Erythropoietin is reduced by combination of diuretic therapy and RAAS blockade in proteinuric renal patients with preserved renal function

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Abstract

Background. Renin–angiotensin–aldosterone system (RAAS) blockade improves prognosis in renal patients, but usually requires diuretic co-treatment. RAAS blockade can decrease erythropoietin (EPO) and/or haemoglobin (Hb) levels. Diuretics decrease EPO in rodents, but their effect on EPO and Hb in humans is unknown.

Methods. Proteinuric renal patients with preserved renal function were treated during 6-week periods with placebo, losartan 100 mg/day (LOS) and LOS plus hydrochlorothiazide 25 mg/day (LOS/HCT), in random order.

Results. Hb was inversely related to proteinuria, and EPO levels were inappropriately low in relation to Hb. Hb was lowered by LOS with and without HCT. EPO was decreased by LOS/HCT, but not by LOS.

Conclusions. EPO and Hb are reduced by HCT added to LOS in proteinuric renal patients with preserved renal function. We hypothesize that EPO reduction by HCT is caused by a decrease in renal oxygen requirement, which is the main stimulus for EPO production, due to the inhibition of active tubular sodium reabsorption. Further studies should explore the exact mechanism of this phenomenon and its clinical impact.

Keywords: diuretics; erythropoietin; haemoglobin; proteinuria; renal disease; renin–angiotensin–aldosterone system blockade

Introduction

Blockade of the renin–angiotensin–aldosterone system (RAAS) with angiotensin-converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) reduces...
hypertension and proteinuria and improves renal and cardiovascular outcome in chronic kidney disease (CKD) [1,2]. For optimal therapeutic efficacy, co-treatment with diuretics is often required [3,4]. ACEI and ARB decrease erythropoietin (EPO) and/or haemoglobin (Hb) levels in different populations [5–8] by blocking the effects of angiotensin II on erythropoiesis [9,10]. Diuretics reduce EPO levels in rodents [11,12], but their effect on EPO and Hb in humans is unknown. We report the effects of the diuretic hydrochlorothiazide (HCT) and the ARB losartan (LOS) on EPO and Hb levels in proteinuric CKD patients with preserved renal function.

Materials and methods

Patients and protocol
This is a post hoc analysis of a randomized, double-blind, placebo (PLA)-controlled cross-over study. The protocol was described in detail elsewhere [13]. In short, 33 non-diabetic CKD patients with overt proteinuria and preserved renal function (Table 1) were included. Patients were treated during 6-week periods with PLA, LOS 100 mg/day, and LOS plus HCT 25 mg/day (LOS/HCT), consecutively combined with a low sodium diet (LS, 92 ± 8 mmol/day) and a high sodium diet (HS, 196 ± 9 mmol/day), in random order (Figure 1).

Measurements and calculations
EPO levels were measured by chemiluminescence immunoassay (Siemens, Los Angeles, CA, USA). To relate the EPO level to the actual Hb, the observed/predicted log EPO ratio (O/P_{EPO}) was calculated as proposed by Westenbrink et al. [14]. O/P_{EPO} in healthy reference subjects (aged 50 ± 5 years) was 0.90 ± 0.029.

Data analysis
Data obtained during PLA plus HS (HS/PLA) were taken as baseline values. Data are given as the mean ± standard error or geometric mean [interquartile range] when skewed. Before statistical testing, skewed variables were natural log transformed to obtain normality. Associations between variables were evaluated with Pearson’s correlation tests. Therapy effects were determined using paired T-tests, with a Bonferroni correction for multiple testing. P < 0.05 was considered statistically significant. SPSS 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used for all analyses.

Results

General parameters
Baseline characteristics are shown in Table 1. During the six different treatment periods, proteinuria decreased from 3.8 ± 0.4 g/day at baseline (HS/PLA) to 1.1 ± 0.2 g/day during RAAS blockade with maximal volume intervention (LS/LOS/HCT, P < 0.001; Figure 2A). Mean arterial pressure decreased accordingly (105 ± 3 mmHg at baseline versus 90 ± 1 mmHg during LS/LOS/HCT, P < 0.001), as previously described in more detail [13]. Creatinine clearance (89 ± 5 mL/min at baseline versus 75 ± 5 mL/min during LS/LOS/HCT, P = 0.001) and body weight (91 ± 3 kg at baseline versus 88 ± 3 kg during LS/LOS/HCT, P < 0.001) decreased as well, consistent with a negative fluid balance during LS and HCT.

Haematological parameters
At baseline (HS/PLA), Hb was inversely related to proteinuria, but was not related to creatinine clearance (Figure 3). O/P_{EPO} was decreased (0.64 ± 0.02 versus 0.90 ± 0.029 in healthy reference subjects, P < 0.001), indicating that EPO levels were inappropriately low in relation to Hb levels. Hb was decreased by LOS with and without HCT (Figure 2B). EPO levels were reduced by the addition of HCT on top of LOS, but not by LOS monotherapy, compared to PLA (Figure 2C). There was no statistical difference, however, between the effect of LOS monotherapy and the effect of HCT on top of LOS on EPO levels. O/P_{EPO} was further decreased by LOS with and without HCT.

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Table 1. Baseline characteristics of renal patients

<table>
<thead>
<tr>
<th>General parameters</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>24</td>
<td>9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>52 ± 2</td>
<td>46 ± 5</td>
</tr>
<tr>
<td>Caucasian race (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28 ± 1</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>144 ± 5</td>
<td>140 ± 10</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>87 ± 3</td>
<td>85 ± 4</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>92 ± 6</td>
<td>81 ± 9</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>3.6 ± 0.5</td>
<td>4.5 ± 0.08</td>
</tr>
</tbody>
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Haematological parameters

| Hb (mmol/L) | 9.4 ± 0.2 | 8.6 ± 0.3* |
| EPO (U/L) | 14.8 [12.3–17.7] | 12.9 [7.4–22.5] |
| Ferritin (μg/L) | 153 ± 22 | 76 ± 28 |

*P < 0.05 versus males.

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Fig. 1. Study design. Renal patients were consecutively treated with PLA, LOS, and LOS plus HCT (LOS/HCT), combined with HS and LS, in random order.
We found that Hb levels are inversely related to proteinuria, and EPO levels are inappropriately low in relation to Hb (low O/P\textsubscript{EPO}) in untreated non-diabetic CKD patients with overt proteinuria and preserved renal function. HCT added to LOS decreases EPO, O/P\textsubscript{EPO} and Hb in these patients.

Hb was inversely related to proteinuria, but was not related to renal function, which strongly suggests an effect of proteinuria \textit{per se} on Hb levels. This is a new finding. Proteinuria can reduce circulating EPO levels through urinary EPO loss [15–17], which may explain the inappropriately low circulating EPO in our patients at baseline. No relationship was, however, found between circulating EPO and proteinuria in these patients, suggesting that other factors such as inflammation may be involved as well [18–20].

Remarkably, HCT added to LOS, while reducing proteinuria, decreased EPO and O/P\textsubscript{EPO} levels compared to PLA. Although RAAS blockade is known to reduce EPO levels [6–8], effects of (add-on) diuretics on EPO were not reported before, besides HCT added to enalapril reducing haematocrit in hypertensive patients [21]. In rodents, diuretics reduce renal EPO production [11,12] via the inhibition of tubular sodium reabsorption which reduces renal oxygen consumption and increases renal oxygen pressure [22,23], causing decreased EPO production.

(Figure 2D). No clear-cut effect of LS on Hb, EPO or O/P\textsubscript{EPO} was observed.

**Discussion**

We found that Hb levels are inversely related to proteinuria, and EPO levels are inappropriately low in relation to Hb (low O/P\textsubscript{EPO}) in untreated non-diabetic CKD patients with overt proteinuria and preserved renal function. HCT added to LOS decreases EPO, O/P\textsubscript{EPO} and Hb in these patients.

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This mechanism might also be involved in our patients. Of note, the effects of LS added to LOS were similar to add-on HCT for proteinuria, blood pressure, renal function and body weight, but LS did not affect EPO levels, suggesting a direct pharmacological effect of HCT on EPO rather than a volume-mediated effect. This notion is supported by the finding that the negative fluid balance with an anticipated reduction of the distribution volume of EPO [16] during HCT was associated with a decrease, instead of an increase, in EPO levels. At present, no direct effects of diuretics on erythroid precursor cells are known.

EPO production was compromised in our patients, as shown by the low O/P_EPO at baseline. Whether HCT can affect uncompromised EPO production cannot be ascertained. It would be relevant to explore this issue in other populations, as (combinations of) diuretics and RAAS blockade are widely used in non-renal conditions such as essential hypertension and heart failure [25,26].

Effects of RAAS blockade on EPO and Hb usually become evident 3–12 weeks after initiation of therapy [5]. Therefore, our treatment periods may have been too short to evaluate the full haematological effect of the treatment regimens. Other limitations are the small sample size, the overall small changes and large variation of EPO levels and the lack of information on HCT monotherapy.

Conclusion

To conclude, Hb levels are inversely related to proteinuria, and EPO levels are inappropriately low in relation to Hb in renal patients with overt proteinuria and preserved renal function. EPO and Hb levels are reduced by HCT added to LOS in these patients. We hypothesize that EPO reduction by add-on HCT is caused by a decrease in renal oxygen requirement, which is the main stimulus for EPO production, due to the inhibition of active tubular sodium reabsorption. Further studies should explore the exact mechanism of this phenomenon and its clinical impact.

Authorship

- M.C.J.S. performed the analyses and wrote the manuscript.
- S.J.S. contributed to the study design.
- M.H.H. supplied the research facilities and wrote the manuscript.
- F.W. performed the clinical intervention.
- L.V. performed the clinical intervention.
- H.C.K.-N. wrote the manuscript.
- G.N. designed the study, supplied the research facilities and wrote the manuscript.
- G.D.L. wrote the manuscript.

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Conflict of interest statement. None declared.

References

A community-based model of care improves blood pressure control and delays progression of proteinuria, left ventricular hypertrophy and diastolic dysfunction in Māori and Pacific patients with type 2 diabetes and chronic kidney disease: a randomized controlled trial

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Abstract
Background. In this study, our main goal was to determine whether an integrated, community-based model of care using culturally appropriate health-care assistants to manage hypertension in Māori and Pacific patients with diabetes and chronic kidney disease (CKD) is more effective than conventional care in achieving blood pressure (BP) targets and delaying progression of cardiac and renal end-organ damage.

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