15 Pharmacological Applications of Copper Amine Oxidases

Emanuela Masini and Laura Raimondi

CONTENTS

15.1 PHARMACOLOGICAL APPLICATIONS

15.1.1 IMPLICATIONS OF CONTROLLING BZAO ACTIVITY IN DIABETES: THE PRESENT

The significance of increased plasma levels of BzAO occurring in diabetes patien is far from clear. Increased plasma BzAO levels are correlated with glycated hem globin (Boomsma et al., 1995 and 2005a; Xu et al., 2005) Moreover circulati BzAO has also been proposed as an independent prognostic marker for mortality

heart failure (Boomsma et al., 2000b), suggesting a relationship of plasma enzyme activity, the duration of diabetes, and its cardiovascular complications.

It is currently accepted that most cardiovascular pathologies have a common base in endothelial dysfunction characterized by metabolic insulin resistance and oxidative stress and impaired flow-mediated vasodilatation aspects. Plasma BzAO may be indicative of the metabolic aspects of endothelial dysfunction and, more generally, of changes in glucose homeostasis. Because subclinical, asymptomatic, endothelial dysfunction is hard to detect, an understanding of BzAO timing in the circulation is crucial to investigating its prognostic value. Whether drug therapies (e.g., statins and renin–angiotensin system blockers) for improving endothelial dysfunction exert beneficial effects by controlling (reducing?) BzAO plasma activity is still unknown. Another unknown factor is whether reduced plasma BzAO levels are among the beneficial effects of these drugs or whether strategies to reduce the onset of type 2 diabetes in at-risk populations may also prevent increases in plasma levels of BzAO.

If BzAO plasma levels serve as markers of diabetes, they may also increase in patients treated with diabetogenic drugs such as β blockers, diuretic thiazides, and corticosteroids. Again, epidemiological data are lacking.

Different perspectives may be predicted for tissue-bound SSAO highly expressed in insulin sensitive cell types. In adipocytes, membrane-bound SSAO dependent substrate deamination via local production of hydrogen peroxide (H_2O_2) has the potential to exert insulin-like effects, even in the absence of insulin, on glucose uptake (Zorzano et al., 2005a), adipose differentiation, and lipolysis (Carpéné et al., 2001). However, even if the production of H_2O_2 is not a peculiarity of membrane-bound SSAO (many other enzyme activities can produce H_2O_2, including DAO), the particular localization of the peroxide produced by membrane bound SSAO seems to facilitate insulin receptor substrate 1 phosphorylation (Zorzano et al., 2005a), thus exerting local beneficial insulin sensitizing effects.

On the other hand, membrane-bound SSAO activity spreads toxic aldehydes in the microenvironment that may initiate a deleterious cycle involving protein and DNA cross linkage related to angiotoxicity (Yu and Zuo, 1997), a typical consequence of hyperglycemia and an index of diabetes complications. However, as a whole, the benefit of reducing membrane bound SSAO activity prevails over the local insulin like effects of the peroxide produced (Stolen et al., 2004a). These authors demonstrated a more favorable role of membrane bound SSAO inhibitors over substrates in reducing the severity of diabetes-related cardiovascular complications with atherosclerotic bases. In particular, in adipocytes and vascular smooth muscle cells, SSAO substrates generate a favorable microenvironment to restore local insulin resistance but remain neutral related to the control of plasma glycemia. Conversely, SSAO inhibitors protect against angiotoxicity. The addition of aminoguanidine (pimagedine), a guanidine like compound, to common antidiabetic treatments, has been used for several years to alleviate the protein aging associated with diabetes (Abdel Rahman and Bolton, 2002; Hou et al., 2998; Yu and Zuo, 1997; Friedman, 1995; Cameron and Cotter, 1995) and the extent of glycated hemoglobin (Yu and Zuo, 2003). Aminoguanidine treatment has a neutral effect on glycemia, even though inhibition of SSAO activity implies increasing tissue levels of enzyme

lationship of plasma enzyme
r complications.
pathologies have a common
metabolic insulin resistance
ation aspects. Plasma BzAO
helial dysfunction and, more
e subclinical, asymptomatic,
ning of BzAO timing in the
thus. Whether drug therapies
by improving endothelial dys
ing?) BzAO plasma activity
reduced plasma BzAO levels
ether strategies to reduce the
o prevent increases in plasma

es, they may also increase in
ockers, diuretic thiazides, and
ge.
ound SSAO highly expressed
ane bound SSAO dependent
gen peroxide (H_2O_2) has the
sence of insulin on glucose
and lipolysis (Carpéné et al.,
e a pecularity of membrane-
ase H_2O_2 including DAO) the
embrane bound SSAO seems
on (Zorzano et al. 2003), thus

ly spreads toxic aldehydes in
s cycle involving protein and
Zuo, 1997), a typical conse
complications. However, as a
AO activity prevails over the
Stolen et al., 2004a). These
rane bound SSAO inhibitors
ated cardiovascular complica-
rocytes and vascular smooth
microenvironment to remove
he control of plasma glycemia.
xicity. The addition of amino
to common antidiabetic treat
protein aging associated with
al., 1998; Yu and Zuo, 1997;
extent of glycated hemoglobin
a neutral effect on glycemia.
easing tissue levels of enzyme

substrates. Aminoguanidine is not selective for SSAO but inhibits the DAO and the NO synthases (Ansar and Ansari, 2006).

15.1.2 Future Diabetes Treatments

Pharmacological treatment of insulin resistance represents a first line approach to reduce diabetes related complications. In the complex picture of future insulin-sensitizing agents, the antidiabetic activity of vanadium compounds has recently garnered immense interest as an oral therapy. Vanadium compounds lowered glycemia and normalized plasma lipid profiles in an animal model of diabetes (Yamazaki et al., 2005; Kordowiak et al., 2005; Mukherjee et al., 2004; Abedin et al., 2003), exerting insulin-like effects downstream of the insulin receptor and probably inhibiting phosphatase activities. Concerns about their safety profiles presently limit clinical applications.

In recent years, major efforts have focused on developing new chemical formulations to minimize the side effects of metal drugs including vanadium esters. A novel combination of amine substrates for membrane-bound SSAO and low concentrations of vanadium esters has been proposed to increase the production of peroxovanadate, the active insulin-mimetic compound of pharmacological interest (Yraola et al., 2004a).

15.1.3 SSAO Activity and Neurodegenerative Diseases: Evidence for Future Pharmacological Implications?

Cerebral amyloid angiopathy characterized by the deposition of β amyloid in brain vessels, inducing the degeneration of vascular smooth muscle and endothelial cells, is considered a crucial event in Alzheimer's disease (AD) pathogenesis. AD patients exhibit increased membrane bound SSAO levels and enzyme activities in their brains are co-localized with β-amyloid protein (Jarrer et al., 2002).

Increased plasma levels of BzAO in AD patients may indicate insulin resistance, and membrane-bound SSAO inhibitors may trigger β amyloid peptide polymerization (Munch et al., 1997) due to increased production of formaldehyde, methylglyoxal, and malonyldialdehyde from endogenous membrane bound SSAO substrate degradation. Membrane bound SSAO activity may act as an initiating factor for protein fibrillation, a typical manifestation of this disease. Therefore, inhibition of membrane bound SSAO may prevent local increases of aldehydes, thus reducing the pro-inflammatory potential of such compounds. If that hypothesis is correct, AD therapy including inhibitors of membrane bound SSAO may become a primary prevention strategy.

If the aldehydes play a role in generating protein cross linkage, diabetic patients may be at risk for AD. The clinical relationship between Type 2 diabetes mellitus and AD has been debated for over a decade. Several studies have failed to show a clear clinical correlation; others have demonstrated that Type 2 diabetes is an independent risk factor for inflammatory-based neurodegenerative diseases including AD (Beeri et al., 2005; Watson and Craft, 2006).

Increasing evidence suggests that insulin contributes to normal brain functioning and that peripheral insulin abnormalities increase risks of memory loss and

neurodegenerative disorders such as AD. Potential mechanisms of these effects include the role of insulin in cerebral glucose metabolism, peptide regulation, modulation of neurotransmitter levels and other aspects of the inflammatory network. AD patients are not routinely evaluated for Type 2 diabetes or hyperinsulinemia.

Current AD treatments produce modest benefits, and several drugs that target metabolic and inflammatory pathways are being evaluated, most notably the statins that reduce low density lipoproteins and inflammation but may not influence amyloid deposition—an important precursor of AD. Although some evidence supports a potentially important role for peroxisome-proliferative activated receptor agonists such as glitazones (Craft, 2007), no current reports cover randomized clinical trials in AD patients of drugs that target insulin or insulin resistance (Williamson et al., 2007). Such patients may benefit from treatment with statins or antidiabetic drugs that target the insulin cascade involved in glucose homeostasis. No data currently indicate that statins or other therapies including amine oxidase inhibitors show potential in ameliorating the severity of AD. On the other hand, no experimental or clinical data demonstrate that inhibition of tissue or plasma SSAO is included in the pharmacological profiles of cholinomimetic or anti-glutamatergic drugs used to treat AD. This point must be considered in assigning a precise role for SSAO in AD pathogenesis and care.

15.1.4 Antiinflammatory Activity of Molecule Inhibiting VAP-1/SSAO Activity

BzAO activity is essential in VAP-1 to mediate leukocyte adhesion to endothelial cells (Yegutkin et al., 2004). Inhibition of SSAO activity reduces the production of reactive and toxic compounds in the endothelium (Yu and Zuo, 1996). In theory, specific and selective inhibitors of VAP-1/SSAO have the potential to reduce tissue damage by reducing accumulation of reactive oxygen species including hydrogen peroxide and toxic aldehydes. In addition, VAP-1/SSAO inhibitors may prevent tissue leukocyte infiltration, an initiating factor for immuno-mediated diseases including Type 1 diabetes.

Whether SSAO plays a role in the preferential entry of leukocytes into an organ or tissue remains unknown. However, based on their possible double role, VAP-1/SSAO inhibitors have been designed, synthesized (Wang et al., 2006), proposed as anti-inflammatory drugs (Salter-Cid et al., 2005), and included in the LJP series. LJP 1586 is a potent amine-based inhibitor for VAP-1/SSAO with good oral bioavailability and therapeutic window. Interestingly, after LJP 1586 supplementation in animals, the enzyme activity recovery time was approximately 72 hr. The pharmacokinetic activity of this compound thus predicts low turnover of enzyme at the cell surface and/or slow reversibility of the interaction with the enzyme (O'Rourke et al., 2008).

From the first study, LJP 1586 has shown benefits as an anti-inflammatory treatment in acute and chronic pulmonary diseases—above and beyond its effects on leukocyte migration and possibly including a favorable impact on fibrosis secondary to local formaldehyde production (O'Rourke et al., 2008). Other SSAO inhibitors of the LJP series have been tested. LJP 1207 has shown potential in a mouse model

of relapsing–remitting expe
shares many characteristics
Animal treatment with LJP
migration across pial vessels
nificantly improved neurolog
given chronic estrogen repla
postischemic inflammation (X
administration of LJP 1207 s
colonic cytokine levels and rev
injury, and ulceration scores (S
also represent a novel strategy
degeneration, and diabetes reti

15.1.5 Assessment of Physi Bound SSAO Endo Enzyme as Strategy

Endogenous substrates for mem
aminoacetone (AA), and β-pher
trace amine precursor of neurotr
abuse (Gass and Olive, 2008), bu
stood. Substrate degradation by
secondary product. Although mar
gen peroxide, the consequences of
ammonia are largely unknown.

Recent evidence suggests that
molecules endowed with signaling
fies feeding behavior, producing s
amine-like effects. Interestingly,
effects, suggesting a common mech
in those of MET.

Although, MET and ammonia pr
MET directly delivered to the cen
injection (i.c.v.) induced different effi
2007). Moreover, MET reduced feed
suggesting that it can freely cross t
inflammation.

MET can therefore be included in
trolling the hypothalamus—a site v
membrane-bound SSAO actively cont
In conditions of SSAO inhibition by a
phagia was produced in both healthy
et al., 2006). MET would have exerted
bound SSAO. In fact, MET hypophag
deamination by SSAO that represents
suggest that MET may be of use in reso

ial mechanisms of these effects
polism, peptide regulation, modu-
of the inflammatory network. AD
etes or hyperinsulinemia.
ts, and several drugs that target
valuated, most notably the statins
tion but may not influence amy-
lthough some evidence supports
rative activated receptor agonists
cover randomized clinical trials
in resistance (Williamson et al.,
vith statins or antidiabetic drugs
homeostasis. No data currently
amine oxidase inhibitors show
he other hand, no experimental
or plasma SSAO is included in
nti-glutamatergic drugs used to
g a precise role for SSAO in AD

LE

kocyte adhesion to endothelial
tivity reduces the production of
Yu and Zuo, 1996). In theory,
e the potential to reduce tissue
en species including hydrogen
AO inhibitors may prevent tis-
nuno-mediated diseases includ-

ry of leukocytes into an organ
r possible double role, VAP-1/
Vang et al., 2006), proposed as
nd included in the LJP series.
P-1/SSAO with good oral bio-
ter LJP 1586 supplementation
pproximately 72 hr. The phar-
low turnover of enzyme at the
n with the enzyme (O'Rourke

as an anti-inflammatory treat-
ove and beyond its effects on
e impact on fibrosis secondary
08). Other SSAO inhibitors of
n potential in a mouse model

of relapsing–remitting experimental autoimmune encephalomyelitis, a model that shares many characteristics with human multiple sclerosis (O'Rourke et al., 2007). Animal treatment with LJP 1017 led to a dramatic reduction in adhesion and transmigration across pial vessels of leukocytes, predominantly neutrophils, and also significantly improved neurological outcomes in diabetic ovariectomized female rats given chronic estrogen replacement therapy—a model associated with increased postischemic inflammation (Xu et al., 2005). In a mouse model of ulcerative colitis, administration of LJP 1207 significantly reduced mortality, body weight loss, and colonic cytokine levels and revealed highly significant suppressions of inflammation, injury, and ulceration scores (Salter-Cid et al., 2005). Inhibition of VAP-1/SSAO may also represent a novel strategy for reducing ocular inflammation of uveitis, macular degeneration, and diabetes retinopathy (Noda et al., 2008).

15.1.5 Assessment of Physiopharmacological Role of Membrane-Bound SSAO Endogenous Substrates: Inhibition of Enzyme as Strategy to Increase Substrate Levels

Endogenous substrates for membrane-bound SSAO include methylamine (MET), aminoacetone (AA), and β-phenylethylamine (β-PEA). The latter is considered a trace amine precursor of neurotransmitters involved in neuronal plasticity and drug abuse (Gass and Olive, 2008), but the significance of MET and AA is poorly understood. Substrate degradation by membrane-bound SSAO produces ammonia as a secondary product. Although many reports cover the effects of aldehydes and hydrogen peroxide, the consequences of membrane-bound SSAO-dependent production of ammonia are largely unknown.

Recent evidence suggests that MET may belong to a series of small endogenous molecules endowed with signaling features. MET supplementation in rodents modifies feeding behavior, producing species-specific effects without eliciting amphetamine-like effects. Interestingly, ammonia supplementation produced similar effects, suggesting a common mechanism of action and inclusion of ammonia effects in those of MET.

Although, MET and ammonia produce hypophagia in mice (Pirisino et al., 2004), MET directly delivered to the central nervous system by intracerebroventricular injection (i.c.v.) induced different effects in rats, depending on dose (Raimondi et al., 2007). Moreover, MET reduced feeding when administered intraperitoneally (i.p.), suggesting that it can freely cross the blood–brain barrier even in the absence of inflammation.

MET can therefore be included in the plethora of endogenous compounds controlling the hypothalamus—a site virtually devoid of SSAO activity. Peripheral membrane-bound SSAO actively controls MET levels that reach the hypothalamus. In conditions of SSAO inhibition by aminoguanadine, a potentiation of MET hypophagia was produced in both healthy and diabetic mice (Pirisino et al., 2001; Cioni et al., 2006). MET would have exerted its own effects independently of membrane-bound SSAO. In fact, MET hypophagia is potentiated by inhibiting the oxidative deamination by SSAO that represents main MET metabolic pathway. These results suggest that MET may be of use in resolving diabetes hyperphagia.

Alimentary disorders are usually included in the clinical aspects of patients suffering neurodegenerative pathologies including AD and senile dementia (Mamhidir et al., 2007; Tamura et al., 2007). Thus, inhibition of membrane-bound SSAO, which is increased in the brain vessels of such patients, would reduce oxidative and carbonyl stress in the central nervous system and help correct alimentary disturbances regulating MET levels. Unfortunately, no information to date indicates the effects of endogenous levels of MET in physiological and pathological conditions.

MET effects in the hypothalamus are linked to the expression of a particular type of voltage-dependent potassium channel of the Shaker-like family known as Kv1.6. This channel is not involved in the hypophagic effects of ammonia. The abilities of ammonia and derivatives to interact with potassium channels are well known (Choi et al., 1993). The identification of Kv1.6 as a possible target for MET effects presents new perspectives. Interacting in these channels in the hypothalamus, MET modulates the releases of NO and dopamine, two key mediators of animal feeding. These and other observations (Carpéné et al., 2007; Prévot et al., 2007) suggest that membrane-bound SSAO inhibitors may be effective as anti-obesity drugs because of their effects on the hypothalamus and also resulting from SSAO localization.

Preferential expression at adipocytes (Raimondi et al., 1991) guarantees a link between enzyme expression and adipose differentiation (Raimondi et al., 1990). Oxidative deamination of SSAO substrates producing hydrogen peroxide in adipocytes induces and sustains adipose differentiation in rodents (Carpéné et al., 2001) and humans (Bour et al., 2007a, b). Again, the double face of this enzyme suggests a crucial role for its ability to regulate factors involved in energy intake and storage.

15.1.6 Drugs Interacting with Plasma BzAO Activity

BzAO, membrane-bound SSAO, and the mitochondrial MAOs may also represent nonmicrosomial phase I enzymes involved in drug metabolism. Although several drugs have shown capacities to inhibit BzAO plasma activity, whether this feature is clinically relevant is still unknown. BzAO inhibition may, however, explain certain drug-related side effects. Two classes can be identified: (1) drugs bearing aminoguanidine-like moieties, and (2) drugs bearing BzAO or SSAO substrate-like moieties.

Aminoguanadine belongs to the first class. Clearly, BzAO and membrane-bound SSAO inhibitions are integral parts of aminoguanidine therapeutic activity. In addition to aminoguanadine, other drugs such as benserazide, a decarboxylase inhibitor used in anti-Parkinson therapy, inhibits plasma BzAO activity. Patients suffering from Parkinson's disease and treated with benserazide exhibited lower plasma BzAO activity than controls. It is possible that side effects following long-term therapy may include modification of circulating levels of the amine substrate for BzAO (Coelho et al., 1985). Whether benserazide therapy reduces oxidative stress of patients or ameliorates their alimentary disorders in consequence of plasma BzAO inhibition is not known.

Phenelzine, a nonselective and irreversible mitochondrial inhibitor of MAO-A and -B, has been used for many years as an antidepressant to treat panic disorders

n the clinical aspects of patients suf-
AD and senile dementia (Mamhidir
on of membrane-bound SSAO, which
nts, would reduce oxidative and car-
help correct alimentary disturbances
mation to date indicates the effects of
d pathological conditions.
l to the expression of a particular type
Shaker-like family known as Kv1.6.
gic effects of ammonia. The abilities
potassium channels are well known
as a possible target for MET effects
channels in the hypothalamus, MET
wo key mediators of animal feeding.
2007; Prévot et al., 2007) suggest that
ctive as anti-obesity drugs because of
lting from SSAO localization.
mondi et al., 1991) guarantees a link
ferentiation (Raimondi et al., 1990).
producing hydrogen peroxide in adi-
entiation in rodents (Carpéné et al.,
gain, the double face of this enzyme
ate factors involved in energy intake

BzAO Activity

ochondrial MAOs may also represent
drug metabolism. Although several
O plasma activity, whether this fea-
zAO inhibition may, however, explain
can be identified: (1) drugs bearing
bearing BzAO or SSAO substrate-like

Clearly, BzAO and membrane-bound
uanidine therapeutic activity. In addi-
benserazide, a decarboxylase inhibitor
ma BzAO activity. Patients suffering
serazide exhibited lower plasma BzAO
fects following long-term therapy may
he amine substrate for BzAO (Coelho
educes oxidative stress of patients or
sequence of plasma BzAO inhibition

e mitochondrial inhibitor of MAO-A
ntidepressant to treat panic disorders

and social anxiety. Its efficacy is the result of MAO inhibition leading to increased sympathetic amine levels at the synaptic cleft and γ-aminobutyric (GABA) acid transaminase inhibition that markedly increases GABA brain levels. Phenelzine also exerts inhibitory activity on BzAO and tissue-bound SSAO. This feature has a role in its neuronal protective effects.

Hydralazine is a guanidine-like drug used in antihypertensive therapy, although its use has been discouraged based on the availability of more selective drugs. Hydralazine is also a potent relaxant of smooth muscle cells; the mechanism of action remained unknown. Hydralazine is a potent and irreversible inhibitor of BzAO and tissue-bound SSAO activities. Recent studies in aortic rings of rats indicate that BzAO and SSAO substrates such as benzylamine, phenylethylamine, and methylamine, by producing H_2O_2, magnify the vasodilation activity of hydralazine. It is speculated that this mechanism may be novel for hydralazine-dependent vasodilation (Vidrio and Medina, 2007).

Isoniazid is a hydrazine derivative used in antitubercular therapy. Because of its structure, isoniazid is an inhibitor of copper-containing amine oxidases (CAOs), including diamine oxidases (DAOs) and membrane-bound semicarbazide-sensitive amine oxidases (SSAOs). No current evidence indicates that inhibition of these enzyme activities is involved in isoniazid's antimicrobial activity. Instead, histamine intoxication after ingestion of histamine-rich foods has been described in isoniazid-treated patients (Uragoda and Lodha, 1979; Uragoda and Kottegoda, 1977). These adverse drug effects result from histamine accumulation, a condition that reduces histamine catabolism.

15.1.7 Diamine Oxidase

15.1.7.1 Role of Diamine Oxidase in Anaphylaxis

Histamine plays a fundamental role in anaphylaxis and is involved in allergic and pseudoallergic reactions. At variance with other metabolic pathways, histaminase activity is not directly upregulated by endogenously released histamine. Plasma histaminase activity increases in anaphylactic shock, but not during histamine injection. In some cases, plasma levels of histaminase may be intrinsically low and its activity further decreased by exogenous histamine, thereby predisposing to anaphylactic reactions.

Enhanced histamine levels in humans may be related to various endogenous and/or exogenous factors. Food-induced histaminosis has been described as the result of high histamine content or histamine releasers in food (Sattler et al., 1989). The first symptom of excess histamine intake and/or release is an increase in gastric secretion followed by tachycardia, headache, and hypotension (Slorach, 1991). The largest amounts of histamine and tyramine (that have similar vasoactive properties) are found in fermented foods such as cheeses, red wines, tinned fish including tuna, fish sauces, sauerkraut, cured pork, and sausages. The histamine content of French cheeses can reach values > 800 μg/g and can cause toxic symptoms (Taylor, 1986). High levels of histamine have also been detected in Oriental food, accounting for the so-called Chinese restaurant syndrome (Chin et al., 1989).