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Ethanol Extract of Soybean Ameliorates Scopolamine-Induced Memory Impairment in Mice

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Abstract – Soy (*Glycine max*, family Leguminosae) contains isoflavones and saponins as main constituents. In our preliminary study, soybean ethanol extract (SE) ameliorated scopolamine-induced memory impairment in mice in the passive avoidance task. Therefore, to confirm its ameliorating effect for memory impairments, we measured its effect in scopolamine-induced memory-impaired mice in Morris water maze task. SE significantly prevented scopolamine-induced memory impairment in the Morris water maze task. SE also increased the swimming time within quadrant section of the platform on the day after the final training session test. SE protected the reduction of brain-derived neurotrophic factor (BDNF) expression and cAMP response element-binding protein (CREB) phosphorylation in the hippocampi of scopolamine-treated mice. However, SE did not inhibit acetylcholinesterase. To understand the possible role of soysaponins in memory impairments, we prepared soyasaponins-rich (butanol) fraction of soybean (SRF) and investigated its protective effect against in the passive avoidance and Morris water maze tasks. SRF ameliorated scopolamine-induced memory impairment in mice. The memory impairment-ameliorating effect of SRF was more effective than that of SE. Based on these findings, soybean may improve memory impairment by regulating CREB phosphorylation and BDNF expression.
Keywords – *Glycine max*, Soybean, Memory, Brain-derived neurotrophic factor, cAMP response element-binding protein

Introduction

Dementia is the most common neurodegenerative disorder characterized by the progressive deterioration of learning and memory as the brain becomes damaged. Of dementia, Alzheimer's disease is irreversible, dislike thyroid disease. Alzheimer's disease (AD) caused by abnormal tau protein and β-amyloid deposition is progressively neurodegenerative (Whitehouse *et al*., 1981; Hardy and Seikoe, 2002; Goedert *et al*., 1991). Memory and cognitive functions in AD are impaired in cortical and hippocampal cholinergic systems areas (Araujo *et al*., 2005). Therefore, to develop the therapeutic agents for AD, many studies have been conducted on acetylcholinesterase (AChE) inhibitors donepezil and tacrine and cholinergic agonists carbachol using scopolamine-induced memory-impaired animals (Kotani *et al*., 2006; Tariot *et al*., 1996). Scopolamine, an anticholinergic drug, causes memory impairments, as well as the reduction of brain-derived neurotrophic factor (BDNF), which regulates memory plasticity and formation.

Soy (*Glycine max*., family Leguminosae), which contains phytic acid, isoflavones and saponins as bioactive ingredients (Kitagawa *et al*., 1976 and 1988), has been reported to show anti-cancer, memory-enhancing, anti-lipidemic, and estrogen-like effects (File *et al*., 2001; Kim *et al*., 2006; Liu *et al*., 2007; Pan *et al*., 2010). Of soybean constituents, isoflavones genistin and daidzin have been reported to exhibit phytoestrogenic, anti-inflammatory, and memory-enhancing effects. However, the memory-enhancing effects of soyasaponins, which are triterpenoid glycosides with one or two polysaccharide chains, have been studied.

In our preliminary study, soybean ethanol extract (SE) ameliorated scopolamine-induced memory impairment. Therefore, to confirm its ameliorating effect for memory impairments, we measured its effect in scopolamine-induced memory-impaired mice in passive avoidance, Y-maze and Morris water maze tasks.

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Experimental

Materials – Tacrine, scopolamine hydrobromide, phosphatase inhibitor cocktail, radioimmuno-precipitation assay (RIPA) buffer, and a protease inhibitor cocktail were purchased from Sigma (U.S.A.). BDNF, cAMP response element-binding protein (CREB), p-CREB, β-actin and secondary antibodies were purchased from Santa Cruz Biotechnology (U.S.A.).

Preparation of SE and its soyasaponin-rich fraction (SRF) from soybeans – Dried soybeans (1 kg), which were purchased in KyungDong Market (Seoul, Korea), were ground to a fine powder, defatted with hexane (10 L), dried (0.8 kg), extracted with 80% ethanol (3 L) twice and concentrated in vacuo [127 g; soy ethanol extract (SE) contained 1.6% soyasaponin Bb, of which contents were analyzed by HPLC]. The concentrated extract was suspended in water, extracted with n-butanol (15 L), evaporated in vacuo [20 g; soyasaponin-rich fraction (SRF) containing 2.0% soyasaponin Ab and 7.1% soyasaponin Bb]

Animals – Male ICR mice (20 - 24 g, 6 weeks) were supplied from the Orient Animal Breeding Center (Korea). All animals were housed in wire cages at 20 - 22 °C and 50 ± 10% humidity, fed standard laboratory mouse chow (Samyang Co., Korea) and water ad libitum. All experiments were performed in accordance with the NIH and Kyung Hee University guidelines for Laboratory Animals Care and Use and approved by the Committee for the Care and Use of Laboratory Animals in the College of Pharmacy, Kyung Hee University.

Passive avoidance task – The passive avoidance task was performed as previously described (Joh *et al*., 2012; Lee *et al*., 2009). SE (100 or 200 mg/kg, *p.o.),* SRF (50 or 100 mg/kg, *p.o.*), tacrine (10 mg/kg, *p.o.*), or vehicle (*p.o.*) were administered 1 h before the test was conducted at every consecutive day. Scopolamine (1 mg/kg, i.p.) was administered 30 min after these oral administrations.

Morris water maze task – The Morris water maze task was performed as previously described (Lee *et al*., 2009; Joh *et al*., 2012). SE (100 or 200 mg/kg, *p.o.*), SRF (50 or 100 mg/kg, *p.o.*), tacrine (10 mg/kg, *p.o.*), or vehicle (*p.o.*) were administered 1 h before the test was conducted at every consecutive day. Scopolamine (1 mg/kg, *i.p.*) was administered 30 min after these oral administrations.

Immunoblotting – Mice were sacrificed by cervical dislocation 30 minutes after the acquisition trial of the passive avoidance task. The hippocampi were then removed and homogenized in 200 μl of protease inhibitor cocktail-contained ice-cold RIPA buffer. After centrifugation for 15 min at 10,000 × g, supernatants were divided into Eppendorf tubes and stored at −80 °C until required for protein assay, which was performed using Bradford protein assay kits (Bio-Rad, USA). The expression levels of CREB, p-CREB, BDNF, and β-actin were measured by immunoblotting according to the previous method of Lee *et al*. (2009).

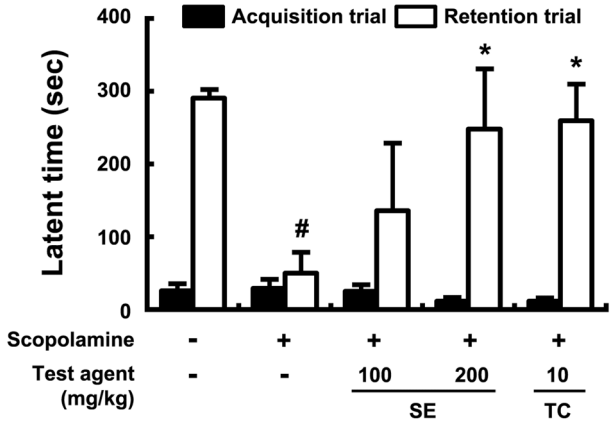

Fig. 1. Effect of SE in Mice against Scopolamine-induced Memory Impairment in Passive Avoidance Task. Memory impairment was induced by treatment with scopolamine (1 mg/kg *i.p.*). Normal control mice were treated with vehicle instead of scopolamine. Acquisition trials were carried out 30 min after a single scopolamine treatment. Test agents (NOR, none; SCO, scopolamine alone; SE100, 100 mg/kg SE with scopolamine; SE200, 200 mg/kg of SE with scopolamine; TC10, 10 mg/kg tacrine with scopolamine) were administered to mice 1 h before treatment with scopolamine. All values are expressed as mean ± S.D (n = 6). #Significantly different from normal mice ($p < 0.05$). *Significantly different from scopolamine-treated control ($p < 0.05$).

Statistics – All values are expressed as means ± SD. For the passive avoidance task, data was analyzed by a Kruskal-Wallis non-parametric ANOVA test. Statistical significance was set at $p < 0.05$.

Results and Discussion

When SE orally administered to mice memory-impaired by scopolamine, its ameliorating effect was evaluated in the passive avoidance task. SE reversed the lowered spontaneous alteration induced by scopolamine in mice (Fig. 1A). SE (200 mg/kg, *p.o.*) significantly reversed it to 85.3 % of normal control mice. During the acquisition trial, latencies between among tested groups were not significantly different. Furthermore, SE significantly shortened the escape latencies prolonged by scopolamine treatment in mice in the Morris water maze task (Fig. 2A). On the day after the last training session test, mean swimming time within quadrant section of the platform in mice treated with scopolamine alone was significantly

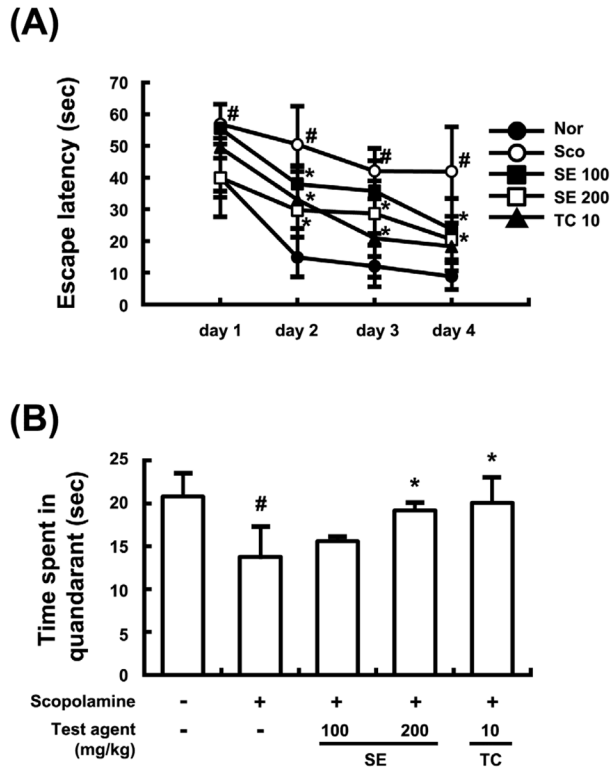

Fig. 2. Effect of SE on Scopolamine-induced Memory Impairment in Morris water maze Tasks. (A) Effect in escape latencies during training trial sessions. (B) Effect in swimming times spent in target quadrant. Acquisition trials were carried out 30 min after a single scopolamine treatment. Test agents (open circle, none; closed, scopolamine alone; open square, 100 mg/kg SE with scopolamine; closed square, 200 mg/kg of SE with scopolamine; closed triangle, 10 mg/kg tacrine with scopolamine) were administered to mice 1 h before treatment with scopolamine. Normal group was treated with vehicle instead of scopolamine and test agents. The training trial and the probe trial sessions were performed as described in MATERIALS AND METHODS. Values are expressed as mean ± S.D (n = 6). #Significantly different from normal mice ($p < 0.05$). *Significantly different from scopolamine-treated control ($p < 0.05$).

lower than in normal mice. SE increased the swimming time within the platform quadrant (Fig. 2 B). Next, we measured the inhibitory effect of SE against AChE in vitro. However, SE did not inhibit AChE (IC_{50}, > 0.2 mM), although tacrine potently inhibit it (IC_{50} = 0.12 μM). We also measured the effect of SE on BDNF expression and CREB phosphorylation in hippocampi of memory-impaired mice induced with scopolamine (Fig. 3). Scopolamine significantly inhibited BDNF expression and CREB phosphorylation, but when scopolamine with SE was given, BDNF expression and CREB phosphorylation were significantly increased. No significant difference in β-actin expression was found.

Of the constituents of soybeans, isoflavones alleviate β-

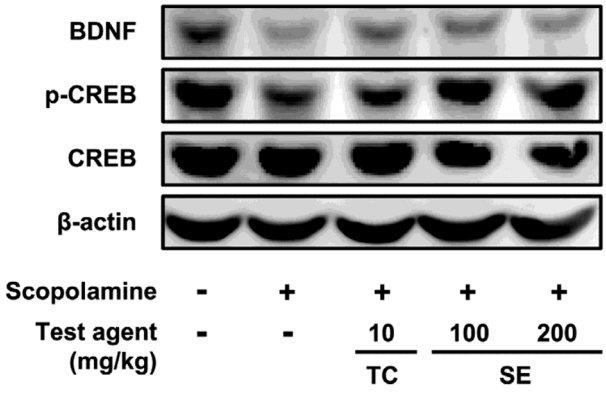

Fig. 3. Effect of SE on BDNF Expression and CREB Phosphorylation in the Hippocampi of Scopolamine-treated Mice. SE (100 or 200 mg/kg, *p.o.*) or tacrine (TC, 10 mg/kg, *p.o.*) was orally administered to mice at 1 h before treatment with scopolamine. Normal control group was treated with vehicle instead of scopolamine and test agents. BDNF, CREB, p-CREB and β-Actin were measured by immunoblotting.

amyloid 1-42 induced learning and memory impairment by down regulation of Toll-like receptor 4 expression (Ding *et al*., 2011) and nuclear factor-κB activity in rats and lecithin also improve memory impairment in aged rats (Su *et al*., 2010; Suzuki *et al*., 2001). However, the ameliorating effect of its saponins has not been reported.

Therefore, to understand the possible role of soysaponins in memory impairments, we prepared SRF and measured its protective effect against in the passive avoidance and Morris water maze tasks (Fig. 4). SRF significantly protected scopolamine-induced memory deficit in the passive avoidance task (Fig. 4 A). During the acquisition trial test, no latency differences were observed among tested groups. SRF (50 and 100 mg/kg, *p.o.*) also significantly shortened the escape latencies prolonged by scopolamine treatment in mice in the Morris water maze task (Fig. 4 B). The memory impairment-ameliorating effect of SRF was more effective than that of SE.

SE and SRF significantly ameliorated scopolamine-induced memory deficit in mice in passive avoidance and Morris water maze tests. Of them, SRF reversed memory deficit more potently than SE. Although SE did not inhibit AChE, SE potently inhibited the reduction of CREB phosphorylation and BDNF expression by scopolamine. BDNF influences neuronal synaptic plasticity by rapidly depolarizing neurons, as glutamate stimulates neuronal synaptic plasticity by activating tyrosine kinase receptors, enhancing glutamatergic synaptic transmission, increasing phosphorylation of the subunits of N-methyl-*D*-aspartate receptors in the hippocampus, facilitating hippocampal LTP (Figurov *et al*., 1996; Hock *et al*., 2000). Particularly, BDNF expression in the hippocampus

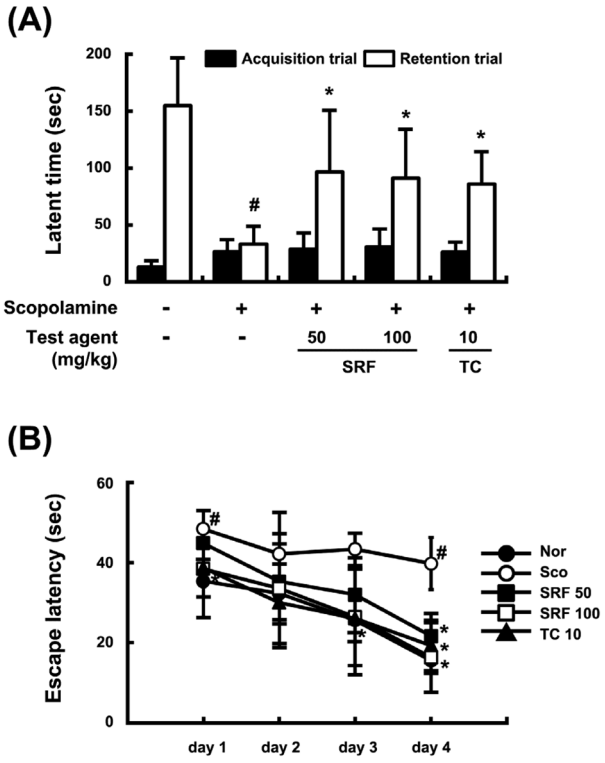

Fig. 4. Effect of SRF on Scopolamine-induced Memory Impairment in Mice in the Passive Avoidance and Morris Water Maze Tasks. (A) Effect in passive avoidance task. (B) Effect in Morris water maze task. Test agents (NOR [open circle], none; SCO [closed circle], scopolamine alone; SRF50 [open square], 50 mg/kg SRF with scopolamine; SRF100 [closed square], 100 mg/kg of SE with scopolamine; TC10 [closed triangle], 10 mg/kg tacrine with scopolamine) were administered to mice 1 h before treatment with scopolamine. Passive avoidance and Morris water maze tasks were performed as described in MATERIALS AND METHODS. Values are expressed means ± S.D (n = 6). #Significantly different from normal mice ($p < 0.05$). *Significantly different from scopolamine-treated control ($p < 0.05$).

and entorhinal cortex in AD is lower than in healthy mans. Of transcription factors for BDNF expression, CREB regulates the expression of tyrosine hydroxylase, BDNF, and many neuropeptides as a cellular transcription factor (Bourtchuladze *et al*., 1994; Pugazhenthi *et al*., 2011; Levine *et al*., 1995). Therefore, in many studies, it was suggested that the increase of BDNF expression and CREB phosphorylation may possess therapeutic potential for patients that have AD (Nagahar *et al*., 2000; Bejar *et al*., 1999). Orally administered SE, particularly SRF, may protect memory impairment as well as the reduction of BDNF expression and CREB phosphorylation by scopolamine. At least, memory impairment-ameliorating effect of soybean may be dependent on isoflavone, lecithin and soyasaponins.

Based on these results, ethanol extract of soybean ameliorates scopolamine-induced memory deficit by increasing BDNF expression and CREB phosphorylation.

References

Araujo, J.A., Studzinski, C.M., and Milgram, N.W., Further evidence for the cholinergic hypothesis of aging and dementia from the canine model of aging. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **29**, 411-422 (2005).

Bejar, C., Wang, R. H., and Weinstock, M., Effect of rivastigmine on scopolamine-induced memory impairment in rats. *Eur. J. Pharmacol*., **383**, 231-240 (1999).

Bourtchuladze, R., Frenguelli, B., Blendy, J., Cioffi, D., Schutz, G., and Silva, A. J., Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. *Cell*, **79**, 59-68 (1994).

Ding, B.J., Ma, W.W., He, L.L., Zhou, X., Yuan, L.H., Yu, H.L., Feng, J.F., and Xiao, R., Soybean isoflavone alleviates β-amyloid 1-42 induced inflammatory response to improve ability by down regulation of Toll-like receptor 4 expression and nuclear factor-κB activity in rats. *Int. J. Dev. Neurosci*., **29**, 537-542 (2011).

Figurov, A., Pozzo-Miller, L.D., Olafsson, P., Wang, T., and Lu, B., Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature,* **381**, 706-709 (1996).

File, S.E., Jarrett, N., Fluck, E., Duffy, R., Casey, K., and Wiseman, H., Eating soya improves human memory. *Psychopharmacology,* **157**, 430-436 (2001).

Goedert, M., Spillantini, M.G., and Crowther, R.A., Tau proteins and neurofibrillary degeneration. *Brain Pathol*., **1**, 279-286 (1991).

Hardy, J. and Selkoe, D.J., The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science*, **297**, 353-356 (2002).

Hock, C., Heese, K., Hulette, C., Rosenberg, C., and Otten, U., Region-specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas. *Arch. Neurol.,* **57**, 846-851 (2000).

Joh, E.H., Yang ,J.W., and Kim, D.H., Gypenoside LXXIV ameliorates scopolamine-induced learning impairment in mice. *Planta Med.,* **76**, 793-795 (2010).

Kato-Kataoka, A., Sakai, M., Ebina, R., Nonaka, C., Asano, T., and Miyamori, T., Soybean-derived phosphatidylserine improves memory function of the elderly Japanese subjects with memory complaints. *J. Clin. Biochem. Nutr*., **47**, 246-255 (2010).

Kim, S.L., Berhow, M.A., Kim, J.T., Chi, H.Y., Lee, S.J., and Chung, I.M., valuation of soyasaponin, isoflavone, protein, lipid, and free sugar accumulation in developing soybean seeds. *J. Agric. Food Chem.,* **54**, 10003-10010 (2006).

Kitagawa, I., Taniyama, T., Nagahama, Y., Okubo, K., Yamauchi, F., and Yoshikawa, M., Structure of acetyl-soyasaponin A_1, A_2 and A_3, astringent partially acetylated bisdesmoside of soyasapogenol A form american soybean, the seed of Glycine max MERRILL. *Chem. Pharm. Bull.,* **36**, 2819-2828 (1988).

Kitagawa, I., Yoshikawa, M., and Yosioka, I., Saponin and sapogenol. XIII. structures of three soybean saponins : soyasaponin I, soyasaponin II, and soyasaponin III. *Chem. Pharm. Bull*. **24**, 121-129 (1976).

Kotani, S., Yamauchi, T., Teramoto, T., and Ogura, H., Pharmacological evidence of cholinergic involvement in adult hippocampal neurogenesis in rats. *Neuroscience,* **142**, 505-514 (2006).

Lee, B., Jung, K., and Kim, D.H., Timosaponin AIII, a saponin isolated from Anemarrhena asphodeloides, ameliorates learning and memory

impairments in mice. *Pharmacol. Biochem. Behav.,* **93**, 121-127 (2009).

Levine, E.S., Dreyfus, C.F., Black, I.B., and Plummer, M.R., Brain-derived neurotrophic factor rapidly enhances synaptic transmission in hippocampal neurons via postsynaptic tyrosine kinase receptors. *Proc. Natl. Acad. Sci. U.S.A.,* **92**, 8074-8077 (1995).

Liu, Y.Q., Xin, T.R., Lü, X.Y., Ji, Q., Jin, Y., and Yang, H.D., Memory performance of hypercholesterolemic mice in response to treatment with soy isoflavones. *Neurosci. Res.,* **57**, 544-549 (2007).

Nagahara, A.H., Merrill, D.A., Coppola, G., Tsukada, S., Schroeder, B.E., Shaked, G.M., Wang, L., Blesch, A., Kim, A., Conner, J.M., Rockenstein, E., Chao, M.V., Koo, E.H., Geschwind, D., Masliah, E., Chiba, A.A., and Tuszynski, M.H., Neuroprotective effects of brain-derived neurotrophic factor in rodent and primate models of Alzheimer's disease. *Nat. Med.,* **15**, 331-337 (2009).

Pan, M., Li, Z., Yeung, V., and Xu, R.J., Dietary supplementation of soy germ phytoestrogens or estradiol improves spatial memory performance and increases gene expression of BDNF, TrkB receptor and synaptic factors in ovariectomized rats. *Nutr. Metab.,* **7**, 75 (2010).

Pugazhenthi, S., Wang, M., Pham, S., Sze, C.I., and Eckman, C.B., Downregulation of CREB expression in Alzheimer's brain and in Aβ-treated rat hippocampal neurons. *Mol Neurodegener.,* **6**, 60 (2011).

Su, Y. F., Mei, L.J., Liu, B., and Yun, Z.Y., Effect of milk powder adding phosphatidylcholine from soybean lecithin on the memory of rats. *China Dairy Industry*, **1**, 29-30 (2011).

Suzuki, S., Yamatoya, H., Sakai, M., Kataoka, A., Furushiro, M., and Kudo, S., Oral administration of soybean lecithin transphosphatidylated phosphatidylserine improves memory impairment in aged rats. *J. Nutr*., **131**, 2951-2956 (2001).

Tariot, P.N., Patel, S.V., Cox, C., and Henderson, R.E., Age-related decline in central cholinergic function demonstrated with scopolamine. *Psychopharmacology* **125**, 50-56 (1996).

Whitehouse, P.J., Price, D.L., Clark, A.W., Coyle, J.T., DeLong, M.R., Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol.,* **10**, 122-126 (1981).

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