Available antifibrotic agents are largely limited to inhibitors of the renin-angiotensin-aldosterone system (RAAS), which are at best only partially effective in delaying the progression of renal disease (96). Understanding the inflammatory mechanisms involved in the development and progression of CKD will enable the identification of new potential targets and facilitate the design of innovative anti-inflammatory therapeutic strategies.

Semicarbazide-sensitive amine oxidase (SSAO; also known as primary amine oxidase, EC 1.4.3.21) has been shown to be involved in the pathogenesis of a number of inflammatory diseases by mediating the migration of leukocytes into tissue and promoting the inflammatory response. However, its direct role in kidney disease is not well established. Previous studies in murine models have demonstrated its pathological role in fibrotic disease, including chronic fibrotic liver injury (97), chronic obstructive pulmonary disease, and vascular remodeling (65). Thus, SSAO inhibition appears to be a logical strategy to limit inflammation and potentially downstream fibrosis in CKD. This review will explore the mechanistic link of SSAO with kidney diseases and the potential role of SSAO inhibitors in kidney fibrosis.

Physiological Role of SSAO

The substrate specificity of SSAO was initially defined by Lewinsohn and coworkers (48). SSAO is found in several tissues, with particularly high activity in highly vascularized tissues, including the kidney (47a). The physiological role of SSAO depends on the tissue where it is expressed (76). SSAO is unique among other endothelial cell-expressed adhesion molecules because it is also an ectoenzyme.
SSAO is encoded by the amine oxidase copper containing 3 gene. It is a type 1 membrane-bound protein with a distal adhesion domain and a catalytic amine oxidase site proximal to the membrane. Membrane-bound SSAO is located in the plasma membrane and has a large extracellular domain containing the active motif. A soluble form of SSAO is present in plasma (60) and is known as vascular adhesion protein (VAP)-1 (88). In vitro studies have demonstrated that membrane-bound SSAO can be proteolytically cleaved by a metalloprotease at the extracellular stalk region, thus giving rise to the active (truncated) circulating form of SSAO (1, 43, 91), a process known to occur for many membrane-bound proteins (34). The VAP-1 molecule is a 170- to 180-kDa homodimeric glycoprotein that comprises two 90-kDa subunits held together by disulfide bonds. Experiments with transgenic and knockout mice have suggested that membrane-bound VAP-1 is the only source of plasma SSAO activity (90). Most serum SSAO activity disappears after the depletion of serum VAP-1 by anti-VAP-1 antibodies, further indicating that VAP-1 is the main source of circulating SSAO. Likewise, in humans, a good correlation has been found between SSAO activity and VAP-1 in serum (83).

Lyles et al. (53) defined the enzymatic characteristics of SSAO and summarized that benzylamine, phenethylamine, tyramine, dopamine, tryptamine, and histamine are SSAO substrates in rat and human tissue. SSAO catalyzes the oxidative deamination of aliphatic and aromatic primary amines to an aldehyde, \( \text{NH}_4^+ \), and \( \text{H}_2\text{O}_2 \) in the presence of complete monoamine oxidase (MAO)-A and MAO-B inhibition, according to the following reaction:

\[
\text{R-CH}_2-\text{NH}_4^+ + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{R-CO} + \text{NH}_4^+ + \text{H}_2\text{O}_2
\]

During the reduction step, a primary amine interacts with topazinone, resulting in a transient covalent interaction (a Schiff base) between the enzyme and substrate. During the oxidation step, the enzyme is reoxidized, and \( \text{NH}_4^+ \) and \( \text{H}_2\text{O}_2 \) are released. The active site, containing one \( \text{Cu}^{2+} \) and one carbonyl cofactor, identified as topazinone connected by a water molecule, is located inside each subunit and communicates with the solvent through a hydrophobic channel (33).

As shown in Fig. 1, endogenous substrates for SSAO include methylamine (a product from adrenaline and creatinine metabolism) and aminoacetone (a product of amino acid catabolism). These soluble products are highly reactive and cytotoxic, leading to protein cross-linking and oxidative stress (9, 31, 102). Yu et al. (102) found that methylamine itself is relatively nontoxic to cultured endothelial cells obtained from both the human umbilical vein and calf pulmonary artery. However, it becomes very toxic in the presence of SSAO (102). Aminoacetone, which may be formed as a result of threonine or glycine metabolism, is oxidized by SSAO to form methylglyoxal, which is both cytotoxic and mutagenic. Methylglyoxal is considered to induce formation of oxygen free radicals and chemical modification of essential proteins by reacting with arginine, lysine, and cystine residues. Methylglyoxal modifies cell proteins nonenzymatically through the Maillard reaction, in which aldehydes and ketones react with ε-amino groups of lysine residues and guanidino groups of arginine residues resulting in stable chemical adducts in proteins, also known as advanced glycation end products (AGEs) (67). This results in the cross-linking of proteins (through the formation of specific covalent and translational bonds) with the resultant alteration of protein structure and resistance to proteolysis, in the end leading to tissue remodeling (35). AGE accumulation is well recognized to correlate with the severity of diabetic nephropathy; this was first described by Monnmier et al. (67). Beisswenger et al. (5) have shown that AGE accumulation precedes and correlates with early manifestations of renal disease.

Mechanistic experiments performed in vitro and in vivo point to the function of SSAO in leukocyte migration from the circulation to sites of inflammation (38). The cell surface expression of SSAO is increased on the vascular endothelium of inflamed tissues, where it partakes in the rolling, firm adhesion, and transmigration phases of leukocyte transmigration (Fig. 2). The current hypothesis suggests that leukocytes initially bind to endothelial VAP-1 using receptors for the anti-VAP-1 antibody-defined surface epitopes of VAP-1. The molecular nature of these putative leukocyte receptors remains to be determined. This interaction and activities of other
adhesion molecule pairs bring leukocytes and endothelial cells into close contact. This allows the penetration of SSAO substrates present on the surface of leukocytes into the enzymatic change of VAP-1. When the catalytic reaction is triggered, a covalent but transient Schiff base forms between the enzyme and substrate, temporarily bringing the two cell types together. Spontaneous cleavage of the Schiff base then allows leukocytes to continue their migration through vessel walls. Such guiding of leukocytes is a result of a cascade of sequential events, sometimes referred to as “homing.” Among mononuclear cells, it specifically directs CD8-positive T killer cells and natural killer cells, but it also supports the adhesion of granulocytes. In addition, the NH$_4^+$ and H$_2$O$_2$ released during the catalytic SSAO reaction may also contribute to leukocyte migration. H$_2$O$_2$ can induce the expression of adhesion molecules, such as P-selectin and E-selectin, and chemokines, such as macrophage chemotactic protein-1 and IL-8, in vitro and in vivo (42, 45, 86). Moreover, SSAO inhibition leads to diminished P-selectin expression in an in vivo model of eye inflammation (75). The modulation of the microenvironment by the end products of VAP-1-driven oxidation may prime the entire leukocyte extravasation cascade.

In adipocytes, where the membrane-bound form of SSAO is abundant (69), SSAO activity stimulates glucose transport, mimicking the insulin effect through H$_2$O$_2$ generated during the catalytic process (23, 68, 106), and is involved in lipid trafficking (89). It also participates in cellular differentiation (21, 64), deposition of the extracellular matrix in smooth muscle cells (46), and control of muscular tone (17) by mechanisms that are not completely understood.

**SSAO in the Normal Kidney**

Similar to other organs, expression of SSAO in human kidneys is localized to endothelial cells, pericytes, and smooth muscle cells of larger vessels. In developing human kidneys, VAP-1 expression can be detected in vascular smooth muscle cells by *embryonic week 13* and also in peritubular capillaries (81). Conversely, glomerular mesangial cells and podocytes are VAP-1 negative in both developing and adult kidneys (44). Whether this differential expression in glomerular and peritubular capillaries (61) is responsible for the differential, prognostic value of glomerular and tubulointerstitial pathology is currently unknown.

**SSAO in Pathology**

SSAO is expressed at low levels in the quiescent endothelium but is significantly upregulated in response to inflammatory stimuli, including inflammatory liver disease, congestive heart failure, diabetes mellitus (DM), and CKD (76), but also in noninflammatory conditions, such as cerebral infarction, uremia, pulmonary and renal fibrosis, and cirrhosis (11, 13, 36, 98). Inflammatory stimuli result in SSAO translocation from storage granules onto the endothelial surface (37, 82), where it catalyzes the oxidative reaction important for the covalent but transient linkage between rolling lymphocytes and endothelial cells (84, 85) during the multistep adhesion cascade (85).

**SSAO in the Vasculature**

Perhaps the highest specific expression of SSAO is found endogenously in vascular smooth muscle cells of elastic and muscular arteries, including renal vessels. SSAO has an important role in vascular smooth muscle cell differentiation (21), organization of the extracellular matrix (95), and regulation of vascular tone (46, 47, 95). At a molecular level, it is conceivable, in view of previous results using molecular modeling (84), that in addition to soluble primary amines, such as methylamine or aminoaacetone, SSAO may act on amino acids, including matrix proteins, and thus contribute to the physiological cross-linking of elastic and collagen fibers. The increase in leukocyte recruitment into the vascular wall induces vascular dysfunction by enhancing the generation of AGEs, causing direct oxidative damage. Similar vasospastic mechanisms also potentially contribute to renovascular disease, with resultant interstitial fibrosis in CKD, especially hypertension. Hypertension is the most important independent risk factor for the development and progression of kidney diseases (4) and has been linked to elevated SSAO activity (15, 28). In patients with stroke and malignant hypertension, average plasma SSAO activities are increased, probably due to the accompanying cardiac dysfunction. Likewise, levels are also elevated in patients with preeclampsia, regardless of whether they have the aggravating complication of HELPP syndrome.

Nelson and Boor (72) found that SSAO metabolized allylamine, a selective cardiovascular toxin in vivo. SSAO inhibitors obviated the oxidative deamination of allylamine to the highly toxic aldehyde acrolein (14, 72). This vascular toxicity was studied by Conklin et al. (17), who examined coronary...
artery spasm mediated by SSAO in physiological preparations of the rat coronary artery. They suggested that SSAO activation in human coronary arteries may well be a site of xenobiotic/endogenous amine metabolism that could serve as a trigger for coronary artery vasospasm. Inhibition of SSAO resulted in vasorelaxation in isolated human arteries (17). However, contradictory results about the influence of SSAO on arterial vascular tone have been reported as Vidrio et al. (95), and they proposed that SSAO-mediated \( \text{H}_2\text{O}_2 \) production could both increase vascular tone and enhance hydralazine-induced vasodilation.

**SSAO in Kidney Disease**

The association between hypertension and the progression of CKD is direct and progressive (40). In humans, Lin et al. (51) showed relationships between serum SSAO levels and existing biomarkers of progressive renal disease, which correlated positively with urinary albumin excretion and negatively with the estimated glomerular filtration rate. In addition, an elevated serum SSAO level is associated with the presence of early CKD (stages 2 and 3) after adjustment for age, sex, and smoking (41).

There are several explanations for an increased serum SSAO level in CKD:

1. In CKD, the accumulation of creatinine and creatine may lead to increased formation of methylamine via sarcosine and conversion by the gut flora (99). This is consistent with a study (3) showing elevated blood methylamine levels in uremic patients to be \( \sim \text{20-fold} \) higher than in the control population. Furthermore, in CKD, there is retention of a large number of small solutes and impaired urinary excretion of methylamine (100).

2. Methylamine undergoes uncontrolled deamination (98) and initiates endothelial injury. This raises toxic aldehyde levels in the blood, enhances oxidative stress, and perpetuates vascular injury and inflammation in blood vessels. Damage to the vascular system results in SSAO leakage, and this creates a vicious cytotoxic cycle contributing toward microangiopathy. Yu et al. (104) found that chronic administration of methylamine enhanced blood prorenin levels, which reflects the activation of the renin-angiotensin system and microvascular damage in the kidney.

3. CKD, particularly at more advanced stages, is associated with impaired immunity and subclinical inflammation involving cytokines derived from adipose tissue (adipocytokines). Increased levels of inflammatory molecules, such as elevated serum C-reactive protein and IL-6 molecules, have shown an inverse correlation with creatinine clearance.

4. Elevated levels of SSAO activity in hemodialysed patients may contribute to the accelerated atherosclerosis observed in uremia through the generation of toxic end products. In contrast, patients on peritoneal dialysis have slightly decreased SSAO activity, which can be explained by the better preserved residual renal function seen in patients on peritoneal dialysis than in patients receiving hemodialysis (73).

In transgenic mice overexpressing SSAO, Stolen et al. (90) found increased glomerulosclerosis. Considering the lack of glomerular VAP-1, this glomerulosclerosis is most likely due to VAP-1-induced hypertension and its proatherogenic effects. Transgenic but not wild-type mice fed with atherogenic diets developed sporoic glomerular cysts, which may signal a disruption of tightly regulated glomerular homeostasis of endothelial and mesangial cells (24, 80). Kidneys of patients with active Wegener’s granulomatosis displayed strong expression of VAP-1 in the peritubular and periglomerular microvasculature. They also had higher numbers of circulating endothelial cells positive for VAP-1 compared with patients in remission. These circulating endothelial cells have a proinflammatory phenotype and impair the capacity of endothelial progenitor cells to repair the endothelium. Their numbers correlate with the inflammatory marker C-reactive protein as well as with the degree of organ damage. After in vitro stimulation with anti-endothelial cell auto-antibodies from patients with Wegener’s granulomatosis, human renal microvascular endothelial cells acquired a proinflammatory phenotype with upregulation of the expression of chemokines and VAP-1. While the proinflammatory effects of anti-endothelial antibodies may be blocked by specific inhibitors of the signaling cascade (such as SAPK/JNK), VAP-1 overexpression cannot (32).

**SSAO in Diabetic Kidney Disease**

Emerging evidence now suggests that inflammatory pathways have a central role in the development of DM as well its complications, including diabetic nephropathy. The relationship of circulating SSAO levels in DM has been well studied, with the common finding that levels are increased (12, 27, 66). Serum SSAO activity is 50% higher in diabetic animals (11, 66, 74, 93) than in control animals (22, 30) and are even more so when microvascular complications of diabetes are present (11, 27). This has been observed in patients with type 1 DM (11) and type 2 DM (27). Boomsma et al. (13) reported high SSAO activity in patients with DM at first clinical diagnosis and preceding the appearance of vascular complications. Taken together, these observations suggest that SSAO inhibition may delay the development of microvascular complications in patients with DM (20, 103).

At low levels, the production of \( \text{H}_2\text{O}_2 \) causes the recruitment of glucose transporters 1 and 4 to the cell surface in an insulin-mimicking way, which accommodates the increased glucose uptake in these cells (21, 56, 68). In this way, SSAO-mediated formation of \( \text{H}_2\text{O}_2 \) has the ability to promote the uptake of glucose into the cells. However, at high levels, SSAO activity can also cause detrimental effects that may potentiate the later complications of diabetes (102). The hyperglycemia seen in patients with DM in conjunction with VAP-1-derived aldehydes can lead to accelerated AGE formation. Li et al. (50) showed that SSAO levels correlated with systemic oxidative stress and AGE production (49). The aldehyde product of methylamine metabolism, formaldehyde, is a potent angiotoxin that can form irreversible adducts by reacting with lysine residues of proteins. Such protein modification is likely to disturb their function (101). Additionally, \( \text{H}_2\text{O}_2 \) generated in SSAO-catalyzed deamination can cross-link vascular proteins and bind various amines (29). Methylglyoxal, which is increased two- to fourfold in the blood and kidneys of diabetics (62), also forms protein adducts, such as with albumin (52), and has been implicated in the pathogenesis of diabetic nephropathy (63, 70, 92). Since AGEs are irreversibly bound to macromolecules, they accumulate in diabetic tissues (55), which cause an overproduction of matrix components as well as an abnormal adhesion of immune cells to the endothelium. Furthermore, the leukocyte-adhesive properties of endothelial...
SSAO worsen the endothelial cell dysfunction, which is recognized as the common initial pathophysiological change in the development of diabetic microangiopathy (87). It is further conceivable that the determination of the enzyme activity in patients with DM may provide a simple and sensitive method for the identification of those who will develop microvascular complications. Proving this hypothesis will require the interrogation of large data sets with defined clinical end points.

**SSAO in the Transplanted Kidney**

Kidney allotransplantation is increasingly being performed as a treatment for end-stage renal failure. Acute and chronic rejection are important complications that may occur after transplantation and are major causes for graft loss. Increased levels of SSAO in patients with end-stage renal disease normalize after transplantation. This demonstrates that VAP-1 potentially contributes to lymphocyte homing into an organ undergoing rejection. The most characteristic histological manifestations of chronic rejection are perivascular inflammation and generalized allograft arteriosclerosis. VAP-1 is highly expressed in the peritubular capillary endothelium of chronically rejected kidney allografts, and lymphocytes adhere to the graft vessels in a VAP-1-dependent manner (44).

**SSAO Inhibitors and CKD**

Several lines of evidence suggest that SSAO is an attractive therapeutic target. As mentioned above, SSAO is upregulated in inflamed vessels, where it mediates the adhesion and transmigration of leukocytes. Therefore, drugs that target SSAO should have preferential effects at sites of inflammation. Additionally, SSAO is an important contributor to leukocyte transport across vascular barriers. Finally, SSAO is readily amenable to an orally administered small-molecule approach. Because the adhesive function of SSAO is intimately related to the enzymatic function, small orally active drug-like molecules can readily inhibit both activities. In summary, nonclinical efficacy data from a variety of disease models suggest that SSAO-based antiadhesion therapy may have clinical utility in a wide spectrum of diseases where inflammation contributes to disease pathology.

The aforementioned studies verify that SSAO is involved in a number of pathophysiological processes, and, as such, SSAO inhibitors may be useful as potential targets for inflammatory and vascular pathways that are upregulated in CKD. The inhibitors described during the last decade represent various structural scaffold-hydradine derivatives, aryalkylamines, propenyl- and propargylamines, oxazolidinones, haloalkylamines, 1,3,4-oxadiazines, 4,5,6,7-tetrahydroimidazo[4,5-c]pyridines, carbox(thi)amides, sulfonamides, and thiazole derivatives (18, 19, 58, 59). Although all these structures are highly hydrophobic small aryl-alkylamine molecules, all of them are potent irreversible inhibitors that interact with the topaquinone cofactor through a covalent bond. Despite the substantial amount of promising pharmacological data with SSAO inhibitors, no end-point trials have been conducted.

Several experiments have shown that SSAO-mediated adhesion and transendothelial migration of leukocytes can be blocked by small-molecule inhibitors (84). BIOTIE, in collaboration with the National Institute for Health Research Liver Biomedical Research Unit of the University of Birmingham, found that in CCl4 mouse models of liver fibrosis, deletion of SSAO and blockade of SSAO using antibody strategies mitigate the fibrotic response (2, 6).

Several compounds known to inhibit SSAO activity have been demonstrated to exert nephroprotective effects (10). However, the specificity of the mechanism of action remains open to question. In general, they were developed as MAO inhibitors and exhibit cross-reactivity with other amine oxidases (58). Few inhibitors have undergone extensive selectivity analyses against SSAO compared with MAO-A and MAO-B (57, 77). Hydralazine, an anti-hypertensive, is known to reduce SSAO activity but may also protect the kidney via a decrease in blood pressure. However, at therapeutic doses, it does not seem to have anti-inflammatory properties (95). Aminoguanidine, a diamine oxidase and nitric oxide synthase inhibitor, exerts its nephroprotective properties by decreasing methylglyoxal concentration and methylglyoxal-induced formation of AGEs. It decreases superoxide formation by suppressing the receptor for AGE activation by interacting with 3-deoxyglucosone, especially in the kidney, where aminoguanidine concentrations are highest. However, its clinical development was halted due to major side effects, including gastrointestinal disturbances, abnormalities in liver function tests, and a rare vasculitis (25), as well as an apparent lack of efficacy (105). Hence, the extent to which inhibition of SSAO is responsible for nephroprotection of these compounds is still largely unknown. No obvious clinical candidates have emerged, although work has been published and a number of patent applications have appeared. There is currently a clinical trial recruiting participants exploring the relationship between VAP-1 and diabetic cardiovascular autonomic neuropathy (16), which will provide useful information regarding its role in diabetic microangiopathy.

Another strategy for SSAO inhibition is based on the observation that VAP-1 function can be blocked with monoclonal antibodies. However, none of these trials were designed with renal end points. In vitro and in vivo experiments performed with chimeric mouse-human antibodies demonstrated that monoclonal antibodies blocked sites used by VAP-1 to promote leukocyte transmigration in humans, without leading to side effects caused by immunogenicity or activation of effector functions. The original mouse anti-human VAP-1 antibody has entered phase I clinical trials and showed no adverse effects (94). Chimeric (39) and fully humanized anti-VAP-1 antibodies have subsequently been developed. A fully human monoclonal antibody that specifically binds to VAP-1 (BTT-1023) developed by BIOTIE is advancing to phase II–III studies for psoriasis and possibly rheumatoid arthritis (6). The benefit of these antibodies in patients with renal disease is, to date, unexplored.

**Summary**

The function of SSAO in the kidney in health and disease is only starting to be elucidated. SSAO is closely linked to processes that are crucial in the development and progression of kidney disease, such as hypertension, inflammation, and endothelial dysfunction. SSAO enzyme inhibitors have shown efficacy in several animal models of inflammation and progressive fibrotic disease. However, to date, relatively few compounds have been discovered with specific substrate, pharmacokinetic, and dynamic properties. The use of monoclonal...
antibodies targeting VAP-1 may offer a feasible novel therapy but requires appropriate interventional studies.

GRANTS

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F1644 SSAO AND KIDNEY DISEASE

Review


