Magnesium Metabolism in Erythrocytes of Patients with Chronic Renal Failure and after Renal Transplantation

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Summary: Plasma and erythrocyte Mg 2+ concentrations were found to be increased in 14 haemodialysis patients with chronic renal failure and in 7 chronic renal failure patients receiving chronic ambulatory peritoneal dialysis. The rate of Na+/Mg 2+ antiport was significantly higher in haemodialysis patients, but not in chronic ambulatory peritoneal dialysis patients (control: 0.15 ± 0.02, haemodialysis: 0.46 ± 0.08, chronic ambulatory peritoneal dialysis: 0.21 ± 0.06; Mg 2+, mmol/30 min ×1 cells). High erythrocyte Mg 2+ content in chronic renal failure results from the increased plasma Mg 2+, which induces elevated Mg 2+ uptake during haematopoiesis. An increased rate of Na+/Mg 2+ antiport, which only performs Mg 2+ efflux, leads to a relatively lower erythrocyte Mg 2+ content in haemodialysis patients compared with chronic ambulatory peritoneal dialysis patients. The elevated Na+/Mg 2+ antiport in erythrocytes from haemodialysis patients was almost normalised after haemodialysis.

Incubation of normal erythrocytes with heat-inactivated plasma from haemodialysis patients led to a doubling of Na+/Mg 2+ antiport, indicating the presence of a heat-stable, dialysable plasma factor. This factor does not accumulate in chronic ambulatory peritoneal dialysis patients. After renal transplantation all changed quantities of Mg 2+ metabolism returned to normal.

Introduction

In patients with chronic renal failure, the plasma Mg 2+ concentration is increased due to reduced renal excretion, and the Mg 2+ content of erythrocytes is higher than in healthy controls (1). In addition, inhibition of Na+/Li + antiport (2), activation of Na+/H + antiport (3), and increased concentration of Na + and Ca 2+ (4) were found in erythrocytes of uraemic patients. The elevated Ca 2+ content is caused by reduced activity of the Ca 2+-pumping ATPase, caused by a substance circulating in uraemic plasma (5). The mechanism of the increase of the erythrocyte Mg 2+ content is not known. It may be caused by increased Mg 2+ influx and/or decreased Mg 2+ efflux. The Mg 2+ uptake may increase during erythropoiesis as a consequence of the increased plasma Mg 2+ concentration, since only haematopoietic precursor cells and reticuloocytes but not mature erythrocytes take up Mg 2+ when incubated at increased extracellular Mg 2+ concentration (6). On the other hand, uraemic plasma may alter Mg 2+ flux across the erythrocyte membrane. In human erythrocytes, net Mg 2+ efflux has been characterised as Na+/Mg 2+ antiport (7). Therefore, we tested Na+/Mg 2+ antiport in erythrocytes from chronic renal failure patients and in erythrocytes after renal transplantation, and investigated the effect of uraemic plasma on the net Mg 2+ efflux and influx of normal erythrocytes.

Methods

Patients

Twenty one patients with chronic renal failure (14 on haemodialysis and 7 on chronic ambulatory peritoneal dialysis treatment) were studied (tab. 1). In haemodialysis patients, an extracorporeal, pump-assisted circulation of blood (200 ml/min during four hours three times a week) is exposed to dialysis solution (Hospal 252,
Hospal Medizintechnik GmbH, Nürnberg, Germany, [Mg\(^{2+}\)] = 0.5 mmol/l. This treatment removes toxic materials and solute by diffusion and ultrafiltration through a high-flux, polysulphon haemodialyser. Blood was collected at the end of haemodialysis. In chronic ambulatory peritoneal dialysis 1.5 liters of a dialysis solution (Fresenius CAPD Standard Lösung, Fresenius AG, Bad Homburg, Germany, [Mg\(^{2+}\)] = 0.5 mmol/l) are introduced four or five times every day into the peritoneal cavity. Toxic materials and solute is continuously eliminated by diffusion and ultrafiltration via the membrane of the peritoneum. All the patients with chronic renal failure had received haemodialysis or chronic ambulatory peritoneal dialysis treatment for more than 6 months.

For a better discrimination between the effect of renal disease and artificial, e.g. mechanical effects of haemodialysis, blood samples were taken from some patients (n = 4) before and after haemodialysis treatment. Furthermore, 10 former chronic renal failure patients, who had received renal transplantation more than one year previously, with subsequent good graft function (serum creatinine concentration < 200 umol/l), were included. All transplant patients were treated with cyclosporin. As controls, blood samples from 11 healthy subjects were analysed.

### Plasma and erythrocyte Mg\(^{2+}\)

Blood was heparinized and centrifuged for 10 min at 1000 g. Plasma was withdrawn and used for the measurement of Mg\(^{2+}\) and Ca\(^{2+}\) by atomic absorption spectrophotometry (AAS, Philips SP9) after appropriate dilution with 100 g/l trichloroacetic acid/1.75 g/l LaCl\(_3\). Part of the erythrocytes was taken for the measurement of the intracellular Mg\(^{2+}\) content. For this purpose, the erythrocytes were washed twice in 150 mmol/l NaCl, deproteinized with 100 g/l trichloroacetic acid/1.75 g/l LaCl\(_3\), centrifuged, and the Mg\(^{2+}\) content of the trichloroacetic acid extract measured by AAS. The Mg\(^{2+}\) content was related to cell volume, determined from the haematocrit.

### Na\(^{+}/Mg\(^{2+}\) antiport

Another part of the washed erythrocytes was used for measurement of Mg\(^{2+}\) efflux, as already described (8). Briefly, the cells were loaded with Mg\(^{2+}\) by incubating a cell suspension (cell volume fraction 0.1) for 30 min at 37 °C in KCl medium (in mmol/l: 140 KCl, 12 MgCl\(_2\), 50 sucrose, 5 glucose, 30 Hepes/Tris, pH 7.4) with the addition of 6 μmol/l of the cation ionophore A23187 (Boehringer Mannheim, Germany) dissolved in dimethyl sulphoxide. For removal of the ionophore, the cells were incubated four times in KCl medium plus 10 g/l bovine serum albumin for 10 min at 37 °C. The KCl medium was removed by washing the cells twice with cold (4 °C) cholinium chloride medium (in mmol/l: 140 cholinium chloride, 5 glucose, 30 Hepes/Tris, pH 7.4). This loading procedure raised the intracellular Mg\(^{2+}\) concentration to about 20 mmol/l cells. Mg\(^{2+}\) efflux was measured by reincubating a cell suspension (cell volume fraction 0.1) at 37 °C in Mg\(^{2+}\)-free NaCl medium (substitution of KCl in KCl medium by 140 mmol/l NaCl) and Mg\(^{2+}\)-free cholinium chloride medium. At the beginning of reincubation and after 30 min, 0.5 ml aliquots of the cell suspensions were centrifuged for 1 min at 10'000 g. Aliquots (100 μl) of the supernatants were diluted with 1 ml 100 g/l trichloroacetic acid/1.75 g/l LaCl\(_3\), and Mg\(^{2+}\) was measured by AAS. Mg\(^{2+}\) efflux was calculated from the increase of Mg\(^{2+}\) in the reincubation media and was expressed in relation to cell volume. Cell volume was determined by measuring the haematocrit. Na\(^{+}\)-dependent Mg\(^{2+}\) efflux (Na\(^{+}/Mg\(^{2+}\) antiport) was determined by subtracting the Mg\(^{2+}\) efflux in cholinium chloride medium from the Mg\(^{2+}\) efflux in Na\(^{+}\) medium. Additionally, erythrocytes from a control were incubated for 18 h with heat-inactivated (30 min, 56 °C), uraemic plasma from four haemodialysis patients or with heat-inactivated homologous plasma. Thereafter, the cells were loaded with Mg\(^{2+}\) and Na\(^{+}/Mg\(^{2+}\) antiport was measured.

### Results

Mg\(^{2+}\) concentrations in plasma and erythrocytes were significantly increased in chronic renal failure patients receiving haemodialysis and chronic ambulatory peritoneal dialysis, thus confirming published data (1, 4). In renal transplant recipients, the Mg\(^{2+}\) content in plasma and erythrocytes was within the normal range, but lower than in controls (tab. 2).

Mg\(^{2+}\) contents and rates of Na\(^{+}/Mg\(^{2+}\) antiport in erythrocytes of controls (tab. 2) were within the same range as reported in previous studies (9). However, Na\(^{+}/Mg\(^{2+}\) antiport was elevated in haemodialysis patients, the mean value being three times that of controls. In chronic ambulatory peritoneal dialysis patients, Na\(^{+}/Mg\(^{2+}\) antiport was not significantly increased. With high Mg\(^{2+}\) loading (20 mmol/l cells), the values represent the \(V_{\text{max}}\) of Na\(^{+}/Mg\(^{2+}\) antiport. The increase in Na\(^{+}/Mg\(^{2+}\) antiport in haemodialysis patients was reversible; immediately after dialysis it was significantly reduced (tab. 3) but not normalised.

After renal transplantation, the erythrocyte Mg\(^{2+}\) concentration and the rate of Na\(^{+}/Mg\(^{2+}\) antiport were not significantly different from those in healthy controls (tab. 2).
Tab. 2 Plasma and erythrocyte [Mg^{2+}] and erythrocyte Na^{+}/Mg^{2+} antiport activity in chronic renal failure patients and renal transplant recipients. Mean ± SEM. Least significant difference to controls according to single factor analysis of variance; *, p < 0.05; ***, p < 0.001.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>[Mg^{2+}] plasma (mmol/l)</th>
<th>[Mg^{2+}] erythrocytes (mmol/l cells)</th>
<th>Na^{+}/Mg^{2+} antiport (Mg^{2+}, mmol/30 min × 1 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11</td>
<td>0.77 ± 0.02</td>
<td>2.12 ± 0.13</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>Haemodialysis patients</td>
<td>14</td>
<td>1.04 ± 0.05***</td>
<td>2.54 ± 0.10*</td>
<td>0.46 ± 0.08***</td>
</tr>
<tr>
<td>Chronic ambulatory peritoneal dialysis patients</td>
<td>7</td>
<td>1.01 ± 0.06***</td>
<td>3.18 ± 0.24***</td>
<td>0.21 ± 0.06</td>
</tr>
<tr>
<td>Renal transplant recipients</td>
<td>10</td>
<td>0.71 ± 0.04</td>
<td>2.10 ± 0.12</td>
<td>0.23 ± 0.06</td>
</tr>
</tbody>
</table>

Tab. 3 Plasma [Mg^{2+}] and erythrocyte [Mg^{2+}] and Na^{+}/Mg^{2+} antiport activity in haemodialysis patients (n = 4) before and immediately after haemodialysis. Significant differences according to paired Student’s t-test; *, p < 0.05; ***, p < 0.01.

<table>
<thead>
<tr>
<th></th>
<th>Before haemodialysis</th>
<th>After haemodialysis</th>
</tr>
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<tbody>
<tr>
<td>Mg^{2+} plasma (mmol/l)</td>
<td>1.43 ± 0.14</td>
<td>0.96 ± 0.01**</td>
</tr>
<tr>
<td>Mg^{2+} erythrocytes (mmol/l cells)</td>
<td>3.33 ± 0.21</td>
<td>3.13 ± 0.17</td>
</tr>
<tr>
<td>Na^{+}/Mg^{2+} antiport (Mg^{2+}, mmol/30 min × 1 cells)</td>
<td>0.65 ± 0.15</td>
<td>0.30 ± 0.15*</td>
</tr>
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</table>

When normal erythrocytes were incubated for 18 h at 37 °C with heat-inactivated, uraemic plasma from haemodialysis patients, the Mg^{2+} efflux via Na^{+}/Mg^{2+} antiport was increased from 0.25 ± 0.02 to 0.51 ± 0.1; Mg^{2+}, mmol/30 min × 1 cells (mean ± SEM, n = 4, p < 0.001). On the other hand, incubation of normal erythrocytes for various periods up to 24 h with heat-inactivated, uraemic plasma from haemodialysis patients at extracellular Mg^{2+} concentrations up to 4.5 mmol/l (uraemic plasma with addition of 3 mmol/l MgCl_2) did not affect the cellular Mg^{2+} content (data not shown). This result shows that in uraemic plasma no factor is present which induces Mg^{2+} uptake in erythrocytes.

Discussion

In chronic renal failure patient plasma, the Mg^{2+} concentration was increased and almost normalised by haemodialysis (tab. 3). The increase of erythrocyte Mg^{2+} is not caused by an altered activity of the Na^{+}/Mg^{2+} antiporter, as this system in human erythrocytes is irreversible, leading only to net Mg^{2+} efflux when the cellular Mg^{2+} content is increased. Hence, Mg^{2+} efflux occurs in both erythrocytes from uraemic patients and in Mg^{2+}-loaded erythrocytes from controls (10).

Human erythrocytes do not take up Mg^{2+} (11, 12). Even during incubation with uraemic plasma at increased extracellular Mg^{2+}, erythrocytes did not accumulate Mg^{2+}. Therefore, the increased Mg^{2+} concentration in uraemic erythrocytes must be induced during haematopoiesis. In agreement with this conclusion, reticulocytes take up Mg^{2+} when incubated at an elevated extracellular Mg^{2+} concentration (6).

The difference in erythrocyte Mg^{2+} content in haemodialysis and chronic ambulatory peritoneal dialysis may depend on the activity of Na^{+}/Mg^{2+} antiport. In haemodialysis, the erythrocyte Mg^{2+} content was lower and the Na^{+}/Mg^{2+} antiport activity was higher than in chronic ambulatory peritoneal dialysis. The Na^{+}/Mg^{2+} antiport of normal erythrocytes was increased when they were suspended in heat-inactivated plasma from haemodialysis patients with a high Na^{+}/Mg^{2+} antiport. As the method of determination of Na^{+}/Mg^{2+} antiport requires plasma-free incubation of the erythrocytes for about 4 hours prior to measurement of transport rates, this effector must have a long lasting but reversible effect on the Na^{+}/Mg^{2+} antiporter. During haemodialysis, this effector is at least partly removed, leading to reduced Na^{+}/Mg^{2+} antiport capacity (tab. 3). In vivo, however, the maximal transport capacity of Mg^{2+}-loaded erythrocytes is not attained and, therefore, only a small (and insignificant) reduction of total intracellular Mg^{2+} content was observed (tab. 3). Also, Na^{+}/H^{+} antiport, which has similar properties to Na^{+}/Mg^{2+} antiport, was increased in chronic renal failure patients (3). A dialysable, heat-stable plasma constituent with a relative molecular mass of M_r < 3000 from chronic renal failure patients has been reported to inhibit Ca^{2+} transport in normal erythrocytes (5). This effector is not identified, but possible candidates are interleukin-1, interleukin-6 and tumour necrosis factor-α. It has been reported that undialysed patients with chronic renal failure and patients on haemodialysis expressed increased plasma levels of these cytokines (for references see l.c. (13)). Isolated peripheral blood mononuclear cells from chronic ambulatory peritoneal dialysis patients produced less tumour necrosis factor-α than cells from haemodialysis patients.
Increased formation of free oxygen radicals, which may occur in erythrocytes of chronic renal failure patients taken before dialysis (tab. 3).

Immediately after the dialysis procedure even had reduced Na\(^+\)/Mg\(^{2+}\) antiport rates compared with those taken before dialysis (tab. 3).

The elevated Na\(^+\)/Mg\(^{2+}\) antiport in haemodialysis patients is also not caused by mechanic stress during haemodialysis, because erythrocytes which were taken immediately after the dialysis procedure even had reduced Na\(^+\)/Mg\(^{2+}\) antiport activity (data not shown).

Increased formation of free oxygen radicals, which may occur in erythrocytes of chronic renal failure patients due to a reduction of protective enzymes (15), can also be excluded. Free oxygen radicals have been shown to decrease rather than increase Na\(^+\)/Mg\(^{2+}\) antiport in human erythrocytes (16).

In chronic renal failure patients receiving chronic ambulatory peritoneal dialysis, Na\(^+\)/Mg\(^{2+}\) antiport was not changed. Probably, this procedure is more effective in eliminating the activating plasma constituent.

In all chronic renal failure patients after renal transplantation, the quantities of Mg\(^{2+}\) metabolism were normal, in parallel with the sufficient function of the transplanted kidney as indicated by the plasma creatinine concentration. As all transplant recipients were treated with cyclosporin, one year after transplantation, this drug does not seem to have an influence on erythrocyte Mg\(^{2+}\) metabolism.

It remains to be established whether cells other than erythrocytes in haemodialysis patients show a high activity of Na\(^+\)/Mg\(^{2+}\) antiport. A generally increased activity of Na\(^+\)/Mg\(^{2+}\) antiport in other cells might lead to an intracellular Mg\(^{2+}\) deficit.

References