Plasma semicarbazide-sensitive amine oxidase is elevated in patients with congestive heart failure

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Abstract

Objective: Semicarbazide-sensitive amine oxidase (SSAO) is present in various mammalian tissues, especially in vascular smooth muscle cells, but also in plasma. The enzyme has been suggested to play a role in vascular endothelial damage through conversion of amines into cytotoxic aldehydes, ammonia and hydrogen peroxide. Endothelial dysfunction is present in diabetes mellitus (DM) and congestive heart failure (CHF). Elevated plasma SSAO activities have been reported in patients with DM, but no data on patients with CHF are as yet available. Methods and Results: Plasma SSAO was measured in 271 patients with CHF and compared to values in 77 controls. SSAO was found to be elevated in patients with CHF compared to controls (589 ± 252 vs. 455 ± 114 mU/l; P = 0.0001). Plasma SSAO was higher in NYHA class III/IV than in class III (662 ± 288 vs. 555 ± 226 mU/l; P = 0.004) and also higher in patients with concomitant DM than in those without (706 ± 248 vs. 557 ± 245 mU/l; P < 0.0001). Plasma SSAO correlated with plasma atrial natriuretic peptide (r = 0.42; P < 0.0001), with plasma norepinephrine (r = 0.27; P < 0.0001) and with left ventricular ejection fraction (r = −0.13; P = 0.0162). Multiple regression analysis showed atrial natriuretic peptide, norepinephrine, DM and cardiothoracic ratio to be the main determinants of plasma SSAO. Conclusion: The finding of elevated plasma SSAO in CHF, increasing with severity of the disease and with the concomitant presence of DM, supports the suggestion that SSAO may be involved in the pathogenesis of vascular endothelial damage. Plasma SSAO may be a useful parameter in assessing severity of CHF and in prognostic evaluation. Pharmacologic manipulation of SSAO activity might be an interesting new concept for prevention of vascular endothelial damage in various vascular disease entities.

Keywords: Heart failure; Diabetes; Neurohormones; Human; Semicarbazide-sensitive amino oxidase

1. Introduction

Semicarbazide-sensitive amine oxidase (SSAO) is a common name for a group of heterogeneous amine oxidases which are present in various mammalian tissues, especially in vascular smooth muscle cells, but also in cartilage, adipose tissue and plasma [1–4]. SSAO differs from the well-known monoamine oxidases MAO-A and MAO-B in co-factor and in substrate and inhibition pattern. The physiological role of SSAO is not clear, but may include protection against various endogenous and exogenous monoamines. In recent years, however, several potentially deleterious effects of SSAO have come to light. The cardiovascular toxin allylamine induces myocardial necrosis and fibrosis; its toxicity has been found to be caused by conversion of allylamine by SSAO to highly cytotoxic acrolein [5]. The endogenous compounds methylamine and aminoacetone have been reported to be readily transformed by SSAO to the corresponding aldehydes formaldehyde and methylglyoxal, while at the same time generating hydrogen peroxide and ammonia. This may cause deleterious effects in 3 ways: (1) direct cytotoxic damage to...
endothelial cells by the aldehydes and hydrogen peroxide formed; (2) reaction of aldehydes with structural and functional proteins to form adducts and advanced glycation endproducts; and (3) increased radical formation and oxidative stress from the hydrogen peroxide generated [6–11]. An increasing body of evidence thus seems to indicate that SSAO may be involved in the pathogenesis of vascular endothelial damage.

Recently, we reported that plasma SSAO was elevated in patients with insulin-dependent diabetes mellitus, a condition in which endothelial function is impaired [12]. Endothelial dysfunction may also be a prominent feature of congestive heart failure (CHF) [13], but whether SSAO is also increased in this disorder is not known. In the present study we therefore investigated plasma SSAO levels in patients with moderate to severe CHF (NYHA class III–IV). Plasma norepinephrine (NE) and atrial natriuretic peptide (ANP), parameters considered to be relevant for assessment of CHF, were also measured and compared with SSAO activities.

2. Methods

2.1. Subjects

Blood was obtained from 271 patients with moderate to severe congestive heart failure who participated in a large multicenter study [14]. The samples of the present study were baseline values of patients who entered this study in the Netherlands. Most patients (95%) were on ACE-inhibitor treatment. Characteristics of the patients are given in Table 1. Left ventricular ejection fraction (LVEF) was measured by radionuclide ventriculography or echocardiography. At baseline an assessment of functional class was made, and patients were divided into NYHA III, III–IV, or IV. As the latter group comprised only 7 patients, these were combined with the 79 patients classified as III–IV to form one group henceforth called III/IV.

Seventy-seven participants of the Rotterdam Study were used as controls. The Rotterdam Study is a population-based follow-up study of 7983 inhabitants aged 55 years or older living in the Rotterdam suburb of Ommoord. The design of the study has been described in detail elsewhere [15]. The 77 controls were randomly sampled from the group of Rotterdam Study participants younger than 71 years at time of baseline examination who were not receiving treatment for heart failure, and whose fractional shortening (calculated from M-mode echocardiography) was higher than the 25th percentile. Patients with diabetes mellitus or previous myocardial infarction were excluded. LVEF in these controls was estimated using Quinones’ prediction formula: LVEF = 1.7 × fractional shortening [16]. The investigations conformed with the principles outlined in the Declaration of Helsinki.

2.2. Methods

For measurement of SSAO and norepinephrine, blood was collected in 10 ml heparinized tubes containing 12 mg of glutathione and centrifuged (4°C, 15 min, 3000 × g). Blood for measurement of ANP was collected in tubes containing EDTA and aprotinin (1.9 mg and 100 kIU/ml blood, respectively). Plasma was stored at −80°C. Plasma norepinephrine was determined by high-performance liquid chromatography with fluorometric detection [17]. Plasma ANP was determined with a commercially available radioimmunoassay kit [18].

Plasma SSAO activity was measured as previously [19]. Briefly, plasma, after preincubation with chloroglyne to inactivate any MAO possibly present, was incubated for 1 h with the SSAO-substrate benzylamine at 37°C. The amount of benzaldehyde generated was quantified by HPLC with fluorometric detection after derivatization with dimedone (5,5-dimethyl-1,3-cyclohexanedione). SSAO activity was expressed as pmol of benzaldehyde formed per min per ml of plasma, or mU/l. The assay shows excellent linearity; inter- and intra-assay variabilities are both 7%. Plasma SSAO activity is stable when stored at −80°C. No gender difference has been found in normal controls.

2.3. Statistics

For comparison between groups Student’s t-test and the Pearson correlation coefficient were used in case of normally distributed variables, and Mann-Whitney’s test and Spearman correlation coefficient in the case of skewed distribution variables. Univariate and stepwise multiple regression analyses were performed on the log-transformed
SSAO values, and evaluated by the F-test statistic. A  
$P$-value of $< 0.05$ was considered statistically significant.  
SAS version 6.10 was used to perform the statistical analyses.

3. Results

In the 271 patients with CHF, plasma SSAO was  
significantly higher than in the controls (Table 1 and Fig. 1A).  
When the patients were divided into two groups  
according to NYHA class III and III/IV (the combination  
III–IV and IV), plasma SSAO was higher in the latter  
group than in the group with NYHA class III (Table 2 and  
Fig. 1B); both subgroups had higher plasma SSAO than  
controls ($P < 0.001$). Fifty-eight of the CHF patients also  
had diabetes mellitus. When a subdivision was made  
according to the absence or presence of diabetes mellitus in  
the CHF patients, those with diabetes mellitus had significantly  
higher plasma SSAO levels than those without (Table 2 and  
Fig. 1C). Plasma SSAO levels in the CHF patients without concomitant diabetes mellitus, however, were still significantly higher than in the controls ($P = 0.0046$). Patients with diabetes mellitus had baseline characteristics similar to patients without diabetes mellitus, except for a slightly higher systolic blood pressure (Table 2).

Plasma NE and plasma ANP were higher in NYHA  
III–IV than in NYHA class III ($P < 0.0001$, Table 2).  
There was no difference in plasma NE or in plasma  
ANP between the groups with and without concomitant  
diabetes mellitus.

Linear regression analysis (Fig. 2) showed significant  
correlations of plasma SSAO with plasma ANP ($r = 0.42$;  
$P < 0.0001$) and weaker correlations with plasma NE ($r = 0.27$;  
$P < 0.0001$) and with LVEF ($r = -0.13$; $P = 0.0162$).  
In a univariate analysis SSAO activity in the CHF  
patients taken as the logarithm because of skewed distri-

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Characteristics and plasma parameters of subgroups of patients</th>
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<tbody>
<tr>
<td></td>
<td>NYHA III</td>
</tr>
<tr>
<td>Number</td>
<td>185</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67±9(69)</td>
</tr>
<tr>
<td>Sex (% males)</td>
<td>78</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74±12(73)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>127±18(126)</td>
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<tr>
<td>DBP (mmHg)</td>
<td>77±9(80)</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>25±7(25)</td>
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<tr>
<td>SSAO (mU/l)</td>
<td>555±226(522)</td>
</tr>
<tr>
<td>NE (nmol/l)</td>
<td>3.0±1.47(2.75)</td>
</tr>
<tr>
<td>ANP (pmol/l)</td>
<td>109±86(89)</td>
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Mean±standard deviation (median). SBP = systolic blood pressure; DBP = diastolic blood pressure; LVEF = left ventricular ejection fraction; SSAO = semicarbazide-sensitive amine oxidase; DM = diabetes mellitus; NE = norepinephrine; ANP = atrial natriuretic peptide. * $P < 0.01$ vs. NYHA III. ** $P < 0.02$ vs. no DM. *** $P < 0.01$ vs. no DM.
correlation was found with sex, age, blood pressure, etiology of CHF and dose of ACE-inhibitor. All parameters with $P < 0.1$ in the univariate analysis were used in a stepwise multiple regression analysis of (log) SSAO activity as the dependent variable in a general linear model procedure. The most important parameters for predicting SSAO activity were plasma ANP ($F = 21.76$, $P < 0.0001$), plasma NE ($F = 10.45$, $P = 0.0014$), cardiothoracic ratio ($F = 10.44$, $P = 0.0014$), the presence of DM ($F = 19.30$, $P < 0.0001$) and the presence of angina pectoris ($F = 16.72$, $P < 0.0001$). The latter is negatively correlated to SSAO activity. The over-all model had an $F$-value of 22.02 ($P < 0.0001$) with a correlation coefficient of 0.55.

4. Discussion

The major finding of the present study is that plasma SSAO is elevated in patients with CHF compared to controls. Age, sex and medication used were not found to be determinants of SSAO activity. The effect of small differences in mean age and sex distribution between the CHF and the control groups therefore appears to be negligible. The higher SSAO activities in CHF are not likely to be due to possibly decreased renal function in the CHF patients, since plasma SSAO activities in 12 patients with chronic renal failure (serum creatinine $1090 \pm 411$ mmol/l) were found to be not elevated ($327 \pm 127$ mU/l).

Plasma SSAO activity appears to increase with severity of the disease (Fig. 1B). This is confirmed by the results of the multivariate analysis, which shows that the most important variables for plasma SSAO activity are plasma ANP and NE, as well as cardiothoracic ratio, all parameters known to increase with increasing severity of CHF. The plasma SSAO activity in CHF is somewhat higher than in patients with uncomplicated diabetes mellitus ($589 \pm 486$ mU/l), but similar to the activity in patients with diabetes mellitus with retinopathy and/or nephropathy ($581-646$ mU/l) [12]. It is noteworthy that patients with both CHF and diabetes mellitus have even higher plasma SSAO activities.

It has been suggested that previous reports on serum MAO activities may in fact have been measurements of SSAO [6,12]. In this respect, it should be noted that McEwen and Harrison [20], as well as Nilsson et al. [21] reported increased serum MAO activities in respectively 79 and 41 patients with heart failure, correlating with the severity of the disease. Matsumoto et al., however, found no significantly increased serum MAO in 6 patients with chronic heart failure compared to controls [22].

The origin of elevated plasma SSAO is not known. Recently the biochemical properties of circulating serum SSAO were found to be identical to those of the SSAO from vascular tissues [23]. It might be hypothesized that increased leakage of SSAO from the membranes of proliferating smooth muscle cells, after vascular endothelial damage, may be the cause of the elevated plasma SSAO levels. Alternatively, the increase may be in response to increased levels of SSAO substrates. Transformation of endogenous amines like methylamine and aminoacetone into aldehydes, ammonia and hydrogen peroxide may lead to (further) vascular endothelial damage. Whether plasma levels of methylamine and/or aminoacetone are increased in CHF, as has been suggested for diabetes mellitus [24,25], has not been investigated. It is of interest to note that methylamine is, amongst others, formed by metabolism of the stress hormone adrenaline, and is a component of cigarette smoke. Both stress and smoking are associated with increased risk for cardiovascular complications.

Apart from cytotoxic damage to the endothelium, aldehydes may also be deleterious due to reaction to and modification of proteins. Methylglyoxal, for example, has been suggested to be involved in the development of diabetic complications, and the methylglyoxal scavenger, aminoguanidine, is presently under investigation as a prophylactic agent for prevention of such complications [11]. Whether methylglyoxal mainly stems from oxidation by SSAO of aminoacetone, or from enzymatic and non-enzymatic elimination of phosphate from dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, remains to be established [26]. In the former case, and/or if formation of formaldehyde from methylamine is substantial, treatment with an SSAO inhibitor (e.g., hydralazine) may prove useful in preventing vascular damage, not only in diabetes mellitus, but possibly also in CHF.

The correlation of plasma SSAO with plasma NE and ANP, which are often regarded as good indicators for severity and prognosis of CHF [27,28], raises the possibility that plasma SSAO may likewise be an indicator. Measurements in long-term survival studies will have to be performed, however, to further establish the potential of plasma SSAO as an indicator for severity and prognosis of CHF. Also, in view of the elevated SSAO in diabetes mellitus, SSAO may turn out to be more generally an indicator for endothelial dysfunction than specifically for severity of CHF.

We conclude that plasma SSAO is increased in CHF, as it is in diabetes mellitus; the elevation is greater with increasing severity of the disease. The possibility that SSAO not only increases after vascular endothelial damage, but may even play a role in the development of vascular endothelial damage, raises interesting questions with regard to diagnosis, follow-up, prognosis and even preventive therapy.

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