Decreased Heme Oxygenase Activity in Patients with Alzheimer’s Disease

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Abstract

Alzheimer’s disease is a neurodegenerative disorder characterized with progressive impairment of cognitive functions. Heme oxygenase is an enzyme that degrades the heme molecule resulting in equimolar amounts of the carbon monoxide, ferrous iron, and biliverdin. Up to now, heme oxygenase activity and its metabolic effects in Alzheimer’s disease have been investigated in so many studies; most of them were performed in post-mortem brain tissues of Alzheimer’s disease patients or in animal models. Therefore, we aimed to investigate heme oxygenase activity in leukocytes of Alzheimer’s disease patients as a peripheral sample. Mean heme oxygenase activity was significantly lower in patients with Alzheimer’s disease (0.53 ± 0.32 nmol/h/mg protein) compared to controls (1.19 ± 0.84 nmol/h/mg protein) (p= 0.001). We think that reduction in leukocyte heme oxygenase activity may limit disease progression through preserving peripheral mitochondrial function by reducing the formation of free iron and carbon monoxide.

Introduction

The term ‘heme’ refers a porphyrin ring which makes up chelate with certain metal ions such as Fe, Mg, Mn, Zn, Sn, Cd, Co, Cu, Cr, Ag, so that a molecule having important biological activities for the continuity of life. Heme molecule can be degraded through different enzymatic and chemical mechanisms. In mammals, the most effective way to enzymatic degradation of heme is heme oxygenase (HO) system. Until now, three isofoms of heme oxygenase have been identified as the inducible HO-1, the constitutive HO-2, and HO-3 (1). HO-1 and HO-2 are catalytically active forms.

In the normal mammalian brain, although HO-1 distributed into neurons, astrocytes, choroid plexus epithelial cells, ependymal cells, neurofibrillary tangles, senile plaques, vascular smooth muscle and endothelial cells, the main brain HO activity is provided by HO-2 (2, 3). It is known that HO-1 shows a progressive increase in cerebral cortex and hippocampus between 3 and 85 years of age in humans (4). Under prolonged oxidative stress conditions, HO system is activated to limit neuronal damage through production of powerful antioxidant bilirubin, which is the end product of heme catabolism (5). However, as a converse effect, heme-derived iron and CO could exacerbate oxidative substrate damage by facilitating the production of reactive oxygen species in the mitochondria (6, 7).

Up to now, HO activity and its metabolic effects have been investigated in so many studies in Alzheimer’s disease. It has been reported that detection of HO-1 increase in the brains of patients with Alzheimer’s disease is compatible with the findings of increased cerebral HO-1 mRNA levels (8), increased bilirubin levels in cerebrospinal fluid (CSF) (9), and an increased local biosynthesis of other stress proteins (HSP 27, HSP 72, α, B crystalline and ubiquitin, etc.) (10-13). Possible stimulation of HO-1 and other stress proteins in patients with Alzheimer’s disease causes accelerated β-amyloid accumulation (14), release of pre-inflammatory cytokines (TNF α, IL 1-β), activated microglial NO-release, and production of reactive oxygen species in aged mitochondria (15).

After all, based on the evidences in brain studies, it has been seen that upregulation of HO system is a...
main response to oxidative stress in neurodegenerative disorders. But most of these studies were performed in postmortem brain tissues of Alzheimer’s disease patients or in animal samples. They do not support any information about HO activity in the live samples. Because we consider that an easy way to analyze the HO system in Alzheimer’s disease patients is needed, we aimed to investigate the peripheral HO activity in leukocytes of Alzheimer’s disease patients in this study.

Subjects and Methods

Subjects

The study started at the May 2010, with the written permission of local ethical committee. Between May 2010 - June 2010, 32 patients with Alzheimer’s disease who agreed to participate in the study were considered for the study from GATA Haydarpasa Training Hospital, Neurology Service. Thirty subjects who do not have any psychiatric or organic neurologic pathology constituted the control group. All patients and healthy individuals signed ‘informed consent’ including informative explanations about the study. Of the patients and controls, who do not wish to participate in this study and have other serious metabolic problems were excluded.

Biological samples

Following 12 hours fasting, blood samples were taken into 10 ml straight tubes for serum and two pieces of 4 ml K2EDTA containing tubes (Greiner Vacuette Bio-One North America Inc., Monroe, NC, 28110 USA) for plasma and leucocytes from both patients and controls. For obtaining leukocyte pellet, one piece of 4 ml K2EDTA containing tube slowly leaked onto 4 ml histopaque (Histopaque -1077, Sigma Aldrich, St Lois, MO, USA) by pasteur pipette and centrifuged at 400 g for 30 min. Between plasma and histopaque layer, leukocyte was taken into another tube by pasteur pipette and 10 ml of saline solution was added. The tube was mixed gently and centrifuged again at 1000g for 10 min. The supernatant was discarded for acquiring leukocyte pellet. Then, all these samples were stored at -80 °C until enzyme activity analysis.

Measurement of heme oxygenase activity

Heme oxygenase activity measurement was performed according to method described by Klemz et al. (16). Briefly; through heme oxygenase activity in the leukocyte pellet, biliverdin product is converted into bilirubin by biliverdin reductase. Measurement of bilirubin formation rate through fluorometer (Shimadzu RF-5301PC, Columbia, MD, 21046 USA) reflects heme oxygenase activity. Results were expressed as amount of composed bilirubin in an hour per milligram of pellet protein (nmol/h/mg protein).

Statistical analysis

The statistical analysis was performed with the SPSS 11.0 (SPSS, Inc., Chicago, IL, USA). The continuous variables were tested for normality with the Kolmogorov–Smirnov test. For specificity and sensitivity values, ROC analysis was performed. A p <0.05 was considered as statistically significant.

Results

In our study, mean age of patients and control groups were 80.5 ± 7.1 (mean ± SD), and 78.0 ± 6.5, respectively (p= 0.127). Mean heme oxygenase activity in the patient group was significantly lower than that in the control group, 0.53 ± 0.32 nmol/h/mg protein and 1.19 ± 0.84 nmol/h/mg protein, respectively (p= 0.001).

In the ROC analysis (Table 1, Figure 1), 0.572 nmol/h/mg protein had the best cut-off value with a sensitivity of 66% and a specificity of 63%.
mg protein value of heme oxygenase activity gave best sensitivity and specificity values with 66% for sensitivity and 63% for specificity. Using 0.572 nmol/h/mg protein value of heme oxygenase enzyme activity as a cut-off value, positive predictive value was 65%, negative predictive value was 63%, and the odds ratio was found to be 3.29.

Discussion

In our study, HO activity in leukocytes of patients with Alzheimer’s was significantly lower compared to healthy controls (p < 0.001). Several studies reported that despite HO-1 mRNA and protein levels increases in the brain parenchyma of Alzheimer’s disease patients, HO-1 levels in CSF, choroid plexus epithelial cells, blood mononuclear cells, and plasma decreases. Smith et al. (1994) reported that HO-1 was significantly higher in the postmortem brain cells of patients with Alzheimer’s disease compared to control group (2). Similarly, the researchers such as Schipper (2004), Hirose et al. (2003), and Prenkumar et al. (1995) also reported increased HO activity in the brain tissue (4, 8, 17). On the other hand, some researchers have detected lower peripheral HO-related mRNA and protein levels in Alzheimer’s disease patients (18-20). In a research, lower plasma HO-1 protein levels in Alzheimer’s disease patients were found with similar to our findings (21). It is understood from above-mentioned studies that HO activity is reduced in the peripheral specimens, although higher in the brain tissue.

It has been suggested that reduction of HO-1 in some cases of Alzheimer’s disease may be caused by increased mRNA heme oxygenase suppressor (HOS) activity (22). HOS is a 50-100 kDa molecular weight, heat-sensitive, and heparin binding glycoprotein that acts as transcription inhibitory factor. Circulating HO-1/HOS ratio is considered as a biomarker that could be used in the diagnosis of Alzheimer’s disease (23). It has been stated that α1-antitrypsin (AAT) exhibits HOS bioactivity, and AAT concentrations were significantly increased in the plasma of patients with Alzheimer’s disease and were positively correlated with HOS activity (24). In addition, AAT immunoreactivity was increased in the brain samples of Alzheimer’s disease patients (22). In response to neurotoxicity in the brain of Alzheimer’s disease patients, there could be large amounts of HOS or other inhibitors in the leukocytes that might cause decreased HO activity. Consequently, HOS could be one reason for the peripheral low activity of HO in Alzheimer’s disease patients of our study, although we did not investigate any HOS activity, as a limitation of our study.

One important result of this reduction in activity of peripheral HO could be for limiting disease in our study. Through this way, the organism might preserve peripheral mitochondrial function by reducing the formation of free iron and CO, thus, might be trying to prevent progression of the disease. It has been proposed that the HO-1 increase in brain cells that releases free iron and CO can cause the excess amount of iron overload concerning with oxidative stress and mitochondrial insufficiency (25). Likewise, another study suggested that iron and CO formed by the reaction of HO could increase intracellular oxidative stress and could provoke the formation of free radicals in mitochondria and other subcellular organelles (26).

In conclusion, we have shown decreased peripheral HO activity in leukocytes of Alzheimer’s disease patients for the first time. Heme oxygenase suppressor factors such as AAT should be further analyzed by comprehensive studies in the leukocytes of Alzheimer’s disease patients. These may support important information about peripheral findings that could facilitate diagnostic processes and that provide early treatment opportunities in the neurodegenerative diseases. In addition, investigation of iron metabolism in both central nervous system and plasma in Alzheimer’s disease patients should be clarified in detail that may supply knowledge about what extent mitochondrial functions in brain are affected through excess amount of iron compared to plasma.

Conflict of interest statement

The authors do not declare any conflict of interest or financial support in this study.

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