

Semicarbazide-sensitive amine oxidase. Its physiological significance*

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Abstract: Although the existence of plasma- and tissue-bound semicarbazide-sensitive amine oxidases (SSAOs) has been recognized earlier, the physiological relevance of the enzyme still remains uncertain. Recent data suggest that elevated serum SSAO activity might cause endothelial injury. Formation of cytotoxic metabolites (e.g., formaldehyde) and increased oxidative stress might lead to initiation or progression of atherosclerosis. Significant positive correlation was found between serum SSAO activity and severity of atherosclerosis, and diabetic macrovascular complications. Effective and selective inhibitors of human SSAO might exert cytoprotective effect on endothelial cells. Compounds, having similar structure to mexiletine, were synthesized and studied relating to SSAO activity. The reference substrate was MDL-72974A. Unfortunately, our new compounds did not reach the potency of the reference substance using human serum samples. In conclusion, we suppose that vascular and soluble SSAO enzymes might have different inhibitor sensitivity. Further studies are required to determine whether the soluble or vascular isoform of SSAO will be the main therapeutic target in the future.

INTRODUCTION

Semicarbazide-sensitive amine oxidase (copper-containing amine:oxygen oxidoreductase EC 1.4.3.6, SSAO) has been widely studied during the past decades, and a lot of information has been accumulated about the tissue distribution and molecular properties of the enzyme, including its substrate specificity and inhibitor sensitivity [1,2].

Distribution and molecular properties of SSAO

SSAO is found in most of the mammalian tissues in two forms: tissue-bound and soluble (plasma SSAO) isoforms. Blood vessels, mainly their smooth muscle layers, serve the major source of the enzyme activity of the tissue-bound form, but endothelial cells, adipocytes, chondrocytes, fibroblasts, retina, sclera, kidney, spleen, placenta, umbilical artery, and bone marrow also contain SSAO activity, associated to the plasma membrane of the cells and in small amount in their microsomal fraction [3].

The tissue-bound isoform has been cloned recently [4]. The enzyme structure revealed that this copper-containing transmembrane glycoprotein has a large extracellular domain, which contains the catalytic site. The cofactor is 6-hydroxy-dopa-quinone (TPQ) [5], and the enzyme has a homodimer structure. Some studies suggest that plasma SSAO might be derived from the cleavage of the extracellular domain of the tissue-bound isoform of the enzyme [4]. Immuno-fluorescence histochemical tech-

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niques could prove the homology of plasma SSAO with the tissue enzyme. The membrane-bound endothelial isoform of SSAO is identical with vascular adhesion protein 1 (VAP-1) [4], and the expression of this unique adhesion protein is strongly regulated in a tissue- and cell-type specific manner [6]. It is possible that advanced glycation end-products, inflammation, shear stress, and/or low-density lipoprotein molecules induce the surface expression of VAP-1 [7,8].

Substrates and inhibitors

SSAO enzymes are capable of deaminating short-chain primary amines, such as methylamine allylamine or aminoacetone (Table 1). Methylamine and aminoacetone are the most important endogenous substrates in humans [9,10]. Methylamine is derived from epinephrine, sarcosine, or creatine catabolism, whereas aminoacetone is a product of glycine and threonin metabolism [11]. A common feature of SSAO enzymes, that they exhibit preference for benzylamine, a nonphysiological substrate. Several well-known biogenic amines are also good substrates of SSAO (e.g., tyramine, histamine, dopamine), however, under normal conditions, the elimination of these amines is due to MAO activity. SSAO might play a scavenger role in the case of overproduction of these biogenic amines.

Table 1 Substrates and inhibitors of SSAO.

Type	Substrates	Inhibitors
Aliphatic	methylamine	semicarbazide
	aminoacetone	hydroxylamine
	allylamine	propargylamine
Aromatic	benzylamine	pyridoxamine
	beta-phenyl-ethylamine	(+)mexiletine
	tyramine	B-24
	dopamine	FLA 336
	mescaline	MDL-72145
	tryptamine	MDL-72974A
	histamine	
Hydrazine derivatives		iproniazid
		phenelzine
		procarbazine
		hydralazine
		carbidopa
		benserazide aminoguanidine

SSAOs are inhibited by carbonyl reagents (e.g., semicarbazide or hydrazine derivatives). SSAO is resistant to acetylenic-type MAO inhibitors (clorgyline, (-)deprenyl) *in vitro*, while *in vivo* it may be inhibited irreversibly by the metabolites of these MAO inhibitors (allylamine) [12]. MDL 72145 and MDL-72974A potently inhibit SSAO and MAO-B [13]. Without highly selective inhibitors of soluble human SSAO, it is difficult to determine the relative importance of the enzyme in the presence of MAO. Procarbazine, a carcinostatic agent, is a selective inhibitor of SSAO, and its potency is greater than that of semicarbazide and benserazide [14,15]. Substantial differences exist between the substrate specificity and inhibitor sensitivity of SSAOs between different species. Even the soluble and membrane-bound isoform might have different inhibitor sensitivity in the same species [16]. 2-bromoethylamine (2-BrEA) was recently found to be a potent and selective SSAO inhibitor on rat lung-bound SSAO [17,18].

Cytotoxic metabolites

The reaction catalyzed by SSAO appears to be of the amino-transferase type producing aldehyde, ammonia, and hydrogen peroxide. These products are potentially cytotoxic and may be involved in the pathogenesis of atherosclerosis and diabetic vascular complications.

From methylamine formaldehyde, and from aminoacetone methylglyoxal are formed, as deaminated metabolites. Allylamine, a xenobiotic, is metabolized also by SSAO to acrolein, which can cause atherosclerosis and acute myocardial necrosis during chronic exposure [3]. Acrolein depletes glutathion and initiates oxidative damage. Inhibitors of SSAO administered before allylamine can prevent the consecutive damage.

It was observed on endothelial cell cultures, that the aforementioned cytotoxic metabolites, especially formaldehyde, might initiate endothelial injury and subsequently atherosclerosis [10]. SSAO inhibitors are capable of preventing endothelial injury on cell cultures [10].

Methylglyoxal has been implicated to form advanced glycation end-products with proteins and collagen. This may be another important step in the pathogenesis of diabetic vascular complications. [19]. A nucleophilic hydrazine, aminoguanidine is capable of blocking the formation of advanced glycation end-products, and this drug is a quite potent inhibitor of SSAO [19, Table 1].

Hydrogen peroxide formed by SSAO may increase oxidative stress or act as a second messenger in peripheral tissues. It can modulate the effects of insulin on glucose transport and stimulate prostaglandin biosynthesis [20,21].

Physiological significance

The exact physiological role of this enzyme is presently not well understood. Developmental, metabolic, scavenger, and immunomodulatory functions are reported in the literature.

The observed developmental toxicity (vascular lesions and aorta dilatation) of SSAO inhibitors suggests a role for SSAO in connective tissue matrix development and maintenance [22]. In rats treated with SSAO inhibitors, uncontrolled proliferation of vascular smooth muscle cells was observed in response to endothelial and intimal injury.

SSAOs might regulate lipolysis influencing histamine metabolism [23] or via hydrogen peroxide formation. Hydrogen peroxide stimulates glucose transport in adipocytes [24], promoting the translocation of GLUT4 carriers to the cell surface.

Protection against exogenous toxic polyamines such as spermine and spermidine might be another function of SSAOs. Nevertheless, the products of polyamine oxidation, the aminoaldehydes, are more toxic than the parent compounds [25].

SSAO activity scavenges the effects of circulating endogenous monoamines (Table 1) in pharmacological concentrations [11]. The oxidative deamination of monoamines reduces the pharmacological activity of these substances. SSAOs also decrease endogenous serum methylamine level, originated from epinephrine, sarcosine, or creatine catabolism. Elevated methylamine level was found in several pathologic sites (such as stress, uremia, and diabetes mellitus). Its oxidative deamination could result in overproduction of formaldehyde in tissues with high SSAO activity, especially blood vessels [26].

Regulation of selectin independent leukocyte adhesion to endothelial cells is also a putative function of SSAO/VAP-1 molecules [4]. It is not clear whether the amine oxidase activity in VAP-1 is required or not for adhesion to leukocytes.

CLINICAL INVESTIGATIONS

The ability of SSAO to metabolize various aliphatic and aromatic monoamines differs between species, which limits the predictive value of the animal studies on human tissues. Therefore, clinical investigations are of great importance in the research of SSAOs.

There are several pathological states where increased serum SSAO activity has been found, such as diabetes mellitus, congestive heart failure, multiple types of cerebral infarction, uremia, and hepatic cirrhosis [27–31]. We presume that increased methylamine, aminoacetone (in patients with diabetes mellitus), creatinine (in patients with uremia) or epinephrine (in stress situations) levels might be responsible for these changes providing excessive substrate concentration. In chronic liver diseases, serum SSAO might be derived from the liver [32]. In diseases with hypoproteinaemia and low serum Cu^{2+} levels (e.g., severe burns, malignancy) the enzyme activity is decreased [33].

This review focuses on human studies of serum and vascular SSAO in healthy subjects (with or without obesity and varicosity) and in patients with diabetes mellitus and atherosclerosis (Fig. 1).

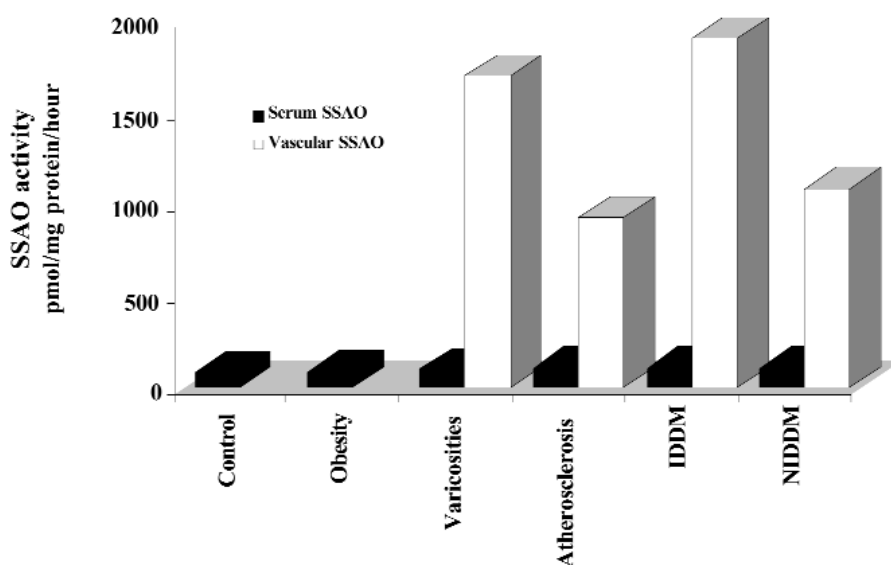


Fig. 1 Human SSAO activity in controls and patients.

SSAO in healthy subjects

Mean SSAO values in control subjects were below 100 pmol/mg protein/hour in all of the clinical studies performed at our university [16,34,35]. In control subjects without obesity ($\text{BMI} \leq 25$), hypertension, atherosclerotic plaques, or varicosities, serum SSAO activity was found to be 74.19 ± 21.27 pmol/mg protein/hour (mean \pm SD, $n = 10$). In obese controls ($n = 14$) and in controls with varicosities ($n = 34$), the serum SSAO activity was higher, however, this difference was not significant (91.12 ± 25.20 and 99.57 ± 30.74 pmol/mg protein/hour, respectively).

In controls with asymptomatic carotid plaques (which were detected by carotid sonography) serum SSAO activity was significantly higher (100.22 ± 28.71 pmol/mg protein/hour, $n = 15$) than in controls without carotid plaques ($p = 0.023$).

Significant positive correlation was found in control patients between serum SSAO activity and total cholesterol levels, body weight, and atherogenic index [34]. Carotid plaque score (Crouse score) and age-corrected intima-media thickness values also showed positive correlation with serum SSAO activity. Other investigated risk factors for systemic atherosclerosis (smoking, age, gender, HDL cholesterol, triglyceride, fasting plasma glucose levels, and presence of hypertension) were not related to SSAO.

In patients with varicosities, the SSAO activity of the surgically removed saphenous vein exhibited significant positive correlation with fasting plasma glucose levels ($R^2 = 0.166$, $p = 0.034$). The mean SSAO activity of the veins was more than 17 times higher than serum SSAO activity (1708.60 pmol/mg protein/hour, $n = 34$).

These results suggest that during the selection of control patients for SSAO studies the presence of obesity, varicosity, asymptomatic carotid plaques, and hypercholesterinemia should be excluded.

SSAO and diabetes mellitus

Elevated plasma SSAO activity has been reported in Type 1 (insulin-dependent) diabetes, and in Type 2 (non-insulin-dependent) diabetes, and even in childhood Type 1 diabetes at first clinical diagnosis [27,36]. Our clinical investigations supported these findings [16,34,35]. In patients with diabetes mellitus without complications, mean serum SSAO activity values were above 110 pmol/mg protein/hour (113.31 ± 52.10 , $n = 10$ in IDDM, and 112.91 ± 60.28 , $n = 21$ in NIDDM).

The presence of hypertension, microvascular (proliferative retinopathy), and macrovascular complications (carotid stenosis) was associated with significant elevation of serum SSAO activity (hypertension: 193.89 ± 84.25 pmol/mg protein/hour, $n = 9$, retinopathy: 163.51 ± 66.02 pmol/mg protein/hour, $n = 16$, macroangiopathy: 194.94 ± 91.10 pmol/mg protein/hour, $n = 8$). Obese patients with Type 2 diabetes exhibited higher serum SSAO values (146.11 ± 64.92 pmol/mg protein/hour, $n = 19$) than non-obese patients, however, this difference was not significant.

In patients with Type 2 diabetes, we found positive correlation between serum SSAO activity and severity of carotid atherosclerosis (carotid plaque score and stenosis score) [35]. Low-density lipoprotein (LDL) molecules, advanced glycation end-products, and shear stress are capable of inducing endothelial VAP-1 expression [7,8] that might result in increased SSAO activity and formation of cytotoxic metabolites in the vascular wall. Increased substrate (methylamine and aminoacetone) supply might also contribute to the elevation of SSAO activity.

It is interesting to note that vascular (carotid artery) mean SSAO activity was almost two times higher in patients with Type 1 diabetes mellitus than in Type 2 diabetes (1907.52 pmol/mg protein/hour, $n = 10$, vs. 1090.20 pmol/mg protein/hour, $n = 21$) (Fig. 1). In patients with diabetes, vascular SSAO did not correlate with fasting plasma glucose (in contrast with controls), body weight, and BMI values.

The exact relationship between vascular and serum SSAO activity has not been elucidated so far. In human beings, serum SSAO activity is relatively low compared to the vascular activity (Fig. 1). Endothelial injury might result in increased expression and liberation of membrane-bound isoform of SSAO. It is also possible that serum SSAO is derived from the liver or adipocytes in pathological conditions, and increased amount and/or activity of the enzyme results in endothelial damage.

SSAO and atherosclerosis

Atherosclerosis has been involved in several life-shortening and degenerative diseases, such as stroke, coronary artery disease, dementia, atherosclerosis obliterans of the lower extremities, etc. In diabetes mellitus and uremia, atherosclerosis exhibits a more severe form and has an early onset. Thus, atherosclerosis might be the common link between a variety of diseases where elevated SSAO activity was observed.

This hypothesis is strongly supported by our results. Significant positive correlation was found between human serum SSAO activity and several risk factors of atherosclerosis (e.g., body mass index, fasting plasma glucose, hemoglobin A_{1c}, and total cholesterol level) [34]. Serum SSAO activity is correlated with carotid plaque score (Crouse score) and intima-media thickness, a reliable indicator of progression of atherosclerosis.

Human umbilical vein SSAO catalyzes endothelial cell-mediated LDL oxidation via enzyme-complexed Cu^{2+} [37]. Increased oxidative stress also promotes this process. LDL oxidation results in increased atherogenicity of the lipoprotein, which might cause accelerated plaque formation.

These results suggest that determination of SSAO activity might be a candidate biochemical marker for screening healthy people with high risk of atherosclerosis for the presence of early atherosclerotic lesions. The determination of the enzyme activity would be a simple and sensitive method for the control of macrovascular complications in diabetic patients.

BASIC INVESTIGATIONS ON SSAO INHIBITORS

Hydrazine compounds, such as semicarbazide, are relatively weak inhibitors of SSAO. Recently, a highly potent, MAO-B inhibitor (E)-2-(4-fluorophenethyl)-3-fluoroallylamine hydrochloride (MDL 72974A), was found to be a very effective inhibitor of SSAO in human serum and human umbilical artery [38,39].

The IC_{50} values of semicarbazide were estimated to be $5 \times 10^{-3}\text{M}$ and $5 \times 10^{-4}\text{M}$ for SSAO from human serum and saphenous vein, respectively [16]. MDL 72974A was more than 1000 times more effective than semicarbazide. The IC_{50} values were 10^{-7}M and 10^{-8}M for SSAO from human serum and saphenous vein, respectively. The IC_{50} values of MDL 72974A were similar in human umbilical artery and human saphenous vein.

Human serum was relatively insensitive to MDL 72974A and semicarbazide compared to vascular tissues. However, human serum SSAO activity was considerably lower than that of the vascular tissues. This finding supports the hypothesis that an endogenous SSAO inhibitor may be present in human serum [40].

Compounds, having similar structure to mexiletine, were synthesized, and their effect was studied on serum SSAO activity in our laboratory. The reference substrate was semicarbazide and MDL-72974A. Unfortunately, our new compounds did not reach the potency of the reference substances.

The potency of the known SSAO inhibitors (hydrazine derivatives, 2-phenyl-3-haloallylamines, 4-picolyamine analogs, aromatic α -methyl-monoamines, etc.) is determined by the species and tissue source of the enzyme [11]. Thus, *in vitro* measurements on human tissues are necessary during development of selective SSAO inhibitors.

We suppose that potent and selective inhibitors of human SSAO enzymes might exert cytoprotective effect on endothelium, and delay the progression of atherosclerosis and late diabetic vascular complications.

POTENTIAL AVENUES FOR FURTHER INVESTIGATION

There are several unresolved questions regarding human SSAO enzymes. From a pharmacological point of view, the following issues have of great importance.

Do the morphological alterations in large vessels precede or follow the changes in the serum level of SSAO? Does serum or vascular SSAO activity correspond to the level of reactive oxygen species in the plasma? What is the cellular source of serum SSAO responsible for the relationship with vascular morphology?

Benserazide inhibits SSAO irreversibly. Is there any additional therapeutic benefit due to the inhibition of SSAO activity during Madopar (benserazide and levodopa) treatment of Parkinson's disease [15]?

MDL compounds (MDL 72974A, MDL 72145) are the most potent irreversible inhibitors of SSAO and MAO-B. It is a question of whether the concomitant inhibition of MAO-B and SSAO means advantage over the selective inhibitors of MAO-B in the treatment of Parkinson's disease.

A further question is whether a long-term treatment with a potent SSAO inhibitor can prevent the endothelial injury and subsequent angiopathy in patients with diabetes mellitus.

The products of SSAO activity (especially formaldehyde) may also play a role in diabetic retinopathy. Can the inhibition of SSAO activity prevent retinopathy in diabetes mellitus?

And the last question is: Does the determination of serum SSAO activity provide a superior marker for systemic atherosclerosis compared to novel risk factors (e.g., C-reactive protein, fibrinogen, homocysteine)?

CONCLUSIONS

Based on our clinical investigations, we suppose that determination of SSAO activity might be a candidate biochemical marker for screening healthy people with high risk of atherosclerosis for the presence of early atherosclerotic lesions. The determination of the enzyme activity would be a simple and sensitive method for the control of macrovascular complications in diabetic patients.

Our *in vitro* investigations on SSAO inhibitors proved that human vascular and soluble SSAO enzymes have different inhibitor sensitivity. Further studies are required to determine whether the soluble or vascular isoform of SSAO will be the main therapeutic target in the future.

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