Induction of Heme Oxygenase-1 by Aluminum and Lack of Effect of Antioxidants

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Introduction

Aluminum (Al) has been implicated as an etiological element in the pathogenesis of various neurodegenerative disorders. There are speculations about the relationship between the accumulation of Al in human brain and the development of Alzheimer's disease (AD). However, involvement of Al in the pathogenesis of AD is still controversial. Senile plaques and neurofibrillary tangles are pathological hallmarks in AD, and it is reported that the expression levels of several mRNA and proteins are altered in AD brains. In our recent study, these mRNAs were detected using reverse transcription-polymerase chain reaction (RT-PCR) analysis of mouse brains after Al treatment for 30 d, and the expression level of heme oxygenase-1 (HO-1) mRNA was remarkably elevated in the brains of these mice.

In the present study we further investigated the induction of HO-1 by Al, focusing on the possibility that the mechanism of the induction might be mediated by oxidative stress.

Methods

Male Wistar rats (5 weeks old; 150 to 170 g) were injected intraperitoneally with a single dose of AlCl₃ (10, 20, 40 mg Al/kg body wt.), and were then injected daily intraperitoneally with Al in the form of AlCl₃ (1 mg Al/kg body wt.) or Al-maltol complex (AlM, 1 mg Al/kg body wt.) for 30 d. Brains and livers were removed and total RNA or nuclear extract was isolated. HO-1 mRNA in the brain and liver was detected by northern blotting using a ³²P-labeled probe of rat HO-1 cDNA (pRHO-1)¹⁾ or RT-PCR using rat HO-1 specific primer. The ability of activating protein-1 (AP-1) or heat shock factor (HSF) to bind DNA sequences specifically was determined by gel shift assay using synthetic double-stranded oligonucleotides.

Results and Discussion

Northern blotting and RT-PCR analysis showed that the level of HO-1 mRNA in rat brain and liver was significantly elevated 3 h after

single administration of Al at doses of 10, 20 and 40 mg Al/kg, and was also elevated 24 h after the last administration with 1 mg/kg Al in the form of AlCl₃ and AlM for 30 d. The single injection of 1 mg Al/kg AlCl₃ and AlM did not promote an increase of HO-1 mRNA level. It is reported that HO-1 protein and mRNA are increased in cerebral cortex and cerebral vessels samples from AD patients.²⁾ HO-1, which is a rate-limiting enzyme in heme catabolism, is a stress response protein, and its induction has been suggested to involve the activation of an oxidative stress through generating reactive oxygen species (ROS). Since several studies have shown that Al treatment results in elevation of lipid peroxidation in animals experimentally, we presumed that the induction of HO-1 in Al-treated rats was mediated by an oxidative stress. To evaluate this hypothesis, we examined the effect of antioxidant agents on this induction. However, pretreatment with the antioxidants *N*-acetylcysteine and α -tocopherol did not suppress the increase of HO-1 mRNA level by Al administration. Next, using gel shift assay, we determined the activity of AP-1 and HSF to bind DNA sequences specifically after Al treatment. It is known that the transcription of HO-1 mRNA is enhanced by a transcription factor, AP-1 or HSF, under oxidative conditions in mammalian cells. Our data showed that AP-1 or HSF DNA-binding activity was not altered in nuclear extracts of rat liver 2 h after Al injection. These results indicated that HO-1 induction after Al treatment was not mediated by an oxidative stress.

References

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