Increased serum N^G-hydroxy-L-arginine in patients with rheumatoid arthritis and systemic lupus erythematosus as an index of an increased nitric oxide synthase activity

Rainer Wigand, Jens Meyer, Rudi Busse, Markus Hecker

Abstract

Objectives—To determine the feasibility of monitoring the serum concentration of N^G-hydroxy-L-arginine (L-NHA) as an index of an increased nitric oxide (NO) synthase activity in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) compared with nitrate (NO_3^-), the major circulating metabolite of NO whose concentration is influenced by dietary intake.

Methods—The serum concentrations of L-NHA, L-arginine (L-Arg), and NO_3^- were determined in 33 patients with RA, 25 patients with SLE and, 29 healthy subjects.

Results—Serum L-NHA was significantly increased in RA patients with high disease activity (287% of control, p<0.01), but not with low disease activity (115%), as well as in patients with SLE (173%, p<0.01). In contrast, serum NO_3^- did not differ significantly between either group of patients and the respective control group.

Conclusion—NO synthase activity or expression, or both, is upregulated in RA patients with high disease activity and in patients with SLE. Serum L-NHA seems to be a more specific and reliable index of an increased activity of this enzyme in patients with acute or chronic inflammatory diseases than NO_3^-.

There is now substantial evidence suggesting a role for nitric oxide (NO) in the pathogenesis of rheumathoid arthritis (RA). Increased production of nitrite (NO_2^-) and nitrate (NO_3^-), as a index of NO formation, has been reported in several animal studies of adjuvant induced arthritis, an experimental immunopathy that is believed to share many features with human RA. Moreover, suppression of NO generation by in vivo administration of NO synthase inhibitors, such as N^G-monomethyl-L-arginine (L-NMA), attenuates or inhibits the development of the disease, whereas supplementation with the NO precursor L-arginine (L-Arg) results in an exacerbation of the inflammatory process. Increased concentrations of NO_3^- or NO_2^- and nitrotyrosine have also been detected in the serum and synovial fluid of patients with RA. In this context, however, it is important to note that the single determination of the concentration of NO_3^- does not provide a reliable index of endogenous formation of NO because of the rapid conversion into NO_2^-.

In endotoxin treated rats, the NO synthase metabolite N^G-hydroxy-L-arginine (L-NHA) is released from NO producing cells and accumulates in the circulating blood, presumably because of a competition of the amino acid with the circulating L-Arg for the y^1 amino acid transport system in vascular cells, hence limiting its metabolism. As there is no source other than NO synthase known to produce L-NHA in the body, we have proposed that monitoring the serum concentration of this amino acid may represent a specific and thus more reliable index of an increased NO synthase activity than serum NO_3^- or NO_2^- in patients with acute or chronic inflammatory diseases.

To substantiate this hypothesis, we have determined the concentration of L-NHA and L-Arg by high performance liquid chromatography (HPLC) analysis in serum samples from patients with RA and systemic lupus erythematosus (SLE) as well as in healthy subjects. In addition, we have determined the concentration of NO_3^- in these serum samples by using the nitrate reductase assay.

Methods

Patients

Serum samples were obtained from 33 patients with RA (19 women, 14 men) and 25 female patients with SLE that fulfilled at least four of the seven American College of Rheumatology 1987 criteria for the classification of rheumatic diseases. Exclusion criteria were inflammatory diseases other than RA or SLE. In addition, blood samples were obtained from 29 healthy subjects (15 women, 14 men). All patients were attending a rheumatology outpatient department. The group of RA patients was divided into two subsets, one comprising patients with RA of low (RA-) and one group with high inflammatory activity of the disease (RA+), the latter defined by involvement (swellings or tenderness, or both) of four or more joints as well as C reactive protein (CRP).
results

Table 1 summarises the demographic and relevant clinical data of the patients. In the group of patients with RA, the Ritchie articular indexV for assessment of joint tenderness was 27.4 (2.1) (mean (SEM), range 15–46) for the RA+ subset and 8.3 (0.7) (range 0–12) in the RA− group. L-NHA and L-Arg were detected in the serum samples from both healthy subjects and patients. Their presence was confirmed indirectly by co-elution with the authentic standards on two additional HPLC columns (Merc LiChrospher 60 RP-select B and HPLC Technology UltraTechsphere 5-ODS) and, in addition, by gas chromatography mass spectrometry analysis (EI mode) of their pentafluoropropionnic anhydride methyl ester derivatives (not shown).

The average concentration of L-NHA in serum samples of patients with low inflammatory activity of RA was not significantly increased compared with the age and sex matched RA− control group (table 1, fig 1). Serum concentrations of L-Arg were also not significantly different between the two groups, while that of NO3− was significantly lower (table 1). In patients with high inflammatory activity of RA, however, serum L-NHA values were significantly higher than in the RA+ control group (table 1, fig 1), while serum NO3− concentrations were not significantly different.

In the group of patients with SLE, which comprised only women, the serum concentra-

<table>
<thead>
<tr>
<th>Group</th>
<th>L-NHA (µM)</th>
<th>L-Arg (µM)</th>
<th>NO3− (µM)</th>
<th>ESR (mm/1st h)</th>
<th>CRP (mg/l)</th>
<th>Age (y)</th>
<th>Number (f/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (RA+)</td>
<td>7.0 (1.2)</td>
<td>101.4 (17)</td>
<td>27.6 (3.8)</td>
<td>40.7 (7.7)*</td>
<td>44.8 (2.1)</td>
<td>8/4</td>
<td></td>
</tr>
<tr>
<td>Patients (RA+)</td>
<td>20.1 (3.3)*</td>
<td>109.7 (5.5)</td>
<td>33.8 (5.1)**</td>
<td>16.0 (3.2)**</td>
<td>54.1 (4.0)</td>
<td>8/4</td>
<td></td>
</tr>
<tr>
<td>Control (RA−)</td>
<td>7.3 (0.9)</td>
<td>94.4 (11.3)</td>
<td>28.6 (3.1)**</td>
<td>40.0 (1.9)*</td>
<td>40.0 (1.9)*</td>
<td>11/10</td>
<td></td>
</tr>
<tr>
<td>Patients (RA−)</td>
<td>8.5 (1.6)</td>
<td>92.6 (5.6)</td>
<td>17.0 (1.6)</td>
<td>17.7 (3.1)</td>
<td>53.3 (2.6)</td>
<td>11/10</td>
<td></td>
</tr>
<tr>
<td>Control (SLE)</td>
<td>9.3 (1.5)</td>
<td>98.5 (13.8)</td>
<td>22.5 (2.6)</td>
<td>37.3 (2.7)</td>
<td>35.5 (2.5)</td>
<td>25/0</td>
<td></td>
</tr>
<tr>
<td>Patients (SLE)</td>
<td>15.9 (1.9)</td>
<td>95.9 (5.5)</td>
<td>21.3 (2.4)</td>
<td>14.8 (2.0)</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal range</td>
<td>—</td>
<td>—</td>
<td>3-8 (m) 6-11 (f)</td>
<td>1-9</td>
<td>—</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are the means (SEM). ESR = erythrocyte sedimentation rate; CRP = C reactive protein; RA+ = rheumatoid arthritis with high inflammatory activity; RA− = rheumatoid arthritis with low inflammatory activity; SLE = systemic lupus erythematosus; ND, not determined. *p < 0.05, **p < 0.01 v patients (RA−); †p < 0.01 (Mann-Whitney U test) v control (RA+).
The serum concentration of L-NHA therefore increased compared with the SLE control group (table 1, fig 1). NO$_3^-$ concentrations, on the other hand, were virtually identical (table 1).

**Discussion**

In this study we present evidence that the serum concentration of L-NHA, an index of an increased NO synthase activity or expression, or both, is significantly raised in patients with RA of high inflammatory activity compared with concentrations found in the serum of healthy subjects or patients with RA of low inflammatory activity. This finding is compatible with earlier observations of an increased concentration of nitrotyrosine in the serum of patients with active RA, but not in the serum of patients without severe inflammation of the joints. An increased concentration of NO$_3^-$ or NO$_2^-$, or both, has also been demonstrated in the serum and urine of patients with RA.

In the group of patients with SLE, L-NHA concentrations were also significantly increased compared with the age-matched female control group, suggesting that an increase in NO synthase activity is also associated with the pathogenesis of SLE in humans, as previously predicted from a SLE-like autoimmune disease model in mice.

It is important to note that we cannot ascertain to what extent our results were influenced by the antirheumatic medication of the patients at the time of blood sampling. The serum concentrations of l-Arg, however, were virtually identical in all groups of patients compared with the respective control group, thus excluding the possibility that the increase in serum L-NHA was based on renal insufficiency in SLE or the use of non-steroidal anti-inflammatory drugs, or both.

Serum L-NHA and NO$_3^-$ were significantly increased only in patients with RA of high inflammatory activity when compared with the respective control group or RA− patients, mainly because the serum concentration of NO$_3^-$ in the group of patients with RA of low inflammatory activity was significantly lower even than that in the group of healthy subjects. The serum concentration of L-NHA therefore seems to be indeed a sensitive and more reliable index of increased NO synthase activity or expression, or both, in patients with RA and SLE and possibly also in other patients with acute or chronic inflammatory diseases.

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