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Increased midkine levels in sera from patients with Alzheimer's disease

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Abstract

Midkine (MK) is a heparin binding growth factor and promotes growth, survival and migration of various cells including neurons. It is also known to accumulate in senile plaques of patients with Alzheimer's disease (AD). To investigate the involvement of serum MK in the pathophysiology of AD, serum MK levels were determined in patients with AD ($n=36$) and age- and sex-matched healthy controls ($n=32$), using an enzyme-linked immunosorbent assay (ELISA). The serum MK values of the patients with AD (median 560 and interquartile range (500–755) pg/ml) were significantly ($U=278.5$, $P=0.0003$, Mann–Whitney U-test) higher than those of the controls (median 500 and interquartile range (385–520) pg/ml). Moreover, 17 patients (47.2%) had abnormally high values of more than 600 pg/ml, but no controls (0%) did. There was no correlation between serum MK level and the mini mental state examination (MMSE) score in the patients. The demonstration of elevated MK levels in sera of patients with AD may contribute toward an understanding the pathophysiology of this disease, and provide a novel potential therapeutic strategy for decreasing neuronal damages in patients with AD. We found that serum MK levels in patients with AD were increased in comparison with those of normal controls.

Keywords: Dementia; Neurotrophic factor; Serum marker

1. Introduction

Midkine (MK), a heparin binding growth factor (Kadomatsu et al., 1988), promotes survival, growth and migration of various cells, including neurons (Muramatsu et al., 1993; Haynes and Rumsby, 2001; Muramatsu, 2002). It is strongly expressed during the midgestation period of embryogenesis, while its expression is nearly undetectable in the normal adult brain (Kadomatsu et al., 1990; Nakamoto et al., 1992; Matsumoto et al., 1994). However, recent evidence suggest that MK may play various roles, not only in the formation of the nervous system, but also in pathological phenomena of the adult brain. Researchers reported that MK expression increases in ischemic brain regions of humans (Yoshida et al., 1995; Wada et al., 2002) as well as rats (Mochizuki et al., 1998). On the other hand, MK has been shown to be neuroprotective (Owada et al., 1999; Harvey et al., 2004). For example, intraventricular administration of MK ameliorates hippocampal delayed neuronal death following transient forebrain ischemia (Yoshida et al., 2001).

Alzheimer's disease (AD) is one of the most prevalent dementing disorders, representing over 50% of all dementia cases in the elderly (Grossberg, 2003). AD is

Abbreviations: AD, Alzheimer's disease; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; HDS-R, Hasegawa Dementia Scale-Revised; MK, midkine; MCI, mild cognitive impairment; MMSE, mini mental state examination; SPECT, single photon emission computed tomography.

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characterized by two pathological hallmarks, namely, senile plaques and neurofibrillary tangles. The former are mainly composed of amyloid-beta peptides (Abeta), while the latter consists mainly of filaments of hyperphosphorylated tau. Yasuhara et al. (1993) reported that senile plaques in the brain of AD were immunoreactive for MK. Furthermore, MK inhibits Abeta fibril formation and Abeta-induced cytotoxicity (Yu et al., 1998; Monji et al., 2000). Since Abeta peptides are believed to play a role in neurodegeneration in AD, MK is likely to play a neuroprotective role in the pathogenesis of AD.

As early detection of AD is beneficial for the treatment of patients, the search for diagnostic markers in serum and cerebrospinal fluid (CSF) has become a rapidly growing field (Teunissen et al., 2002, 2003). Researchers have focused on CSF biochemical markers; total-tau, phospho-tau and Abeta(1–42), to detect AD (Clark et al., 2003; Zetterberg et al., 2003). On the other hand, serum levels of MK are measurable in human samples (Muramatsu et al., 1996), and we have developed highly sensitive enzyme-linked immunosorbent assays to determine MK levels in previous studies (Song et al., 1997; Ikematsu et al., 2000; Shimizu et al., 2003). Because MK is a senile plaque-associated protein, it is of considerable interest to examine serum MK levels in AD cases and healthy controls. Here, the authors report increased MK levels in the sera of AD patients and discuss its significance.

2. Methods

2.1. Subjects

The ethics committee of Chiba University Graduate School of Medicine approved the present study. All of the subjects provided written informed consent for participation in the study after the procedure had been fully explained. Thirty-six patients with AD (mean age: 73.5 years [S.D. 5.8], range: 63–83 years; 13 men and 23 women) were recruited from the Chiba University Hospital and Chiba City Aoba Hospital, Chiba, Japan. Thirty-two age- and gender-matched healthy subjects (mean age: 72.0 years [S.D. 6.5], range: 61–84 years; 8 men and 24 women) also participated in this study as normal controls. All patients were diagnosed as probable AD according to the National Institute of Neurological and Communicational Disorders and Stroke (NINCDS)–Alzheimer's Disease and Related Disorders Association (ADRDA) criteria (McKhann et al., 1984), and underwent a mini mental state examination (MMSE) (Folstein et al., 1975) and/or the Hasegawa Dementia Scale-Revised (HDS-R) (Hosokawa et al., 1994). Patients with cerebral infarcts, hemorrhage, normal pressure hydrocephalus or neoplasm were excluded by brain CT scan or MRI imaging. Single photon emission computed tomography (SPECT) with *n*-isopropyl-*p*-[^{123}I]iodoamphetamine (IMP) or [^{99m}Tc]-labelled L,L-ethyl cysteinate dimer (ECD) was performed as a supplementary means to evaluate the cause of dementia (Johnson et al., 1998). Laboratory tests eliminated the possibility that factors known to change serum MK levels, such as carcinoma, infection, dehydration and hemolysis, were active in the present participants. Patients with any other previous mental or physical illnesses were also excluded from the study. The healthy controls had no history of psychiatric or neurological disorder, and no abnormalities were observed upon routine clinical examination, including a MMSE.

2.2. Procedure

Serum samples of the patients and the controls were collected from 11:00–12:00 am, and stored at −80 °C until assay. The concentration of serum MK was determined by using an enzyme-linked immunosorbent assay (ELISA) as described previously (Muramatsu et al., 1996; Ikematsu et al., 2000). Serum homocysteine content was also determined by homocysteine microplate STE assay Kit (Diazyme laboratories, San Diego, CA) according to the manufacturer's procedure.

2.3. Data analyses

Normally distributed data are presented as mean±standard deviation (S.D.); data that were not normally distributed are reported as medians with interquartile ranges. Calculations were performed using the statistical software package Statview (Abacus Concepts, Berkeley, CA) and SPSS for windows (SPSS, Chicago, IN). The Fisher's exact test was used for categorical variables, and the Student's *t*-test was employed for the continuous variables. As the serum MK values were not found to have a normal distribution, the difference between the two groups was examined using the non-parametric Mann–Whitney *U*-test. Spearman correlation coefficients were used to examine the association of the serum MK value with the MMSE score or age. A *P* value of less than 0.05 was considered to be statistically significant.

3. Results

There was no significant difference between the AD patients and the healthy controls (Table 1). The serum MK values of the patients with AD (median 560 and interquartile range (500–755) pg/ml) were significantly (U=278.5, P=0.0003, Mann–Whitney *U*-test) higher than those of age-matched controls (median 500 and interquartile range (385–520) pg/ml) (Table 1, Fig. 1). Based on our previous study (Ikematsu et al., 2000), in which none of 135 healthy control subjects showed MK serum values reaching 600 pg/ml, a subject with an MK level higher than 600 pg/ml was defined as having an abnormally high MK. Seventeen out of 36 (47.2%) patients exhibited abnormally high values of