Does circulating erythropoietin reflect progression of IgA nephropathy? Comparison with urinary N-acetyl-β-D-glucosaminidase

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Abstract

Background. Recent reports describe that erythropoietin (Epo) is produced by peritubular interstitial fibroblast-like cells in response to a hypoxic stimulus. We studied serum Epo levels as a possible marker of tubulointerstitial damage in the progression of IgA nephropathy (IgAN), in comparison with urinary (u-) levels of N-acetyl-β-D-glucosaminidase (NAG), which is mainly derived from proximal tubular cells and is used as a marker of tubular damage.

Methods. Thirty-eight patients with IgA nephropathy (IgAN) with relatively preserved renal function (serum creatinine: sCr, 0.5–2.2 mg/dl) were examined. The severity of glomerulosclerosis and interstitial fibrosis of the renal biopsy tissue was expressed by semiquantitative grading scores. Clinical parameters including serum creatinine (sCr), blood pressures, and 24-h proteinuria levels were obtained at the renal biopsy. Epo was measured by a radioimmunoassay (RIA) of sera obtained in the morning and u-NAG was measured by colorimetric method of 24-h urine samples.

Results. The mean Epo level of the patients (17.7 ± 6.3 mU/ml) was not different from the control level (19.3 ± 3.7 mU/ml). There were no significant correlations between Epo levels and red blood cell (RBC) counts, haematocrit (Hct), or haemoglobin (Hb) levels. The mean u-NAG level of the patients (6.7 ± 6.2 U/gCr) was significantly higher than the control level (1.9 ± 0.5 U/gCr). There was an inverse quantitative correlation between Epo and u-NAG levels in the patients (P < 0.02). The u-NAG levels showed quantitative positive correlations with sCr (P < 0.001), u-proteins (P < 0.001), systolic (SBP) (P < 0.001), and diastolic blood pressures (DBP) (P < 0.05). Conversely, Epo levels were inversely correlated with sCr, SBP and DBP (each P < 0.05). The patients with higher u-proteins (> 2.0 g/day) showed significantly decreased Epo levels (P < 0.05) than those with lower u-proteins (<2.0 g/day). The both scores of glomerulosclerosis and interstitial fibrosis were positively correlated with the u-NAG levels (each P < 0.001), but were not correlated with the Epo levels.

Conclusions. The significant correlation between u-NAG and serum Epo levels suggests that tubulointerstitial damage and interstitial cell dysfunction are associated each other in the progression of IgAN. Serum Epo levels bearing inverse correlations with sCr, blood pressure levels and heavy proteinuria seem to reflect clinical severity of IgAN, whereas u-NAG can be more useful progression marker of IgAN bearing correlations with both clinical and histological findings.

Key words: anaemia; erythropoietin; glomerulosclerosis; IgA nephropathy; N-acetyl-β-D-glucosaminidase; tubulointerstitial damage

Introduction

Tubulointerstitial lesions, characterized by tubular atrophy, interstitial fibrosis, and inflammatory cell infiltration, play a pivotal role in the progression of renal diseases including primary glomerulopathies [1–4]. In the development of chronic tubular damage in primary glomerulonephritis, it has been suggested that filtered proteins and inflammatory cytokines released from nephritic glomeruli stimulate tubular cells, which may subsequently produce and release chemoattractant substances and fibrogenic factors. Consequently, interstitial fibroblast activation and macrophage infiltration may be induced, leading to interstitial fibrosis with tubular damage. In addition, the following mechanisms have been considered as accelerating factors; (i) the increase of oxygen consumption and production of reactive oxygen species in surviving nephrons, which causes further ammoniagenesis and complement activation, and (ii) hypertensive microvascular injuries followed by the narrowing and obliteration of postglomerular peritubular capillaries [5–7].
Recently, it has been shown that fibroblast-like interstitial cells which exist between peritubular capillaries and tubular cells are the source of circulating Epo [8,9] and that the production of Epo, dependent on the extent of low oxygen pressure, may be attenuated in association with the decreased number of the corresponding fibroblastic cells and the phenotypic change to myofibroblasts in experimental renal fibrosis [10,11]. These findings suggest the presence of a correlation between serum Epo levels and the degree of tubulointerstitial lesions in IgA nephropathy. The measurement of the urinary levels of NAG, a lysosomal enzyme abundant in proximal tubular segments [12], has been a useful marker to monitor tubular damage in patients undergoing nephrotoxic therapy and in those with nephrotic syndrome, glomerulonephritis, or diabetic nephropathy [13,14]. In the present study, to determine whether circulating Epo levels reflect the severity of IgA nephropathy, with emphasis on interstitial damage, we measured serum Epo levels of IgA nephropathy patients and compared them with clinical and histological parameters, in parallel with urinary NAG levels.

Subjects and methods

Patients

Thirty-eight IgAN patients, 17 males and 21 females, admitted for renal biopsy to Himeji National Hospital, were registered in this study. The diagnosis of IgAN was based on the detection of mesangial IgA deposition by immunofluorescence methods. Informed consent was obtained from each patient. The patients with overt urinary tract infection were excluded to determine the importance of tubulointerstitial damage in IgA nephropathy. The A portion of each 24-h urine sample was centrifuged, and the supernatant was used for the urinary NAG test within 4 h of the collection. The urinary NAG levels were measured by a spectrophotometric method using 6-methyl-2-pyridyl-methenamine (PAM) stainings. Immunofluorescence staining was applied for the detection of IgA, IgG, IgM, C3c, and fibrinogen. Glomerular sclerosis was expressed by the mean grade of each glomerulus: grade 0, normal or minor abnormalities; grade 1, sclerosed area less than 1/3 of the glomerulus; grade 2, sclerosed area from 1/3 to 2/3; grade 3, sclerosed area more than 2/3; and grade 4, complete obsolescence. Interstitial fibrosis was also semiquantitatively expressed as the mean grade of each view on a microscopy-linked TV monitor at ×64 magnification: grade 0, no fibrosis; grade 1, fibrosis less than 10% of tubulointerstitial area; grade 2, fibrosis from 10 to 30%; and grade 3, fibrosis more than 30%. These findings were recorded after the observations by three renal pathologists.

Urinary NAG test and serum Epo measurement

A portion of each 24-h urine sample was centrifuged, and the supernatant was used for the urinary NAG test within 4 h of the collection. The urinary NAG levels were measured by a two-antibody radioimmunoassay using 125I-labelled Epo and standard recombinant Epo samples with a commercial kit (Recombin Epo kit, Japan DPC Co., Tokyo, Japan). The results are expressed as units per gram of urine creatinine (U/gCr). Circulating serum Epo levels were measured by an anti-Epo Epo kit. Circulating serum Epo levels were measured by a two-antibody radioimmunoassay using 125I-labelled Epo and standard recombinant Epo samples with a commercial kit (Recombin Epo kit, Japan DPC Co., Tokyo, Japan, courtesy of SRL Inc., Tokyo).

Statistical analysis

The results are expressed as the mean ± SD. A regression analysis and unpaired Student’s t test were used. P values less than 0.05 by the two-tailed method were considered significant.

Results

Comparison between clinical parameters and histological scores

To determine the importance of tubulointerstitial lesions in IgA nephropathy, clinical parameters were compared with the glomerulosclerosis index and interstitial fibrosis index. The interstitial fibrosis index showed highly significant correlations with the sCr, u-protein, SBP, and DBP levels (each P < 0.001). The glomerulosclerosis index showed similar correlations (P < 0.001) with sCr and u-proteins, and a correlation with SBP (P < 0.05); however, it was not correlated with DBP (Table 1).

Relationship of Epo with RBC, Hct, and Hb levels

The mean values of RBC, Hct, and Hb in the patients were 428 ± 56 × 10⁴/μl (range 319–555 × 10⁴), 38.9 ± 5.4% (range 27.6–48.3) and 13.1 ± 1.9 g/dl (range 9.4–16.7) respectively. There were no significant relationships between the Epo levels and any of these parameters.

Relationship between NAG and Epo

The mean NAG level in the IgA nephropathy patients was 6.7 ± 6.2 U/gCr, significantly higher than the level of the healthy controls (1.9 ± 0.5 U/gCr); (P < 0.002). The mean Epo level in the patients was 17.7 ± 7.0 mU/ml (range 9.5–36.2), which was not different from
Table 1. Correlations between clinical parameters and histological scores

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Glomerulosclerosisa</th>
<th>Interstitial fibrosisb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P value</td>
</tr>
<tr>
<td>sCr (mg/dl)</td>
<td>0.68</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>U-proteins (g/day)</td>
<td>0.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>0.34</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>0.29</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

aGlomerulosclerosis was expressed by the mean grade from 0 to 4.
bInterstitial fibrosis was expressed by the mean grade from 0 to 3 (see text).
sCr, serum creatinine; U-proteins, urinary proteins; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Relationship of NAG and Epo levels with clinical parameters

Urinary NAG showed a significant quantitative correlation (P<0.001) with sCr (Figure 2A), and serum Epo showed an inverse correlation (P<0.05) with sCr, i.e., lower Epo levels were seen in the patients with higher sCr levels (Figure 2B). The NAG levels showed a significant quantitative correlation with the u-protein levels (P<0.001) (Figure 3A). On the other hand, the Epo levels did not show significant correlation with the u-protein levels (Figure 3B); however, when the patients were divided into two groups based on u-protein levels, the mean Epo level in patients with u-proteins higher than 2.0 g/day (13.4±3.0 mU/ml) was significantly decreased (P<0.05) than the mean in patients with u-proteins lower than 2.0 g/day (18.9±6.5 mU/ml) (Figure 3D). In the similar comparison, the patients with higher u-proteins (>2.0 g/day) showed much more increased u-NAG (P<0.001) than those with lower u-proteins (Figure 3C). With regard to the blood pressure values, NAG was positively and Epo was inversely correlated with both SBP (P<0.001 and P<0.05 respectively) and DBP (P<0.05 each) (Figure 4).

Relationship of NAG and Epo levels with histological severity

The patients with higher scores of glomerulosclerosis (Figure 5A) or interstitial fibrosis (Figure 5C) showed more increased NAG levels in a significant relationship between these parameters (P<0.001 each). On the other hand, the Epo level was not correlated with the glomerulosclerosis score (Figure 5B) nor with the interstitial fibrosis score (Figure 5D).

Discussion

In this study we first compared the degree of glomerulosclerosis and interstitial fibrosis with clinical parameters such as sCr, u-proteins, SBP, and DBP. We found that the interstitial fibrosis score showed strong correlations with all of these parameters (each P<0.001), whereas the glomerulosclerosis score showed similar correlations with sCr and u-proteins and, to a lesser extent, with SBP, with no relationship with DBP. These findings emphasize the clinical importance of tubulointerstitial damage in the progression of IgAN. We next examined the clinical usage of u-NAG as a marker of tubular damage in IgAN. We measured NAG levels in urine samples collected over 24 h in clean bottles from patients without urinary tract infection, although fresh urine samples are preferable for the enzyme activity. With this regard, Wellwood et al. have shown that NAG activity was not changed after storage of urine samples for 24–48 h at 37 °C if bacterial growth was avoided [15], and the comparable results have been described utilizing 24-h u-NAG data in patients with various renal diseases [14,16,17]. In these papers it has been shown that there were positive correlations between u-NAG and u-protein levels in various types of primary glomerulonephritis [14,17], and the NAG levels were different according to the histological subtype of glomerulonephritis [16,18]. These data seem to indicate that tubular injury may be caused by protein resorption and that u-NAG may reflect the activity of glomerulonephritis. In the present study of IgAN we found positive correlations of u-NAG not only with u-proteins but also with sCr, SBP, and DBP, and with the interstitial fibrosis score. These results indicate that u-NAG measurement may be a highly beneficial clinical marker of the severity of IgAN, reflecting the degree of tubulointerstitial damage.
Fig. 2. Relationship between serum creatinine (sCr) and urinary NAG levels (A) and between sCr and serum erythropoietin (EPO) levels (B) in the IgA nephropathy patients. There was a positive correlation between sCr and NAG ($P < 0.001$), and an inverse correlation between sCr and EPO ($P < 0.05$).

Fig. 3. Relationship between urinary protein (u-Protein) and urinary NAG levels (A) and between u-Protein and serum erythropoietin (EPO) levels (B) in the IgA nephropathy patients. There was a significant correlation between u-Protein and NAG ($P < 0.001$); u-Protein was not correlated with EPO. Comparison of the mean levels of NAG (C) and EPO (D) between the patients with high (>2.0 g/day) and low (<2.0 g/day) u-Proteins. The vertical bars indicate mean $\pm$ SD. There were significant differences in comparison with NAG ($P < 0.001$) and EPO ($P < 0.05$).

The beneficial effects of recombinant human Epo in the treatment of anaemia in chronic renal failure have been established [19], but there are only a few papers on the endogenous circulating Epo levels in the progression of renal disease [20–22]. Inomata et al. reported that low serum Epo might be a new predicting marker of the progression of diabetic nephropathy [20], and the laboratories of Nielsen and Thaysen have shown Epo deficiency in patients with acute renal failure, including crescentic glomerulonephritis [21,22]. However, these reports did not describe the relationship between Epo levels and tubular damage, notwithstanding the fact that Epo is produced in peritubular interstitial cells. Recent molecular studies have shown that Epo production responsive to hypoxia is mediated by hypoxia-inducible factor-1 (HIF-1), which also stimulates platelet-derived growth factor (PDGF) and vascular endothelial growth factor [23,24]. Other inflammatory cytokines such as tumour necrosis factor, IL-1 and transforming growth factor $\beta$ (TGF $\beta$) are known as antagonists for Epo production [25,26].

Toxic metabolites produced in renal failure are thought to be antagonists of Epo synthesis [19]. Taking into account the accumulating evidence that different cytokines contribute to the progression of IgAN; for example, PDGF in the mesangial cell proliferation and TGF $\beta$ in the fibrogenic process [6], and that Epo stimulates vascular endothelial cells and causes vasoconstriction [27,28], the circulating Epo levels in patients with IgAN should be determined.

We examined the relationship between u-NAG and serum Epo levels, and the relationship of Epo with u-proteins, sCr, blood pressures, and the histological scores of glomerulosclerosis and interstitial fibrosis. Serum samples for the Epo measurement were obtained early in the morning at the renal biopsy, because a study of the circadian rhythm of serum Epo levels has shown the basal level in the morning and the highest level (a 60% increase) at 8.00 p.m. [29]. In contrast to the discrepancy between the Epo levels in the patients being in a range similar to the control levels and the u-NAG levels in the patients being higher than the
When the patients were divided into four groups of high and low levels of both Epo and NAG based on the mean levels, most of the patients with high Epo showed low NAG, and most of those with high NAG showed low Epo. This finding suggests that in mild injury of tubular cells, interstitial cells may be activated, contributing to the relatively higher Epo levels, whereas in advanced tubular damage as shown by increased urinary NAG levels, the interstitial fibroblasts may be in a state of dysfunction, bearing phenotypic changes similar to those of myofibroblasts producing lower Epo levels [30,31]. In this regard, Maxwell et al. [11] proposed that in experimental models of interstitial renal disease, the proportion of Epo-producing cells may be decreased among interstitial fibroblast-like cells bearing the myofibroblast phenotype, and these cells are partially refractory to hypoxic stimulation for Epo production.

In comparison with clinical parameters, Epo showed inverse correlations with the sCr, SBP, and DBP (each P < 0.05). Because sCr, SBP, and DBP are important clinical parameters reflecting the progression of renal disease, such inverse correlations seem to suggest that the advancement of interstitial cell dysfunction causing depressed Epo levels may be associated with the progression of IgAN. Recent studies have shown a positive quantitative correlation between endogenous Epo levels and blood pressures in essential hypertension, indicating that Epo may be a pathogenetic factor through a vasoconstricting effect in the development of essential hypertension [32,33]. However, such a relationship was not observed in the present IgAN patients. Rather, it seems likely that interstitial damage substantially enough to exhibit decreased Epo levels contributes to the development of renal hypertension. The positive correlation between urinary proteins and the NAG levels suggests the pathogenic role of urinary

control levels, we found an inverse quantitative correlation between the serum Epo and the u-NAG levels. The present data on Epo in the nearly normal range might be a reflection of the basal levels, because the serum samples were obtained in the morning, which might be easily stimulated to a wider range than the present levels by diurnal endocrine factor [29] or tissue hypoxia based on histological and haemodynamic changes [10,11]. Additional study based on Epo levels obtained at the peak level at 8.00 p.m. would be beneficial in more elaborate comparisons with various clinical and histological parameters.
proteins in the development of proximal tubular damage through absorption. On the other hand, heavy proteinuria seems to suppress Epo production, because the patients with u-proteins higher than 2.0 g/day showed significantly lower Epo levels ($P<0.05$) than those with u-proteins lower than 2.0 g/day. These findings suggest harmful effects of u-proteins not only on tubular cells but also on interstitial cells.

In histological comparison, the NAG levels seemed to reflect the severity of glomerulosclerosis and interstitial fibrosis in IgAN; however, the Epo levels did not show significant relationship with both histological scores. Further study on various histological markers of phenotypic changes of fibroblasts and tubular cells, such as smooth muscle actin, desmin, and various cytokines as well as Epo gene expression will clarify the abnormalities of Epo production in the progression of tubulointerstitial lesions in IgAN.

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References


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T. Machiguchi et al.