

OSTA BIOTECHNOLOGIES INC.

DIAGNOSTIC TEST FOR ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a dementing illness characterized by progressive neuronal degeneration, gliosis, and the accumulation of intracellular inclusions (neurofibrillary tangles) and extracellular deposits of amyloid (senile plaques) in discrete regions of the basal forebrain, hippocampus, and association cortices. Although the precise mechanisms responsible for neuronal demise in AD remain largely unknown, there exists a substantial body of evidence implicating oxidative stress (free radical injury) and mitochondrial insufficiency (bioenergetic failure) in the pathogenesis of this condition. These pathological features may, in turn, be related to the aberrant deposition of redox-active iron, an important generator of damaging reactive oxygen species (ROS), in the affected brain tissues. In the AD cerebral cortex and hippocampus, increased deposition of non-heme iron occurs in neurofibrillary tangle (NFT)-bearing neurons, astrocytes, microglia, and in the vicinity of senile plaques.

Osta's technology is based on a key protein called Heme Oxygenase-1 (HO-1). The presence of a plasma HO-1 suppressor (HOS) activity has been shown for the first time to distinguish AD patients from normal young controls (NYC), normal elderly controls (NEC), people with mild cognitive impairment (MCI) and people suffering from Parkinson's Disease (PD). In a clinical study conducted at the JGH involving a total of 82 subjects, the % HOS activity was found to be 9% in the plasma of NYC subjects, 13% in the plasma of NEC subjects, 38% in the plasma of MCI subjects, 72% in the plasma of AD patients ($P < 0.001$ relative to NEC, $P < 0.001$ relative to MCI) and 29% in the plasma of PD patients. Our results to date indicate that measurement of plasma HOS activity may provide an exciting new biological marker for the evaluation of patients with dementing diseases and form the basis of a novel and revolutionary blood test for the accurate diagnosis/prognosis of sporadic (non-familial) AD.

HO-1 is a highly-inducible 32 kDa member of the stress protein superfamily that catalyzes the oxidative degradation of heme to biliverdin in brain and other tissues. The regulatory region of the mammalian *ho-1* gene is comprised of a 500bp promoter, a proximal enhancer and two or more distal enhancers. These regulatory regions contain heat shock consensus (HSE) sequences, metal (MtRE, CdRE) and antioxidant (ARE) response elements and AP-1, AP-2, nuclear factor kappa B (NF κ B) and HIF-1 binding sites. These response elements render the *ho-1* gene exquisitely sensitive to up-regulation by heme, dopamine, β -amyloid, H_2O_2 and other oxidants, UV light, transition metals, pro-inflammatory cytokines, lipopolysaccharide and prostaglandins.

Using immunolabeling techniques, our researchers demonstrated marked over-expression of immunoreactive HO-1 in neurons and astrocytes of the hippocampus and temporal cortex of AD brain relative to control specimens matched for age and post-mortem interval. Furthermore, our researchers and others reported co-localization of the HO-1 protein to neurofibrillary tangles, senile plaques and corpora amylacea in the AD specimens. In response to oxidative stress, induction of HO-1 may protect cells by promoting the catabolism of pro-oxidant metalloporphyrins, such as heme, to bile pigments (biliverdin, bilirubin) with free radical scavenging capabilities. These and other data from our researchers indicate that free iron and carbon monoxide generated from HO-1-mediated heme catabolism in astrocytes may contribute to the abnormal patterns of brain iron deposition, intracellular oxidative stress and mitochondrial insufficiency amply documented in AD brain.

Contrary to our observations in brain parenchyma, we found that plasma HO-1 protein concentrations are significantly *suppressed* in subjects with probable early sporadic AD compared to normal elderly controls (NEC) and individuals with various neurological and medical disorder. We also observed that HO-1 protein levels are lower in post-mortem specimens of CSF and choroid plexus epithelium derived from pathologically-proven cases of AD relative to neurohistologically-normal controls. The suppression of the HO-1 activity in AD plasma compared to controls and other neurodegenerative conditions forms the basis of our company's diagnostic blood test for AD.