Renalase, a Novel Enzyme Involved in Blood Pressure Regulation, Is Related to Kidney Function but Not to Blood Pressure in Hemodialysis Patients

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Key Words
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Abstract
Renalase, secreted by the kidney, degrades catecholamines and may play a role in the regulation of sympathetic tone and blood pressure. The aim of this study was to assess serum renalase levels in hemodialysis patients and their relationship to blood pressure control, type of antihypertensive therapy and the presence of residual renal function. Results: The mean serum renalase in the study cohort was significantly higher than in the control group (27.53 ± 7.18 vs. 3.86 ± 0.73 μg/ml, p < 0.001). The serum renalase concentration was significantly lower in patients with residual renal function when compared to the anuric patients. The type of hypotensive treatment (β-blockers, ACE inhibitors or AT1 receptor blockers) did not affect renalase levels. There was a significant inverse correlation between the serum renalase and age (r = −0.28, p = 0.023) and residual renal function (r = −0.327, p = 0.001). Renalase was not related to blood pressure, heart rate or hemodialysis vintage. Conclusion: Elevated renalase levels in HD patients may be due to impaired kidney function. Further studies are needed to prove or disprove the possible role of renalase in the pathogenesis of hypertension in patients with kidney diseases.

Introduction
There are multiple factors involved in blood pressure control and cardiovascular disease developing in ESRD [1]. Most dialysis patients suffer from hypertension and blood pressure control is often very poor in this group [1, 2]. Recent investigations have clearly supported the notion that activation of the sympathetic nervous system is commonly associated with chronic renal failure and substantially contributes to the poor prognosis in this group [2, 3]. The elevated level of catecholamine in ESRD patients is the result of not only overspill, in the mechanisms that involve inhibition of nitric oxide followed by increased angiotensin II and increases in sympathetic afferent outflow from diseased kidneys, but also of reduced catecholamine clearance [1, 4]. Norepinephrine clearance is reduced by 20% in mild renal failure and by up to 40%
in hemodialysis patients. Among the various functions of the kidney, it is also the endocrine organ [5]. Xu et al. [6] recently indicated that the new hormone renalase, secreted by the kidney and circulating in blood, degrades catecholamines and may play a role in the regulation of sympathetic tone and blood pressure.

The aim of the study was to assess the serum concentration of renalase in a cohort of 104 hemodialysis patients and its relationship to blood pressure control, type of antihypertensive therapy and presence of residual renal function.

Patients and Methods

We included in the study 104 hemodialysis patients with a mean age of 62 years (50.76% male) from the dialysis center in Bielystok. The mean duration of hemodialysis was 25.46 months. All patients were informed about the aim of the study and gave their informed consent. The study was approved by the medical university’s ethics committee. Blood pressure was measured before and after the hemodialysis session in the sitting position using an automatic manometer. The arithmetic average of three measurements taken on different days was used for analysis. Blood pressure level was assessed according to K/DOQI guidelines [7] as lower than 140/90 mm Hg before the hemodialysis session and lower than 130/80 mm Hg afterwards. The body weight gain was calculated according to the dry weight and the weight measured before the hemodialysis session (the arithmetic average from three hemodialysis sessions). The presence of residual renal function was defined as 24-hour urine collection above 100 ml. Data on all hypotensive drugs and kind of dialyzers (low-flow or high-flow) were collected from the individual prescription cards. The blood for the estimation of the renalase serum concentration was taken once before the hemodialysis session in the middle of three dialysis sessions (when also blood pressure and weight were assessed). The enzyme-linked immunosorbent assay (ELISA) kit made by Uscn Life Science Inc. (China), using a monoclonal antibody specific to renalase, was used.

To obtain the normal ranges for renalase in this ELISA assay, 27 healthy volunteers were studied. The study cohort was divided into two groups: one according to blood pressure control, and the other according to the presence of residual renal function. The Statistica 9.0 Poland program was used for statistical analysis, the Shapiro-Wilk test was used to determine the normal distribution, and Student’s t test and Mann-Whitney U test was used for comparison of the two groups.

Results

According to the K/DOQI guidelines (lower than 140/90 mm Hg before hemodialysis session and lower than 130/80 mm Hg afterwards), the preliminary results showed abnormal blood pressure in 67% (n = 65) of the patients in the study cohort. The main antihypertensive medications used in this population were β-blockers (61.16%), and calcium channel blockers (60.57% on the second position). The high-flow dialyzers were used in 13.46% of the patients. The mean serum renalase concen-
tration in the study cohort was significantly higher compared to the control group (fig. 1). There were no differences in the renalase serum concentration between the two groups divided according to blood pressure control: one with good blood pressure control and the other with uncontrolled blood pressure. In turn, we found the differences between the two subgroups divided according to the presence of residual renal function. The serum renalase concentration was significantly lower in patients with residual renal function when compared to the anuric patients (diuresis rate lower than 100 ml per day; fig. 2). There was no difference in the serum renalase concentration between patients with diuresis rate ≥1,000 ml (n = 15) and <1,000 ml (25.29 ± 5.4 vs. 27.91 ± 7.4 μg/ml, respectively). The time of hemodialysis in group 1 (patients with residual renal function), was significantly shorter in comparison to the patients without residual renal function (table 1). There were also no significant differences in blood pressure control and type of dialyzer between the two groups with and without residual renal function. In addition, we did not find differences in the serum renalase concentration between patients treated

Table 1. Basal clinical characteristics of the studied groups (divided according to the presence of residual renal function)

<table>
<thead>
<tr>
<th></th>
<th>Whole group (n = 104)</th>
<th>Presence of residual renal function (n = 52)</th>
<th>No residual renal function (n = 52)</th>
<th>&gt;p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>62.4 ± 15.08</td>
<td>63.5 ± 14.9</td>
<td>60.6 ± 15.9</td>
<td></td>
</tr>
<tr>
<td>Time of HD, months</td>
<td>39.22</td>
<td>29.59</td>
<td>74.41</td>
<td>0.003</td>
</tr>
<tr>
<td>Min.</td>
<td>2.53</td>
<td>2.53</td>
<td>2.56</td>
<td></td>
</tr>
<tr>
<td>Max.</td>
<td>277</td>
<td>93.97</td>
<td>277</td>
<td></td>
</tr>
<tr>
<td>Mean BP before HD, mm Hg</td>
<td>138/74 ± 20/12</td>
<td>138/74 ± 21/12</td>
<td>138/75 ± 21/13</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Mean BP after HD, mm Hg</td>
<td>129/70 ± 11/1.5</td>
<td>129/69 ± 11/10</td>
<td>129/70 ± 12</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Hypertension</td>
<td>65 (67)</td>
<td>28 (84)</td>
<td>24 (85)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Low-flow dialyzer</td>
<td>90 (86.5)</td>
<td>42 (87.5)</td>
<td>40 (83.3)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>High-flow dialyzer</td>
<td>14 (13.5)</td>
<td>6 (12.5)</td>
<td>8 (16.6)</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

Values given are means ± SD, median and ranges, or n (%). BP = Blood pressure; HD = hemodialysis.
with β-blockers or ACE inhibitors/angiotensin II receptor antagonists. However, we did find that the serum renalase level was higher in males compared to females (29.8 ± 7.28 vs. 25.92 ± 7.79 μg/ml, p < 0.05). There was a significant inverse correlation between the serum renalase concentration and age of patients (r = –0.2836, p = 0.023) and residual renal function (r = –0.327, p = 0.001). No correlation between serum renalase concentration and blood pressure rate, heart rate (before and after hemodialysis session) and duration of hemodialysis was observed.

Discussion

We found for the first time that the concentration of renalase was significantly higher in hemodialyzed patients when compared to the healthy volunteers. Even more interesting, it was significantly elevated in the group of patients without residual renal function and maintained hemodialysis for a longer time, and there was a strong correlation between serum renalase concentration and residual renal function. It is probably caused by much higher sympathetic nervous system activity and much lower renalase clearance presented in these patients.

There is virtually no data on renalase concentration and/or activity in humans. Xu et al. [6] found that in hemodialyzed patients (n = 8), renalase expression was decreased compared with healthy volunteers (n = 4). Similar findings were presented by Wang et al. [8]. They reported decreased renalase expression in 1 CKD patient and 1 hemodialysis patient compared with 2 healthy controls. In a recent study [9], serum renalase was significantly higher in kidney transplant recipients relative to healthy volunteers. In this study, the predictors of serum renalase were kidney function, age, time after transplantation and diastolic blood pressure. Przybylowski et al. [10] showed similar findings in heart transplant recipients. In this study the only predictor of serum renalase was kidney function. In addition, a recent study [11] reported highly elevated serum renalase in patients on peritoneal dialysis compared with healthy volunteers. Moreover, renalase was positively related to the duration of peritoneal dialysis and negatively to residual renal function. No correlation between serum renalase concentration and blood pressure, age, gender or adequacy of dialysis was found.

Boomsma and Tipton [12] question the method used by Xu et al. [6] to measure renalase activity. They considered that the (patho)physiological concentrations of catecholamines were lower than the concentrations used in the experiments. They even concluded that it was unlikely that renalase was a catecholamine-metabolizing enzyme. They suggest that it may have important cardiovascular functions, but through another mechanism. On the other hand, Luft [13], according to the studies he quotes, noted that dopamine is associated with lower blood pressure and decreased cardiovascular risk, which is why diminishing dopaminergic tone (by renalase for example) would increase blood pressure and cardiovascular risk. Patients on dialysis are generally hypertensive and have increased sympathetic nerve traffic [14]. He underlined that after bilateral nephrectomy, their hypertension generally went away. In our population we found 2 patients after bilateral nephrectomy and their renalase were much higher than mean values (39.32 and 34.47 μg/ml, whereas the mean value was 27.53 μg/ml).

We measured renalase concentration, whereas in the only available study by Xu et al. [6] renalase was assessed in ESRD patients using Western blot with polyclonal antibodies and found to be lower than in the healthy volunteers. In general, Western blot is not used to assess the activity, but rather the presence of a protein. Renalase activity was measured using the Amplex Red Monoamine Oxidase Assay Kit from Invitrogen, based on the detection of H₂O₂ in a horseradish peroxidase-coupled reaction by Xu et al. [6]. They extrapolated their results to the renalase activity. Pandini et al. [15], using two different methods, were unable to prove that renalase exhibited monoamine oxidase activity. Nevertheless, when administered to rats it exerted its hypotensive properties, despite being catalytically inactive. Therefore, the question whether renalase is really monoamine oxidase is still valid. Moreover, in their recent paper, Milani et al. [16] pointed out that renalase was not a monoamine oxidase and most probably not an oxidase. Due to the growing criticism on potential monoamine oxidase activity and structure of renalase [15–17], interpretation of data on renalase activity is rather difficult. Xu et al. [6] assessed renalase expression, whereas in our study we measured renalase level using a commercially available assay. The manufacturers of the assay claim that only renalase 1 levels are measured. Since there are no published data on the specificity of the ELISA used in the study, we may also take into account that the apparent increase in serum renalase in ESRD could be explained by cross-reactivity with breakdown products of serum renalase. However, there are no data on possible cross-reactivity. Since renalase is secreted not only by the kidney, but also by cardiomyocytes, liver and adipose tissue [6], in the case of
end-stage renal failure, other organs and tissues may oversecrete renalase, thus leading to very high levels. When expression of renalase is assessed, we cannot be sure that the protein is active. To explain this situation, we suggest the different method of the estimation used in our study and different antibodies, i.e. monoclonal versus polyclonal, in the study of Xu et al. [6].

Gu et al. [18] found that the concentration of renalase (using ELISA with anti-renalase antibody 1:32,000, Abcam) was higher in heart failure, and they suggested that more activated renalase would degrade the increased norepinephrine levels. They proposed that the kidney might synthesize and secrete more renalase to compensate for the increased catecholamine levels in the early phase of acute myocardial infarction in animal models. Therefore, the problem of kidney function and renalase activity/levels still requires further studies.

In conclusion, the preliminary results of the present study suggest the elevated renalase levels in hemodialysis patients may be due to sympathetic nervous system hyperactivity found in this population and may have an impact on the development of cardiovascular complications. Further studies are needed to prove or disprove the possible role of renalase in the pathogenesis of hypertension in patients with kidney diseases. It seems that regulation of blood pressure and development of cardiovascular complications are far more complex than they appear and the role of renalase is far from resolved.

References