Influence of standard haemodialysis treatment on transcription of human serum- and glucocorticoid-inducible kinase SGK1 and taurine transporter TAUT in blood leukocytes

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Abstract

Background. Standard haemodialysis (HD) rapidly alters osmolality and composition of extracellular fluid and, thus, challenges cell volume constancy. Cell volume-sensitive genes upregulated by osmotic cell shrinkage include those encoding for taurine transporter TAUT as well as for serum- and glucocorticoid-inducible kinase SGK1.

Methods. Six HD patients were haemodialysed for 4 h with high-flux dialysers. Blood was drawn from the arterial section of the fistula immediately prior to start of HD and subsequently after 60, 120 and 240 min of HD treatment and, in addition, 120 min after HD treatment. Taurine plasma concentrations ([taurine]p) and erythrocytic taurine content ([taurine]e) were determined by high-performance liquid chromatography. SGK1 and TAUT transcript levels in leukocytes were quantified by real-time polymerase chain reaction.

Results. The [taurine]p was significantly higher in HD patients before HD treatment when compared with healthy controls and it decreased significantly during 4 h of HD. The ratio of SGK1/GAPDH and of TAUT/GAPDH transcript levels increased significantly by 50% or 27%, respectively, during HD.

Conclusions. Standard HD treatment decreases plasma taurine concentration and upregulates leukocyte SGK1 and TAUT transcription. As SGK1 is a potent regulator of ion channels and transporters in nervous system, heart muscle and epithelial cells, the deranged regulation of SGK1 may contribute to acute side effects of HD treatment.

Keywords: cell volume regulation; osmolytes; real-time polymerase chain reaction; SGK1; taurine transporter

Introduction

During a standard 4-h HD treatment the enhanced pre-dialytic plasma osmolality is usually decreased to isotonicity [1]. This decrease is attributed mainly to urea clearance while the extracellular (plasma) Na⁺ concentration remains largely constant. Nevertheless, the rapid alterations of plasma osmolality and composition during dialysis may challenge cell volume regulatory mechanisms [2].

To counteract osmotic shrinkage, cells have to gain osmolality by uptake of electrolytes and organic osmolytes [2]. This is achieved by activation of a set of membrane transporters, including the Na⁺/H⁺ exchanger in parallel to the Cl⁻/HCO₃⁻ exchanger, the Na⁺, K⁺, 2Cl⁻ co-transporter, Na⁺ channels and Na⁺, Cl⁻ coupled uptake mechanisms for inositol, taurine and betaine [2,3]. In addition, cells may generate organic osmolytes by degrading intracellular proteins to the osmotically more active amino acids [2] and by stimulating sorbitol [3] and glycerophosphorylcholine formation [4]. To counteract osmotic cell swelling, cells release electrolytes and organic osmolytes through activation of K⁺/Cl⁻ symport and by opening of osmolyte channels [2]. However, cells not only activate the respective carriers, channels and enzymes, but adapt to osmotic challenges by an altered expression of the respective genes [2].

Osmosensitive genes include the Na⁺ taurine co-transporter TAUT [5], which serves to accumulate the organic osmolyte taurine in shrunken cells [3],
and the serum- and glucocorticoid-inducible kinase SGK1 [6]. In contrast to TAUT, SGK1 is an early gene upregulated within minutes upon osmotic cell shrinkage [7,8].

The aim of this study was to explore the influence of a standard 4-h HD treatment on the mRNA transcription of cell volume-sensitive genes. The transcript levels of SGK1 were taken as those of a rapidly regulated gene, while the Na\textsuperscript{+}taurine co-transporter TAUT represents a slowly regulated gene [5]. Expression of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was taken as the reference gene. Transcript levels were determined by real-time polymerase chain reaction (PCR) of blood leukocyte mRNA before, during and at the end of standard HD treatments. In addition, taurine concentrations in plasma and erythrocytes were determined by high-performance liquid chromatography (HPLC).

Subjects and methods

Patients

Six HD patients (average age: 63 ± 14 years; mean time on HD: 36 ± 28 months) with stable cardiovascular parameters were included. All patients were haemodialysed with a Fresenius 4008 dialysis machine containing a diasafe device for water pureness and using F-series high-flux dialyser membranes (Fresenius Medical Care, Bad Homburg, Germany). The iso-osmolar dialysate contained glucose (1 g/l) and sodium (140 mM), which was kept constant throughout the 4-h sessions. For real-time PCR, blood samples were withdrawn at 0, 60, 120 and 240 min of the 4-h HD sessions and, in addition, 120 min after HD treatment for HPLC measurements at 0 and 240 min.

Exclusion criteria were severe comorbidities (e.g. malignancy and active allergies), blood haemoglobin <9 mg/dl, C-reactive protein >5 mg/dl and active infections as indicated by fever >39°C. Drop-out criteria were acute febrile episodes (T > 39°C), intradialytic hypotensive episodes with intravenous application of a sodium bolus, an oral fluid volume of >500 ml during HD and patients not complying with the study protocol (e.g. dialyser and blood samples).

All patients gave informed consent and the study was approved by the ethics committee of the University of Tübingen.

HPLC measurements

Taurine concentrations in plasma and erythrocytes were measured by HPLC. Erythrocytes were separated from plasma by low-spin centrifugation (3500 g, 10 min, 15°C) and hypotonically lysed in distilled water (500 µl per 10\textsuperscript{10} cells). The lysate was spun again (15000 g, 10 min, 4°C) and 80 µl of the clear supernatant was deproteinized using 20 µl sulphosalicylic acid (10%). Amine acid concentrations, including taurine in plasma and erythrocyte lysates, were determined using an amino acid analyser (Eppendorf LC 3000, Hamburg, Germany), which includes ion exchange chromatography with successive ninhydrin derivatization and photometric analysis of stained amino acids at 440 and 570 nm. Amino acids were determined in comparison with authentic standards.

Quantitative real-time PCR measurements

Total RNA of blood leukocytes was stabilized immediately and extracted using the PaxGene System\textsuperscript{®} (Qiagen, Hilden, Germany). Subsequently, 1 µg total RNA was reverse transcribed into cDNA utilizing the reverse transcription system (Bioscience, USA) with oligo(dT) primers according to the manufacturer’s protocol. To determine SGK1 mRNA levels, quantitative real-time PCR with the LightCycler System\textsuperscript{™} (Roche Diagnostics, Mannheim, Germany) was established. PCR reactions for SGK1 were performed in a final volume of 20 µl containing 2 µl cDNA, 2.4 µl MgCl\textsubscript{2} (3 mM), 1 µl primer mix (0.5 µM of both primers), 2 µl cDNA Master SybrGreen I mix (Roche Molecular Biochemicals, Mannheim, Germany) and 12.6 µl DEPC-treated water.

The following primers for SGK1 (Gene Bank accession no. NM005627) were used:

SGK1 upper: 5'-TTC TCT TTC CAG ACT GCT GA-3'
SGK1 lower: 5'-TGG ATG TTG TGC TGT TGT GT-3'

Transcript levels of the housekeeping GAPDH were determined for each sample using a commercial LightCycler primer kit (Search LC, Heidelberg, Germany). Here, the PCR reactions were performed in a final volume of 20 µl containing 2 µl cDNA, 2 µl primer mix (Search LC, Heidelberg, Germany), 2 µl cDNA Master SybrGreen I mix (Roche Molecular Biochemicals, Mannheim, Germany) and 14 µl DEPC-treated water.

Amplification of the target DNA was performed during 35 cycles of 95°C for 10 s, 68°C for 10 s and 72°C for 16 s, each with a temperature transition rate of 20°C/s and a secondary target temperature of 58°C with a step size of 0.5°C. Melting curve analysis was performed at 95°C for 0 s, 58°C for 10 s and 95°C for 10 s to determine melting temperatures of primer dimers and the specific PCR products. Melting curve analysis confirmed the amplified products, which were then separated on 1.5% agarose gels to confirm the expected size (270 bp; data not shown). Finally, results were calculated as a ratio of target vs housekeeping gene transcript levels.

Transcript levels for human Na\textsuperscript{+}taurine co-transporter TAUT were determined using a commercial LightCycler primer kit (Search LC, Heidelberg, Germany). PCR reactions for TAUT were performed in a final volume of 20 µl containing 2 µl cDNA, 2 µl primer mix (Search LC, Heidelberg, Germany), 2 µl cDNA Master SybrGreen I mix (Roche Molecular Biochemicals, Mannheim, Germany) and 14 µl DEPC-treated water. Real-time PCR was performed with the same protocol as for SGK1.

Quantitative analysis of SGK1/TAUT expression in leukocytes before/after HD ± plasma exchange

To explore whether altered SGK1/TAUT transcription levels were secondary to an altered composition of plasma rather than to mechanical stress of the blood cells during HD treatment, SGK1/TAUT transcript levels were quantified in leukocytes before and after HD with plasma obtained before and after dialysis. To this end, blood samples taken from HD patients before and after 240 min of HD were centrifuged (10 min, 3000 r.p.m.) to separate corpuscular and plasma components. Sedimented blood cells before or after...
HD were then mixed with plasma isolated both before and after standard HD. After an incubation period of 1 h, mixed blood samples were processed as described above.

**Cell culture**

HepG2 human hepatoma cells were maintained in DMEM/5% CO2/5 mM glucose at 37°C, pH 7.4, supplemented with 10% (vol/vol) fetal calf serum. Prior to RNA isolation, the cells were grown to 90% confluence and shifted into basal Eagle medium (BME, GIBCO/BRL) without FCS for 12 h. As indicated, the cells were incubated for 180 min with different urea concentrations (0, 10, 30, 100 and 300 mmol/l). For measurement of time dependence, HepG2 cells were incubated with 300 mmol/l urea at different time points (0, 30, 60, 120 and 180 min). Furthermore, SGK1 mRNA was determined following inhibition of transcription by actinomycin D (5 μg/ml). For comparison of the effect of hypertonic urea, hypertonic NaCl or raffinose were applied for 2 h. In each of the experiments, the mRNA abundance was determined by northern blot analysis.

**Northern blot analysis**

Digoxigenin (DIG)-labelled probes were generated by direct PCR labelling of the differential amplicons using the appropriate primers and conditions noted above, except for dNTP concentrations: 200 μM dATP, 200 μM dCTP, 200 μM dGTP, 190 μM dTTP and 10 μM DIG–dUTP (Boehringer Mannheim, Mannheim, Germany). Northern blots were prepared with 20 μg total RNA or with 2 μg poly(A) RNA that had been electrophoresed through 1% agarose gels in the presence of 2.2 M formaldehyde. Equivalent loading of samples was verified by ethidium bromide staining of the ribosomal RNA bands or by using a DIG-labelled antisense ribonucleoprotein C1 as internal standard when poly(A) RNA was examined. The size of RNA was estimated by the DIG-labelled Molecular Weight Marker I (Boehringer Mannheim, Mannheim, Germany). Vacuum blotting (Appligene Oncor Trans DNA Express Vacuum Blotter; Appligene, Illkirch Graffenstaden, France) was used for transfer on positively charged nylon membranes (Boehringer Mannheim, Germany), which were then cross-linked under ultraviolet light (Stratagene UV Stratalinker 2400; Stratagene, Amsterdam, the Netherlands). Hybridization overnight was performed in DIG-Easy-Hyb (Boehringer Mannheim, Mannheim, Germany) at a probe concentration of 25 or 100 ng/ml and at a temperature of 50 or 65°C for DNA probes or RNA probes, respectively.

**Measurement of serum electrolytes, bicarbonate, pH and osmolality**

Sodium, chloride and potassium concentrations were determined utilizing the respective electrodes (Advia 1650; Bayer Leverkusen, Leverkusen, Germany) and calcium concentrations were determined by photometric determination of cresolphthalein (Advia 1650; Bayer Leverkusen, Leverkusen, Germany). For determination of pH and HCO3− an ABL 725 analyser was used (ABL 725 Radiometer; ABL, Willich, Germany). Osmolality was measured using the ultracooling method at an Osmo Station OM-6050 (A. Menarini Diagnostics, Neuss, Germany).

**Statistical analysis**

Results are expressed as means ± SEM. Statistical analysis was performed using unpaired Student’s t-tests. A P-value of <0.05 was considered significant.

**Results**

**Plasma osmolality, electrolyte, glucose and urea concentrations**

Standard haemodialysis (HD) decreased plasma osmolality from 302 ± 4 to 281 ± 3 mOsm/kg, paralleled by a significant (P < 0.01) decrease of serum urea concentration from 132 ± 10 to 39 ± 3 mg/dl (n = 6), values typical for standard HD treatment in Tübingen (Figure 1A and B). At the same time, serum sodium concentration increased significantly (P < 0.05) from 138.8 ± 0.9 to 141.8 ± 0.4 mmol/l (Figure 1C). Plasma potassium concentration decreased and bicarbonate concentration increased during 4 h of HD (n = 4), alterations being statistically significant in paired comparison (P < 0.05). The pH and plasma calcium and glucose concentrations did not change significantly during 4 h of HD (Table 1).

**Taurine plasma concentration and taurine content of erythrocytes**

As illustrated in Figure 2A, taurine plasma concentration tended to be higher in HD patients prior to HD (74 ± 12 μM; n = 6) when compared with healthy control individuals (52 ± 4 μM; n = 5). A 4-h HD treatment led to a significant (P < 0.01) decrease of plasma taurine concentration to 33 ± 5 μM.

The taurine content of erythrocytes was significantly (P < 0.01) higher in HD patients (74 ± 12 μM) than in healthy volunteers (28 ± 2 μM) (Figure 2B). A 4-h HD treatment did not significantly influence the erythrocytic taurine content (81 ± 11 μM).

**Transcript levels of the Na+/taurine co-transporter TAUT**

The ratio of TAUT transcript levels in relation to GAPDH transcript levels in total blood leukocytes from HD patients (n = 12, from six different patients) increased significantly (P < 0.05) by 9 ± 5%, 18 ± 7% and 27 ± 12% at 60, 120 and 240 min of HD, respectively (Figure 3). In addition, the TAUT transcript levels were still enhanced 2 h after completion of HD (39 ± 26%, n = 5; P = NS).

**Transcript levels of the serum- and glucocorticoid-inducible kinase SGK1**

In HD patients (n = 12, from six different patients) the ratio of SGK1 transcript levels in relation to GAPDH
Fig. 1. Plasma osmolality, serum urea and serum sodium concentrations. Plasma osmolality (A), serum urea (B) and serum sodium (C) in dialysis patients (n = 6). HD increased the transcript levels of SGK1 despite a significant (P < 0.01) simultaneous decrease of plasma osmolality from 302 ± 4 to 281 ± 3 mOsm/kg. Serum urea dropped significantly (P < 0.01) from 132 ± 10 to 39 ± 3 mg/dl during HD. Serum sodium concentration increased significantly (P < 0.05) from 138.8 ± 0.9 to 141.8 ± 0.4 mmol/l.

Table 1. Serum electrolytes, glucose, bicarbonate and pH. A standard 4-h HD treatment decreased plasma potassium concentration and increased bicarbonate concentration significantly (P < 0.05). The pH, plasma calcium and glucose concentrations did not change significantly during HD (n = 4).

<table>
<thead>
<tr>
<th>Glucose (mg/dl)</th>
<th>0 min</th>
<th>60 min</th>
<th>120 min</th>
<th>240 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium (mmol/l)</td>
<td>4.8 ± 0.5</td>
<td>4.2 ± 0.4</td>
<td>4.0 ± 0.3</td>
<td>3.7 ± 0.2a</td>
</tr>
<tr>
<td>Calcium (mmol/l)</td>
<td>1.17 ± 0.09</td>
<td>1.13 ± 0.04</td>
<td>1.12 ± 0.03</td>
<td>1.10 ± 0.04</td>
</tr>
<tr>
<td>HCO3 (mmol/l)</td>
<td>21.0 ± 3.0</td>
<td>24.0 ± 0.8</td>
<td>25.0 ± 0.5</td>
<td>25.0 ± 0.9a</td>
</tr>
<tr>
<td>pH</td>
<td>7.34 ± 0.08</td>
<td>7.39 ± 0.06</td>
<td>7.42 ± 0.07</td>
<td>7.41 ± 0.08</td>
</tr>
</tbody>
</table>

aP < 0.05.

Fig. 2. Taurine plasma and erythrocyte content. (A) Taurine plasma concentration in HD patients (n = 6) vs control (n = 5). Taurine concentration in plasma was not significantly (P > 0.08) different in HD patients prior to HD (74 ± 9 μM) as compared with healthy individuals (52 ± 4 μM). A 4-h HD treatment led to a significant (P < 0.01) decrease of plasma taurine concentration to 33 ± 5 μM. (B) Taurine content of erythrocytes in HD patients (n = 6) vs control (n = 5). In contrast to taurine plasma concentration, erythrocyte taurine content was significantly (P < 0.01) higher in HD patients (74 ± 12 μM) as compared with healthy volunteers (28 ± 2 μM). A 4-h HD treatment did not change the erythrocyte taurine content (81 ± 11 μM).

Fig. 3. Regulation of TAUT transcription in blood leukocytes. Taurine transporter transcript levels in HD patients (n = 12, from six different patients). The ratio of TAUT transcript levels in relation to GAPDH transcript levels increased significantly (P < 0.05) by 18 ± 7% and 27 ± 12% within 120 and 240 min of HD, respectively. The transcript levels still were enhanced 2 h after completion of HD (39 ± 26%, n = 5; P = NS).
transcript levels increased significantly during a standard 4-h HD treatment \((P<0.01)\) by 43\(\pm 7\)% and 59\(\pm 12\)% within 60 and 120 min of HD, respectively. SGK1 transcript levels were 50\(\pm 15\)% higher immediately after HD treatment as compared with the respective values prior to HD \((P<0.01)\). The transcript levels were still significantly enhanced 2 h after completion of HD \((79\pm 31\,\% , n=5; P<0.05)\).

Quantitative analysis of SGK1/TAUT expression in leukocytes before/after HD±plasma exchange

As illustrated in Fig. 5A, SGK1 transcript levels were significantly \((p<0.05)\) lower in leukocytes gathered after HD and exposed to plasma obtained prior to HD \((-5.5\pm 12\%)\) than in leukocytes incubated with plasma after HD \((\text{increase of } 39\pm 10\%).\) The observation points to inhibition of SGK1 transcription by a component of pre-dialytic plasma. SGK1 transcript levels in leukocytes gathered prior to HD tended to increase following exposure to post-dialytic plasma, an effect, however, not reaching statistical significance. Measurement of TAUT expression showed no significant difference in the examined groups (Figure 5B).

Influence of urea on SGK1 expression in HepG2 cells

In vitro experiments on cultured HepG2 cells were performed to test whether the increase of SGK1 transcript levels during HD could possibly be attributed to the simultaneous decrease of serum urea concentrations. The mRNA abundance was determined by northern blot analysis. Urea led to a rapid decline of SGK1 transcript levels in a dose- (Figure 6A) and time-dependent manner (Figure 6B), indicating that the declining urea concentrations during HD may contribute to the increase of SGK1 transcript levels. Following inhibition of SGK1 transcription with actinomycin D \((5\mu\text{g/ml})\) or urea \((300\text{mmol/l})\), the decrease of SGK1 transcript levels was comparable (Figure 6C), indicating that urea does not significantly destabilize SGK1 mRNA and that the effect of urea is due to inhibition of transcription. Exposure of the cells to hypertonic NaCl or raffinose increased SGK1 mRNA abundance, indicating that the effect of urea was not due to hypertonicity (Figure 6D).

Discussion

The present observations confirm earlier data [9] showing that renal insufficiency leads to enhanced intraerythrocyte levels of the organic osmolyte taurine. The present observations further present evidence
that HD treatment leads to efficient elimination of plasma taurine, but does not normalize the enhanced erythrocyte taurine levels. While intraerythrocytic taurine levels remain elevated, plasma taurine concentration decreases from values significantly above to values significantly below those observed in healthy controls. Altered plasma taurine concentrations have been observed in HD patients previously [10].

More importantly, the present study reveals a significant increase of transcript levels of two genes (SGK1 and TAUT) during a standard 4-h HD session. Both genes are known to be upregulated by osmotic cell shrinkage [2,3]. TAUT expression is regulated slowly [5], whereas SGK1 is an early gene upregulated within minutes upon osmotic cell shrinkage [7,8]. HD leads to a significant decrease of plasma osmolality. This decrease is, however, largely due to a decrease of plasma urea concentration. Urea rapidly permeates through cell membranes and, thus, does not usually create osmotic gradients between cells and extracellular fluid [2]. Nevertheless, urea has been shown to shrink cells, an effect thought to be due to destabilization of proteins resulting in a shift of the cell volume regulatory set point [11,12]. Earlier experiments indicated that SGK1 transcription is stimulated by cell shrinkage and not by hyperosmolarity [8]. Accordingly, concentrative uptake of amino acids decreases SGK1 transcription by inducing cell swelling [8]. Thus, urea was expected to stimulate, not to decrease SGK1 transcription. However, in contrast to the effect of hypertonic

Fig. 6. SGK1 transcript levels in cultured human hepatoma (HepG2). (A, B) HepG2 cells were exposed for 3 h to different concentrations of urea (A) or for different times to 300 mmol/l urea (B). (C) Decrease of SGK1 expression after incubation of HepG2 cells with RNA polymerase-inhibitor actinomycin D (5 µg/ml) or 300 mmol/l urea. (D) Increase of SGK1 expression following exposure to hypertonic NaCl (+50 mM NaCl) or raffinose (+20, 50 or 100 mM raffinose). The lower lanes in (A–C) show ethidium bromide staining of total RNA and reflect RNA loading (10 µg total RNA/lane). The 2.6 kb band corresponds to human SGK1 [this is double-loaded in (C) for a better comparison].
NaCl or hypertonic raffinose, high urea concentrations decrease SGK1 transcript levels. The effect of urea requires excessive concentrations to be significant and may not be relevant for the increase of SGK1 transcript levels during HD. Instead, several of the toxic plasma components accumulated during uraemia [13] may contribute to the derangement of SGK1 transcription prior to and during HD.

Irrespective of the underlying cause, altered transcriptional regulation of SGK1 may influence diverse cellular functions. The kinase has been shown to modify a wide variety of ion channels, including the renal epithelial Na\(^+\) channel ENaC [14], the renal outer medulary K\(^+\) channel ROMK1, the voltage-gated Na\(^+\) channel SCN5A, the voltage-gated K\(^+\) channel KCNE1/KCNQ1 and the voltage-gated K\(^+\) channel Kv1.3 [15–17]. In addition to these channels, SGK1 regulates the Na\(^+\)/H\(^+\) exchanger NHE3 [6,18], the glutamate transporter SN1, the glutamate transporter EAAT1 and the Na\(^+\)/K\(^+\)-ATPase [19]. Moreover, compelling evidence points to a role of SGK1 in fibrosing diseases, such as diabetic nephropathy, Crohn’s disease, fibrosing pancreatitis, glomerulonephritis, liver cirrhosis and lung fibrosis [19]. However, the altered SGK1 transcription observed in leukocytes does not necessarily reflect respective alterations of SGK1 expression in other tissues and expressed SGK1 is not necessarily functional, but requires activation through a signalling cascade involving phosphoinositol-3-kinase and phosphoinositide-dependent kinase [20]. To the extent that the respective tissues are affected, any alterations of SGK1 expression may affect neuronal excitability, cardiac action potential, epithelial transport and blood pressure control.

In conclusion, standard HD decreases the plasma taurine and urea concentration and upregulates leukocyte SGK1 and TAUT transcription. As SGK1 is a potent regulator of ion channels and transporters in the nervous system, as well as in heart muscle and epithelial cells, the deranged regulation of SGK1 may participate in the immediate, but potentially also in the long-term, response to the extracorporeal HD treatment setting.

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Conflict of interest statement. T.P.S. and J.P.-D. are employees of Fresenius Medical Care Deutschland GmbH.

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