



Milk Containing BF-7 Enhances the Learning and Memory, Attention, and Mathematical Ability of Normal Persons

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Abstract

Previous studies indicate that BF-7 enhances learning and memory in normal and elderly individuals. Here, we evaluated whether milk containing BF-7 (BF-7 milk) could improve the brain function, with thirty normal university students (21 ± 1.2 years). Two versions of the Paced Auditory Serial Addition Test were used under double-blinded conditions to measure the efficacy of BF-7 milk on learning and memory, especially working memory and attention, and on mathematical ability. As a result, BF-7 milk improved the accuracy of the task more than 3-fold. Furthermore, BF-7 milk protected cultured neuronal cells from 3-hydroxykynurenine, a normal endogenous brain stress agent. These results indicate that BF-7 milk enhances memory, attention and mathematical ability in normal persons.

Key words : learning and memory, attention, BF-7, mathematical ability, milk

Introduction

Our brains are constantly stressed by various neuronal stresses. Many lines of evidence, including those from controlled clinical studies, suggest that BF-7, a natural extract from *Bombyx mori*, can significantly improve learning and memory in normal persons and can enhance the function of the nervous system (Chae *et al.*, 2004; Kim *et al.*, 2005; Lee *et al.*, 2004a). BF-7 has been shown to decrease the infarct size of rat hippocampal neurons (Lee *et al.*, 2004b), to increase the acetylcholine concentration in brain of preclinical rat models (Kim *et al.*, 2004; Lee *et al.*, 2004a), and to protect neurons from

amyloid beta (A β) toxicity and oxidative stress (Kim *et al.*, 2005). It has also been suggested that BF-7 can improve the memory function of demented persons and can protect human neuroblastoma cells from reactive oxidative stress (Kim *et al.*, 2005; Lee *et al.*, 2004a). BF-7 reportedly improves the memory and attention of healthy high school students and significantly attenuates A β induced-apoptosis by reducing ROS accumulation and diminishing caspase-like protease activity (Chae *et al.*, 2004). BF-7 was also shown to increase the mean IQ of 4 persons from 103 to 114 and to increase blood flow in the parahippocampal gyrus and the medial temporal area (Lee *et al.*, 2004c).

Omega-3 fatty acids such as EPA/DHA have been widely used to make brain functional food including functional milk. However, the clinical effects of DHA on brain function are not approved from Korea Food and Drug Administration (FDA), because the role of DHA on

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the function is not identified clinically. Moreover, polyunsaturated fatty acids (PUFAs) like as DHA and EPA is easily oxidized with a little exposure to the air and its oxidized form is known to be toxic (Choi *et al.*, 2008; McClements and Decker, 2000). In addition, peroxidation of PUFAs led to the formation of cytotoxic lipid-aldehyde species (Esterbauer *et al.*, 1990). It has been also suggested that DHA-derived lipid-aldehyde species are causative agents of CNS disorders such as Alzheimer's disease with significant components of oxidative damage (Milne *et al.* 2006; Montine *et al.* 2002; Musiek *et al.* 2004; Reich *et al.* 2001). And it has been reported that these peroxidation products from PUFAs like as omega-3 fatty acids also damaged DNA (Takasu *et al.*, 2007).

Based on these previous studies, we conducted a clinical study with thirty university students to examine whether milk supplemented with BF-7 (BF-7 milk) could improve the cognitive functions of concentration and cognition using two versions of the Paced Auditory Serial Addition Test (PASAT) (Gronwall version and the Levin version) (Gronwall and Sampson, 1974; Levin *et al.*, 1987) under double-blinded conditions. We also investigated whether BF-7 milk could effectively protect neurons from 3-hydroxykynurenine (3-HK), one of the most common neuronal stress agents (Ogawa *et al.*, 1992; Pearson and Reynolds, 1992; Okuda *et al.*, 1996; Sardar *et al.*, 1995).

Materials and Methods

Preparation of BF-7

BF-7 was presented from Rural Development Administration, in Suwon, Korea, and prepared as previously described (Yeo *et al.*, 2004). In brief, *Bombyx mori* and its cocoon were dissolved and proteolyzed with specific set of proteases, followed by separation and purification of the specific peptide portion using various chromatography.

Cell culture and pharmacological treatment

SK-N-SH human neuroblastoma cells were cultured at 37°C in Dulbeccos Modified Eagles Medium (DMEM) (GIBCO, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (GIBCO) in a humidified 95% air, 5% CO₂ incubator. 3-hydroxykynurenine (3-HK) was obtained from Sigma Chemical Co. (USA) and dissolved in DMSO. The cells were transferred to a low serum media (1% FBS) 2 h before treatment with 3-HK (250 μM, which induces 50% cell death within 36 h) to assure

neuronal survival and morphological integrity of the cells.

Clinical subjects and BF-7 milk administration

Thirty normal healthy university students (21±1.2 years) were recruited at the Seoul Medical School and the Chung-Ang Medical School, Seoul, Korea. Ten milligrams of BF-7 powder were mixed in 200 mL of ordinary commercial plain milk, BF-7 milk was maintained at 4°C for 5 d, and 200 mL of BF-7 milk per day were administered to clinical subjects for 30 d.

Clinical procedures

Clinical data were obtained through questionnaires and interviews with the Levin version of PASAT and the computerized Gronwall version. During the study period, the investigator and the study participants were double-blinded. In both versions of PASAT, a random series of numbers from 1 to 9 were presented, and the subject was instructed to consecutively add pairs of numbers such that each number is added to the one that immediately precedes it. The test is scored from the number of errors summed over all trials (Strauss *et al.*, 2006).

Gronwall and Sampson (1974) manipulated the speed of information processing by presenting the same sequence of digits (61 items per trial) at different rates, thereby assessing visual attention and mathematical ability. We used a modified Gronwall version with three presentation rates (2.4, 2.0, 1.6, and 1.2).

We used the original Levin version (50-item-per-trial for four trials) developed by Levin *et al.* (1987) to assess auditory attention and mathematical ability. In brief, the subjects listened to the series of numbers and added pairs of numbers.

A Student's *t*-test was used to compare variables before and after intake of BF-7, and statistical significance was defined as $p < 0.05$.

Results and Discussion

Enhancement of learning and memory, attention, and mathematical ability by BF-7 milk

Many lines of evidence have shown that by itself, BF-7 improves brain functions such as learning and memory. To evaluate if BF-7 remains effective when mixed with milk, BF-7 milk was clinically administered to thirty university students who were tested with two versions of the PASAT. The PASAT is one of the tests most frequently used by neuropsychologists to assess learning and mem-

ory such as working memory, attention, information-processing ability, and mathematical ability (Chronicle and MacGregor, 1998; Strauss *et al.*, 2006). Fortunately, this test was sensitive enough to detect the neurofunction of BF-7 milk in this study.

BF-7 milk significantly enhanced the memory, attention, and mathematical abilities of the students. The number of errors made by students taking the Gronwall version of the PASAT was dramatically reduced from an average of 1.46 (before BF-7 milk intake) to 0.42 (after BF-7 milk intake for 30 d) (Fig. 1A), corresponding to a ~3.5-fold increase in accuracy (Fig. 1B). Enhanced accuracy following the intake of BF-7 milk was also found when students were assessed using the Levin version, with a reduction in the number of errors from 19.25

(before BF-7 milk intake) to 5.17 (after BF-7 milk intake) (Fig. 2A). Consistent with the Gronwall version, the accuracy was improved by ~3.73-fold. The improvement of accuracy in both tests indicates that the attention, memory, and mathematical ability are increase by intake of BF-7 milk.

Since BF-7 continued to function well when mixed with milk, our results indicate that BF-7 function is not attenuated by various kind molecules, including milk proteins. Thus, BF-7 can be used to produce functional milk that enhances brain functions including learning and memory, attention, and mathematical ability.

The PASAT activates a variety of areas of the brain related to many diverse functions (Audoin *et al.*, 2003; Lazeron *et al.*, 2003; Lockwood *et al.*, 2004; Staffen *et*

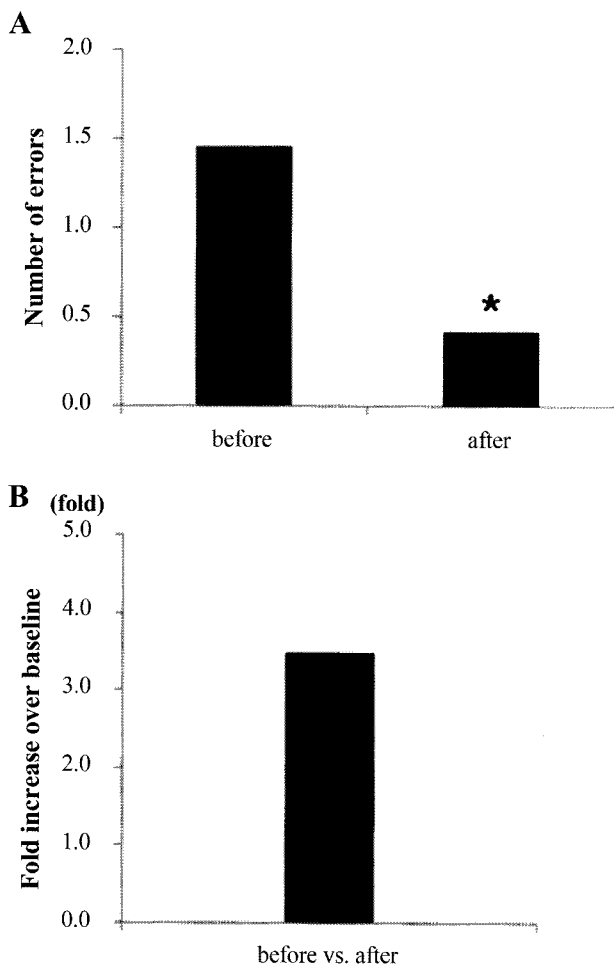


Fig. 1. BF-7 milk enhances the accuracy of normal persons assessed with the Gronwall version of the PASAT. (A) Accuracy on the Gronwall version of the PASAT was determined before and after a 30 d treatment with 200 mL of BF-7 milk per d. (B) Fold reduction of error compared to baseline. The scores before and after administration of BF-7 milk were compared using Student's *t*-test. Significant differences are shown as * ($p < 0.05$).

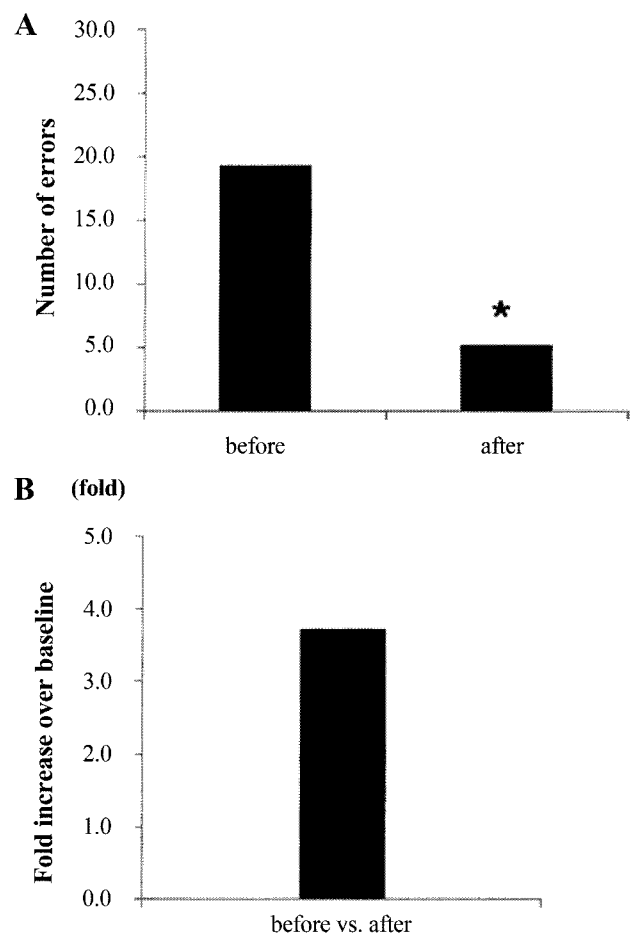


Fig. 2. BF-7 milk enhances the accuracy of normal persons assessed with the Levin version of the PASAT. (A) Accuracy on the Levin version of the PASAT was determined before and after a 30 d treatment with 200 mL of BF-7 milk per d. (B) Fold reduction of error compared to baseline. The scores before and after administration of BF-7 milk were compared using Student's *t*-test. Significant differences are shown as * ($p < 0.05$).

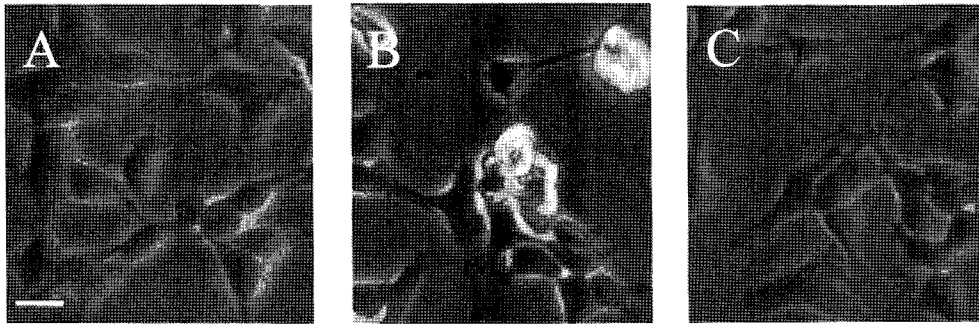


Fig. 3. Morphological assessment of neuronal stress by phase-contrast microscopy reveals that neuronal stress *in vitro* was prevented by BF-7 milk. (A) Control; (B) 3-HK; and (C) BF-7 milk + 3-HK. SK-N-SH cells were treated with 250 μ M of 3-HK for 24 h without (B) or with pretreatment (C) with 2 μ L of BF-7 milk (10 mg of BF-7 in 200 mL of plain milk). Scale bar, 10 μ m.

al., 2002). The PET study by Lockwood *et al.* (2004) showed that the PASAT activates dispersed non-contiguous foci in the superior temporal gyri, bifrontal, biparietal, anterior cingulate, and bilateral cerebellar sites, which regulate auditory perception and processing, speech production, cognitive domains of attention and executive control, working memory, and the speed of information processing. Functional MRI studies have suggested that the PASAT activates a broad range of brain networks, including those important for attention and working memory (Lazeron *et al.*, 2003).

The protective effect of BF-7 on 3HK-induced neuronal stress

In order to better understand the role of BF-7 milk in the brain, we next investigated whether BF-7 milk could protect neuronal cells from stress. BF-7 milk protected the neuronal cell line SK-N-SH from the general neuronal stress molecule, 3-HK. Cellular stress mediated by 3-HK was detected morphologically using phase-contrast microscopy. The administration of 250 μ M of 3-HK for 24 h induced morphological changes, such as membrane blebbing and cell shrinkage, compared to the untreated control (Fig. 3). Interestingly, pretreatment with BF-7 milk effectively prevented these neuronal changes by 3-HK (Fig. 3C), indicating that BF-7 milk protects neurons from neuronal stress.

Generally, milk is considered as an ideal food with essential nutrients required for the healthy development of a person (Bae and Nam, 2006; Lee and Hwangbo, 2001).

In conclusion, BF-7 is excellent substance for making better neuro-functional milk, since BF-7 itself and BF-7 milk clinically enhanced the learning, memory, attention, and mathematical abilities of normal persons, and effectively protected brain from neuronal stress.

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