J Neural Transm (2017) 124:1055–1066
DOI 10.1007/s00702-017-1740-9

TRANSLATIONAL NEUROSCIENCES - ORIGINAL ARTICLE

Type B and A monoamine oxidase and their inhibitors regulate the gene expression of Bcl-2 and neurotrophic factors in human glioblastoma U118MG cells: different signal pathways for neuroprotection by selegiline and rasagiline

Keiko Inaba-Hasegawa[1] · Masayo Shamoto-Nagai[1] · Wakako Maruyama[1] · Makoto Naoi[1]

Received: 29 March 2017 / Accepted: 30 May 2017 / Published online: 2 June 2017

Abstract Type B monoamine oxidase (MAO-B) in glial cells has been considered to be associated with neuronal death in Parkinson's disease. MAO-B inhibitors, rasagiline and selegiline [(−)deprenyl], protect neurons in animal and cellular models of neurodegeneration. However, the role of MAO-B itself in the regulation of cell death processing remains elusive, whereas type A MAO (MAO-A) mediates the induction of anti-apoptotic Bcl-2 genes by rasagiline and selegiline. In this paper, the involvement of MAOs in the induction of neuroprotective genes by MAO inhibitors was investigated in human glioblastoma U118MG cells expressing mainly MAO-B. Selegiline significantly increased *Mao-B*, which was suppressed by *Mao-A* knockdown with short interfering (si)RNA, whereas rasagiline less markedly increased *Mao-B*, which was not affected by *Mao-A* knockdown. *Mao-A* mRNA was also markedly increased by rasagiline and selegiline, and *Mao-B* knockdown significantly enhanced the induction by selegiline, but not by rasagiline. *Mao-B* knockdown also significantly increased mRNA levels of Bcl-2, brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF). Selegiline synergistically enhanced the expression of these genes in *Mao-B* knockdown cells, but *Mao-A* knockdown suppressed the increase. Rasagiline increased BDNF and GDNF, which *Mao-B* and *Mao-A* knockdown inhibited. These results show that MAO-B might function as a repressor and MAO-A as a mediator in the constitutional expression of pro-survival genes, and that MAO-B and MAO-A might regulate different signal pathways for rasagiline and selegiline to induce neuroprotective genes. The novel role of glial MAOs in the regulation of gene expression is discussed.

Keywords Type B and A monoamine oxidase · Neuroprotective gene induction · MAO-B inhibitor rasagiline–selegiline · Neurotrophic factors BDNF–GDNF · Bcl-2

Abbreviations

MAO-A and MAO-B	Type A and B monoamine oxidase
MAOB-SH cells	MAO-B overexpressed SH-SY5Y cells
siRNA	Short interfering RNA
siMao-A and *siMao-B*	siRNA against *Mao-A* and *Mao-B*
siNS	Non-specific siRNA

Introduction

Monoamine oxidase (MAO, amine: oxygen oxidoreductase, EC 1.4.3.4) catalyzes oxidative catabolism of monoamines and regulates the homeostasis of neurotransmitter monoamines in the brain. MAO is classified as type A and B (MAO-A, MAO-B), depending on the selective affinity to substrates and the sensitivity to distinct

✉ Makoto Naoi
mnaoi@dpc.agu.ac.jp

Keiko Inaba-Hasegawa
ponykei@yahoo.co.jp

Masayo Shamoto-Nagai
nagaim@dpc.agu.ac.jp

Wakako Maruyama
maruyama@dpc.agu.ac.jp

[1] Department of Health and Nutrition, Faculty of Psychological and Physical Science, Aichi Gakuin University, 12 Araike, Iwasaki-cho, Nisshin, Aichi 470-0195, Japan

inhibitors (Shih et al. 1999). MAO-A and MAO-B are coded by different genes located on the X chromosome (Xp11.23) and are composed of different protein species, but they share many similar properties, such as 70% sequence identity and covalent FAD as the prosthetic group. MAO-A is expressed in catecholaminergic neurons and MAO-B in serotonergic and histaminergic neurons and glial cells, suggesting their different role in brain functions. MAO-B expression increases age dependently in the brain, suggesting the association of MAO-B with neuronal loss in age-related disorders, such as Parkinson's disease by increased production of reactive oxygen species (ROS) (Jenner and Olanow 1996). However, age-dependent MAO-B increase was absent in the substantia nigra of control subjects (Saura et al. 1997), and MAO-B knockdown could not protect mice from ischemic injury, suggesting that MAO-B might be not directly involved in neuronal loss (Holschneider et al. 1999). More direct evidences are required to support the association of MAO-B itself with the pathogenesis of Parkinson's disease (Damier et al. 1996).

Irreversible MAO-B inhibitors, selegiline [(−)deprenyl, (2*R*)-*N*-methyl-1-phenyl-*N*-pro-2-ynyl-propan-2-amine] and rasagiline [(1*R*)-*N*-prop-2-ynyl-2,3-dihydro-1*H*-amine] protect neurons from cell death in animal and cellular models of neurodegeneration, which is independent of MAO inhibition (Magyar 2011; Naoi et al. 2012, 2013a; Youdim and Bakhle 2006; Youdim et al. 2006). Rasagiline and selegiline directly suppress mitochondrial apoptotic signaling (Naoi et al. 2013b; Wu et al. 2015, 2016) and induce anti-apoptotic Bcl-2 and pro-survival neurotrophic factors and antioxidant enzymes (Maruyama et al. 2004; Weinreb et al. 2007; Maruyama and Naoi 2010). The role of MAO in neuroprotection by MAO-B inhibitors has been investigated using MAO-knockdown animals and cellular models treated with short interfering RNA (siRNA), microRNA (miRNA) and antisense oligonucleotides. In the cortex, striatum and corpus callosum of MAO-B-knockout mice, selegiline lost almost all the specific binding and the remaining 2–4% of ^{3}H-selegiline was bound to MAO-A, suggesting that the selegiline still targets MAO in the brain (Ekblom et al. 1998). MAO-A expression was knocked down with siRNA against MAO-A (*siMao-A*), which inhibited rasagiline-induced increase in the mRNA and protein of Bcl-2 and MAO-A, and the enzymatic activity of MAO-A itself (Inaba-Hasegawa et al. 2012, 2013).

Glial cells, especially microglia and astrocytes, play a vital role in the disease progress of neurodegenerative disorders (Mosley et al. 2006). Microglia and astrocytes promote neuronal survival through removal of toxic molecules (α-synuclein, glutamate, potassium and calcium) in the extracellular space and control synaptogenesis and plasticity by release of trophic factors [nerve growth factor (NGF), neurotrophin-3 (NT-3) and basic fibroblast growth factor (bFGF)] and antioxidant glutathione (Rappold and Tieu 2010). However, it is not well documented whether MAO-B is associated with gene induction by the MAO-B inhibitors in glial cells.

This paper presents the results on the involvement of MAO-B in the constitutive expression of genes coding MAO, Bcl-2 and neurotrophic factors in human U118MG cell line composed of a mixture of neuroblastoma and astrocyte cells. Gene induction by rasagiline, selegiline and an MAO-A inhibitor, clorgyline [3-(2,4-dichlorophenoxy)-*N*-methyl-*N*-prop-2-ynyl-propan-1-amine], was also investigated in MAO-knockdown cells, by use of siRNA against *Mao-B* and *Mao-A* (*siMao-B, siMao-A*), respectively. The role of MAO observed in glial U118MG cells was discussed by comparison with those in neuronal SH-SY5Y cells. Regulation of neuroprotective genes by MAOs and the inhibitors is discussed with respect to the role of glial cells in neuroprotection for Parkinson's disease and other neurodegenerative disorders.

Materials and methods

Materials

Professor J. Knoll (Semmellweis University, Budapest, Hungary), TEVA (Netanya, Israel) and May-Baker (Dagenham, UK) kindly donated selegiline, rasagiline and clorgyline, respectively. Kynuramine was purchased from Sigma (St. Louis, MO, USA), and Dulbecco's modified Eagle's medium (DMEM) and other chemicals were from Wako (Osaka, Japan).

Culture of U118MG cells and wild and MAO-B-overexpressed SH-SY5Y cells

U118MG cell line was purchased from American Type Culture Collection (Manassas, VA, USA) and cultured in DMEM containing 10% fetal calf serum (PBS, ATCC 30-2020), 4 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin. SH-SY5Y cells were cultured in Cosmedium-001 tissue culture medium (CosmoBio, Tokyo, Japan) supplemented by 5% fetal calf serum in 95% air–5% CO_2. Stable clone overexpressing MAO-B was established in SH-SY5Y cells (MAOB-SH cells) by DNA transfection of MAO-B gene, as reported (Yi et al. 2006).

MAO-B and MAO-A knockdown with siRNA

Expression of MAO-B and MAO-A was knocked down in U118MG and SH-SY5Y cells by the use of siRNA targeting *Mao-B* and *Mao-A* mRNA (sc-35849, sc-35847,

Santa Cruz Biotechnology, Dallas, Texas, USA), as reported previously (Inaba-Hasegawa et al. 2012, 2013). The cells (1.5×10^5 cells/well) were cultured in the six-well culture flask for 24 h and treated with siRNA for 48 h for the following experiments.

Quantitation of mRNA of MAO-A, MAO-B, Bcl-2 and neurotrophic factors

Induction of genes was quantitatively determined by the measurement of mRNA using the real-time reverse transcription-polymerase chain reaction (RT-PCR) method. U118MG and SH-SY5Y cells (3×10^5 cells/well) pre-treated without or with siRNA against MAO were cultured in the six-well culture flask and then treated with MAO inhibitors (10^{-6}–10^{-10} M) for 24 h. The mRNA levels of MAOs, Bcl-2 and neurotrophic factors were quantitatively determined by the real-time RT-PCR method. The cells were gathered, washed with phosphate-buffered saline and the total RNA was extracted by use of Trizol plus RNA purification kit according to the manufacture's protocol (Invitrogen, San Diego, CA, USA). The cDNA was generated by reverse transcription of the total RNA (100 ng) using PrimeScript RT reagent kit (TaKaRa Bio, Ohtsu, Japan), and the cDNA fragments were amplified using the PCR primers and SYBR Premix EX Taq^{TM} II (TaKaRa Bio). The used primers were as follows: *Bcl-2*, HA032558; *Mao-A*, HA071085; *Mao-B*, HA032947; *BDNF*, HA10999); *NGF*, HA115875; *NT-3v1* and *NT-3v2*, HA1500423 and HA15558040; *GDNF*, HA003973; and *β-action,* HA067803-F/R.

The relative concentrations of PCR products were determined from the standard curve prepared by real-time PCR of serial dilutions of template cDNAs. The relative mRNA levels were quantitatively determined from the cycle threshold (C_t) of the PCR reaction analyzed by Real time system software (TaKaRa Bio) and the standard curve. The mRNA level was normalized by comparison of *β-actin* levels in each sample and expressed as the percent of those in control cells treated without siRNA (non-treated cells).

Quantitative measurement of MAO activity in the mitochondrial fraction

MAO activity in the mitochondrial fraction was determined with kynuramine as a substrate by a microassay method modified from the method of Kraml (1965). The mitochondrial fraction (0.1–25 μg protein) was reacted with 1 mM kynuramine in 10 mM sodium phosphate buffer, pH 7.4 (the total volume, 100 μl) at 37 °C for 1 h, then 50 μl of 10% $ZnSO_4$, 10 μl of 1 M NaOH and 40 μl of distilled water were added. After heating at 100 °C for 2 min, the sample was centrifuged at 1000*g* for 10 min and the supernatant (50 μl) was mixed with 50 μl NaOH in a 96-well microplate and the fluorescence at 450 nm was measured with excitation at 340 nm, using a Corona MTP-600F microplate fluorometer (Corona Electrics, Hitachinaka, Japan). MAO activity was expressed as pmoles of produced 4-hydroxyquinone per minute per microgram protein. MAO-A and MAO-B activity were differentiated by 20 min pre-treatment with 10 μM selegiline or clorgyline, respectively.

Quantitation of Bcl-2 protein

Bcl-2 protein was quantitatively determined by Western blot analysis, as reported (Inaba-Hasegawa et al. 2012). The cells (3×10^5 cells/well) were cultured for 24 h in a six-well poly-L-lysine-coated culture flask (Iwaki, Asahi Glass, Tokyo, Japan). The cells were treated with 10^{-5}–10^{-11} M MAO inhibitors for 24 h, gathered, washed with phosphate-buffered saline (PBS) and suspended in RIPA buffer [10 mM Tris–HCl buffer, pH 7.5, containing 1% NP-40, 0.1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 150 mM NaCl and 1 mM EDTA 2 Na] and a protease inhibitor cocktail (Roche, Mannheim, Germany). The lyzed protein was separated by SDS-PAGE using 10–20% gradient polyacrylamide gel (Bio-Rad Lab., Hercules, VA, USA) and electroblotted onto a PVDF membrane (Amersham Hybond-P, GE Healthcare, Buchinghamshire, UK). After blockage with 5% nonfat milk in 10 mM Tris–HCl buffer, pH 7.5, containing 0.1% Tween 20 and 150 mM NaCl, the membrane was incubated overnight at 4 °C with anti-human Bcl-2 antibody (500× dilution, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-β-actin antibody for control (5000× dilution, Sigma). The membrane was incubated further with horseradish peroxidase (HRP)-linked anti-mouse IgG at room temperature. The immunoblots were visualized by use of Amersham ECL plus western blotting detection reagents (GE Healthcare) or Immunostar LD (Wako). The amounts of Bcl-2 protein were quantitatively determined using a Fujifilm LAS-4000 luminescent image analyzer (Tokyo, Japan). The protein amounts were quantified by densitometry, normalized against β-actin and expressed as the percent of those in non-treated cells.

Statistics

Experiments were repeated at least three to four times in triplicate or quadruplicate measurements, and the results were expressed as the mean and SD. Differences were statistically evaluated by analysis of variance (ANOVA) followed by Scheffe's *F* test. A *p* value less than 0.05 was considered to be statistically significant.

Results

MAO-B in U118MG cells and effects of siRNA treatment on MAO levels

MAO protein in U118MG cells was detected as a single band with molecular mass of 520 kDa corresponding to MAO-B, but the amount was quite lower than MAO-A protein in wild SH-SY5Y cells (data not shown). MAO-B activity was the major part of the MAO activity in the mitochondrial fraction of U118MG cell: 15.5 ± 0.3 versus 1.5 ± 0.4 pol/min/mg mitochondrial protein, for MAO-B and MAO-A, respectively (Fig. 1a). In wild SH-SY5Y cells, MAO-A activity was 97.5% of the total MAO activity: 913 ± 57 out of 937 ± 44 pol/min/mg protein. In MAOB-SH cells MAO-B activity increased to 12 450 ± 1 020 pmol/min/mg protein from 31.3 ± 14.0 pmol/min/mg protein in wild SH-SY5Y cells, but MAO-A activity remained unchanged, 1 113 ± 43 pmol/min/mg protein.

In U118MG cells, *Mao-B* mRNA level was deceased by treatment with 20–60 nM *siMao-B*, whereas *siMao-B* at 40 and 60 nM did not affect *Mao-A* expression and increased at 20 nM, 136 ± 18% of that in non-treated cells (Fig. 1b). Vice versa, *siMao-A* treatment downregulated *Mao-A* mRNA to 8% of non-treated cells, but did not affect *Mao-B* level. In SH-SY5Y cells, *siMao-A* (20 nM) suppressed only *Mao-A* expression, but *siMao-B* neither affected *Mao-A* nor *Mao-B* levels (Fig. 1c). In MAOB-SH cells, *siMao-B* significantly suppressed *Mao-B* expression, whereas *siMao-B* did not affect *Mao-A* levels (Fig. 1d). These results indicate that siRNA against *Mao-B* and *Mao-A* selectively decreased targeted *Mao-B* and *Mao-A* levels in these cells.

Selegiline and rasagiline increased *Mao-B* and *Mao-A* mRNA, and *siMao-B* synergistically increased selegiline-enhanced *Mao-A* expression

In SH-SY5Y cells, rasagiline and selegiline induced MAO-A mRNA, protein and enzyme activity, and the induction by rasagiline was mediated by MAO-A itself (Inaba-Hasegawa et al. 2013). The effects of rasagiline and selegiline on *Mao-B* levels were investigated in U118MG cells treated with 20 μM *siMao-B*, *siMao-A,* or non-specific siRNA (*siNS*) (Fig. 2). The effects of rasagiline and selegiline on *Mao-A* and *Mao-B* were not affected by *siNS* treatment, which was also the case with Bcl-2 and neurotrophic factors. Rasagiline at 10^{-7}–10^{-10} M increased *Mao-B* mRNA in *siMao-A*- and *siNS*-treated cells virtually by the same extent, except at 10^{-9} M of *siMao-A*. Selegiline upregulated *Mao-B* mRNA in *siNS*-treated cells, which was suppressed by *siMao-A,* suggesting that MAO-A might mediate *Mao-B* mRNA increase by selegiline, but not by rasagiline in U118MG cells (Fig. 2a).

Effects of rasagiline and selegiline on *Mao-A* mRNA levels were also examined. In *siNS*-treated cells, rasagiline

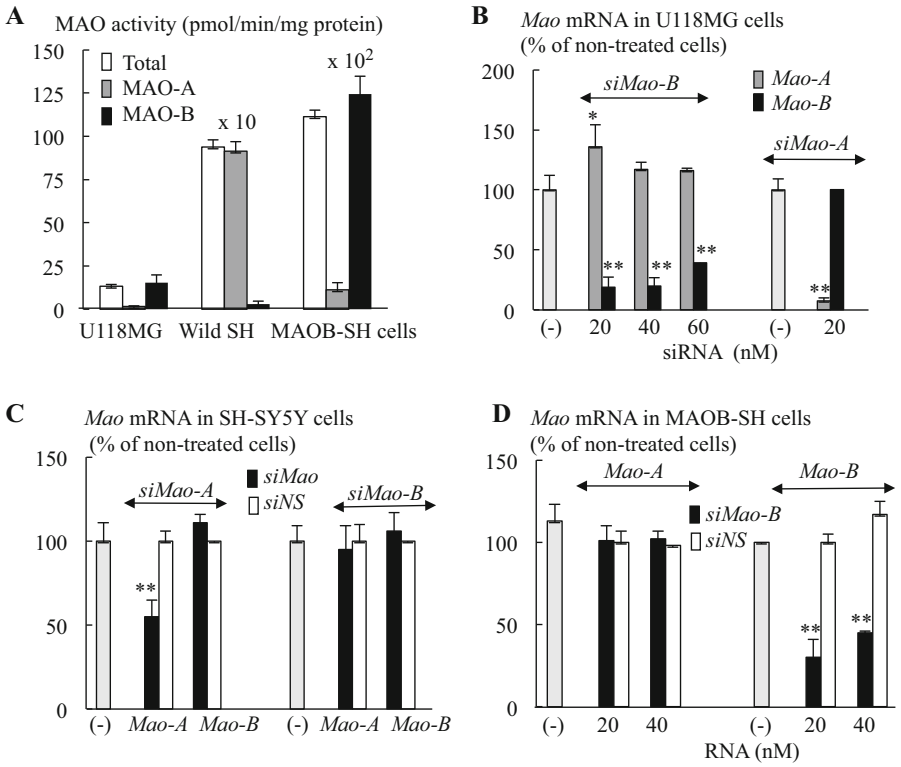

Fig. 1 The enzymatic activity of MAO-B and MAO-A in U118MG cells and wild and (Wild SH) and MAO-B overexpressed SH-SY5Y (MAOB-SH) cells. **a** MAO activity was expressed as pmol/min/mg of mitochondrial protein. **b** *Mao* mRNA levels in U118MG cells treated with *siMao-B* at 20, 40 and 60 nM or *siMao-A* at 20 nM. The mRNA levels were expressed as percent of the values in cells treated without siRNA (non-treated cells). **c** *Mao* mRNA levels in wild SH cells treated with 20 nM *siMao-A* and *siMao-B*. **d** *Mao* mRNA levels in MAOB-SH cells treated with 20 and 40 nM *siMao-A* and *siMao-B*. *Column and bar* represent the mean and SD. *Asterisk* and *double asterisk* statistically different from non-treated cells, $p < 0.05$ and 0.01, respectively

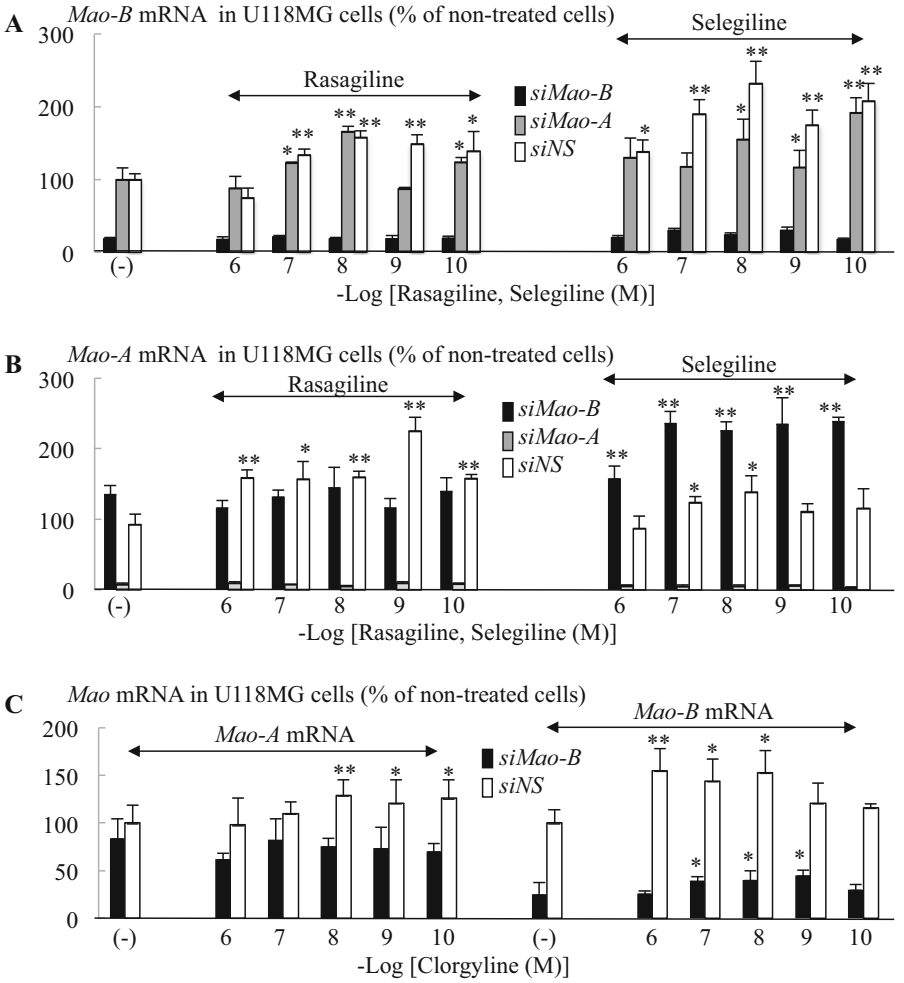

Fig. 2 Effects of rasagiline, selegiline and clorgyline on *Mao-B* and *Mao-A* mRNA in *siMao-B*-, *siMao-A*- and *siNS*-treated U118MG cells. Cells were treated with 20 nM *siMao-B*, *siMao-A* or *siNS* and then with 10^{-6}–10^{-10} M MAO inhibitors. **a**, **b** *Mao-B* and *Mao-A* mRNA levels in cells treated with siRNA and then rasagiline and selegiline. **c** *Mao-A* and *Mao-B* mRNA levels in cells treated with *siMao-B* and then with clorgyline. The mRNA levels were expressed as percent of the values in non-treated cells. *Column and bar* represent the mean and SD. *Asterisk* and *double asterisk* statistically different from the corresponding siRNA-treated cells without MAO inhibitor [(−)], $p < 0.05$ and 0.01, respectively

at 10^{-6}–10^{-10} M and selegiline at 10^{-7} and 10^{-8} M increased *Mao-A* mRNA levels (Fig. 2b). In *Mao-B*-knockdown cells, selegiline at 10^{-6}–10^{-10} M increased *Mao-A* mRNA synergistically, but rasagiline did not affect the levels, suggesting that MAO-B might suppress selegiline-dependent *Mao-A* induction (Fig. 2b).

The effects of clorgyline, an MAO-A inhibitor, on *Mao-A* and *Mao-B* expression were investigated. Clorgyline at 10^{-8}–10^{-10} and 10^{-6}–10^{-8} M increased *Mao-A* and *Mao-B* mRNA levels in *siNS*-treated cells (Fig. 2c). In *Mao-B*-knockdown cells, clorgyline did not change *Mao-A* levels, but increased *Mao-B* levels, which were significantly downregulated by *siMao-B*.

Rasagiline and selegiline increased anti-apoptotic Bcl-2 in U118MG cells

Rasagiline and selegiline increased Bcl-2 mRNA and protein in SH-SY5Y cells by mediation of MAO-A (Inaba-Hasegawa et al. 2012). In U118MG cells, the effects of rasagiline and selegiline on Bcl-2 protein levels were examined. Rasagiline at 10^{-6}–10^{-10} M and selegiline at 10^{-5}–10^{-7} and 10^{-10} M increased Bcl-2 protein (Fig. 3a, b). Treatment with 20 and 40 nM *siMao-B* increased *Bcl-2* mRNA levels significantly, suggesting the suppression of the constitutional Bcl-2 levels by MAO-B (Fig. 3c). In cells treated with 20 nM *siMao-B*, the *Bcl-2* mRNA levels were increased to 147 ± 13% of those in non-treated cells, whereas *siMao-A* treatment reduced those to 60 ± 10%.

In cells pretreated with 20 nM *siNS*, rasagiline and selegiline at 10^{-7}–10^{-10} and 10^{-6}–10^{-10} M markedly increased *Bcl-2* mRNA. In *siMao-B*-treated cells, selegiline further increased *Bcl-2* mRNA levels, but rasagiline did not. In *siMao-A*-treated cells rasagiline- and selegiline-dependent Bcl-2 expression was suppressed, except at 10^{-7} and 10^{-10} M of selegiline (Fig. 3d). These results indicate again that MAO-B negatively regulated Bcl-2 expression by selegiline, and MAO-A positively that by selegiline and rasagiline in U118MG cells.

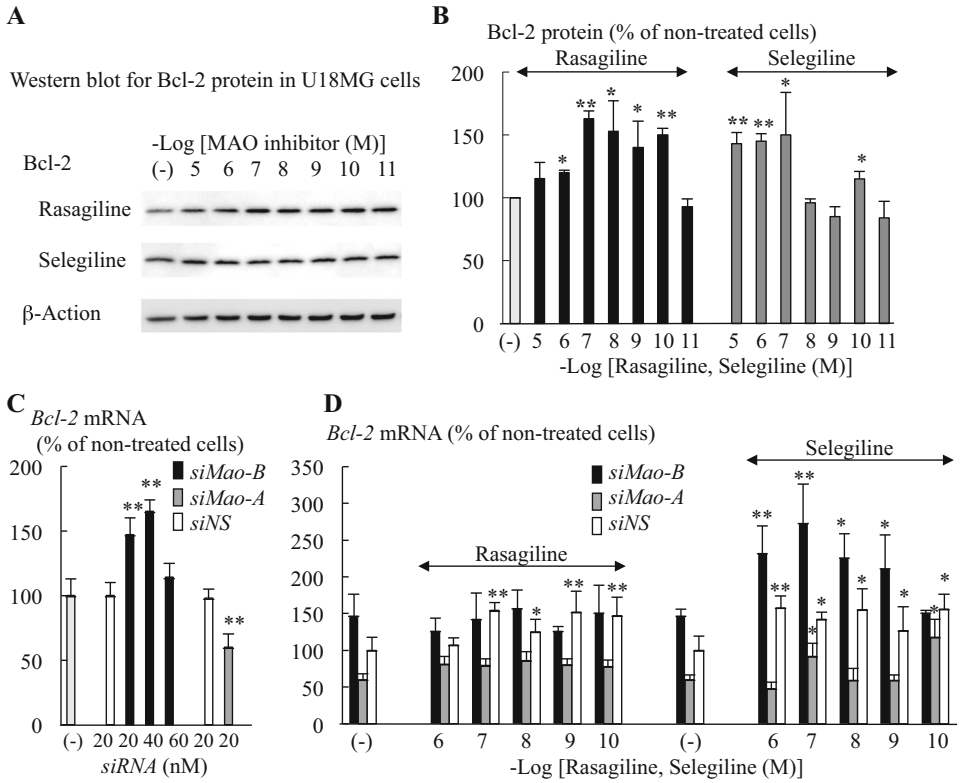

Fig. 3 Effects of rasagiline, selegiline, and *siMao-B*- and *siMao-A*-treatment on Bcl-2 protein and mRNA levels in U118MG cells. Cells were treated with rasagiline and selegiline at 10^{-5}–10^{-11} M and subjected to quantitative analysis for Bcl-2. **a**, **b** Western blot analysis and quantitative results for Bcl-2 protein levels. The value was expressed as the percent of that in non-treated cells. **c** Bcl-2 mRNA in U118MG cells treated with 20, 40 and 60 nM *siMao-B*, 20 nM *siMao-A* or 20 nM *siNS*. Bcl-2 mRNA level was represented as percent of the value in non-treated control cells. **d** Bcl-2 mRNA levels in cells pretreated with 20 nM *siMao-B, siMao-A* or *siNS*, and then with 10^{-6}–10^{-10} M rasagiline or selegiline. The value was expressed as percent of that in non-treated cells. *Column and bar* represent the mean and SD. *Asterisk* and *double asterisk* significantly different from corresponding siRNA-treated cells without MAO inhibitor, $p < 0.01$ and 0.05, respectively

Mao-B knockdown increased the mRNA levels of neurotrophic factors

In U118MG cells, treatment with 20 and 40 nM *siMao-B* significantly increased the mRNA levels of *BDNF*, *NGF*, *NT-3v1*, *NT-3v2* and *GDNF*, whereas *siNS* at 20 nM was not affected, except for the downregulation of *GDNF* expression (Fig. 4a). The treatment with 60 nM *siMao-B* increased only *NT-3* levels and did not affect *BDNF*, *NGF* and *GDNF* mRNA levels. On the other hand, the *siMao-A* treatment downregulated *BDNF*, *NGF* and *GDNF* mRNA levels (Fig. 4b). The constitutional expression of pro-survival neurotrophic factors was regulated by MAO-B and MAO-A in an opposite way.

Mao-B knockdown and selegiline synergistically enhanced *BDNF* and *GDNF* expression

Rasagiline and selegiline induced BDNF and GDNF protein and mRNA in SH-SY5Y cells (Maruyama and Naoi 2010). In U118MG cells, the effects of these MAO-B inhibitors on the expression of neurotrophic factors were investigated. In *siNS*-treated cells, rasagiline at 10^{-8}–10^{-10} M and selegiline at 10^{-6}–10^{-10} M increased *BDNF* mRNA significantly (Fig. 5a). As described above, *siMao-B* increased the basal expression of neurotrophic factors, whereas *siMao-A* inhibited that of *BDNF*, *NGF* and *GDNF*. In *siMao-B*-treatment cells, selegiline synergistically enhanced *BDNF* levels, but not in *siMao-A*-treated cells. Rasagiline did not increase *BDNF* levels in *siMao-B*- and *siMao-A*-treated cells, except at 10^{-7} M of *siMao-A*.

GDNF levels in *siNS*-treated ells were increased by rasagiline at 10^{-6}–10^{-10} M, which was inhibited by *siMao-A* and *siMao-B* treatment. Selegiline also enhanced *GDNF* levels at 10^{-6}–10^{-9} M in *siNS*-treated cells and also significantly increased them at 10^{-6}–10^{-10} M in *Mao-B*-knockdown cells, but did not increase *GDNF* in *siMao-A*-treated cells (Fig. 5b).

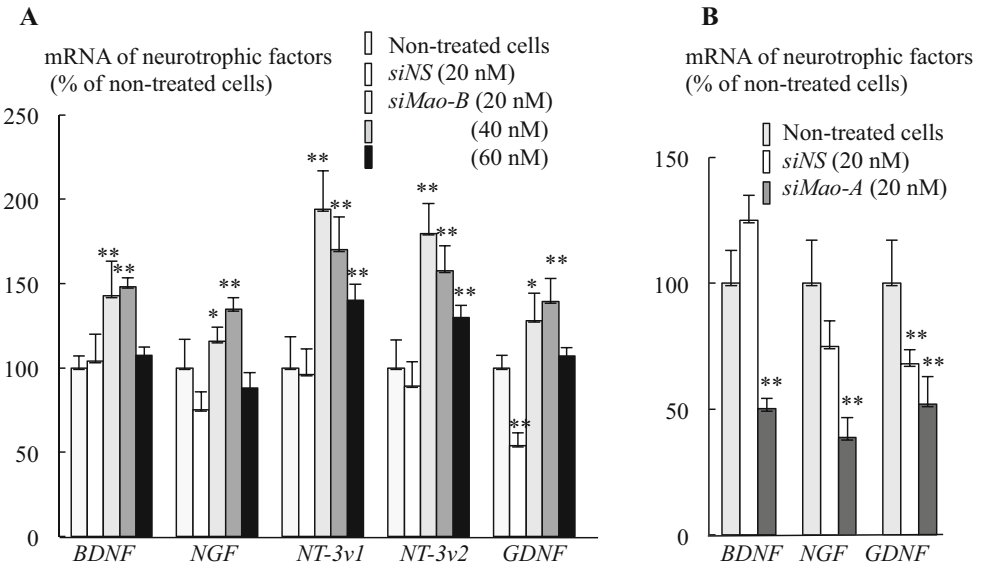

Fig. 4 Effects of *Mao-B* and *Mao-A* knockdown on mRNA levels of neurotrophic factors. **a** U118MG cells treated with 20, 40 and 60 nM *siMao-B* or 20 nM *siNS*, and the mRNA levels of neurotrophic factors were quantified by real-time RT-PCR method. The value was presented as percent of the each neurotrophic factor levels in non-treated cells. **b** U118MG cells treated with 20 nM *siMao-A* or *siNS*, and then BDNF, NGF and GDNF mRNA levels were quantified. *Asterisk* and *double asterisk* significantly different from non-treated cells, $p < 0.05$ and 0.01

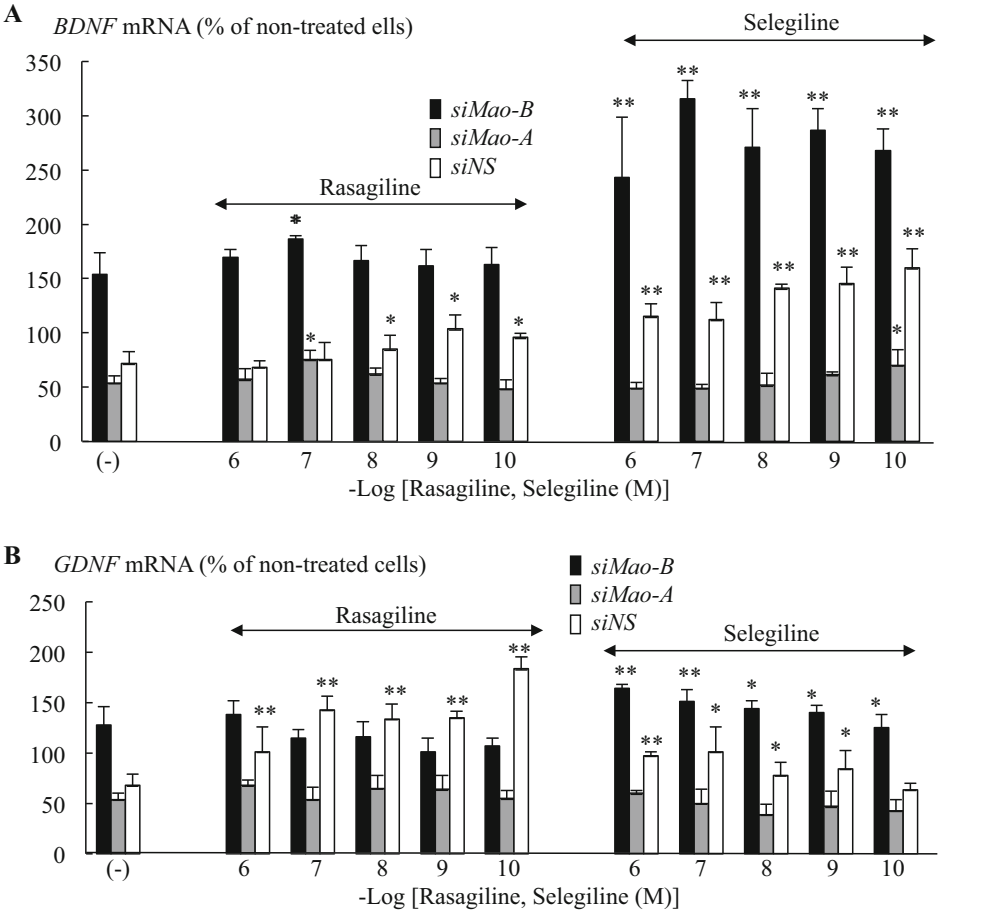

Fig. 5 Effects of rasagiline and selegiline on *BDNF* and *GDNF* mRNA in *siMao-B*- or *siMao-A*-treated U118MG cells. Cells were treated with 20 nM *siMao-B, siMao-A* or *siNS,* and then 10^{-6}–10^{-10} M rasagiline or selegiline. The mRNA levels were quantified, and the value was expressed as percent of that in non-treated cells. *Asterisk* and *double asterisk* significantly different from corresponding siRNA-treated cells without MAO inhibitor, $p < 0.05$ and 0.01, respectively

Clorgyline increased *BDNF* and *GDNF* in *Mao-B*-knockdown U118MG cells

The effects of clorgyline on the expression of neurotrophic factors were investigated in U118MG cells treated with *siMao-B* and *siNS* (Fig. 6). In *siNS*-treated cells, clorgyline did not affect *BDNF* and *GDNF* mRNA levels, except *BDNF* increase at 10^{-8} M. In *siMao-B*-treated cells, clorgyline further upregulated *BDNF* and *GDNF* levels, suggesting the association of MAO-B with the induction

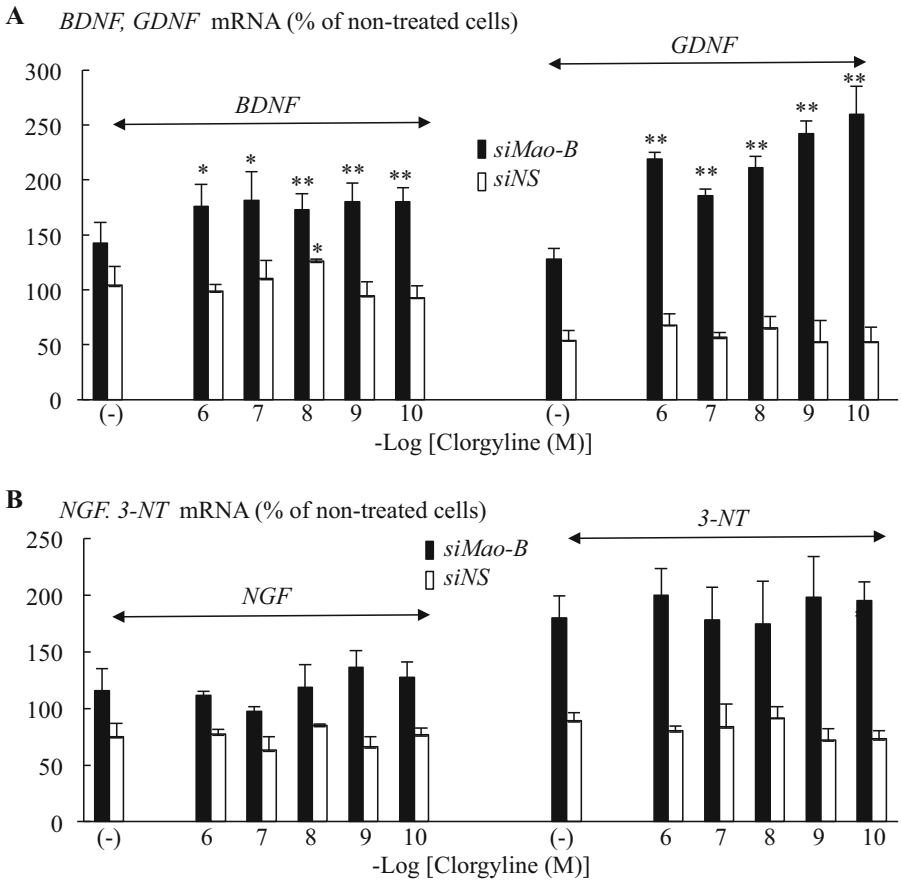

Fig. 6 Effects of clorgyline on mRNA of neurotrophic factors in *siMao-B*-treated U118MG cells. Cells were treated with 20 nM *siMao-B* or *siNS* and then with 10^{-6}–10^{-10} M clorgyline. The results were expressed as percent of that in non-treated cells. *Asterisk* and *double asterisk* statistically different from the corresponding siRNA-treated cells without clorgyline, $p < 0.05$ and 0.01, respectively

(Fig. 6a). NGF and 3-NT levels were not affected by clorgyline in *siNS*-treated cells. *siMao-B* treatment increased the basal levels of NGF and 3-NT, but clorgyline did not further affect them (Fig. 6b).

In SH-SY5Y cells, MAO-A controversially regulated BDNF induction by rasagiline and selegiline

The association of MAO-A with *BDNF* induction by rasagiline and selegiline was examined in *siMao-A*-treated SH-SY5Y cells. Rasagiline at 10^{-6}–10^{-8} and 10^{-10} M increased *BDNF* levels in *siNS*-treated cells, and *siMao-A* treatment inhibited the induction, whereas rasagiline increased *NGF* only at 10^{-6} M (Fig. 7a). Selegiline at 10^{-6}–10^{-10} M increased *BDNF* in *siNS*-treated cells, and *Mao-A*-knockdown further enhanced *BDNF*, but did not increase *NGF* in *siNS*- and *siMao-A*-treated cells, except at 10^{-8} M (Fig. 7b). On the other hand, clorgyline did not affect *BDNF* and *NGF* mRNA levels in *siMao-A*- and *siNS*-treated SH-SY5Y cells, except *BDNF* increase at 10^{-7} M in *siMao-A*-treated cells (Fig. 7c). MAO-B overexpression in SH-SY5Y cells decreased the induction of *Bcl-2*, *BDNF* and *GDNF* gene by rasagiline and selegiline (data not shown).

Discussion

In this paper, the role of MAO-B and MAO-A in the induction of anti-apoptotic and pro-survival genes in glial U118MG cells was confirmed, as summarized in Fig. 8. In the mouse model of Parkinson's disease, where MAO-B expression was induced in astroglia at adulthood, MAO-B was directly involved in neuronal loss (Mallajosyula et al. 2008). Neurotoxicity of MAO-B against dopamine neurons is due not only to oxidative stress, but also to dopaminochrome produced from dopamine with hydrogen peroxide, a reaction product by MAO, resulting in impaired mitochondrial function (Siddiqui et al. 2010). On the other hand, selegiline, desmethylselegiline and dopamine agonists induce pro-survival BDNF and GDNF in cultured mouse astrocytes (Mizuta et al. 2000; Ohta et al. 2010). These results present bilateral function of glial MAO-B in neurodegeneration.

MAO-A regulates the homeostasis of serotonin, norepinephrine and dopamine in the brain, and its activity is closely associated with emotional, cognitive, perceptive and behavioral functions (Shih et al. 1999; Bortolato et al. 2008; Naoi et al. 2016, 2017). MAO-A mRNA and activity were increased in apoptosis induced in SH-SY5Y and

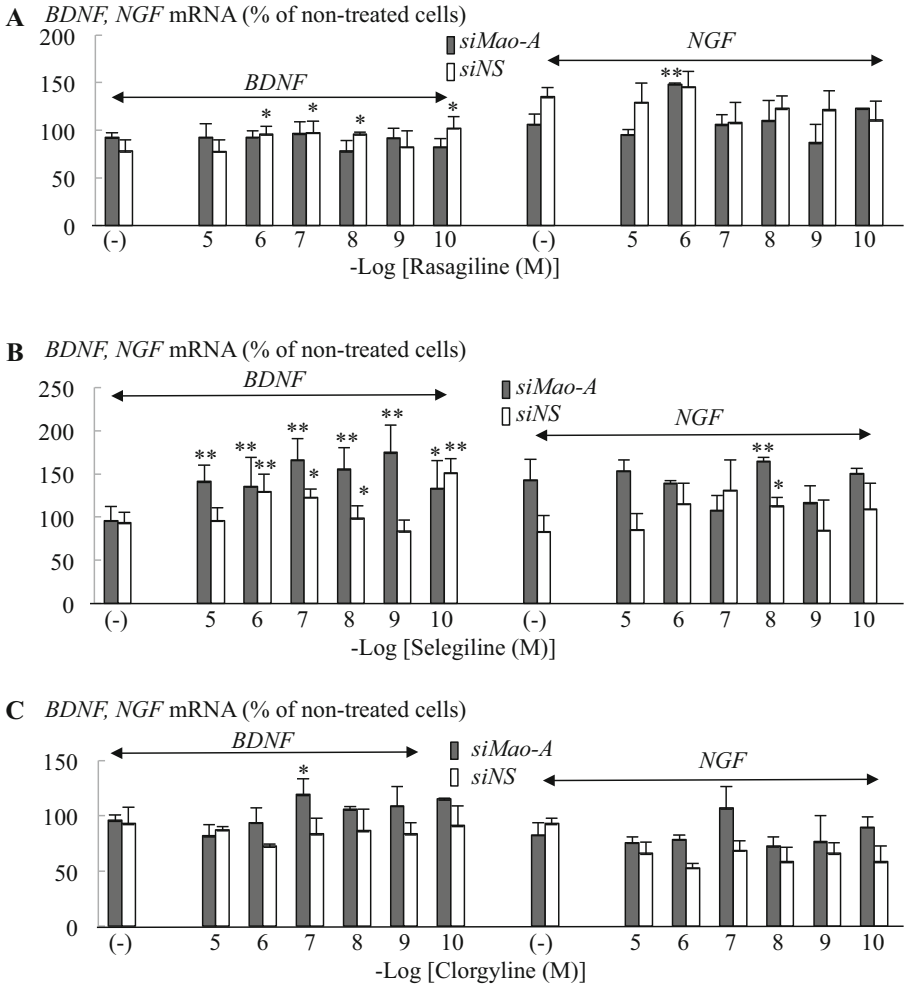

Fig. 7 Effects of *Mao-A* knockdown on *BDNF* and *NGF* induction by rasagiline, selegiline and clorgyline in wild SH-SY5Y cells. **a–c** Cells were treated with 20 nM *siMao-A* or *siNS*, and then with 10^{-5}–10^{-10} M rasagiline, selegiline and clorgyline. The mRNA was expressed as percent of that in non-treated cells. *Asterisk* and *double asterisk* statistically different from the corresponding siRNA-treated cells without MAO inhibitor, $p < 0.05$ and 0.01, respectively

PC12 cells, suggesting the involvement of MAO-A in neuronal death (De Zutter and Davis 2001). An endogenous dopaminergic neurotoxin, *N*-methyl(*R*)salsolinol, binds to MAO-A and induces apoptosis in SH-SY5Y cells (Yi et al. 2006). MAO-A knockdown with microRNA protected SH-SY5Y cells from cell death by mitochondrial toxins (Fitzgerald et al. 2014). However, protection by clorgyline has been reported only in non-neuronal cells: human melanoma M14 cells (Malorni et al. 1998) and mouse N2a neuroblastoma cells (De Girolamo et al. 2001). Clorgyline prevented tyramine-induced mitochondrial permeability transition and calcium release in rat liver mitochondria (Marcocci et al. 2002). Clorgyline increased Bcl-2 in irradiated HaCaT cells, a human keratinocyte cell line, but the protective effects were limited (Seymour et al. 2003). In this paper, clorgyline induced BDNF and GDNF in U118MG cells, suggesting that MAO-A inhibitors may show neuroprotective activity through induction of neuroprotective genes in glial cells.

In in vivo and in vitro experiments, selegiline and rasagiline increase Bcl-2 and neurotrophic factors. In non-human primate, rasagiline increased GDNF levels in the cerebrospinal fluid (CSF) more markedly than selegiline, and vice versa selegiline increased BDNF levels in the CSF from parkinsonian patients (Maruyama and Naoi 2010). These results are comparable to our results on the preferential induction of GDNF and BDNF by rasagiline and selegiline in SH-SY5Y cells. In clinical studies, selegiline shows the beneficial effects in depression, and rasagiline in Parkinson's disease and other neurodegenerative disorders. The difference is considered to ascribe to their metabolites, namely cytotoxic methamphetamine from selegiline, and protective aminoindan and *N*-propargylamine from rasagiline (Bar Am et al. 2004). Our results in U118MG cells present that MAO-B functioned as a repressor for the constitutive expression of neuroprotective genes, whereas MAO-A as an enhancer, and that selegiline and rasagiline induced BDNF or GDNF by different signal pathways, either depending on MAO-A or MAO-B.

Molecular mechanism underlying how MAOs are associated with gene induction by MAO inhibitors remains elusive. Increase of monoamines by MAO inhibitors

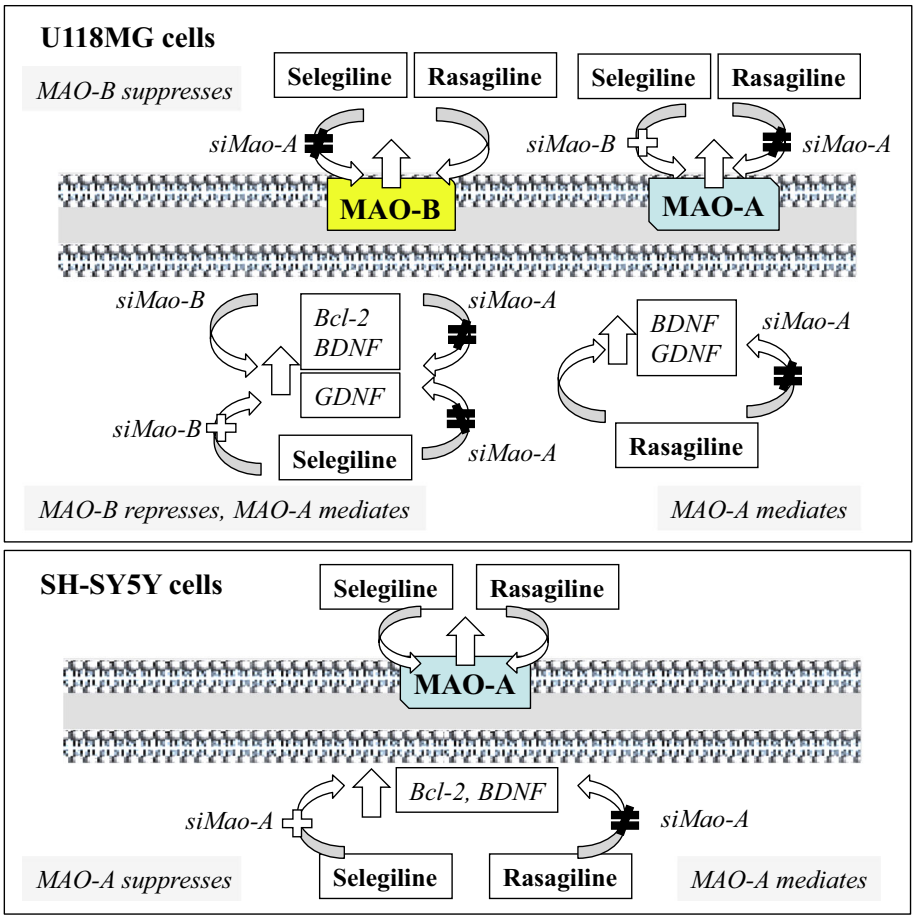

Fig. 8 MAO-B and MAO-A contradictorily regulate gene induction by selegiline and rasagiline in U118MG and SH-SY5Y cells. Selegiline and rasagiline increased *Mao-B*, *Mao-A* and *Bcl-2* expression. *siMao-A* suppressed mRNA induction of MAO-B by selegiline, and *siMao-B enhanced* that of MAO-A. *siMao-A* treatment inhibited rasagiline-dependent *Mao-A* induction, but not *Mao-B*, whereas *siMao-b* did not affect *Mao-A* and *Mao-B* induction. *siMao-B* treatment enhanced and *siMao-A* suppressed the mRNA levels of Bcl-2 and neurotrophic factors. Selegiline synergistically enhanced *BDNF* and *GDNF* in *Mao-B*-knockdown cells, and *siMao-A* treatment inhibited *BDNF* and *GDNF* induction. Rasagiline also increased *BDNF* and *GDNF* in U118MG cells, which was inhibited by *siMao-A* treatment. In SH-SY5Y cells, rasagiline induced *Bcl-2* and *BDNF*, which was suppressed by *siMao-A* treatment. Selegiline increased *BDNF*, which *siMao-A* treatment synergistically increased

induced BDNF and GDNF in glial cells (Juric et al. 2006). Dopamine upregulated BDNF protein levels in cultured rat astrocytes (Miklic et al. 2004), serotonin induced *GDNF* mRNA in rat C6 glioma cells via fibroblast growth factor receptor 2 (FGFR2) (Tsuchioka et al. 2008) and norepinephrine stimulated BDNF synthesis in cultured rat cortical astrocytes through α_1- and β_1/β_2-adrenergic receptors (Juric et al. 2008). In U118MG cells, these monoamines were not detected in the cell lysate and culture medium, as observed in the increased neurotrophic factors of rat astrocytes by antidepressants (Kajitani et al. 2012). These results suggest that selegiline and rasagiline might increase GDNF and BDNF expression through signal pathway not dependent on monoamines.

Rasagiline increased the mRNA, protein and enzymatic activity of MAO-A in SH-SY5Y cells though suppression of R1 (RAM2/CDCA7L/JPO2) (Inaba-Hasegawa et al. 2013). R1 represses MAO-A promoter activity by competing the zinc finger transcription factor specificity protein 1 (Sp1) for the binding to Sp1 sites (Chen et al. 2005; Shih et al. 2011). Expression of MAO-A and MAO-B is activated by a transcription factor, Kruppel-like factor 11 [KLF11, also called as transforming growth factor-β-inducible early gene (TIEG2)], which forms a transcriptional complex with Sp1 and enhances MAO transcription (Ou et al. 2004). The KLF11–Sp1 pathway was involved in MAO-B induction by ethanol in U118MG and SH-SY5Y cells, and the induction was inhibited by selegiline and rasagiline (Ou et al. 2010). These results suggest that this KLF11–Sp1 pathway might be associated with regulation of gene expression by MAO-B and MAO-A, and also the gene induction by MAO-B inhibitors. This issue should be further investigated.

Future study on the more detailed molecular mechanism of gene induction by MAO-B and MAO-A inhibitors may promote the development of novel anti-apoptotic and neuroprotective drugs applicable for the treatment of neurodegenerative disorders and depression.

Compliance with ethical standards

Conflict of interest The authors declare that there are no competing financial interests in relation to the work described.

References

Bar Am O, Amit T, Youdim MBH (2004) Contrasting neuroprotective and neurotoxic actions of respective metabolites of anti-Parkinson drugs rasagiline and selegiline. Neurosci Lett 355(3):169–172

Bortolato M, Chen K, Shih JC (2008) Monoamine inactivation: from pathophysiology to therapeutics. Adv Drug Deliv Rev 60(13–14):1527–1533

Chen Z, Trapp B (2016) Microglia and neuroprotection. J Neurochem 136(Suppl 1):10–17

Chen K, Ou XM, Chen G, Choi SH, Shih JC (2005) R1, a novel receptor of the human monoamine oxidase A. J Biol Chem 280(12):11552–11559

Damier P, Kastner A, Agid Y, Hirsch EC (1996) Does monoamine oxidase type B play a role in dopaminergic nerve cell death in Parkinson's disease? Neurology 46(5):1262–1269

De Girolamo LA, Hargreaves AJ, Billett EE (2001) Protection from MPTP-induced neurotoxicity in differentiating mouse N2a neuroblastoma cells. J Neurochem 78(3):650–660

De Zutter GS, Davis RJ (2001) Pro-apoptotic gene expression mediated by the p38 mitogen-activated protein kinase signal transduction pathway. Proc Natl Acad Sci USA 98(11):6168–6173

Ekblom J, Oreland L, Chen K, Shih JC (1998) Is there a "non-MAO" macromolecular target for L-deprenyl?: studies on MAOB mutant mice. Life Sci 63(2):181–186

Fitzgerald JC, Ugun-Klusek A, Allen G, De Cirolamo LA, Hargreaves I, Ufer C, Abramov AY, Billett EE (2014) Monoamine oxidase-A knockdown in human neuroblastoma cells reveals protection against mitochondrial toxins. FASEB J 28(1):218–229

Holschneider DP, Scremin OU, Huynh L, Chen K, Shih JC (1999) Lack of protection from ischemic injury of monoamine oxidase B-deficient mice following middle cerebral artery occlusion. Neurosci Lett 259(3):161–164

Inaba-Hasegawa K, Akao Y, Maruyama W, Naoi M (2012) Type A monoamine oxidase is associated with induction of neuroprotective Bcl-2 by rasagiline, an inhibitor of type B monoamine oxidase. J Neural Transm 119(4):405–414

Inaba-Hasegawa K, Akao Y, Maruyama W, Naoi M (2013) Rasagiline and selegiline, inhibitors of type B monoamine oxidase, induce type A monoamine oxidase in human SH-SY5Y cells. J Neural Transm 120(3):435–444

Jenner P, Olanow CW (1996) Oxidative stress and the pathogenesis of Parkinson's disease. Neurology 47(Suppl 3):S161–S170

Juric DM, Miklic S, Carman-Krzan M (2006) Monoaminergic neuronal activity up-regulates BDNF synthesis in cultured neonatal rat astrocytes. Brain Res 1108:54–62

Juric DM, Loncar D, Carman-Lrzan M (2008) Noradrenergic stimulation of BDNF synthesis in astrocytes: mediation via α1- and β1/β2-adrenergic receptors. Neurochem Int 52(1–2):297–306

Kajitani N, Hisaoka-Nakanishi K, Morioka N, Okada-Tsuchiuoka M, Kaneko M, Kasai M, Shibasaki C, Nakata Y, Takebayashi M (2012) Antidepressant acts on astrocytes leading to an increase in the expression of neurotrophic/growth factors: different regulation of FGF-2 by noradrenalin. PLoS One 7(12):e51197

Kraml M (1965) A rapid microfluorometric determination of monoamine oxidase. Biochem Pharmacol 14:1684–1686

Magyar K (2011) Pharmacology of selegiline. Int Rev Neurobiol 100:65–84

Mallajosyula JK, Kauer AD, Chinta SJ, Rajagopalan S, Rane A, Nicholis DG, Di Monte DA, Macarthour H, Andersen JA (2008) MAO-B elevation in mouse brain astrocytes results in Parkinson's pathology. PLoS One 3:e1616

Malorni W, Giammarioli AM, Matarrese P, Pieryrangeli P, Agostinelli E, Cianccio A, Grassilli E, Mondovi B (1998) Protection against apoptosis by monoamine oxidase A inhibitors. FEBS Lett 426(1):155–159

Marcocci L, de Marchi U, Salvi M, Milella ZG, Nocera S, Agostinelli E, Mondovi B, Toninello A (2002) Tyramine and monoamine oxidase inhibitors as modulators of the mitochondrial membrane permeability transition. J Membr Biol 188(1):12–31

Maruyama W, Naoi M (2010) "70th birthday Professor Riederer" induction of glial cell-line-derived and brain-derived neurotrophic factors by rasagiline and (−)deprenyl: a way to a disease-modifying therapy? J Neural Transm 120(1):83–89

Maruyama W, Nitta A, Shamoto-Nagai M, Hirata Y, Akao Y, Youdim M, Furukawa S, Nabeshima T, Naoi M (2004) *N*-Propargyl-1-(*R*)-aminoindan, rasagiline, increases glial cell line-derived neurotrophic factor (GDNF) in neuroblastoma SH-SY5Y cells through activation of NF-κB transcription factor. Neurochem Int 44(6):293–400

Miklic S, Juric DM, Caman-Krzan M (2004) Differences in the regulation of BDNF and NGF synthesis in cultured neonatal rat astrocytes. Int J Dev Neurosci 22(3):119–130

Mizuta I, Ohta M, Ohta K, Nishimura M, Mizuta E, Hayashi K, Kuno S (2000) Selegiline and desmethylselegiline stimulate NGF, BDNF and GDNF synthesis in cultured mouse astrocytes. Biochem Biophys Res Commun 279(3):751–755

Mosley RL, Benner EJ, Kadiu I, Thomas M, Boska MD, Hasan K, Laurie C, Gendelman HE (2006) Neuroinflammation, oxidative stress and the pathogenesis of Parkinson's disease. Clin Neurosci Res 6(5):261–281

Naoi M, Maruyama W, Inaba-Hasegawa K (2012) Type A and B monoamine oxidase in age-related neurodegenerative disorders. Their distinct roles in neuronal death and survival. Curr Top Med Chem 12(20):2177–2188

Naoi M, Maruyama W, Inaba-Hasegawa K (2013a) Revelation in neuroprotective functions of rasagiline and selegiline: the induction of distinct genes by different mechanisms. Expert Rev Neurother 13(6):671–684

Naoi M, Maruyama W, Yi H (2013b) Rasagiline prevents apoptosis induced by PK11195, a ligand of the outer membrane translocator protein (18 kDa), in SH-SH5Y cells through suppression of cytochrome c release from mitochondria. J Neural Transm 120(11):1539–1551

Naoi M, Riederer P, Maruyama W (2016) Modulation of monoamine oxidase (MAO) expression in neuropsychiatric disorders: genetic and environmental factors involved in type A MAO expression. J Neural Transm 123(2):91–106

Naoi M, Maruyama W, Shamoto-Nagai M (2017) Type A monoamine oxidase and serotonin are coordinately involved in depressive disorders: from neurotransmitter imbalance to impaired neurogenesis. J Neural Transm. doi:10.1007/s00702-017-1709-8

Ohta K, Kuno S, Inoue S, Ikeda E, Fujinami A, Ohta M (2010) The effect of dopamine agonists; the expression of GDNF, NGF, and

BDNF in cultured mouse astrocytes. J Neurol Sci 291(1–2):12–26

Ou X-M, Chen K, Shih JC (2004) Dual functions of transcription factors, transforming growth factor-beta-inducible early gene (TIEG)2 and Sp3, are mediated by CACCC element and Sp1 sites of human monoamine oxidase (MAO) B gene. J Biol Chem 279(20):21021–21028

Ou X-M, Stockmeier CA, Meltzer HY et al (2010) A novel role for GADPDH-MAO B cascade in ethanol-induced cellular damage. Biol Psychiatry 67(9):855–863

Rappold PM, Tieu K (2010) Astrocytes and therapeutics for Parkinson's disease. Neurotherapeutics 7(4):413–423

Saura J, Andres N, Andrade C, Ojuel J, Eriksson K, Mahy N (1997) Biphasic and region-specific MAO-B response to aging in normal human brain. Neurobiol Aging 18(5):497–507

Seymour CB, Mothersill C, Mooney R, Moriarty M, Tipton KF (2003) Monoamine oxidase inhibitors *l*-deprenyl and clorgyline protect nonmalignant human cells from ionising radiation and chemotherapy toxicity. Br J Cancer 89(10):20

Shih JC, Chen K, Ridd MJ (1999) Monoamine oxidase: from genes to behavior. Ann Rev Neurosci 22:197–217

Shih JC, Wu JB, Chen K (2011) Transcriptional regulation and multiple functions of MAO genes. J Neural Transm 118(7):979–986

Siddiqui A, Mallajosyula JK, Rane A, Anderson JK (2010) Ability to delay neuropathological events associated with astrocytic MAO-B increase in a Parkinsonian mouse model: implications for early intervention on disease progression. Neurobiol Dis 40(2):444–448

Tsuchioka M, Takebayashi M, Hisaoka K, Maeda N, Nakata Y (2008) Serotonin (5-HT) induces glial cell line-derived neurotrophic factor (GDNF) mRNA expression via the transactivation of fibroblast growth factor 2 (FGR2) in rat C6 glioma cells. J Neurochem 106(1):244–257

Weinreb O, Amit T, Bar-Am O, Youdim MB (2007) Induction of neurotrophic factors GDNF and BDNF associated with the mechanism of neurorescue action of rasagiline and ladostigil: new insights and implications for therapy. Ann N Y Acad Sci 1122:155–168

Wu Y, Kazumura K, Maruyama W, Osawa T, Naoi M (2015) Rasagiline and selegiline suppress calcium efflux from mitochondria by PK11195-induced opening of mitochondrial permeability transition pore: a novel anti-apoptotic function for neuroprotection. J Neural Transm 122(10):1399–1407

Wu Y, Shamoto-Nagai M, Maruyama W, Osawa T, Naoi M (2016) Rasagiline prevents cyclosporin A-sensitive superoxide flashes induced by PK11195, the initial signal of mitochondrial membrane permeabilization and apoptosis. J Neural Transm 123(5):491–494

Yi H, Akao Y, Maruyama W, Chen K, Shih J, Naoi M (2006) Type A monoamine oxidase is the target of an endogenous dopaminergic neurotoxin, *N*-methyl(*R*)salsolinol, leading to apoptosis in SH-SY5Y cells. J Neurochem 96(2):541–549

Youdim MBH, Bakhle YS (2006) Monoamine oxidase: isoforms and inhibitors in Parkinson's disease and depressive illness. Br J Pharmacol 147(Suppl 1):S287–S296

Youdim MBH, Edmondson D, Tipton KF (2006) The therapeutic potential of monoamine oxidase inhibitors. Nat Rev Neurosci 7(4):295–309