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Pre-Treatment Effect of Different Doses of Soy Isoflavones on Spatial Learning and Memory in an Ovariectomized Animal Model of Alzheimer's Disease

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Abstract: The aim of this study was to evaluate the effects of different doses of dietary soy meals (with or without isoflavone) on dementia in ovariectomized (OVX) animal model of Alzheimer's disease. Female Wistar's rats with the exception of intact group were ovariectomized at the first line of study. Animals were divided into 2 main groups: control (c) and pre-treatment groups. Animals in pre-treatment groups received one of five types of diet during four weeks prior Nucleus Basalis Magnocellularis (NBM) electrical lesion normal diet (0), 10 g soy with isoflavone (10), 20 g soy with isoflavone (20), 10 g soy without isoflavone (-10) and 20 g soy without isoflavone (-20) in 30 g daily diet. The spatial learning and memory were tested using Morris water maze after electrical lesion. Rats were trained in water maze to find a hidden escape Platform. Rats received 6 blocks that each block consisted of 3 trials. Following acquisition trials, one probe trial was conducted in which the platform was removed. Soy meal diet (with or without isoflavone) in ovariectomized rats with Alzheimer's disease caused improvement of performance across 18 trials of Acquisition. Our results suggest that soy meal is a potential alternative to estrogen in the prevention and treatment of Alzheimer's disease.

Key words: Alzheimer's disease, isoflavone, Morris water maze, ovariectomy, rat

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease, which is the main cause of dementia in elder subjects (Tanzi *et al*., 1996; Wisniewski *et al*., 1997; Auld *et al*., 2002; Kandel *et al*., 2002; Gauthier and Quirion, 2001). Patients mostly show abnormalities of memory, problem solving, calculation, judgment, disorientation to time and place, language problems, depression, agitation and delusions (Tanzi *et al*., 1996; Kandel *et al*., 2002; Greenberg, 2002; Morris, 2003). AD affects approximately 7% of people older than 65 years of age and perhaps 40% of people over the age of 80 years of age (Kandel *et al*., 2002). Genetic (Auld *et al*., 2002; Gauthier and Quirion, 2001; Braunwald *et al*., 1987; Lassmann, 1996), β-amyloid (Kandel *et al*., 2002; Gauthier and Quirion, 2001; Lannfelt *et al*., 1993; Perez, 1998) and decrease in acetylcholine (Tanzi *et al*., 1996; Kandel *et al*., 2002; Gauthier and Quirion, 2001; Lannfelt *et al*., 1993; Perez, 1998; Behl, 1999) are the main factors in pathogenesis of AD. Furthermore, recent studies suggest that insulin resistance, excess free radicals, inflammatory metabolites, hyperhomocysteinemia and estrogen deficiency also are the risk factors of AD (Morris, 2003; Berrino, 2002). In addition, postmenopausal women are at greater risk of developing AD than men (Markham *et al*., 2002; Bang *et al*., 2004). On the other hand, estrogen replacement therapy (ERT) in postmenopausal women is associated with delayed onset and reduced risk of AD (Markham *et al*., 2002; Bang *et al*., 2004; Green and Simpkins, 2000; Ishunina *et al*., 2001; Heikkinen *et al*., 2004; Day and Good, 2004). Several studies indicate that usage of estrogen increase performance on some tests of memory/cognition (Markham *et al*., 2002; Green and Simpkins, 2000; Heikkinen *et al*., 2004; El-Bakri *et al*., 2004; Fernandez and Frick, 2004; Daniel and Lee, 2004). However, estrogen has proliferative and oncogenic effects on non-neuronal cells which are responsive to estrogen, such as breast and endometrium cells. A careful analysis of both positive and negative effects showed a balanced number of risks and benefits (Bang *et al*., 2004). Thus, the use of estrogen as a treatment for AD is limited. Hence, other estrogenic agents with fewer side-effects are needed to develop alternative treatment strategies. For the

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CNS, the ideal estrogen-like compound would have activity in the brain and none in the periphery (Cyr *et al*., 2002). Soy bean is a rich source of genistein. Unlike estrogen, genistein did not trigger proliferation of cells. Because genistein is a selective ER agonist, it is possible that ER, but not ER, mediates the proliferation of endometrium (Bang *et al*., 2004; Epizorno and Murray, 1999; Kim *et al*., 2000; Chang, 2002; Lephart *et al*., 2002). In the other hand, ER has a higher level of expression than ER in brain regions critical to memory function (Zeng *et al*., 2004). So, the main objective of the present study was to evaluate the effect of dietary soy meals (with and without isoflavones) for improving of postmenopausal dementia in an animal model of AD.

MATERIALS AND METHODS

Subjects: Forty-two female Wistar rats, approximately five months of age, obtained from animal house of Ahwaz Jondishapur University of Medical Sciences (AJUMS), at the beginning of the experiment. The study was conducted on July 2006. All rats were singly housed and kept under conditions of controlled temperature (20-23°C) and humidity (40-70%) and to a light/darkness cycle of 12/12 h (lights on at 7:00 am). Food and water were available ad libitum.

Groups and ovariectomy surgery: Animals were divided into 2 main groups: intact/control (c) and pre-treatment. Before surgery, rats were randomly assigned to one of the following five groups of diet during four weeks prior nucleus basalis magnocellularis (NBM) electrical lesion: (1) normal diet; (2) 10 g soy with isoflavone; (3) 20 g soy with isoflavone (20); (4) 10 g soy without isoflavone (-10) and (5) 20 g soy without isoflavone (-20) in 30 g daily diet. All the rats were subjected to ovariectomy surgery (OVX) under general anesthesia (i.p.) with a dose of 90 mg kg^{-1} Ketolar® (ketamine chlorohydrate, Parke-Davis, Dublin, Ireland) and 10 mg kg^{-1} Xylazine® (Miles laboratories, Shawnee, Kansas, USA). All efforts were made to minimize the number of animals used.

Diet preparation: In order to prepare isoflavone free soy ethanol (80 degree) was added to soy powder. After passing 24 h, the soy was dried in suitable place. Before and after alcohol washing, Total isoflavone concentration of soy was determined by high performance liquid chromatography (HPLC) (Frank *et al*., 1998).

Stereotaxic surgery: Four weeks after OVX, In order to create animal model of postmenopausal and Alzheimer's disease-induced dementia. The NBM (Nucleus Basalis Magnocellularis) of animals in pre-treatment groups, was destroyed bilaterally with electrical lesion (0.5 mA for 3 sec) while under anesthesia induced by injection of ketamine (90 mg kg^{-1} i.p. RotexMedica, Trittau, Germany) and Xylazine (10 mg kg^{-1} i.p. Miles laboratories, Shawnee, Kansas, USA) and stereotaxic surgery (Ap = -1.4 mm, M1 = -2.3 mm, DV = -6.8 mm) (Paxinos and Watson, 1986). In final stage of experiment animals were scarified under deep anesthesia and their brains were perfused with 5% formalin in normal saline via left carotid artery and then histological study was done on prepared stained brain slices in order identify the location of lesion. Correct lesioned samples were used for statistical analysis.

Morris water maze: The Morris water maze was a black circular pool (140 cm in diameter and 70 cm in height) located in a well lit room and filled with water (50 cm height) with 27°C. The maze performance was recorded by a video camera suspended above the maze and interfaced with a video (Tivanich instruments tracking system, Tehran, Iran). Numerous extra-maze cues surrounding the maze were fixed at specific locations and were visible to the rats. A platform (12 cm in diameter), was located in the center of north-east guardant of the pool, allowed rats to escape the water. The escape platform was positioned 2 cm below the water surface.

Acquisition trials: One week following NBM electrical lesion, water maze training began. In this task, the rats were trained to find a submerged platform using extra maze cues. Prior to water maze testing, all rats were habituated to the water using a three-trial shaping procedure. This procedure habituated the rat to the water and taught them to escape from the water by climbing on to a platform. Subjects were trained across one day. Each rat received 18 trials per day. There was a 20 min break between each 3 trials (6 blocks, each block consist of 3 trials). The location of submerged platform did not change through out the experiment. For each trial, the subject was placed in water facing the edge of the tank from random start points. On each trial, the subject was allowed 60 sec to escape to the submerged plat form; rats that failed to escape were led to the platform and were allowed to remain on it for 15 sec before being removed from the maze and dried off (Norris and Foster, 1999).

Probe trial: Following the one day acquisition period, a probe trial was order. The probe trial was identical to the acquisition trials with one exception. During the probe trial, the submerged plat form was removed. Multiple measures of water maze performance were recorded.

Swim distance (cm), quadrant time (percent time that each subject spent in the quadrant containing the plat form) and swim speed (cm sec^{-1}) were recorded during 18 trials and one probe trial (Norris and Foster, 1999).

Body weight and plasma estrogen: Animal's body weight at the baseline and four weeks later was recorded. Plasma estrogen was measured by ELIZA test.

Statistical analysis: A paired t-test analysis was used to determine whether significant differences existed in the OVX group weight at the baseline and one month after ovariectomy. One-way analysis of variance (ANOVA) was run to determine whether group differences existed in terms of percent time spent in the target quadrant and path length during acquisition and probe trials with SPSS v11.5 software. To further explore the effect of treatment across blocks, separate one-way repeated measures of ANOVAs were conducted for each block. One-way analysis of variance (ANOVA) was run to determine whether group differences existed in plasma estrogen. All post hoc comparisons were computed using the least significant difference method. P-value less than 0.05, was assumed to denote a significant difference.

RESULTS

Acquisition trials-path length: The total path length of pre-treatment group received normal diet (0) had significantly longer ($p<0.001$) than other groups (Fig. 1). In order to further exploration of the effect of treatment across blocks, separate one-way repeated measures ANOVAs were conducted for each block.

Probe trials test: There were no significant differences in percent of total time spent in target (goal) quarter of probe trial between all groups except between group (c) and group (-10) ($p<0.05$) (Fig. 2).

Probe trials-swim speed: Lesion of NBM had no significant effect on swim speed in the water maze. There were no significant differences ($p<0.15$) between swim speed in all groups during probe trials.

Body weight: The body weight at the baseline (weight 1) was significantly lower ($p< 0.05$) than body weight at four weeks later (weight 2) in pre-treatment group received normal diet (0), pre-treatment group treated with 10 g isoflavone free soy (-10) and pre-treatment group treated with 20 g isoflavone free soy (-20) in daily diet for four weeks (Table 1).

Table 1: Mean body weight (Mean±SEM) at the baseline (weight 1) and four weeks later (weight 2) for all groups

Group's label	Definition	No.	Weight 1 (g) (Mean±SEM)	Weight 2 (g) (Mean±SEM)
C	Control	7	262.00±4.940	267.57±6.97
Zero (0)	Normal Diet ψ	7	264.29±6.410	299.71±6.65*
10	pre-treated with 10 g soy ψ	7	297.14±12.88	296.71±12.61
20	pre-treated with 20 g soy ψ	7	248.43±9.860	255.29±9.89
-10	pre-treated with 10 g isoflavone free soy ψ	7	244.14±8.920	247.86±11.47*
-20	pre-treated with 20 g isoflavone free soy ψ	7	242.00±2.780	273.17±4.70*

Control group(c), pre-treatment group received normal diet (0), pre-treated with 10 g soy (10), pre-treated with 20 g soy (20), pre-treated with 10 g isoflavone free soy (-10) or pre-treated with 20 g isoflavone free soy (-20) in daily diet for four weeks, *Includes all p-value less than 0.05 vs. weight, ψ: All groups include OVX and NBM Lesion except control group

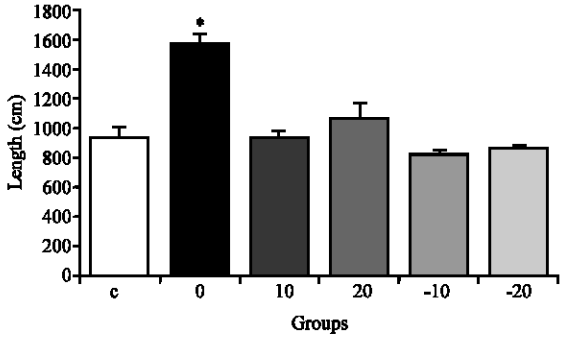

Fig. 1: Mean±SEM path length to locate the escape platform for total acquisition trials in all groups: control group (c), pre-treatment group received normal diet (0), pre-treated with 10 g soy (10), pre-treated with 20 g soy (20), pre-treated with 10 g isoflavone free soy (-10) and pre-treated with 20 g isoflavone free soy (-20) in daily diet for four weeks (*$p<0.001$ vs. other groups)

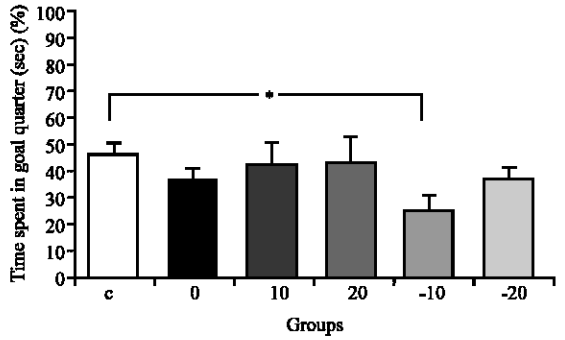

Fig. 2: Mean percent (Mean±SEM) of total time spent in target quarter for probe trial in all groups: control group (c), pre-treatment group received normal diet (0), pre-treated with 10 g soy (10), pre-treated with 20 g soy (20), pre-treated with 10 g isoflavone free soy (-10) or pre-treated with 20 g isoflavone free soy (-20) in daily diet for four weeks (*$p<0.05$)

Table 2: Mean plasma estrogen (Mean±SEM) for all groups

Group's label	Definition	No.	Estrogen (pg mL^{-1}) (Mean±SEM)
C	Control	5	1758.4±410.40**
Zero (0)	Normal Diet ψ	5	109.8±27.50
10	pre-treated with 10 g soy ψ	5	21.2±4.28
20	pre-treated with 20 g soy ψ	5	108.2±23.31
-10	pre-treated with 10 g isoflavone free soy ψ	5	71.4±32.44
-20	pre-treated with 20 g isoflavone free soy ψ	5	46.2±10.30

Control group(c), pre-treatment group received normal diet (0), pre-treated with 10 g soy (10), pre-treated with 20 g soy (20), pre-treated with 10 g isoflavone free soy (-10) or pre-treated with 20 g isoflavone free soy (-20) in daily diet for four weeks. **Includes highly significant p-value less than 0.001 for control group vs. other groups. There were no significant different between all five groups, ψ all groups include OVX and NBM Lesion except control group,

Plasma estrogen: The plasma estrogen levels in pre-treatment (that were OVX) groups were significantly lower ($p<0.001$) than plasma estrogen in control group (Table 2).

DISCUSSION

The results of the present study indicate that soy consumption apart from containing isoflavone or not in an ovariectomized animal model of Alzheimer's disease improve performance of acquisition in the Morris water maze. Pre-treatment groups receiving soy with or without isoflavone spent a significantly greater percentage of their total swim time in the quadrant in which the platform was located than pre-treatment group that not receiving soy. Pre-treatment groups receiving soy with or without isoflavone spent a similar time in the target quadrant in comparison with control group. Mean path lengths to reach the platform were longer in pre-treatment group not receiving soy than control and pre-treatment groups that receiving soy. These data suggest that NBM lesion impairs performance of acquisition in the Morris water maze and soy can prevent impairment induced by NBM lesion. There were no differences between groups at performance on a probe trial, suggesting that by the end of testing all groups had learned the task to the same degree. It is not clear whether the positive effect of soy that has been seen in this study is due to its isoflavone or other constituents. It is possible that this little amount of isoflavone is responsible for beneficial effects that have been seen in our study. Previous studies have shown that at the nanomolar level (5, 10 and 100 nM), genistein has neuroprotective effects against beta amyloid-induced neurotoxicity (Bang *et al*., 2004; Zeng *et al*., 2004). Our findings in this study are consistent with other previous studies (Lephart *et al*., 2002; Celec *et al*., 2004; Kritz-Silverstein *et al*., 2003; Lund *et al*., 2001; Lee *et al*., 2004). There are proposed mechanisms for the neuroprotective effects of isoflavones against Alzheimer's disease. Evidences from a variety of sources implicate those amyloid-β (Aβ) peptide have important roles in AD neuropathology. It is indicate that Aβ peptide can negatively regulate various steps in the synthesis and release of acetylcholine, thus suggesting a link between amyloid burden and cholinergic impairment in AD (Auld *et al*., 2002). The amyloid fragments can also induce production of free radicals in cell cultures which in turn increase Ca^{++} influx viaCa^{++} channels, thereby inducing neurotoxicity (Holscher, 1998). Genistein, a phytoestrogen that is capable of crossing the blood-brain barrier, has been reported to have an antioxidative effect (Zeng *et al*., 2004). Genistein inhibits the elevation of intracellular free Ca^{++} and the production of oxidant free radicals caused by A (Zeng *et al*., 2004). Genistein protects cells from H_2O_2-induced toxicity (Bang *et al*., 2004). H_2O_2 is a Reactive Oxygen Species (ROS) which can damage the neurons (Morris, 2003; Behl, 1999; Holscher, 1998; Ramassamy *et al*., 2000). This antioxidative effect of soy can protects human from neurodegenerative diseases such as AD (Zeng *et al*., 2004). Furthermore, phytoestrogens significantly affect the brain calcium-binding protein calbindin (CALB), which acts as a buffer by binding intracellular calcium and plays an important role in mediating cell proliferation, programmed cell death (apoptosis) and neurotoxicity (Lund *et al*., 2001). Previous findings suggest that the mechamisms by which phytoestrogens especially genistein protect neuronal cells include not only by the physiological properties of genistein, such as its antioxidative activity, but also activation of Estrogen Receptors (ERs) and upregulation of brain-derived neurotrophic factor (Zeng *et al*., 2004). In ovariectomized female rats, on the other hand, phytoestrogen treatments resulted in a dose-dependent improvement of VSM (Pan *et al*., 1999). This improvement in cognitive ability in phytoestrogen treated females may be due in part to the increased presence of choline acetyltransferase messenger RNA in the frontal cortex, which has been shown to be associated with protection and enhancement of cognitive function (Pan *et al*., 1999). As mentioned earlier, beside isoflavone, other soy constituents may act as neuroprotective agent. Other soy constituents include: Protease inhibitors (that have anti-cancer andanti-inflammatory effects), Lignans (that have phytoestrogenic, anti-tumorand anti-viral activity), Comestans (with phytoestrogenic effects), Saponins (that have anti-cancer, antioxidant and anti-mutagenic properties) and Phytates (with antioxidant and anti-cancer effects) (Epizorno and Murray, 1999). In conclusion, although the present study suggests the potential use of soy in the prevention of AD, future studies will address the effects of soy constituents on AD distinctly.

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