Effects of angiotensin II blockade on nitric oxide blood levels in IgA nephropathy

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Abstract

**Background.** The effects of renin–angiotensin system blockade on nitric oxide (NO), especially in pathological conditions, are far from being established. The influence of kinins and angiotensin type 2 receptor are largely speculative and based mainly on animal studies. This study was aimed to address these aspects in humans.

**Methods.** Eight IgA nephropathy patients with documented clinical and histological indicators of poor prognosis were given 50 mg of losartan, 10 mg of enalapril, and 40 mg of the NO donor isosorbide 5 mononitrate (as a control of NO generation) in randomized order for 7 days each. Treatment periods were separated by washout periods of 7 days each. Laboratory investigations were performed before and after each study period. Seven healthy controls received losartan and enalapril according to the same study design.

**Results.** Glomerular filtration rate remained stable while effective renal plasma flow increased with each treatment (P<0.05). Under losartan and enalapril, filtration fraction fell (P=0.02), plasma renin activity increased (P<0.05) and urinary aldosterone concentration decreased (P=0.02). Angiotensin-converting enzyme activity was reduced to the limit of detection under enalapril (P<0.001). Blood NO, detected as nitrosylhaemoglobin by a recently developed technique of spin-trap electron paramagnetic resonance, increased significantly, as expected, during treatment with isosorbide 5 mononitrate (P=0.01), with enalapril (P<0.05), and also with losartan (P<0.05). Unlike losartan, enalapril significantly reduced albuminuria (P=0.01) in this short-term period. In the seven healthy controls, neither enalapril nor losartan were able to increase blood NO levels significantly.

Conclusions. Blood levels of nitrosylhaemoglobin, a surrogate marker of NO, increased under blockade of the renin–angiotensin system in patients with IgA nephropathy, but not in healthy volunteers. This increase could contribute to changes of effective renal plasma flow in renal disease states.

**Keywords:** angiotensin receptor antagonists; haemodynamics; IgA nephropathy; nitric oxide

Introduction

The renal haemodynamic actions of angiotensin II (AII) blockade have been extensively studied in both normal volunteers [1,2] and in non-diabetic patients with moderate renal insufficiency [3]. The action of AII subtype 1 (AT1) receptor antagonism generally seems to be comparable with ACE inhibition, although some differences have been reported, including effects on bradykinin, prostaglandins, and renal sympathetic nerve activity (reviewed in [4]).

The effect of AII blockade on nitric oxide (NO) generation represents a still incompletely examined aspect. The role of NO in regulation of vascular smooth muscle relaxation should critically involve blood pressure response and renal plasma flow. Indirect evidence suggests that, contrary to AT1 receptor antagonism, ACE inhibition causes NO release via activation of the bradykinin B2-receptor [5]. However, technical difficulties in detecting this unstable molecule have prevented extensive studies.

We recently developed a method of spin-trap electron paramagnetic resonance detection of nitrosylhaemoglobin as a surrogate marker of NO blood levels [6] and focused on the haemodynamic effects of the ATII blockade in patients with IgA nephropathy (IgAN). These patients progress to renal failure through a relatively silent course histologically related to prevalent sclerotic changes [7]. Decreased glomerular filtra-
tion rate (GFR), persistent proteinuria, hypertension, and interstitial fibrosis are generally considered indicators of poor prognosis in these patients [8,9]. Identification of the haemodynamic mechanism sustaining this evolutive course is presently mandatory in determining therapeutic strategies that interfere with progressive glomerular sclerosis. Among them, blockade of the renin–angiotensin system is currently thought to be the most rational approach. Indeed, AII induces mesangial contraction [10], intracellular trapping and mesangial uptake of macromolecules [11,12], glomerular hypertension [13], and changes in glomerular membrane permeability [14,15]. Local hyperactivity of the renin–angiotensin system in patients with high risk of progression has previously been reported [16].

The role of haemodynamic factors in disease progression is also supported by studies on endothelin 1 (ET1). Apart from its powerful vasoconstrictor effects, ET1 induces mitogenesis of mesangial cells and matrix production [17] and is likely to be involved in sclerotic processes. Patients with IgAN have significantly higher plasma ET1 levels than normals [18] and those with initial decrease in renal function often have increased urinary ET1/cGMP ratio [19], which suggests a local imbalance between vasoconstrictor and vasodilatory factors in some IgAN patients with high risk of progression. NO, originally described as endothelium-derived relaxing factor [20], is produced by the glomerular endothelium which, in part, is in contact with the mesangium. NO released from endothelial cells increases cGMP in mesangial cells, antagonizes the constrictor action of AII and ET1 [21], and regulates glomerular haemodynamics.

No definite data are presently available on the influence of blockade of the renin–angiotensin system on NO production. Understanding this effect could be of particular interest in patients with relentless progressive renal insufficiency due to haemodynamic factors.

Subjects and methods

Eight patients with IgAN were given standard doses over a 7-day period of the AII type 1 receptor antagonist losartan, the angiotensin converting enzyme inhibitor enalapril, or the NO donor (as a control) isosorbide 5 mononitrate (ISO5M). Treatment periods were randomized and separated by a week of study-drug discontinuation in order to clear the pharmacological effects of the previously administered compound before starting the new treatment. Laboratory investigation included detection of GFR, effective plasma renal flow (ERPF), filtration fraction (FF), blood NO, plasma renin activity, angiotensin converting enzyme (ACE) activity, plasma and urinary aldosterone, and albuminuria. All these parameters were assessed before and after each 7-day period.

Seven healthy volunteers from the staff were given enalapril and losartan according to the same protocol in order to allow evaluation of NO generation under pharmacological challenge in normal individuals.

Patients' enrolment

Case selection among patients biopsied in the 2 months preceding the start of the study was based on knowledge of patients' therapeutic compliance and acceptance of the experimental aim of the study. Patients, who gave informed consent for participation in this protocol in accordance with the guidelines of the local Medical Ethics Committee, were chosen among those having clinical and histological features generally accepted [22] as poor prognostic indices.

Table 1 summarizes the main histological data. Patients (seven males and one female, mean age 49.37±14.87 years, range 32–73) had persistent proteinuria (>1 g/day) in the last 6 months no history of recurrent macroscopic haematuria and, with the exception of a female (no. 8 of Table 2), a reduced GFR (<75 ml/min). Four patients (nos 2, 3, 5, 7) were hypertensive (blood pressure ≥150/95 mm Hg) and four (nos 3–6) had increased urinary ET1/cGMP ratio (> 0.08), known to be associated with ISO5M-dependent decrease in FF in IgAN patients [19]. Patients and healthy volunteers were treated according to randomized protocol. Drug doses were: 10 mg enalapril, 50 mg losartan, and 40 mg ISO5M. Arterial pressure values were measured by a physician twice a day. In each treatment period, hypertensive patients were given clonidine in order to maintain blood pressure values close to previously optimized levels. The range of tolerated values was ±5% of the previous mean blood pressure.

ISO5M, an active metabolite of isosorbide-dinitrate currently used for treatment of angina pectoris, is an esterification product of nitric acid, characterized by complete enteral absorption and prolonged half-life. Losartan is an AII receptor antagonist, phenyl tetrazole substituted imidazole, non-peptide of low molecular weight, which binds with high affinity and specificity to the type 1 AII membrane receptors and displays no intrinsic agonist properties [23].

Laboratory investigation

Electron paramagnetic resonance (EPR) was used to detect blood NO using haemoglobin (Hb) as a ‘spin trap’ to overcome the radical’s instability. The identity of the new compound’s spectra was defined by a triplet feature at 3.250–2.400 gauss with g values of 2.02, which was found to be specific for NO–Hb. Extensive details of this method are given elsewhere [19]. A computer system on-line with the EPR spectrometer (Bruker ESP 300) allowed calculation of spectra. Each spectrum was measured as a derivative of the signal integral of interest normalized for the signal background and expressed as arbitrary units (AU)×10^-4 [24].

A radioenzymatic test was used for direct determination of ACE in serum (Buhmann Labs AG, Switzerland). The minimum detectable ACE level, determined as the activity at twice the blank value using a 2-h incubation time, was found to be 2 units. Plasma renin activity and plasma and urine human aldosterone were detected by specific radioimmunoassay (Biodata, Guidonia Montecelio, Italy).

The detection limit of the assays, defined as the concentration of angiotensin I or aldosterone equivalent to the mean counts of 20 replicates of the zero standard minus 2 SD, was 0.039 ng/ml and 6.0 pg/ml respectively. Urinary albumin levels were detected by standard nephelometric method on 24-h urine collection. Radioisotopic clearances for estimation of GFR and ERPF were performed as described in detail elsewhere [25].
Table 1. Histological risk factors for chronic renal insufficiency in the patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Crescents</th>
<th>A-sclerosis</th>
<th>G-sclerosis</th>
<th>I-fibrosis</th>
<th>Capillary Ig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>+</td>
<td>50%</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>10%</td>
<td>+</td>
<td>50%</td>
<td>1</td>
<td>−</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>+</td>
<td>30%</td>
<td>3</td>
<td>−</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>+</td>
<td>20%</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>+</td>
<td>40%</td>
<td>3</td>
<td>−</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>+</td>
<td>40%</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>+</td>
<td>35%</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>+</td>
<td>35%</td>
<td>2</td>
<td>−</td>
</tr>
</tbody>
</table>

A-sclerosis, arteriolosclerosis; G-sclerosis, glomerular sclerosis; I-fibrosis, interstitial fibrosis; Capillary Ig, immunoreactants along the peripheral glomerular capillary wall.

Presence of crescents and glomerular sclerosis is expressed as percentage of involved glomeruli.

Degrees in interstitial fibrosis and tubular atrophy are 1, focal; 2, diffuse; 3, diffuse with interstitial leukocyte infiltration.

Biopsies were performed 2–7 weeks before starting the study.

Table 2. Clinical features of the patients

<table>
<thead>
<tr>
<th>Patient (age)</th>
<th>Macro</th>
<th>Hypert</th>
<th>Proteinuria (&gt;1 g/day)</th>
<th>&lt;75 ml/min GFR</th>
<th>ET1/cGMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (36)</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>2 (53)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>3 (32)</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>4 (69)</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5 (73)</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6 (42)</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7 (48)</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>8 (42)</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

data obtained during the study of randomized administration of the AT1 receptor antagonist, losartan, ACE inhibitor (ACE-i), enalapril, and NO-donor, ISO5M.

Serum creatinine and GFR did not change with any treatment. ERPF showed an increase \( (P<0.05) \) and FF a decrease \( (P=0.02) \) with treatment. Plasma renin activity increased \( (P<0.05) \) and urinary aldosterone decreased \( (P=0.02) \) under AT1 receptor antagonist and ACE-i. ACE levels reached almost undetectable levels under ACE-i treatment \( (P<0.001) \).

Blood nitrosylhaemoglobin, a surrogate marker of NO, increased with each treatment \( (P<0.05) \), especially under ISO5M \( (P=0.01) \). NO profiles under pharmacological challenge in the IgAN patient sample are shown in Table 3 and Figure 1a. At variance from patients, no significant influences of both enalapril and losartan on nitrosylhaemoglobin levels were observed in controls (Figure 1b). Only ACE-i was able to significantly reduce albuminuria during this 1-week treatment \( (P=0.01) \) in IgAN patients. However, all four patients with increased (i.e. >0.08) urinary ET1/cGMP ratio and one out of four with low ratio responded to ISO5M with at least 30% reduction of albuminuria.

Statistics

Results were analysed by a commercial software package (Abacus Concepts, Stat View, Abacus Concepts Inc., CA, 1992). Comparisons in normally distributed variables were addressed by analysis of variance (ANOVA) to compare multiple groups. If statistically significant differences were found, differences between individual groups were tested by Student’s t-test. Linear regression analysis was used to assess correlation between variables.

Results

Significant relationships were found between NO levels and ERPF (positive correlation, \( P=0.02 \)) and FF values (negative correlation, \( P=0.04 \)) detected at the starting point of the study and at the end of the wash-out periods in patients. Table 3 summarizes general

Discussion

This short-term study focused on the possibility of modifying glomerular haemodynamics by means of cur-
AT II blockade in IgAN

Table 3. General data from the study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>AT1 R ant</th>
<th>Wash-out 1</th>
<th>ACE-i</th>
<th>Wash-out 2</th>
<th>NO-donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>96.4 ± 1.6</td>
<td>95.6 ± 1.9</td>
<td>94.4 ± 3.5</td>
<td>95.0 ± 2.2</td>
<td>92.0 ± 3.8</td>
<td>96.2 ± 1.8</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>63.5 ± 7.8</td>
<td>61.0 ± 6.1</td>
<td>57.6 ± 9.1</td>
<td>57.7 ± 9.0</td>
<td>57.0 ± 7.9</td>
<td>58.5 ± 9.0</td>
</tr>
<tr>
<td>ERPF (ml/min)</td>
<td>314.1 ± 36.3</td>
<td>343.0 ± 47.8*</td>
<td>298.5 ± 44.5</td>
<td>320.2 ± 43.2*</td>
<td>294.5 ± 29.4</td>
<td>352.1 ± 36.2*</td>
</tr>
<tr>
<td>FF (%)</td>
<td>0.20 ± 0.01</td>
<td>0.18 ± 0.01**</td>
<td>0.19 ± 0.01</td>
<td>0.18 ± 0.02**</td>
<td>0.20 ± 0.01</td>
<td>0.17 ± 0.02**</td>
</tr>
<tr>
<td>Alb (g/24 h)</td>
<td>1.8 ± 0.5</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.1 ± 0.1***</td>
<td>1.5 ± 0.2</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>PRA (ng/ml/h)</td>
<td>1.7 ± 0.2</td>
<td>4.7 ± 1.4*</td>
<td>1.5 ± 0.2</td>
<td>6.1 ± 2.7*</td>
<td>1.9 ± 0.5</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>ACE (mmol/min)</td>
<td>40.5 ± 2.7</td>
<td>40.6 ± 3.5</td>
<td>43.1 ± 3.2</td>
<td>3.3 ± 0.7***</td>
<td>38.4 ± 6.1</td>
<td>41.0 ± 4.2</td>
</tr>
<tr>
<td>pALDO (pg/ml)</td>
<td>156.6 ± 19.3</td>
<td>126.5 ± 27.3</td>
<td>149.7 ± 21.0</td>
<td>102.3 ± 20.7</td>
<td>150.3 ± 22.0</td>
<td>130.8 ± 23.3</td>
</tr>
<tr>
<td>uALDO (mg/24 h)</td>
<td>8.5 ± 1.3</td>
<td>5.9 ± 1.0**</td>
<td>7.6 ± 1.3</td>
<td>4.9 ± 0.6**</td>
<td>7.0 ± 1.4</td>
<td>6.5 ± 1.3</td>
</tr>
<tr>
<td>NO (AU × 10⁴)</td>
<td>4.0 ± 0.8</td>
<td>6.0 ± 1.0*</td>
<td>4.2 ± 0.6</td>
<td>6.3 ± 0.9*</td>
<td>4.5 ± 0.6</td>
<td>8.6 ± 0.8***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard error.

MAP, mean arterial pressure; GFR, glomerular filtration rate, as determined by [¹²⁵I]hippurate clearance; ERPF, effective renal plasma flow, as determined by [¹²⁵I]hippurate clearance; FF, filtration fraction; PRA, plasma renin activity; ACE, angiotensin converting enzyme; pALDO, plasma aldosterone; uALDO, urinary aldosterone; NO, blood nitric oxide, as detected as NO–Hb by electron paramagnetic resonance and expressed in arbitrary units (AU).

*P < 0.05; **P < 0.02; ***P < 0.01.

Currently available compounds able to influence with renin–angiotensin system and NO production.

Results obtained from this sample of IgAN patients with definite risk of non-immunological progression toward renal failure showed that the AT1 receptor antagonist losartan and the ACE-i enalapril comparably increased blood NO levels and ERPF and reduced filtration fraction. However, albuminuria was reduced at significant levels only by ACE-i treatment. These data confirmed results of the acute response to captopril previously observed by our group in this subset of IgAN patients [16].

In acute and chronic experimental settings, AT1 receptor antagonist was substantially less potent than the ACE inhibitor in lowering glomerular pressure in the kidney [27,28]. Specifically, our findings were in agreement with the results of the experimental puromycin aminonucleoside nephrosis [28], in which an early agreement with the results of the experimental puromycin aminonucleoside nephrosis [28], in which an early antiproteinuric effect, (channelled, at least in part, through activation of bradykinin), was found only with ACE-i. However, in other experimental conditions AT1 receptor antagonist and ACE inhibitor were comparable [29].

Gansevoort et al. [3] examined 11 patients with mild renal failure, moderate hypertension, and non-diabetic proteinuria. Renal haemodynamic response was similar to that described in the present paper (including ERPF, GFR, FF, ACE activity, and PRA) except for urinary protein excretion. Apart from differences in underlying nephropathy, baseline proteinuria (greater) and time of administration (longer in the Gansevoort report), it is worth noticing that, at variance with the earlier study using placebo in washout periods, mean blood pressure values were kept constant throughout the present study, which allowed observation of drug-mediated, non-antihypertensive-related effects of the two compounds on proteinuria.

At the dose used, a prominent action of ACE-i on membrane permselectivity [14] in this short-term course of treatment could have played a critical role.

The need of a long-term drug administration for
optimal antiproteinuric effects of losartan has been recently emphasized [30]. Blood levels of nitrosylhaemoglobin, as a surrogate marker of NO, were determined by EPR in the present study. Correlations between amount of the NO–Hb signals as detected by EPR and both ERPF and FF values during non-treatment periods were consistent with the role of NO in regulation of a steady-state renal haemodynamics, at least in IgAN patients with moderate renal failure.

Since the effects of the ACE inhibitor enalapril, and the AT1 receptor antagonist losartan, on NO generation were found to be weak in healthy subjects, one could speculate that either NO-independent actions of ACE inhibitors and ATIII receptor blockers on ERPF and FF or a disease-dependent vulnerability of the renal haemodynamic regulation system accounted for this apparently disease-limited phenomenon. Similar results as in the IgAN patients examined in the present study have been obtained in a few patients with focal glomerulosclerosis (data not shown), suggesting a role for reduced renal mass in response to these pharmacological challengers.

In the study group, NO–Hb levels increased with each treatment, including losartan. A remarkable difference between ACE-i and AT1 receptor antagonists (which generally share comparable actions on renal haemodynamics) is the specific ACE-i-mediated increase bradykinin levels [31]. Bradykinin causes vasodilation through activation of the bradykinin B2-receptor, which acts as a selective effenter arteriolar vasodilator [32,33] and enhances the release of NO [31]. Since AT1 receptor antagonism does not presumably have a direct effect on kinins or prostaglandins, the increase in the amount of NO–Hb signal, shown in the present study under losartan treatment, suggests the possible involvement of other factors. The AT1 receptor antagonist administration upregulates angiotensin and activates AT2 receptor [34]. Genetic engineering revealed that the role of AT2 receptor includes transduction of the blood-pressure-lowering effect of angiotensin [35]. The activation of the renin–angiotensin system, for instance during sodium depletion, increased renal nitric oxide production in experimental animals [36]. Stimulation by AII at the angiotensin AT2 receptor could be the mechanism [36]. However, the possible role of a receptor subtype distinct from AT1 and AT2, but recognizable by the competitive non-selective AT1 antagonist [Sar^1–Thr^5] AII has been proposed [37]. We speculate that a similar mechanism can be operative in humans as well, at least in patients with established moderate renal failure.

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References


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