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Inhibition of cognitive decline in mice fed a high-salt and cholesterol diet by the angiotensin receptor blocker, olmesartan

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Abstract

The metabolic syndrome is closely related to dietary habits and seems to be associated with impairment of cognitive function in humans. Angiotensin receptor blockers are widely used with the expectation of preventing cardiovascular events and stroke and potential amelioration of the metabolic syndrome. We examined the diet-induced changes of cognitive function in mice treated with a high-salt and high-cholesterol diet. C57BL/6J mice were fed a high-salt (2% NaCl in drinking water) and high-cholesterol (1.25% cholesterol, 10% coconut oil) diet (HSCD) or a normal diet (ND), and subjected to 20 trials of a passive avoidance task every week from 8 weeks of age. An age-dependent decline of the avoidance rate starting from 10 weeks of age was observed in HSCD mice, whereas the avoidance rate gradually increased in the ND group. Oral administration of an angiotensin receptor blocker, olmesartan, at a dose of 3 mg/kg per day in drinking water from 8 weeks of age prevents this decline of avoidance rate in HSCD mice (49% vs. 82% at 12 weeks of age). Treatment with olmesartan significantly decreased serum glucose and cholesterol levels in HSCD mice, with a slight decrease in blood pressure. Administration of olmesartan in HSCD-fed mice showed a 1.6-fold increase in mRNA expression of a neuroprotective factor, MMS2, compared to HSCD-fed mice without olmesartan. Olmesartan attenuated the increase in superoxide anion production detected by dihydroethidium staining in the brain of HSCD mice. Our results suggest that olmesartan could be therapeutically effective in preventing the impairment of quality of life in persons on a high-fat and high-salt diet.

Keywords: Angiotensin II receptors; High-salt diet; High-cholesterol diet; Cognitive impairment

1. Introduction

Cognitive function is one of the major determinants of quality of life in older age. The metabolic syndrome seems to be closely associated with impairment of cognitive function in humans. Accumulating evidence has demonstrated an association between the metabolic syndrome and the onset of cardiovascular disease. Despite an increasing awareness that cardiovascular risk factors exaggerate the risk of cognitive decline and dementia, the detailed relationship between the metabolic syndrome and cognitive decline has not been well defined. Yaffe et al. (2004b) reported a contribution of the metabolic syndrome to cognitive impairment in the elderly. Moreover, blood pressure-related pathophysiological processes adversely affecting the brain may begin earlier in the adult lifespan than previously thought (Elias et al., 2004), indicating that earlier prevention of the metabolic syndrome may be necessary to improve quality of life in aged people. The metabolic syndrome seems to be closely associated with the daily diet, and the changes in cognitive function in mice fed with a high-salt intake for hypertension and a high-cholesterol diet for hyperlipidemia have not been well defined.

Recent major clinical research such as the Perindopril pROtection aGainst REcurrent Stroke Study (PROGRESS) (Schiffrin, 2002), the Study on COgnition and Prognosis in the Elderly (SCOPE) (Lithell et al., 2003), the Losartan Intervention for Endpoint Reduction in Hypertension Trial (LIFE) (Dahlof et al., 2002), and the Acute Candesartan Cilexetil Therapy in Stroke Survivors study (ACCESS) (Schrader et al., 2003)

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indicate that blockade of the renin–angiotensin system is effective in preventing a first or recurrent stroke, or cardiovascular event, beyond the blood-pressure-lowering effect. Furthermore, blockade of the renin–angiotensin system is expected to prevent cognitive decline more effectively compared to other anti-hypertensive drugs. Indeed, in the SCOPE study, an angiotensin receptor blocker, candesartan, improved cognitive function in hypertensive patients with mild cognitive impairment (Skoog et al., 2005). The renin–angiotensin system also plays an important role in the pathophysiology of the metabolic syndrome, such as salt sensitivity, insulin resistance (Shiuchi et al., 2004), obesity (Engeli et al., 2003) and adipogenesis (Matsushita et al., 2006). Our recent paper demonstrated that an angiotensin receptor AT1 specific blocker, olmesartan etc. reduced glucose intolerance, with an increase in translocation of glucose transporter 4 (Iwai et al., 2006). Moreover, we have reported that angiotensin II type 2 (AT_2) receptor stimulation prevents cognitive impairment in mice after focal cerebral ischemia, which is at least partly due to an increase in a neuroprotective factor, methyl methanesulfonate sensitive 2 (MMS2) one of the ubiquitin conjugating enzyme variants (Mogi et al., 2006), leading us to examine whether AT_1 receptor blockers are also effective in preventing possible hypertension- and hyperlipidemia-associated cognitive decline, which are at least partly due to relative stimulation of the AT_2 receptor. Therefore, we examined cognitive function in mice fed a high-salt and high-cholesterol diet, and the possible inhibition of metabolic syndrome-induced cognitive decline by olmesartan.

2. Materials and methods

2.1. Animals and treatment

This study was performed in accordance with the National Institutes of Health guidelines for the use of experimental animals. All animal studies were reviewed and approved by the Animal Studies Committee of Ehime University. Adult male 6 weeks of age C57BL/6J mice (average weight 22 g) were purchased from CLEA (Tokyo, Japan). The animals were housed in a room where lighting was controlled (12 h on, 12 h off) and the temperature was kept at 25 °C. After 2 weeks of adjustment, mice at 8 weeks of age were fed a standard diet (MF, Oriental Yeast Co. Ltd., Osaka, Japan) or a high-cholesterol diet (1.25% cholesterol, 10% coconut oil) (Oriental Yeast Co. Ltd.) and water containing 2% NaCl ad libitum. At the same time, we started administration of an angiotensin receptor blocker olmesartan (provided by Sankyo Pharmaceutical Co. Ltd., Tokyo, Japan). Mice were housed individually and their weight and water intake were checked three times per week during the period of HSCD treatment. However, there was no significant difference in body weight change and water intake between ND and HSCD treatment. The final calculation of the exact dose of olmesartan was estimated to be 3 mg/kg per day. Plasma cholesterol level was measured by the cholesterol oxidase method (Cholesterol E-test, WAKO Chemical Industries, Ltd., Osaka, Japan), and blood glucose level was measured by the glucose oxidase method (Glucose CII-test, WAKO Chemical Industries, Ltd.). Plasma insulin level was measured by ELISA (Ultra Sensitive Rat Insulin Kit, Morinaga Institute of Biological Science, Inc., Kanagawa, Japan).

2.2. Blood pressure measurement

Systolic blood pressure was monitored in conscious mice by the tail-cuff method (MK-1030, Muromachi Co. Ltd. Tokyo, Japan) twice before and after 4 weeks of treatment. Mice were held in a small plastic holder on a warming pad thermostatically controlled at 37 °C, as described in a previous paper (Krege et al., 1995). Mean systolic blood pressure of ten measurements in each group was shown.

2.3. Passive avoidance test

A shuttle avoidance cage (32 × 12. × 15 cm; Melquest, Toyama, Japan) and an isolation cabinet (48 × 42 × 37 cm; Melquest) were used as previously reported (Mogi et al., 2006). The shuttle avoidance box was divided into equal-size chambers by a stainless steel divider. The floor of the shuttle box consisted of stainless steel rods. Scrambled shocks were delivered by a shock generator (SG-200, Melquest). Mice were individually placed in a chamber and given 20 inescapable electric shocks (0.3 mA) of 3 s duration at intervals of 2 s. A tone signal was presented during the first 5 s of each trial. If there was no avoidance response within this period, the tone signal remained on and a 0.3 mA shock was delivered for a duration of 3 s through the grid floor. In the case of no escape response within this period, both the tone and shock were automatically terminated. The inter-trial interval was 10 s. The number of escape failures was recorded. Escape failure was defined as a non-crossing response during shock delivery. Passive avoidance tests were performed in the morning once a week.

2.4. Real-time reverse transcription-polymerase chain reaction (RT-PCR) method

RT-PCR was performed with a SYBR green I kit (MJ Research, Inc., Waltham, MA). PCR primers were as follows: for MMS2, 5′-ATG GCA GTC TCC ACA GGA GT-3′ (forward) and 5′-GCC CAA TAA TCA TGC CTG TC-3′ (reverse); for AT_1 receptor, 5′-GTT CCT GCT CAC GTG TCT CA-3′ (forward) and 5′-CAT CAG CCA GAT GAT GAT GC-3′ (reverse); and for AT_2 receptor, 5′-CCT GGC AAG CAT CTT ATG TAG TTCC-3′ (forward) and 5′-TGG TCA CGG GTA ATT CTG TTC TTCC-3′ (reverse).

2.5. Detection of superoxide anion in brain sections

Detection of superoxide anion was carried out as described previously (Szocs et al., 2002). In brief, frozen, enzymatically intact, 10-μm-thick sections were prepared from mouse brain and incubated immediately in dihydroethidium in PBS for 30 min at 37 °C in a humidified chamber protected from light. Dihydroethidium is oxidized on reaction with superoxide to ethidium, which binds to DNA in the nucleus and fluoresces red. For detection of ethidium, samples were examined with an Axioskop microscope (Axioskop 2 Plus with AxioCam, Carl Zeiss, Oberkochen, Germany) equipped with a computer-based imaging system. The intensity of the fluorescence was analyzed and quantified using computer-imaging software (Densitograph, ATTO Corporation, Tokyo, Japan).

2.6. Statistical analysis

All data are expressed as mean ± SE in the text and figures. The data were analyzed by two-way ANOVA. When a statistically significant effect was found, post-hoc analysis was performed to detect the difference between the groups. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Treatment with olmesartan inhibited cognitive decline and improved cognitive function in mice fed a high-salt and high-cholesterol diet

C57BL6 mice fed a high-salt and high-cholesterol diet (HSCD) exhibited significant failure to increase the avoidance rate and a time-dependent cognitive decline after 12 weeks of age compared to normal diet (ND)-fed C57BL6 mice (Fig. 1).