>
<td>THF (µg/24 h)</td>
<td>0.64 ± 0.6</td>
<td>1.87 ± 1.2</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>α-THF (µg/24 h)</td>
<td>0.33 ± 0.5</td>
<td>1.23 ± 1.0</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>THE (µg/24 h)</td>
<td>1.01 ± 0.8</td>
<td>3.64 ± 2.1</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>THFs/THE ratio</td>
<td>1.46 ± 1.2</td>
<td>0.96 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>THFs/THE ratio ≥ 1.5</td>
<td>44% (14/32)</td>
<td>24% (6/25)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SD. Statistical significance (P < 0.05) was defined by χ² test for qualitative variables (*) or by Student’s t test (†). SBP, systolic blood pressure; DBP, diastolic blood pressure; ARR, aldosterone to renin ratio; THFs/THE ratio, tetrahydrocortisol- versus tetrahydrocortisone-metabolites ratio.

3. Results

3.1. Subjects

Fifty-seven subjects were evaluated, 32 undergoing glucocorticoid therapy and 25 EH patients. Of the 32 patients treated with prednisone, 15 developed hypertension. No major differences in terms of duration, doses of prednisone or the major clinical–biochemical features were seen between the two groups of glucocorticoid-treated subjects or between the EH and those who developed hypertension on glucocorticoid treatment. In particular, potential differences between the two groups were evaluated for age, serum creatinine, plasma renin and aldosterone, plasma ACTH, serum cortisol, serum and urinary electrolytes, urinary steroids profile (data not shown).

3.2. Relationships between HSD11B2 promoter methylation, urinary THFs/THE and hypertension

The methylation index was correlated to the absolute values of THFs/THE ratios (Pearson’s correlation coefficient r = 0, 49, P < 0.0001). Table 1 shows the clinical and biochemical features of glucocorticoid-treated and essential hypertensive patients. As expected, within the rheumatoid arthritis glucocorticoid-treated patients group, there was a higher number of females whereas more males were within the EH group. Furthermore, EH patients showed higher creatinine levels and glucocorticoid-treated subjects had lower serum cortisol and ACTH compared to the EH group, as expected for the inhibitory effect of prednisone therapy. Urinary THF, α-THF and THE were also significantly lower in the glucocorticoid-treated compared to the EH patients group (Table 1).

In the whole group (n = 57), an elevated THFs/THE ratio (defined as a ratio ≥ 1.5) was associated with higher HSD11B2 promoter methylation (52.0% versus 43.75%, P < 0.0001) (Fig. 1). When glucocorticoid-treated subjects were stratified according to urinary THFs/THE ratio ≥ 1.5, a statistically significant larger number of patients with a high ratio had hypertension (60.0% versus 29.4%, P = 0.036) (Fig. 2A). Moreover, glucocorticoid-treated patients who developed hypertension showed a higher HSD11B2 promoter methylation than those who did not (53.63 versus...
Fig. 2. (A) Subjects under glucocorticoid treatment with high THFs/THE ratio ($n = 15$) according to hypertensive status. As shown, a higher percentage of subjects (9/15, 60%) with an elevated THFs/THE developed hypertension ($P = 0.036$). (B) Promoter HSD11B2 methylation index, expressed as percent density (intensity/mm²) of [methyl-band/(methyl-band + unmethyl-band)], in glucocorticoid-treated patients according to the presence of hypertension. As shown, HSD11B2 promoter methylation was higher in those glucocorticoid-treated patients who developed hypertension. ($P = 0.018$).

45.72%, $P = 0.018$) (Figure 2B). Table 2 shows the differences in promoter methylation and THFs/THE ratio between essential and prednisone-treated hypertensive patients. As indicated in Table 2, glucocorticoid-treated hypertensive subjects had higher HSD11B2 promoter methylation and THFs/THE ratios than EH patients (1.5, 95% CIs 1.1–2.06 versus 0.8, 95% CIs 0.65–1.04, $P = 0.002$). A higher percentage of glucocorticoid-treated hypertensive patients showed a high THFs/THE ratio ($\geq 1.5$) compared with EH patients (60% versus 24%, $P = 0.023$).

### Table 2

Characteristics of the subjects affected by either essential or prednisone-treatment associated hypertension

<table>
<thead>
<tr>
<th>Variable</th>
<th>Glucocorticoid-treated hypertensive patients ($n = 15$)</th>
<th>Essential hypertensive patients ($n = 25$)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSD11B2 promoter methylation index</td>
<td>53.63%</td>
<td>43.08%</td>
<td>$&lt;0.0001^*$</td>
</tr>
<tr>
<td>THFs/THE ratio</td>
<td>1.5$^a$ (1.1–2.1)</td>
<td>0.8$^b$ (0.6–1.0)</td>
<td>0.002$^a$</td>
</tr>
<tr>
<td>THFs/THE ratio $\geq 1.5$</td>
<td>60.0% (9/15)</td>
<td>24.0% (6/25)</td>
<td>0.023$^a$</td>
</tr>
</tbody>
</table>

Statistical significance ($P < 0.05$) was defined by Student's $t$-test ($^*$), Pearson’s $\chi^2$ test was used for qualitative variables ($^\dagger$).

$^a$ Promoter methylation index in HSD11B2 is expressed as percent density (intensity/mm²) of [methyl-band/(methyl-band + unmethyl-band)].

$^b$ Geometric mean; 95% CI in parentheses (all such values).

4. Discussion

The present study shows that the HSD11B2 promoter region in PBMC DNA has an elevated methylation status in glucocorticoid-treated patients who develop hypertension. Furthermore, the higher DNA methylation at HSD11B2 promoter sites parallels a higher urinary THFs/THE ratio. The urinary THFs/THE ratio is an index of 11beta-HSD2 activity which is known to play a role in hypertension [1–4]. Promoter methylation regulates gene transcription and is, therefore, an indicator of gene expression and 11beta-HSD2 enzyme activity [11]. The epigenetic regulation of HSD11B2 gene promoter region by methylation highlights, therefore, a potential new mechanism whereby 11beta-HSD2 exerts its role in the pathogenesis of glucocorticoid-induced hypertension and in some patients with essential hypertension. The present results thus strongly support the view that even mild changes in the 11beta-HSD2 activity due, at least in part, to aberrant site-specific methylation, may affect blood pressure control [1,4]. The methylation status at HSD11B2 promoter site in PBMC DNA may reflect a global rather than a tissue-selective status and be a potentially useful molecular biomarker to characterize hypertensive patients.

The study of patterns of DNA methylation has recently received increasing interest as a major mechanism for the regulation of gene expression [15]. In mammalian cells, DNA methylation usually occurs at cytosine bases located 5’ to a guanosine in CpG dinucleotide sequences, the so called CpG islands [15]. The human HSD11B2 gene has been recently shown to contain two CpG islands located in the promoter and exon 1 and two islands in exon 5 and further downstream region [11].

The genesis of the higher HSD11B2 promoter methylation observed in some prednisone-treated patients and essential hypertensives is open to speculation. DNA methylation is catalyzed by methyltransferases which use S-adenosylmethionine, a product of one-carbon metabolism, as methyl donor [16]. In a rodent experimental model [17,18], a role for glucocorticoids in affecting one-carbon metabolism has been suggested [17–19] and this pathway is known to modulate DNA methylation [16,20]. Such a possibility that cortisol thus modulates methylation of DNA at this site could be only proven by determining whether such patients show reduced HSD11B2 promoter methylation after prednisone withdrawal together with normalization of the ratio.
DNA methylation at promoter sites has to date been largely addressed in cancer, and epigenetically mediated transcriptional-silencing events demonstrated [15]. The present study is an example of the role of DNA methylation in modulation of HSD11B2 gene expression in the pathogenesis of hypertension, with a clear relationship between patterns of methylation and clinical outcome. Considering that epigenetic phenomena such as DNA methylation are potentially reversible, the present results may have both etiopathogenetic and therapeutic implications.

Acknowledgments

This work was supported by grants from the Cariverona Foundation, Verona, Italy to S. Friso; the Ministry of the University and Scientific and Technological Research and the Veneto Region Department of Health to O. Olivieri.

References