Supplementary Materials

Supplementary Methods

Osmotin purification

Osmotin was purified to apparent homogeneity (≥ 99 %) from NaCl-adapted cultured cells of *Nicotiana tabacum* cv. Wisconsin 38 as described[84] and then further purified by repeated recrystallization. The endotoxin content of osmotin was <0.03 EU/mg protein as measured with an endotoxin estimation kit (LAL assay kit, GenScript).

Materials

The fluorescent carboxyl magnetic particles, Nile Red, 1% w/v, were purchased from Spherotech. The nanoparticle size was 0.20-0.39 μm in diameter. $A\beta_{1-42}$ peptides were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The Magnetic Fluid-Carboxyl (Catalog Number, MF-COO-0090) with a mean diameter of 94.00 ± 22.85 nm were purchased from MagQu Co., Ltd. Taiwan. The magnetic beads consist of single-crystal Fe_3O_4 magnetic sphere core and being coated with dextran. Osmotin was purified with some modification as previously described.[84]

Transmission electron microscopy (TEM)

The nanoparticle surface morphology was examined using transmission electron microscopy (Tecnai-12, 200 kV) and negative staining with an aqueous solution of 10% uranyl acetate.

MTT assay

The in vitro cytotoxicity of the magnetic nanoparticles was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Four different

concentrations of the sample were tested in a normal HT-22 cell line and normal SH-SY5Y cells. The cells were seeded in 96-well plates with 1×10^4 cells/well and incubated with increasing concentrations of nanoparticles and osmotin ranging from 50 to 200 μg/mL incubated at 37°C for 24 h. The cells treated with the growth medium served as control. The cytotoxicity of the cells was measured as described previously.[85]

Animals

Male wild type C57BL/6J mice (25–30 g, 8 weeks old) were purchased from Jackson Laboratory (Bar Harbor, ME, U.S.A). The mice were acclimatized for 1 week in the university animal house under a 12-h/12-h light/dark cycle at 23 °C with 60 ± 10% humidity and provided with food and water ad libitum. The mice maintenance and treatment were carried out in accordance with the animal ethics committee (IACUC) guidelines issued by the Division of Applied Life Sciences, Department of Biology at Gyeongsang National University, South Korea. All efforts were made to minimize the number of mice used and their suffering. The experimental methods with mice were carried out in accordance with the approved guidelines (Approval ID: 125) and all experimental protocol were approved by the animal ethics committee (IACUC) of the Division of Applied Life Sciences, Department of Biology at Gyeongsang National University, South Korea.

Evaluation of BBB integrity in normal mice by Evans blue

The BBB permeability was assessed by measuring the extravasation of intravenously injected Evans blue.[86] Briefly, 20 μL of a 2% solution of Evans blue in saline was injected intravenously into the tail vein after MNP administration and exposure to the functionalized magnetic field and allowed to circulate for 60 minutes prior to sacrifice. After perfusion with 100 ml of saline via a catheter inserted into the left ventricle of the heart, the brains were immediately removed and

then homogenized in 1 mL of 50% trichloroacetic acid in distilled H_20. Thereafter, the samples were centrifuged for 20 minutes at 10,000 rpm, and the supernatants were removed and diluted 1:4 in 100% ethanol. After vigorous mixing, an aliquot of each sample was diluted three-fold in a solution of 50% trichloroacetic acid/l00% ethanol. For fluorescence measurement, a Perkin-Elmer LS-5 fluorospectrophotometer (Norwalk, CT, U.S.A.) was used at an excitation wavelength of 620 nm and an emission wavelength of 680 nm. A serial dilution of 2% stock Evans blue was taken as a standard curve, which was linear from 25 to 500 ng/mL. The concentrations of the samples were normalized against the brain tissue weight and calculated as nanograms of Evans blue per gram of brain tissue.

Morris water maze (MWM) test

Behavioral study was performed on mice using MWM and Y-maze test. Experimental apparatus consisted of a circular water tank (100 cm in diameter, 40 cm in height), containing water (23 ± 1 °C) to a depth of 15.5 cm, which was made opaque by adding white ink. A transparent escape platform (10 cm in diameter, 20 cm in height) was hidden 1 cm below the water surface and placed at the midpoint of one quadrant. Each mouse received training per day for 5 consecutive days using a single hidden platform in one quadrant with three quadrants of rotational starting. Latency to escape from the water maze (finding the hidden escape platform) was calculated for each trial. After 24 hr of 5th day the probe test was performed for the evaluation of memory consolidation. The probe test was carried out by removing platform and allowing each mouse to swim freely for 60 sec. The number of crossing over platform place (where the platform was located during hidden platform training) was measured. All data were recorded using video-tracking software (SMART, Panlab Harward Apparatus, Bioscience Company, Holliston, MA, USA).

Y-maze test

The Y-maze was made of black-painted wood. Each arm of the maze was 50 cm long, 20 cm high and 10 cm wide at the bottom and 10 cm wide at the top. Each mouse was placed at the center of the apparatus and allowed to move freely through the maze for three 8-min sessions. The series of arm entries was visually observed. Spontaneous alteration was defined as the successive entry of the mice into the three arms in overlapping triplet sets. Alteration behavior percentage (%) was calculated as [successive triplet sets (entries into three different arms consecutively) / total number of arm entries-2] x 100. A higher percentage of spontaneous alteration behavior was considered to be enhanced cognitive performance.

Protein extraction from mouse brain

Brain tissues from all the above-mentioned groups in table 1 were collected after FMNP administration and exposure to the FMF. In the second part of our study that is in the case of MNP-OSM treatment to the $A\beta_{1-42}$ treated mice, after behavioral analysis in the 40 days post-injection $A\beta_{1-42}$ mice, the mice were killed without anesthesia to avoid hypothermic condition which triggered tau hyper phosphorylation.[87] The brains were immediately removed and hippocampus was dissected carefully and the tissues were frozen on dry ice and stored at − 80 °C. The hippocampal tissue were homogenised in 0.01 M phosphate buffered saline (PBS) with phosphase inhibitor and protease inhibitor cocktail. The samples were then centrifuged at 10,000 Xg at 4 °C for 25 minutes. The supernatants were collected and stored at − 80 °C.

Western blot analysis

Western blot analysis was performed as previously described[10] with some modifications. The protein concentration was measured (Bio-Rad protein assay kit, Bio-Rad Laboratories, CA,

U.S.A). Equal amounts of protein (15–30 μg) were electrophoresis using 4–12% BoltTM Mini Gels (Novex; Life Technologies, Kiryat Shmona, Israel). The membranes were blocked in 5% (w/v) skim milk to reduce nonspecific binding and incubated with primary antibodies overnight at 4°C with a 1:1000 dilution. After reaction with a horseradish peroxidase-conjugated secondary antibody, as appropriate, the proteins were detected using an ECL detection reagent according to the manufacturer's instructions (Amersham Pharmecia Biotech, Uppsala, Sweden). The X-ray films were scanned, and the optical densities of the bands were analyzed through densitometry using the computer-based Sigma Gel program, version 1.0 (SPSS Inc., Chicago, IL, USA).

Antibodies

The following primary antibodies were used. The primary antibodies Aβ, BACE-1, p-Tau, poly (ADP-ribose) polymerase-1(PARP-1) were purchased from Santa Cruz Biotechnology, CA, USA. Primary antibodies caspase-3, synaptophysin, PSD-95, and β-actin were from Cell Signaling Biotechnology Beverly, MA, USA. The secondary antibodies used in our experiments were goat anti-mouse IgG, goat anti-rabbit IgG, and rabbit anti-goat IgG, were purchased from Santa Cruz Biotechnology CA, USA.

Tissue collection and sample preparation

For the tissue analysis the mice were perfused transcardially with 4% ice-cold paraformaldehyde, and the brains were post-fixed for 72 hr in 4% paraformaldehyde and transferred to 20% sucrose for 72 hr. The brains were frozen in O.C.T compound (A.O, USA), and 14-μm coronal sections were cut using a CM 3050C cryostat (Leica, Germany). The sections were thaw-mounted on probe-on plus charged slides (Fisher, Rockford, IL,USA).

Immunofluorescence staining

The slides were washed twice for 15 minutes in 0.01 M PBS, followed by blocking for 1 hr in 5% normal goat or bovine serum and 0.3% Triton X-100 in PBS. Primary antibodies (Aβ and PSD95) diluted 1:100 in PBS containing 2 % (v/v) normal serum and 0.1 % (v/v) Triton X-100 were applied at 4 °C overnight. The slides were rinsed twice with PBS and FITC or TRITC (anti-rabbit or anti-mouse) secondary antibodies diluted 1:50 in PBS were applied at room temperature for 90 min. Tissue slides were washed twice with PBS and 4′,6-diamidino-2-phenylindole (DAPI) nucleus counterstaining solution was applied for 10 min. Slides were rinsed with PBS and glass cover slips were mounted on glass slides with fluorescent mounting medium (Dako 53023). The Aβ (red), PSD95 (green) and DAPI (blue) staining patterns were examined using a confocal laser-scanning microscope (Flouview FV 1000, Olympus, Japan). The integrated density was evaluated using Image J software.

Thioflavin S staining

The sections were washed twice for 10 minutes in 0.01 M PBS, then immersed in a Coplin jar containing fresh 1% thioflavin S (Sigma Chemical Co., St. Louis, MO, USA), and stained at room temperature for 10 min. Sections were incubated into 70% ethanol for 5 min, rinsed 2 times in water, and glass coverslips were mounted with propidium iodide (PI) (Invitrogen, Carlsbad, CA, USA). Strong green fluorescence of thioflavin S was observed on confocal laser-scanning microscope. For quantitative analysis, a percentage of plaque area/number of plaques was calculated by using the ImageJ analysis program.

Nissl staining

Nissl staining was used for the histological examination and measurement of neuronal loss. The slides with 14-μm sections were washed twice for 15 minutes in 0.01 M PBS and stained with a 0.5% cresyl violet solution (containing a few drops of glacial acetic acid) for 10–15 min. The sections were washed with distilled water, dehydrated in graded ethanol (70%, 95%, and 100%), placed in Xylene and coverslipped using mounting medium.