

Apomorphine Treatment in Alzheimer Mice Promoting Amyloid- β Degradation

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Objective: Intracellular amyloid β -protein (A β) contributes to neurodegeneration in Alzheimer disease (AD). Apomorphine (APO) is a dopamine receptor agonist for Parkinson disease and also protects against oxidative stress. Efficacy of APO for an AD mouse model and effects of APO on cell cultures are studied.

Methods: The triple transgenic AD mouse model (3xTg-AD) has 2 familial AD-related gene mutations (*APP*_{KM670/671NL}/*PS1*_{M146V}) and a *tau* gene mutation (*Tau*_{P301L}). Six-month-old 3xTg-AD mice were treated with subcutaneous injections of APO once a week for 1 month. Memory function was evaluated by Morris water maze before and after the treatment. Brain tissues were examined by immunohistochemical staining and Western blotting. Effects of APO on intracellular A β degradation, activity of A β -degrading enzymes, and protection against oxidative stress were studied in cultured SH-SY5Y cells.

Results: After APO treatment, short-term memory function was dramatically improved. Significant decreases in the levels of intraneuronal A β , hyper-phosphorylated tau (p-tau), p53, and heme oxygenase-1 proteins were observed. Moreover, APO promoted degradation of intracellular A β , increased activity of proteasome and insulin-degrading enzyme, protected against H₂O₂ toxicity, and decreased p53 protein levels in the cultured cells.

Interpretation: 3xTg-AD mice show intraneuronal A β accumulation and memory disturbances before extracellular A β deposition. Our data demonstrating improvement of memory function of 3xTg-AD mice with decreases in intraneuronal A β and p-tau levels by APO treatment strongly suggest that intraneuronal A β is an important therapeutic target and APO will be a novel drug for AD.

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Alzheimer disease (AD) is a devastating disease characterized by disturbances of memory and other cognitive functions in elderly people. There are 2 major hallmarks of AD, neurofibrillary tangles (NFTs) and senile plaques (SPs). NFTs consist of hyperphosphorylated tau protein (p-tau), whereas SPs consist of the 4kD amyloid β -protein (A β). A β aggregation may be an early pathogenic event in AD, and A β ending at residue 42 (A β 42) is a highly aggregative species that deposits in SPs (amyloid cascade theory).¹ Increases in the levels of A β oligomers and synaptic impairment are thought to play a pivotal role in AD pathogenesis.² Recent clinical trials of

Ginkgo biloba, nonsteroidal anti-inflammatory drugs, phenserine, statins, tarenfluril, tramiprosate, and xaliproden in AD patients have not demonstrated clear efficacy in phase 3 trials, although many other compounds are still under study in phase 2.³ Also, long-term clinical efficacy of A β immunization has not been observed despite certain improvements in pathology.⁴ Many of these therapies target extracellular A β fibrils, and new therapeutic strategies for AD are still under development. Some previous reports have shown that, in AD patients and mouse models, A β 42 accumulation in neurons precedes extracellular A β deposition.^{5–8} Thus, intraneuronal

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A β 42 accumulation may represent an important therapeutic target for the treatment of AD.⁹

Apomorphine (APO) is a nonspecific dopamine agonist for Parkinson disease (PD),¹⁰ and is also used for erectile dysfunction (ED).¹¹ Some previous reports suggest that APO may protect neurons from oxidative stress in PD mouse models and from brain infarction in a gerbil stroke model.^{12–16} The antioxidative stress mechanism of APO may be mediated by the NF-E2-related factor-2/antioxidant response element pathway.¹⁷ Oxidative stress is a major pathogenic factor in AD, and antioxidative stress drugs may be beneficial for AD patients.¹⁸ In addition, APO inhibits fibril formation by A β 40 *in vitro*.¹⁹ Thus, we tested the efficacy of APO in an AD mouse model. The triple transgenic AD (3xTg-AD) mouse is a familial AD (FAD) mouse model that has 2 FAD-related gene mutations (*APP_{KM670/671NL/PS1_{M146V}}*) and a *tau* gene mutation (*Tau_{P301L}*).²⁰ In homozygous 3xTg-AD mice, A β 42 accumulation in neurons and cognitive impairment begin at around 4 months of age, and decreases in the levels of intraneuronal A β 42 improve memory function.²¹ Thus, 3xTg-AD mice represent an appropriate model with intraneuronal A β pathology in which to test the efficacy of various candidate drugs for AD.^{22–25} We observed a dramatic improvement in memory function and decreases in A β and p-tau pathology in 3xTg-AD mice treated with APO injections.

Materials and Methods

Mice and APO Injection

We used hemizygous and homozygous 3xTg-AD mice, and nontransgenic (non-Tg) mice that had the same genetic background as 3xTg-AD mice.²¹ All mice were kept on a 12-hour light and 12-hour dark schedule. All experiments were approved by the ethical committee of Kyushu University. Apomorphine hydrochloride (Sigma, St. Louis, MO) was dissolved in saline, and was subcutaneously injected once a week, 5 \times , at concentrations of 5 or 10mg/kg, according to previous reports of APO injection into PD model mice.^{12,14} Injection with saline only was used as a control.

Morris Water Maze Analysis

At 6 months of age, before APO treatment, the learning and short-term memory functions were analyzed in a Morris water maze (MWM) using a DV-Track Video Tracking System (Muromachi Kikai, Tokyo, Japan). On the day after the last injection, a second evaluation of MWM test was performed. The MWM analysis was similar to those described in a previous report.²¹ A circular tank (90cm diameter) was filled with water at 24°C. Mice

were trained to swim to a 10cm-diameter circular clear platform that was submerged 1.5cm beneath the surface. The platform location was selected randomly for each mouse, but was kept constant throughout training. In each trial, the mouse was placed into the tank at 1 of 4 designated start points. If a mouse failed to find the platform within 60 seconds, it was manually guided to the platform and remained there for 10 seconds. An overhead camera recorded the swimming paths. Mice underwent 4 trials a day for as many days as were required to satisfy 1 of the following criteria: <20-second mean escape latency for homozygote 3xTg-AD mice; <10-second mean escape latency for hemizygous 3xTg-AD and non-Tg mice. To evaluate the retention of spatial memory, a probe trial of a 60-second swim starting on the opposite side of the pool (without the platform) was performed 24 hours (homozygous 3xTg-AD) or 48 hours (hemizygous 3xTg-AD and non-Tg) after each criterion was satisfied. The parameters measured by the probe trial were initial latency to cross the platform location (seconds), number of platform location crosses (n), and time spent in the quadrant containing the platform location (%).

Immunohistochemistry

After the last MWM analysis, mice were fixed by perfusion with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS). Brain tissues were immersed in 4% PFA in PBS at 4°C over 24 hours, followed by freezing and preparation of 16 μ m-thick sections. Anti-A β 17-24 (1:1,000, 4G8; Millipore, Bedford, MA), anti-p-tau (1:200, AT8, AT180; Pierce, Rockford, IL), anti-p53 (1:100; Santa Cruz Biotechnology, Santa Cruz, CA), and anti-heme oxygenase-1 (HO-1) (1:500; Stressgen Biotechnologies, Victoria, Canada) antibodies were used. Autoclave pretreatment was employed for intracellular A β .²⁶ Antigens were detected using the Mouse to Mouse HRP (DAB) Staining System (ScyTek Laboratories, Logan, UT). Cultured cells on coverslips were fixed with 4% formaldehyde and were incubated with 4G8 (1:1,000) or anti- β -tubulin antibody (1:500, Sigma), followed by detection using the DAB substrate kit (Vector Laboratories, Burlingame, CA) or secondary antibody conjugated with green fluorescence (Invitrogen, Camarillo, CA).

Immunoprecipitation and Western Blotting Analysis

A β was first immunoprecipitated with anti-A β 42 (BC-05) or anti-A β 40 (BA-27).^{26,27} Fifteen micrograms of protein from brain tissues was dissolved in radioimmunoprecipitation assay buffer (10mM Tris, pH 8.0, 150mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 5mM ethylenediaminetetraacetic acid) containing the protease inhibitor

mixture Complete Mini (Roche Applied Science, Tokyo, Japan), and mixed with 2 μ g of BC-05 and 25 μ l of Protein G-coupled Dynabeads (Invitrogen) in a 1.5ml tube. After rotation at 4°C overnight, A β was eluted and analyzed by Western blotting (WB) with anti-A β 1-16, 6E10 (1:1,000; Covance/Signet, Berkeley, CA).²⁸ A similar method was used for analysis of the conditioned medium and PBS extract. For other WB analysis, brain tissues and cultured cells were lysed in 2% SDS.²⁸ After blotting, the polyvinylidene difluoride membrane (Millipore) was blocked with 5% skim milk in TBST (25mM Tris-HCl, pH 7.6, 150mM NaCl, 0.1% Tween-20) for 1 hour, and was incubated with AT8 (1:3,000), AT180 (1:3,000), anti-APP C-terminal (1:1,000, Invitrogen), anti-cleaved Notch1 (1:1,000, Val1744; Cell Signaling Technology, Danvers, MA), anti-p53 (1:500), anti-HO-1 (1:1,000), or anti- β -actin (1:4,000, Sigma) antibodies. We used appropriate secondary antibodies conjugated to horseradish peroxidase (Pierce) or Can Get Signal (Toyobo, Osaka, Japan), and the ECL Western Blotting System (Amersham Bioscience, Piscataway, NJ) or Supersignal West Dura Extended Duration Substrate (Pierce). The band density was measured using the ChemiDoc XRS system (Bio-Rad, Hercules, CA) and corrected by the β -actin band.

Intracellular Accumulation of A β Peptides

Synthetic A β 40 or A β 42 peptide (Bachem, Budendorf, Switzerland) was accumulated in the cytosol using the Influx Pinocytotic Cell-loading Reagent (Invitrogen). Cells were exposed to hypertonic medium containing A β peptides, which were carried into the cells via pinocytotic vesicles. Replacement of medium with hypotonic medium induced release of A β peptides into the cytosol. Cells were treated with APO and a proteasome inhibitor, MG132 (Enzo Life Sciences, Plymouth Meeting, PA) 2 hours before loading of A β .

Activity Assay of 20S Proteasome, Insulin-Degrading Enzyme (IDE) and Nephilysin

Activity of intracellular proteasome and insulin-degrading enzyme (IDE) was measured using the 20S Proteasome Assay Kit (Cayman Chemical, Ann Arbor, MI) and the InnoZyme Insulysin/IDE Immunocapture Activity Assay Kit (Calbiochem, San Diego, CA), respectively. Activity of nephilysin in the membrane was measured according to a previous report.²⁹ Fluorescence of the specific substrates was measured at 360nm (ex)/480nm (em) (proteasome), at 320nm/405nm (IDE) and at 390nm/460nm (nephilysin).

Cell Cultures and Cell Viability Assay

A human neuroblastoma cell line SH-SY5Y was used. Details of the APP-transfected cells have been described previously.³⁰ Primary neuronal cultures were prepared

from embryonic day 15 mouse brains and maintained in serum-free Dulbecco modified Eagle medium containing Neurobasal-A+B27 (Gibco BRL, Rockville, MD) for 3 to 5 days.^{9,27} Cultured cells were treated with H₂O₂ and APO for 24 hours. Cell viability was assessed using a CellTiter-Blue Fluorometric Viability Assay kit (Promega, Madison, WI) as described in our previous report.³¹

Statistical Analysis

All data obtained from MWM and WB, activity of enzymes, and cell viability were expressed as means \pm standard error of the mean, and were analyzed by StatView Software version 5.0 (SAS Institute, San Francisco, CA). Differences between 2 groups were analyzed using an unpaired 2-tailed Student *t* test (MWM, activity of enzymes, cell viability), and differences among three groups (WB) were analyzed by the Kruskal-Wallis test followed by the Mann-Whitney *U* test. Values of *p* < 0.05 are considered statistically significant.

Results

We first evaluated the short-term memory function in the same 3xTg-AD mice by MWM analysis before and after treatment. In hemizygous 3xTg-AD mice, the latency to the platform after 3 days of training was decreased significantly (*p* < 0.05) on the first day after 1 month of APO treatment compared with pretreatment values (Fig 1). In hemizygous 3xTg-AD mice, probe trials revealed a significant decrease in latency (*p* < 0.05), a significant increase in crossing counts (*p* < 0.05), and a significant increase in the percentage of time spent in the quadrant containing the platform (*p* < 0.05) after 5mg/kg APO treatment. In homozygous 3xTg-AD mice, the latency to the platform on the first day of training was longer than that in hemizygous 3xTg-AD mice before treatment. After 1 month of APO treatment, a significant decrease in latency to the platform was observed relative to the pretreatment values. In homozygous 3xTg-AD mice, probe trials revealed a significant decrease in latency (*p* < 0.05), a significant increase in crossing counts (*p* < 0.01), and a significant increase in percentage of time spent in the quadrant of the platform location (*p* < 0.01) after APO treatments. Interestingly, APO efficacy appeared to be more significant in the 5mg/kg APO treatment group than in 10mg/kg APO treatment group. Figure 2 shows representative swimming tracks from the 60-second probe trials of 5 homozygous 3xTg-AD mice that responded well to APO treatment. Untreated 3xTg-AD mice and 3xTg-AD mice injected with pramipexole (1mg/kg)³² in the same manner showed no significant improvement in memory function (n = 8, data not

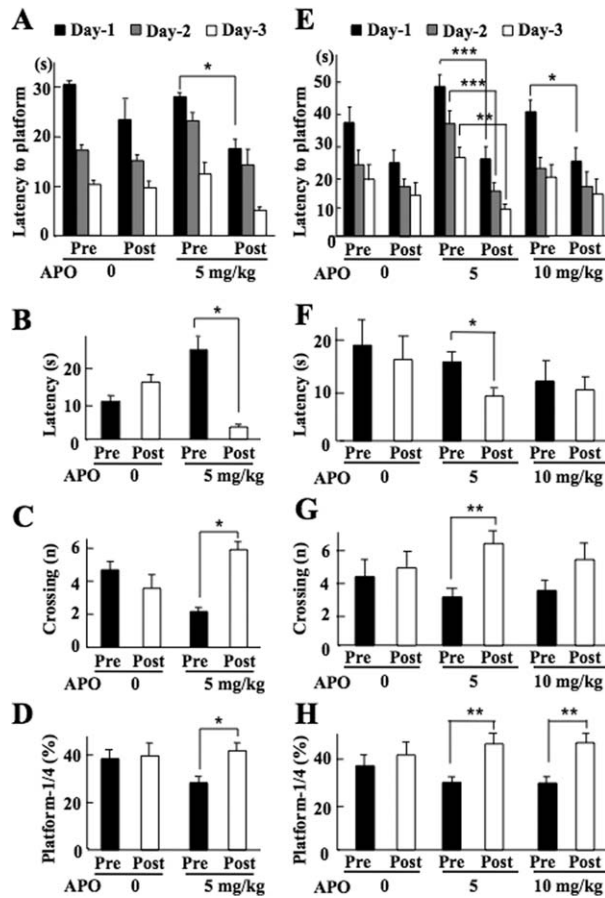


FIGURE 1: Effects of apomorphine (APO) on memory function in 3xTg-AD mice in Morris water maze tests. Latency to reach the platform during 3 days of training of hemizygous (A) and homozygous (E) mice was significantly improved by APO treatment. In 48-hour or 24-hour probe tests of hemizygous (B–D) and homozygous (F, G) mice, latency to the platform location (B, F), crossing counts of the platform location (C, G), and percentage of time spent in the quadrant of the platform location (D, H) were significantly improved by APO treatment. Pre = pre-treatment; Post = post-treatment; n = 4 (A–D) and 8 (E–H); *p < 0.05, **p < 0.01, ***p < 0.001.

shown). Six-month-old non-Tg mice showed better memory functions than 3xTg-AD mice, and showed no significant alteration in memory function after 5mg/kg APO treatment (n = 8, data not shown).

Immunostaining demonstrated almost complete disappearance of intraneuronal Aβ immunoreactivity in hemizygous 3xTg-AD mice and marked decrease of intraneuronal Aβ immunoreactivity in homozygote 3xTg-AD mice after treatment with APO compared with untreated mice (Fig 3). No apparent difference in the numbers of neurons in the same cortical areas was observed between treated mice and untreated mice (data not shown). Immunoprecipitation/WB detected Aβ42 in brain tissues of untreated mice but not in those of treated mice. We then checked the PBS extract of brain tissues. Levels of soluble

Aβ40 were not altered by APO treatment, and Aβ42 was not detected (data not shown). Next, immunostaining with AT8 and AT180 revealed decreases in p-tau levels in treated mice compared with untreated mice (Fig 4). The axons of neurons in the hippocampus were stained intensely by AT180. WB analysis with AT8 and AT180 showed decreases in p-tau levels in brain tissues of treated mice, and these were statistically significant (p < 0.01).

To investigate the mechanism by which APO decreases intracellular Aβ levels, we examined the effects of APO on APP expression and Aβ generation in cultured cells. Using APP-transfected SH-SY5Y cells,^{28,30} we found no apparent alteration in levels of APP or secreted Aβ40/42 24 hours after 10μM APO treatment (Fig 5).

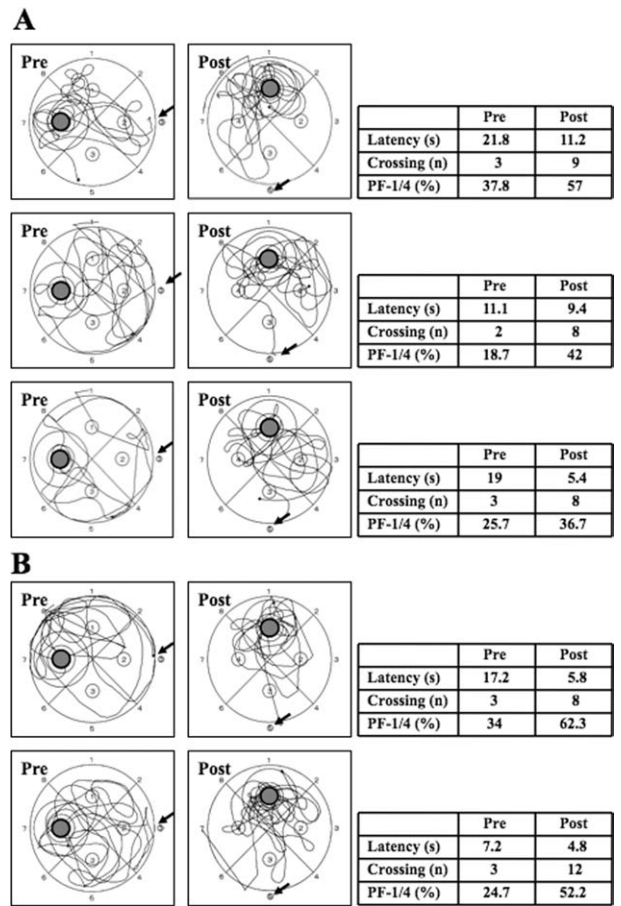


FIGURE 2: Representative tracks of homozygous 3xTg-AD mice treated with apomorphine (APO) in the probe test. (A) Treatment with 5mg/kg APO. (B) Treatment with 10mg/kg APO. A probe trial of a 60-second swim started on the opposite side (arrows) 24 hours after 3 days of training on the day before APO treatment (Pre) and on the day after the last injection (Post). All mice demonstrated apparent improvements in latency, crossing counts, and percentage of time spent in the quadrant containing the platform (PF-1/4) after APO treatment (Post) compared to pretreatment (Pre). Those values are shown in the right tables. Circles indicate location of platforms during 3-day training.

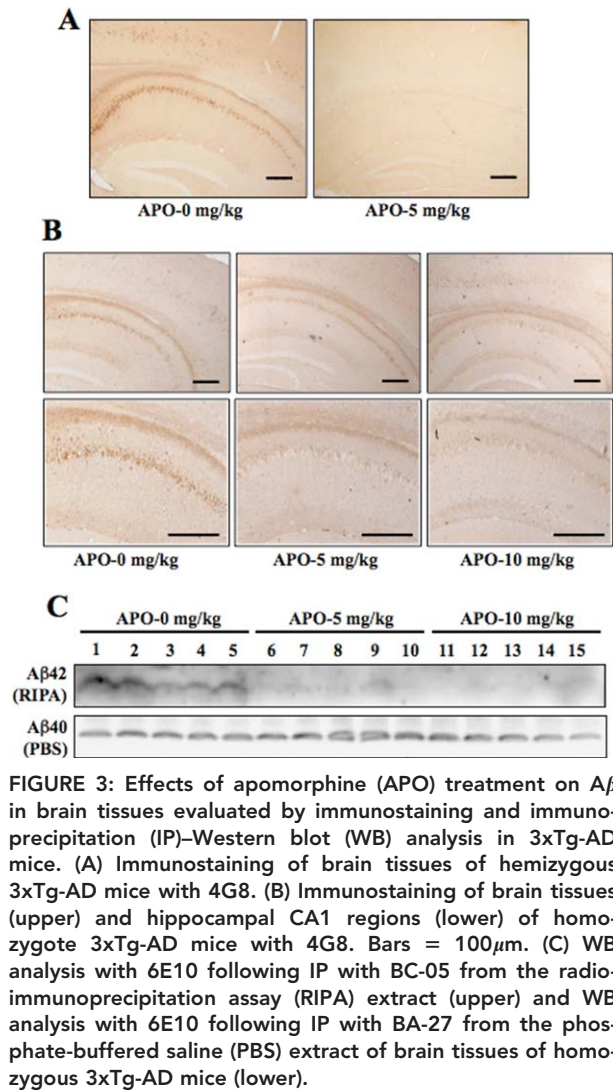


FIGURE 3: Effects of apomorphine (APO) treatment on A β in brain tissues evaluated by immunostaining and immunoprecipitation (IP)–Western blot (WB) analysis in 3xTg-AD mice. (A) Immunostaining of brain tissues of hemizygous 3xTg-AD mice with 4G8. (B) Immunostaining of brain tissues (upper) and hippocampal CA1 regions (lower) of homozygote 3xTg-AD mice with 4G8. Bars = 100 μ m. (C) WB analysis with 6E10 following IP with BC-05 from the radioimmunoprecipitation assay (RIPA) extract (upper) and WB analysis with 6E10 following IP with BA-27 from the phosphate-buffered saline (PBS) extract of brain tissues of homozygous 3xTg-AD mice (lower).

increased by APO treatment ($p < 0.05$), and marked inhibition of proteasome activity by MG132 was partially counteracted by APO ($p < 0.01$). Interestingly, IDE activity was increased by APO treatment ($p < 0.01$), especially with MG132 treatment ($p < 0.01$). Neprilysin activity was not significantly altered by MG132 and APO treatment.

We have previously reported that intracellular A β 42 accumulation promotes H₂O₂-induced p53-related apoptosis,^{9,27,28} and APO might thus attenuate p53-related apoptosis. A significantly protective effect of APO against H₂O₂ toxicity was observed, and a concentration of 10 μ M was found to be the most effective treatment (Fig 6). Although H₂O₂ at concentrations <0.5mM did not decrease cell viability of SH-SY5Y, p53 protein levels were elevated by treatment with H₂O₂ at concentrations

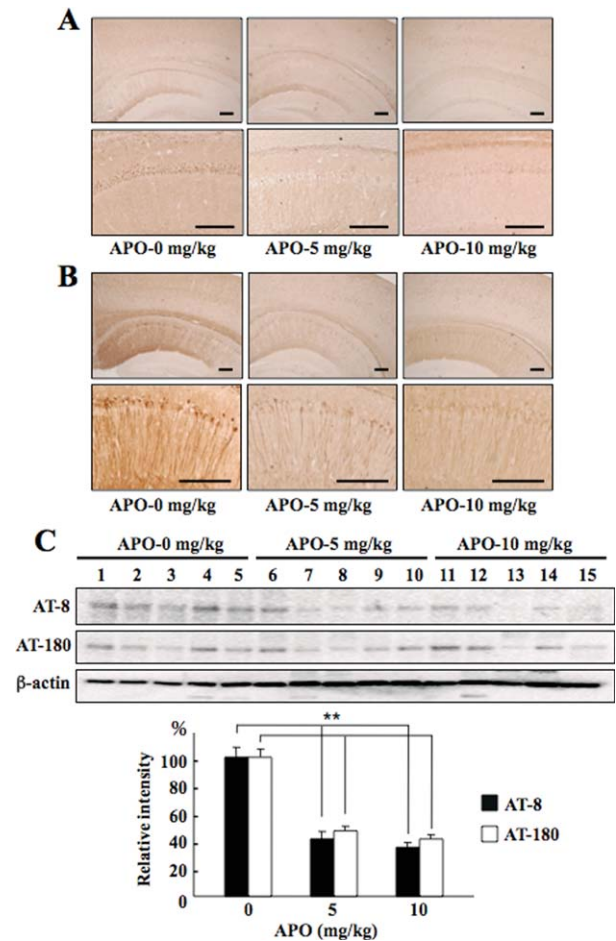


FIGURE 4: Effects of apomorphine (APO) treatment on p-tau in brain tissues evaluated by immunostaining and Western blot (WB) analysis in homozygous 3xTg-AD mice. (A) Immunostaining of brain tissues (upper) and hippocampal CA1 regions (lower) with AT8. (B) Immunostaining of brain tissues (upper) and hippocampal CA1 regions (lower) with AT180. Bars = 100 μ m. (C) WB analysis of the brain tissues with AT8, AT180, and anti- β -actin antibody (upper), and the relative intensities of the specific bands for p-tau (lower, n = 5). ** $p < 0.01$.

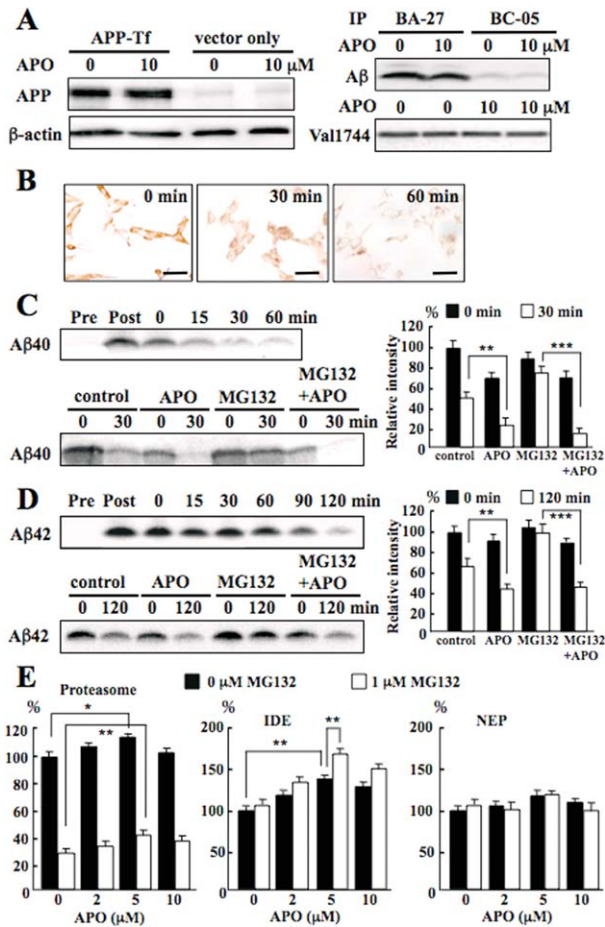


FIGURE 5: Effects of apomorphine (APO) treatment on intracellular A β degradation. (A) Western blot (WB) analysis of APP holoprotein and β -actin in APP-transfected (APP-Tf) and control (vector only) SH-SY5Y cells (left). WB analysis with 6E10 following immunoprecipitation (IP) with BA-27 (A β 40) or BC-05 (A β 42) from medium conditioned with APP-Tf SH-SY5Y cells (right upper). WB analysis of cleaved form of Notch1 with Val1744 antibody in SH-SY5Y cells (right lower). APO treatment did not alter the levels of APP, secreted A β 40/42, and cleaved form of Notch1. (B) Immunostaining analysis of A β at 0, 30, and 60 minutes after the artificial accumulation of A β 40 peptide in SH-SY5Y cells, demonstrating time-dependent degradation of cytosolic A β 40. Bars = 20 μ m. (C) WB analysis of intracellular A β preinflux (Pre), postinflux (Post), and 0, 15, 30, and 60 minutes after the artificial accumulation of A β 40 peptide (left upper), and 0 and 30 minutes after the accumulation (left lower). Relative intensity of WB bands of the left lower panel (right, n = 5). (D) WB analysis of intracellular A β preinflux (Pre), postinflux (Post), and 0, 15, 30, 60, 90, and 120 minutes after the artificial accumulation of A β 42 peptide (left upper), and 0 and 120 minutes after the accumulation (left lower). Relative intensity of WB bands of the left lower panel (right, n = 5). 0 min = 10 minutes after the time point of "Post." The cells were treated with 2 μ M MG132 and 10 μ M APO 2 hours before A β accumulation. APO treatment enhances intracellular A β 40 and A β 42 degradation, counteracting the inhibitory effect of MG132 on A β degradation. (E) Relative activity of proteasome (left panel), insulin-degrading enzyme (IDE; middle panel), and neprilysin (NEP; right panel) in SH-SY5Y cells 2 hours after treatment with MG132 and APO (n = 6). * p < 0.05, ** p < 0.01, *** p < 0.001.

of 0.3 and 0.5mM, and this elevation was attenuated by APO treatment. Similarly, in primary cultured neurons, APO treatment at 10 μ M was the most protective treatment in terms of cell viability and morphology. To examine the possibility that APO attenuates p53 upregulation and oxidative stress in 3xTg-AD mice, we studied p53 and HO-1, an oxidative stress marker.³⁶ Immunostaining (Fig 7A) and WB analysis (see Fig 7B) demonstrate that both p53 and HO-1 are significantly decreased in the 3xTg-AD mice treated with APO (p < 0.05).

Discussion

Here we have shown that APO accelerates intracellular A β degradation and protects neurons from oxidative

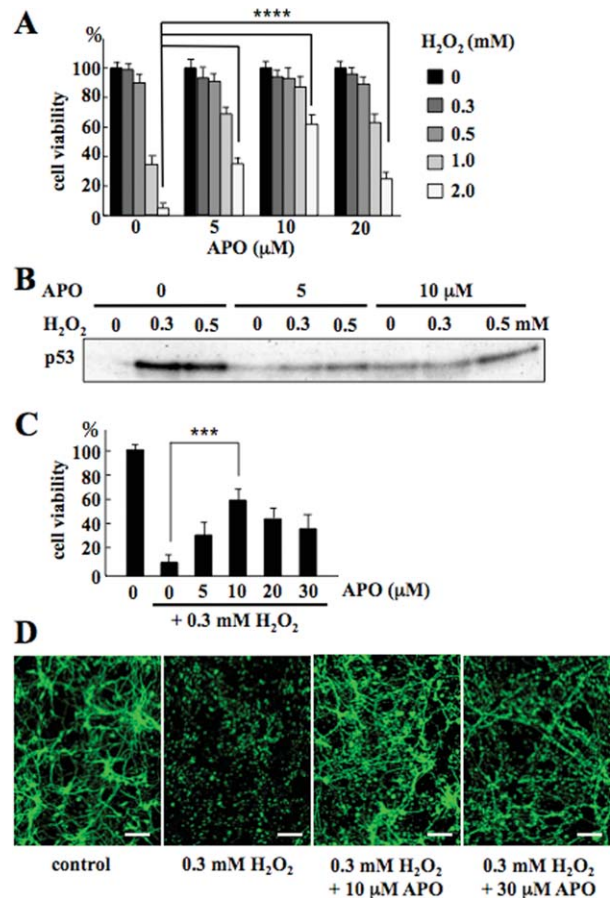


FIGURE 6: Effects of apomorphine (APO) treatment on cultured cells damaged by oxidative stress. (A) Cell viability of SH-SY5Y cells 24 hours after treatment with H₂O₂ and APO (n = 6). APO protects the cells from H₂O₂ toxicity significantly. (B) Western blot analysis for p53 in SH-SY5Y cells 24 hours after treatment with H₂O₂ and APO. APO attenuates an increase in p53 protein levels by H₂O₂ treatment. (C) Cell viability of primary cultured neurons 24 hours after treatment with 0.3mM H₂O₂ and APO (n = 6). APO protects the neurons, as well as SH-SY5Y cells, from H₂O₂ toxicity significantly. (D) Immunostaining of β -tubulin in primary cultured neurons 24 hours after treatment with 0.3mM H₂O₂ and APO. Bars = 20 μ m. *** p < 0.001, **** p < 0.0001.

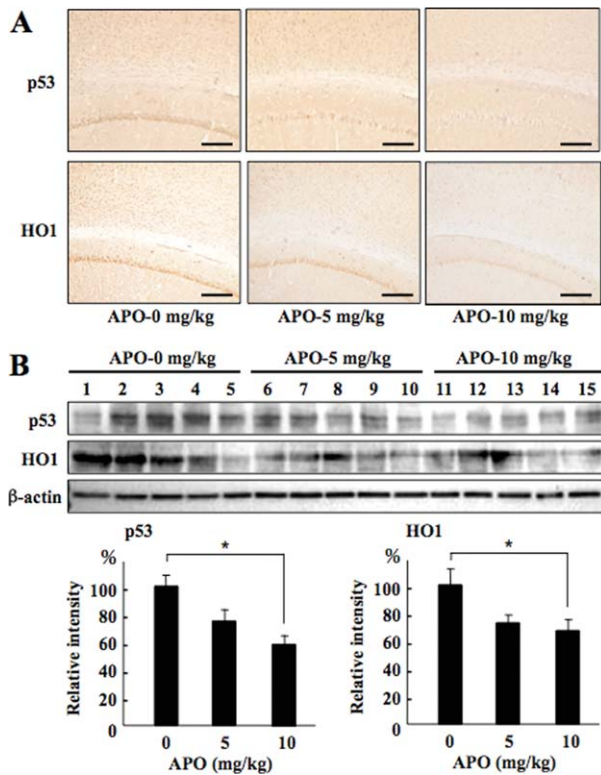


FIGURE 7: Effects of apomorphine (APO) treatment on p53 and HO-1 in brain tissues evaluated by immunostaining and Western blot (WB) analysis in homozygous 3xTg-AD mice. (A) Immunostaining of brain tissues with anti-p53 (upper) and anti-HO-1 (lower) antibodies. Bars = 100µm. (B) WB analysis of the brain tissues with anti-p53, anti-HO-1, and anti-β-actin antibodies (upper), and the relative intensity of the specific bands for p53 and HO-1 (lower, n = 5). *p < 0.05.

stress, and that APO restores memory dysfunction and improves the major pathological hallmarks in 3xTg-AD mice. Memory dysfunction of 3xTg-AD mice is associated with intraneuronal Aβ accumulation²¹ and soluble tau accumulation.³⁷ We found APO treatment to decrease both intraneuronal Aβ and p-tau levels. However, APO injection at 15 and 20mg/kg was less effective on memory function compared with APO injection at 5mg/kg, despite apparent decreases in intraneuronal Aβ and p-tau immunoreactivities (data not shown). Thus, the improvement in cognitive function induced by APO treatment may not only be due to decreases in the levels of Aβ and p-tau; alternatively, the overuse of APO may be toxic rather than protective. In support, treatment with APO at concentrations >10µM was less effective than treatment with 10µM APO. Also, treatment with 1 to 10µM APO was shown to protect rat PC12 cells from oxidative stress in a previous report.¹³

Oxidative stress may increase Aβ generation,³⁸ may enhance tau phosphorylation,^{39,40} and may be accelerated in AD mouse models.^{36,41} Here, we have found decreases in HO-1 protein levels in the 3xTg-AD mice treated with APO, indicating that the efficacy of APO may be

due to an antioxidative stress mechanism. However, H₂O₂ treatment causes Aβ₄₂ accumulation and p53 up-regulation,^{9,27,28,42} and enhancement of intracellular Aβ degradation may thus be an alternative protective mechanism of APO. Moreover, intracellular Aβ₄₂ may be a source of Aβ oligomers, which are linked to tau pathology in 3xTg-AD mice.⁴³⁻⁴⁵ Intracellular Aβ oligomerization was observed in cultured neurons, other AD mouse models, and AD patients.⁴⁶ Because extracellular and intracellular Aβ pools may be dynamically associated with each other in 3xTg-AD mice,^{47,48} it is possible that reducing Aβ levels inside neurons reduces the levels of Aβ oligomers outside neurons. Thus, APO may decrease the levels of intracellular Aβ and possibly extracellular Aβ oligomers, leading to a decrease in p-tau protein levels.

Intracellular Aβ may be degraded by the proteasome system³⁴ and by some Aβ-degrading enzymes.³⁵ In our study, APO treatment attenuated the effect of MG132 treatment, increasing activity of proteasome and IDE. Neprilysin activity was not altered, because neprilysin may degrade extracellular Aβ preferably. Increases in IDE activity were remarkable when treated with MG132, indicating that APO may partially restore an abnormal condition. Proteasome function, which is involved in AD,⁴⁹ may be affected by both intracellular Aβ²⁸ and extracellular Aβ oligomers.⁵⁰ Because p53 and p-tau are also degraded by proteasome, Aβ degradation promoted by APO treatment may enhance the degradation of these pathogenic proteins. Intracellular Aβ affects multiple

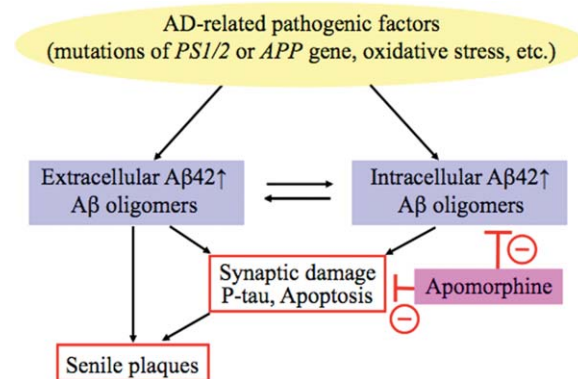


FIGURE 8: Scheme of Aβ pathogenesis inside and outside neurons and effects of apomorphine (APO). Genetic and environmental pathogenic factors, for example, mutations of PS1/2 or APP gene, ischemia, and oxidative stress, increase Aβ₄₂ levels in both extracellular and intracellular space. Many recent therapeutic strategies target extracellular Aβ oligomers, which are reported to cause synaptic damage and cognitive impairment. In addition to inhibition of oxidative stress, APO promotes degradation of intracellular Aβ₄₂ and Aβ oligomers, and might also decrease extracellular Aβ₄₂ and Aβ oligomers. Also, APO treatment decreases p-tau and attenuates p53-related apoptosis indirectly or possibly directly.

organelles,⁹ and starting APO therapy in the early stage may be more beneficial than starting it in the late stage of AD. We emphasize that major advantages of APO are its safety, relatively low cost, and high feasibility. APO injections and oral tablets are currently used in PD and ED patients, respectively. Because APO may play a unique role in AD therapeutics (Fig 8), appropriate combination of APO therapy with other antiextracellular A β therapies may be safer and more effective than monotherapies alone.

Stimulation of the dopamine D4 receptor may be protective,⁵¹ but dopamine signaling may exacerbate tau phosphorylation⁵² and modulate A β release via signaling by protein kinase C.^{53,54} We did not find positive immunoreactivity of the dopamine D1–D4 receptors on neurons in the cortices and hippocampi of 3xTg-AD mice (data not shown) and found no improvement of memory function after the injection of pramipexole, another dopamine agonist. Thus, the effects of APO may be mediated, at least in part, by some dopamine-independent pathways. Identification of novel pathways or receptors that mediate the therapeutic effects of APO may contribute to the development of a novel drug for AD.

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Authorship

E.H. and Y.O. contributed equally to this work.

Potential Conflicts of Interest

Nothing to report.

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