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Basic cell physiological activities (cell adhesion, chemotaxis and proliferation) induced by selegiline and its derivatives in Mono Mac 6 human monocytes

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Abstract Selegiline (*R*-deprenyl), a monoamine oxidase-B (MAO-B) inhibitor, has complex pharmacological effect that contributes to treatment of neurodegenerative diseases such as Parkinson's and presumably Alzheimer's disease and might work as an inhibitor of tumor growth. In respect of tumorigenesis and metastasis formation, the controlled modifications of adhesion and migration have high therapeutic significance. In the present study, our purpose was to investigate cell physiological responses (adhesion, chemotaxis and proliferation) induced by selegiline, its metabolites and synthetic derivatives and to find some correlations between the molecular structure and the reported antitumor behavior of the derivatives. Our results demonstrated that both *R*- and *S*-deprenyls have the potency to elicit increased adhesion and a chemorepellent activity in monocyte model (Mono Mac 6 cell line derived from monoblastic leukemia); however, only the *R*-enantiomer proved to be cytotoxic. Among the metabolites *R*-amphetamine has retained the adhesion inducer and the chemorepellent effect of the parent drug on the most significant level. In contrast, a reversed chemotactic effect and an improved cytotoxic character were detected in the presence of fluoro group (*p*-fluoro-*S*-deprenyl). In summary, the adhesion inducer activity, chemorepellent and advantageous cytotoxic effects of selegiline and some derivatives indicate that these drug molecules might have inhibitory effects in metastasis formation in primary tumors.

Keywords Chemotaxis · Cell adhesion · Monocyte · Tumor · Selegiline

Introduction

Selegiline (*R*-deprenyl, phenyl-isopropyl-methyl-propargylamine) the selective, irreversible inhibitor of MAO-B (Knoll et al. 1965; Magyar et al. 1967; Knoll and Magyar 1972) has been used for decades, as an antiparkinsonian drug. Since dopamine (DA) is a good substrate for MAO-B in the central nervous system (CNS), the enzyme inhibition raises the concentration of DA, the missing transmitter, at least primarily responsible for the development of Parkinson's disease (PD). The increase of antioxidant capacity due to the decrease of H_2O_2 formation is also the consequence of MAO-B inhibition (Riederer and Youdim 1986; Magyar et al. 2004). Chronic treatment with the inhibitor leads to a rise of superoxide dismutase (SOD1 and SOD2) capacity (Carrillo et al. 1991). In addition, selegiline inhibits the age-dependent increase of MAO-B activity and prevents the subsequent oxidative damages of the aging brain (Fowler et al. 1997; Lamensdorf et al. 1996).

Selegiline possesses other functions, not related to inhibition of MAO-B, such as antiapoptotic (Tatton et al. 1994, 1996) and transport inhibitory effects (Knoll and Magyar 1972; Buu et al. 1987), which play essential role in synaptic function. It was postulated that the transport (re-uptake) of transmitters represents the most effective inactivation process of the synapse. In addition, Jenei et al.

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(2005) published that selegiline increased cell to cell adhesion of PC12 and NIH3T3 cells. The cell adhesion is provided by adhesion molecules, which mediate cell-extracellular matrix and cell–cell binding. Cell adhesion molecules allow cells to communicate either with one another or with the extracellular environment and underlie the cell migration and spreading. (Moh and Shen 2009; Schmidt and Friedl 2010). The cell adhesion and migration are cell type-, concentration- and time-dependent events, and play a key role in number of physiological processes, including differentiation, embryonic development, and neuronal function. Antitumorigenic potential of selegiline was reported by several papers (ThyagaRajan et al. 1995, 1999; Magyar and Szende 2004). Importantly, deregulations in adhesion and migration contribute to the development of neurodegenerative diseases and metastatic dissemination. The effect of selegiline on the cell adhesion and migration might contribute to its well-known neuroprotective effect and the inhibition of tumorigenesis, metastasis formation.

Selegiline is metabolized with mixed function oxidases, the microsomal CYP-450 enzymes in the presence of NADPH. The main metabolites are *R*-methamphetamine, *R*-amphetamine *N*-desmethyl-*R*-deprenyl (Reynolds et al. 1978; Heinonen et al. 1989; Barrett et al. 1996b) and their corresponding para-hydroxylated products (Shin 1997). Flavin-containing monooxygenase (FMO) enzyme is responsible for the formation of deprenyl-*N*-oxide (DNO) metabolite, which has a new chiral center with positive charge on the tertiary nitrogen (Wu and Ichikawa 1995; Szökő et al. 2004). Among the metabolites *N*-desmethyl-*R*-deprenyl, deprenyl-*N*-oxide preserved their propargyl groups, simultaneously with neuroprotective action. Our findings with selegiline, *N*-desmethyl-*R*-deprenyl and *R*-deprenyl-*N*-oxide possessing propargyl group strengthen the concept of Youdim; namely the propargyl moiety is a pre-requisite of antiapoptotic and neuroprotective action (Youdim and Weinstock 2002; Youdim et al. 2006). It is well-known from the literature that *N*-oxide undergoes retro reduction, back to the parent compound. The formed new chiral center with positive charge seems to modify the biological effect of *R*-deprenyl-*N*-oxide (Clement et al. 2000). *R*-amphetamine and *R*-methamphetamine without propargyl groups lose their neuroprotective effects and increase the apoptotic activity. This complication consists of a limitation of selegiline in clinical application (Haberle et al. 2001; Tatton and Chalmers-Redman 1996; Szende et al. 2001).

It is hypothesized that some of the well-established therapeutic effects of the *R*-deprenyl are partly due to the metabolic transformation of the drug. Therefore it seems to be also grounded to examine the involvement of the products of the two metabolic pathways (CYP-450 and FMO) in the cell biological actions of selegiline.

Chemotactic responsiveness is one of the most ancient signaling mechanisms of a single cell. It is a well-conserved type of regulation, can be observed in invertebrate and vertebrate level. In the last decade, novel lab-on-chip laboratory techniques were developed including the method measuring electric cell-substrate impedance (ECIS). This technique is suitable to measure such basic cell physiological activities as cell adhesion, migration/chemotaxis and proliferation (Giaever and Keese 1984). Due to the technical developments mentioned above, migratory activity of cells (monocyte, tumor cells, etc.) could be analyzed during the whole experimental period (Atienza et al. 2005). Because of the formers more and more attention are focused on the ancient signaling of a single cell in pharmacology and mainly in tumor pathology.

The above-mentioned observations prompted us to investigate whether the antitumoral function of selegiline can be attributed to modulator actions of the drug on adhesion. It is good reason to believe that in addition to adhesion, chemotactic and proliferation capacities might play important role in drug effects. Consequently during the present studies, the effects of selegiline and its metabolites were studied on the adhesion, chemotaxis and proliferation using a tumor modeling cell line. According to the putative stereoselective and metabolism-dependent pharmacological effects of *R*-deprenyl, it seemed to be reasonable to involve the enantiomer *S*-deprenyl and the racemic *RS*-deprenyl into our studies. In addition, six synthetic derivatives of selegiline were also comprehended: optical isomers of *p*-fluoro-selegiline, *R*-methyldeprenyl, *R*-dimethyldeprenyl, and *R*-ethylamphetamine (Terleckyj and Heikkila 1992; Magyar 1994; Magyar et al. 1979). Our aim was to have better insight into the complex pharmacological activity of *R*-deprenyl, metabolites and novel synthetic derivatives by the hope to find some relation to these essential cell functions and neuroprotective and antitumor action of selegiline and to evaluate the relationships between structure and detected biological effects of *R*-deprenyl and its derivatives.

Experimental procedure

Cell culture

Cultures of Mono Mac 6 (MM6) cells (human monocytic cell line) were maintained in RPMI 1640 (Sigma Ltd., St. Louis, USA) containing 10% FCS (fetal calf serum) (Lonza

Group Ltd., Basel, Switzerland), L-glutamine (2 mM/mL) (Gibco®/Invitrogen Corporation, New York, USA), penicillin/streptomycin (1%) (Gibco®/Invitrogen Corporation, New York, USA) at 37°C in a humidified 5% CO_2 atmosphere.

Chemicals

The assayed 13 derivatives of deprenyl were: *R*-, *S*-, racemic deprenyl, *p*-fluoro-deprenyl, *R*-methyl-, *R*-dimethyl- and *N*-desmethyl-*R*-deprenyl, *R*-deprenyl-*N*-oxide, *R*-amphetamine, *R*-methamphetamine and *R*-ethylamphetamine. All the drugs tested were synthesized in Chinoin Pharmaceutical and Chemical Works/Sanofi-Aventis (Budapest, Hungary); the derivatives were provided to us as a generous gift of the firm.

Cell adhesion assay

The effects of the deprenyl and its derivatives on adhesion of the MM6 cells were examined by xCELLigence System (Roche Applied Science, Indianapolis, USA). The main part of this system, regarding the direct events and monitoring of cell adhesion, is the 96-well tissue culture E-plate. In this tool interdigitating gold micro-electrodes are fabricated onto the bottom of each well. The xCELLigence System is label-free assay and measures the change in impedance of gold micro-electrodes to AC current flow in real time. The presence of the cell on the electrodes alters the local ionic environment at the electrode/solution interface, leading to an increase in the electrode impedance. The impedance depends on the number of the attached cells on the surface of the electrode, and the degree of adhesion. More cells attached on electrode, or spreading cause larger increase in the impedance. The change in impedance represents as cell index (CI). The CI is a relative and dimensionless value, and is calculated by the following formula: $CI = (Z_i - Z_0)/15$, where Z_i is the impedance at an individual point of time during the experiment, Z_0 is the impedance at the start of the experiment.

To prepare the electrode, all of the wells were coated with human fibronectin (Chemicon® International Inc., Temecula, Canada). Mixture of 2 μg/cm^2 human fibronectin in 0.1% gelatine (Sigma Ltd., St. Louis, USA) was dropped to the bottom of each well. After 20-min incubation at 4°C, the protein solution was removed and the wells were desiccated for 5 min. Then 100-μL culture medium was added to each well and the background value of impedance was registered for 1 h. The given time was sufficient in each experiment to gain constant background curves of impedance. In the following two steps, the wells were prepared with deprenyl derivatives (10^{-12}, 10^{-9}, and 10^{-6} M), and finally were loaded with MM6 (10^4 cells/well) monocytes. Compound-free wells served as a control. Data of impedance were recorded at 10 kHz, with 20 s interval. The adhesion of the MM6 cells was monitored for 24 h. Each data represent the mathematical average of three parallels.

Chemotaxis assay

Chemotactic responsiveness of the MM6 cell line was measured by a modified Boyden chamber technique in a NeuroProbe® MBB 96 chamber (NeuroProbe, Gaithersburg, MD, USA) in 10^{-12}, 10^{-9} and 10^{-6} M concentrations. The tested substances were placed into wells of a 96-well, flat bottom, suspension microtitration plate (Sarstedt Inc., Newton, USA) which were used as lower chambers. MM6 cells were applied into the upper chamber separated from the lower one by polycarbonate filter (pore size 8 μm) (NeuroProbe, Gaithersburg, MD, USA). The incubation time was 3 h at 37°C in a humidified 5% CO_2 atmosphere. The number of the positive chemotactic responder cells was determined by MTT assay using 12-h incubation with MTT salt (3-4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium) (Sigma Ltd., St. Louis, USA). The MTT crystals were solved in DMSO. The absorbance was measured at 540 and 620 nm by ELISA reader (Labsystems Multiskan MS, Finland). Identical points of the concentration course study represent average of 15 parallel measurements. In chemotaxis experiment mentioned above, compound-free wells served as controls. The evaluated value was normalized to the control and this value is given as 'chemotaxis activity' (Chtx. act.) in percent.

Cell proliferation/cytotoxicity assay

Effects of deprenyl derivatives on the proliferation of MM6 monocyte model cells were investigated in logarithmic phase cell cultures. Groups were growing in sterile 96-well tissue culture plates for suspension cells (83.1835.500, Saarstedt, Germany) in triplicates; 100 mL of deprenyl derivative was added to 100 mL of cell culture. Effect of all deprenyl derivatives was analyzed on 10^{-12}, 10^{-9}, and 10^{-6} M concentrations prepared in tissue culture medium composed of RPMI 1640 (Sigma Ltd., St. Louis, USA), 10% FCS (fetal calf serum) (Lonza Group Ltd., Basel, Switzerland), L-glutamine (2 mM/mL) (Gibco®/Invitrogen Corporation, New York, USA), penicillin/streptomycin (Gibco®/Invitrogen Corporation, New York, USA). Control groups were treated with culture media without deprenyls. Final cell density of cell culture was 10^5 cell/mL. Cell number of samples was evaluated in 48- and 72-h cultures. For evaluation of cell densities, CASY TT (Roche, Germany) cell counter and analyzer was used.

Method pulse area analysis is applied in the system to count the cells and to characterize viability and morphometric properties of the samples. The main setting parameters provided us to measure 400 µL samples (100-µL sample of cell culture diluted in 5-mL CASYton) in triplicates. Cell viability was tested in the same system by CASYblue exclusion assay. For evaluation of data, CASYexcell 2.3 was used.

Statistical evaluation of data

Data shown in the figures represent averages and ±SD values. Statistical analysis of data was done by the application of ANOVA of Origin 8.0. Histograms provided by CASY were further analyzed online by Kolmogorov–Smirnov statistical assay (http://www.physics.csbsju.edu/stats/KS-test.html). The level of significance is shown as follows: *x*: $P < 0.05$, *y*: $P < 0.01$, and *z*: $P < 0.001$.

Results

Cell adhesion

Deprenyl and its metabolites

Cell adhesion induced by the optical enantiomers of deprenyl shows significant differences (Fig. 1Aa, Ba, Ca). The *R*-isomer proved to be the most effective in the lowest concentration (10^{-12} M) used in these studies however, the highest level of *R*-deprenyl (10^{-6} M) over-exceeded the control value after 3 h of incubation. In 10^{-9} M concentration, *R*-deprenyl proved to have negative (0–5 h) or neutral effects (5–10 h) (Fig. 1Aa). In contrast to the concentrations course study of *S*-deprenyl shows a mirror arrangement of effectiveness: 10^{-9} M *S*-deprenyl results in a constant increase of the cell adhesion in the 0–10 h period; the positive effect of in 10^{-6} M *S*-deprenyl was

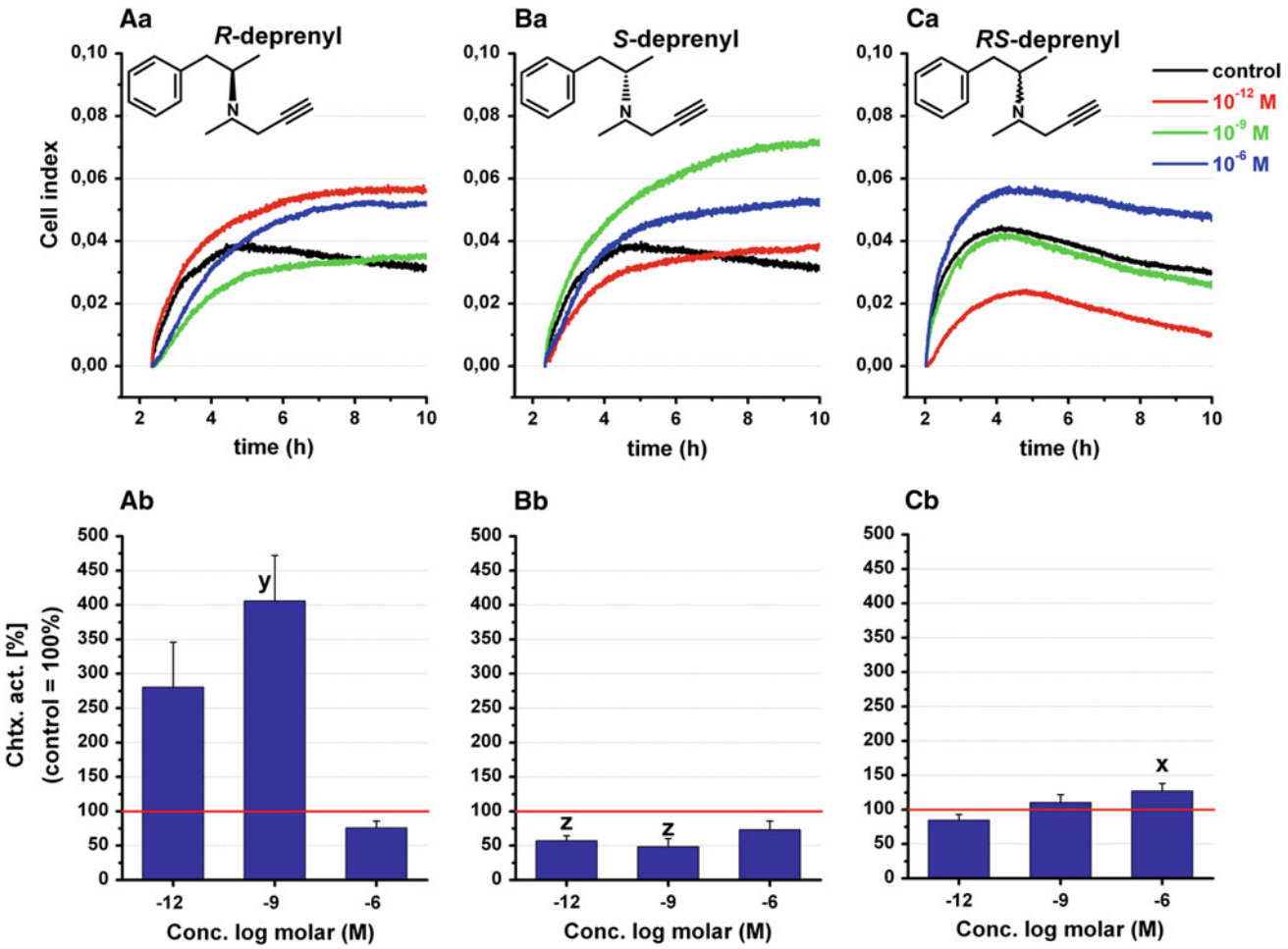

Fig. 1 Adhesion (**a**) and chemotaxis (**b**) induced by deprenyl enantiomers on MM6 human monocytic cell line. **a** The cell index was computed by the integrated software of xCELLigence system. Data represent the mean of 3 parallels. **b** Chemotactic activity (Chtx. act.) was expressed as a percentage of the control. Data represent the mean of 15 parallels ±SD. The level of significance is shown as follows: *x*: $P < 0.05$, *y*: $P < 0.01$, and *z*: $P < 0.001$

detectable only after 1.5 h incubation time, while at 10^{-12} M concentration *S*-deprenyl—which was the most effective one in selegiline—has a neutral effect (Fig. 1Ba). *RS*-deprenyl shows that the maximal 10^{-6} M concentration has a positive effect on cell adhesion, independently of the effect of each optical enantiomer. In lower concentrations no positive effect was recorded, at 10^{-12} M racemic deprenyl elicited the most significant inhibitory effect on cell adhesion regarding the three enantiomers tested (Fig. 1Ca).

R-Amphetamine proved to be the most effective ligand examined in this study (Fig. 2Aa). The significantly positive cell adhesion elicited by the lowest concentrations (10^{-12} and 10^{-9} M) of the ligand convincingly shows that cell adhesion is sensitive to the metabolites, as well. The strong sensitivity of this signaling mechanism to amphetamine indicates an essential role in the pharmacological effect of amphetamine. It seems interesting that *R*-methamphetamine—possessing an extra methyl group on the primary amino position—on the contrary could not reach the potency of *R*-amphetamine, in spite of an increase in *R*-methamphetamine was a strong inhibitor of cell adhesion (Fig. 2Ba).

The absence of the methyl group from the amino moiety of deprenyl (*N*-desmethyl-*R*-deprenyl) resulted a neutral (10^{-12} M) or a concentration-dependent and significant adhesion inhibitory effect (10^{-9} and 10^{-6} M) (Fig. 2Ca). Substitution of the amino moiety of deprenyl significantly decreases cell adhesion, which was proved by the results gained with *R*-deprenyl-*N*-oxide, too. In this ligand the oxidation of the tertiary amino group results a positive charge (quaternary-N) and an additional chiral centre. The result of the change, described above, is an unambiguously negative one, and *R*-deprenyl-*N*-oxide has a significant adhesion blocker effect in all the three representative concentrations tested (Fig. 2Da).

Synthetic derivatives of deprenyl

One of the promising modifications of deprenyl structure as a MAO-B inhibitor is the para substitution to the phenyl ring with fluoro-moiety. The *p*-fluoro-*R*-deprenyl was strong inhibitors of adhesion in 10^{-6} and 10^{-9} M concentrations, while the optical enantiomer (*p*-fluoro-*S*-deprenyl) led to a significant increasing potency on cell adhesion in all the three representative concentrations

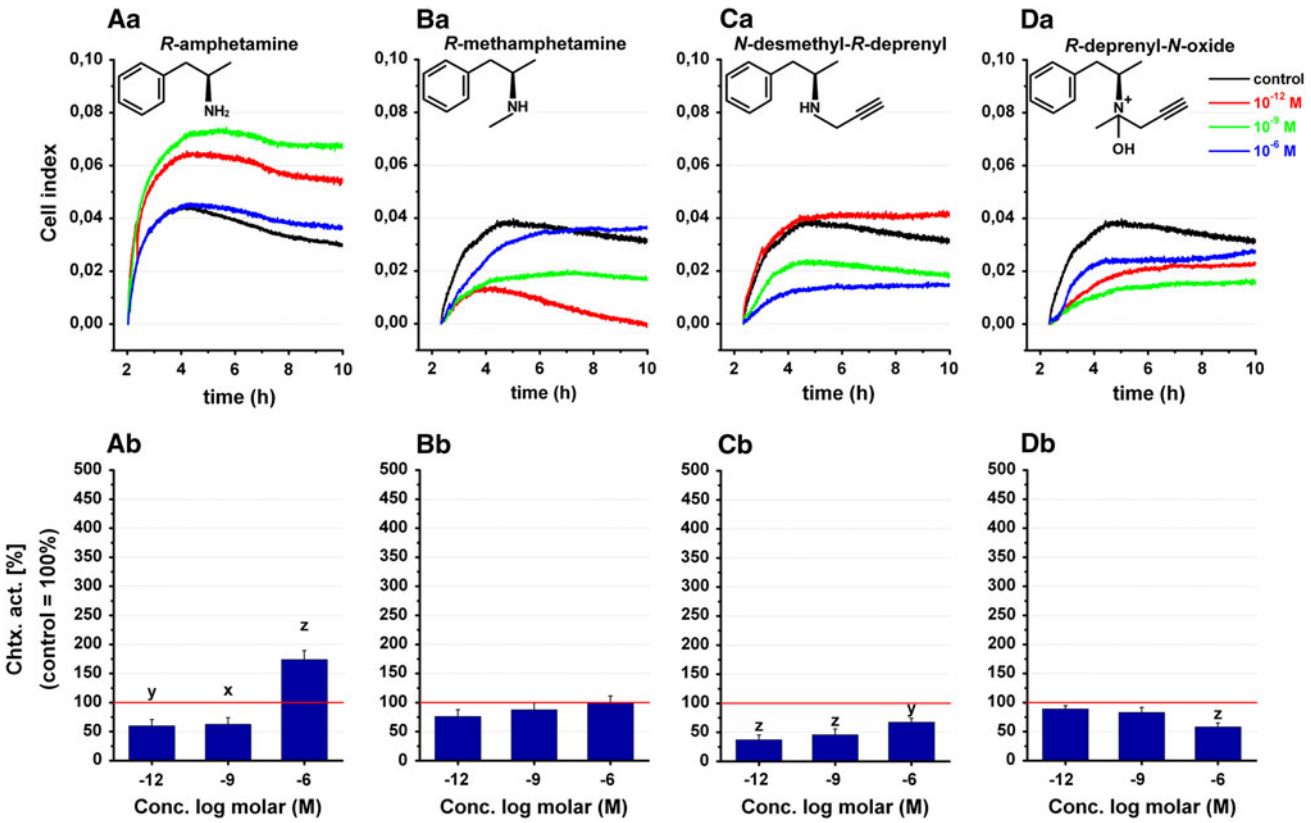

Fig. 2 Adhesion (**a**) and chemotaxis (**b**) induced by the metabolites of *R*-deprenyl on MM6 human monocytic cell line. **a** The cell index was computed by the integrated software of xCELLigence system. Data represent the mean of 3 parallels. **b** Chemotactic activity (Chtx. act.) was expressed as a percentage of the control. Data represent the mean of 15 parallels ±SD. The level of significance is shown as follows: *x*: $P < 0.05$, *y*: $P < 0.01$, and *z*: $P < 0.001$

(Fig. 3Aa, Ba). The racemic *RS*-enantiomer in all the concentration studied elicits adhesion inhibitory effect (Fig. 3Ca).

Synthetic deprenyl derivatives modified in the C_1 position (see *R*-methyldeprenyl, *R*-dimethyldeprenyl, and *R*-ethylamphetamine) were also studied in this experiment. Cell adhesion was enhanced most effectively, when the alkyl side chain at C_1 position in deprenyl was changed with a single methyl group. In a concentration of 10^{-12} M, *R*-methyldeprenyl could elicit a rapid (0–2 h) and significantly increasing response, while the higher concentrations (10^{-9} and 10^{-6} M) were strong inhibitors of adhesion (Fig. 4Aa). Substitution of the two geminal methyl groups in *R*-dimethyldeprenyl (on the alkyl group of the C_1 position) could not increase the effect described above, but all the three characteristic concentrations were neutral (10^{-6} M) or inhibitory (10^{-12} and 10^{-9} M) on cell adhesion of monocyte cells (Fig. 4Ba).

In case of *R*-ethylamphetamine, the elongation of the side chain with ethyl-moiety reduces the adhesion enhancer activity of the *R*-amphetamine, but could slightly increase the adhesion only with a long-term characteristic, after 6–10 h (Fig. 4Ca).

Chemotaxis

Deprenyl and its metabolites

Significant differences were detected in chemotactic abilities of deprenyl enantiomers. In the case of selegiline, the low concentrations of the ligand could elicit significant chemoattractant responses (10^{-12} M, 280.5% and 10^{-9} M, 406.2%) in monocytes. In contrast to the former, 10^{-6} M deprenyl, which is the effective concentration regarding MAO-B inhibition, proved to be chemorepellent (76%); however, this effect was statistically not significant

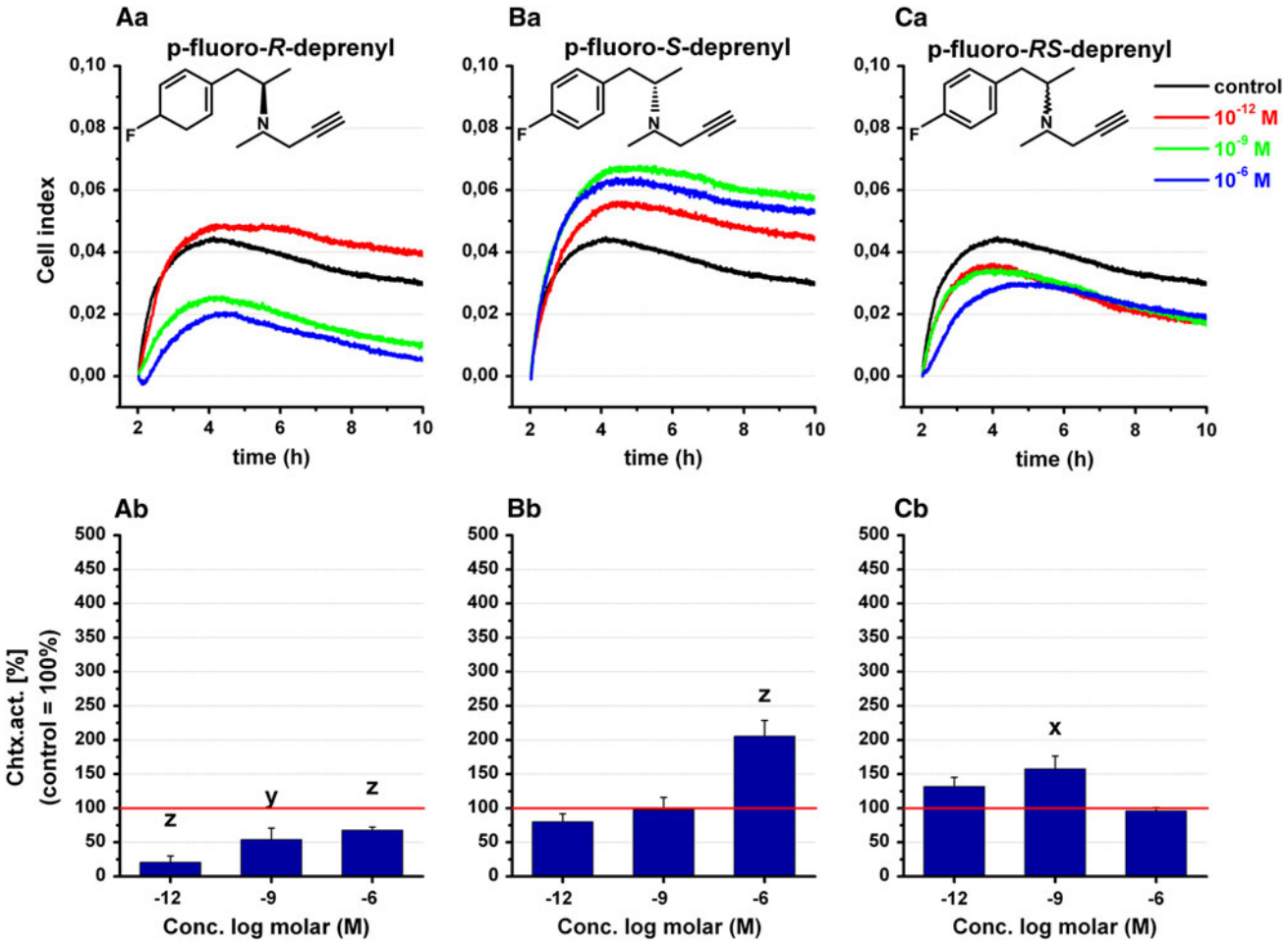

Fig. 3 Adhesion (**a**) and chemotaxis (**b**) induced by the *p*-fluoro-deprenyl enantiomers on MM6 human monocytic cell line. **a** The cell index was computed by the integrated software of xCELLigence system. Data represent the mean of 3 parallels. **b** Chemotactic activity (Chtx. act.) was expressed as a percentage of the control. Data represent the mean of 15 parallels ±SD. The level of significance is shown as follows: *x*: $P < 0.05$, *y*: $P < 0.01$, and *z*: $P < 0.001$

(Fig. 1Ab). The *S*-deprenyl has a repellent chemotactic effect in all concentrations tested (10^{-12} M, 57.4%; 10^{-9} M, 48.6%; 10^{-6} M, 73.2) (Fig. 1Bb). The effect of racemate shows that in this respect the chemorepellent character of *S*-deprenyl could be even manifested at low concentration (10^{-12} M, 84.7%) (Fig. 1Cb). The chemorepellent character of both enantiomers at 10^{-6} M turned to be a weak, but significant chemoattractant (127.3%) effect in case of racemic deprenyl.

Our observations registered on the metabolites of the selegiline have underlined the fact that substitutions of $-NH_2$ residue influence notably cell migration inducer capacities of the ligands. In the case of *N*-desmethyl-*R*-deprenyl, the removal of the methyl group from the propargyl-methyl-amino moiety results a wide range chemorepellent derivative (10^{-12} M, 37.%; 10^{-9} M, 45.9%; 10^{-6} M, 67.5%) (Fig. 2Cb). Presence of methyl residue and lack of propargyl group (*R*-methamphetamine) result weak chemorepellent (10^{-12} M, 76.1%) or neutral effects (10^{-9} M, 87.7%; 10^{-6} M, 100.5%) (Fig. 2Bb). In contrast, when the $-NH_2$ residue is free of both methyl and propargyl groups (*R*-amphetamine), we could detect characteristic changes, in concentrations the ligand had significant chemorepellent activity (10^{-12} M, 60.1%; 10^{-9} M, 62.6%) and in the highest concentration tested it had a significant chemoattractant effect (10^{-6} M, 174%) (Fig. 2Ab).

Significance of the tertiary amine group in respect of migration inducer capacity of selegiline is supported by our observations registered on *R*-deprenyl-*N*-oxide, too. The oxidation of amine residue could diminish the enhanced chemoattractant ability of selegiline to neutral level in the lower concentrations (10^{-12} M, 89%; 10^{-9} M, 83%), while the native chemorepellent character of selegiline was sustained at 10^{-6} M (58.1%) (Fig. 2Db).

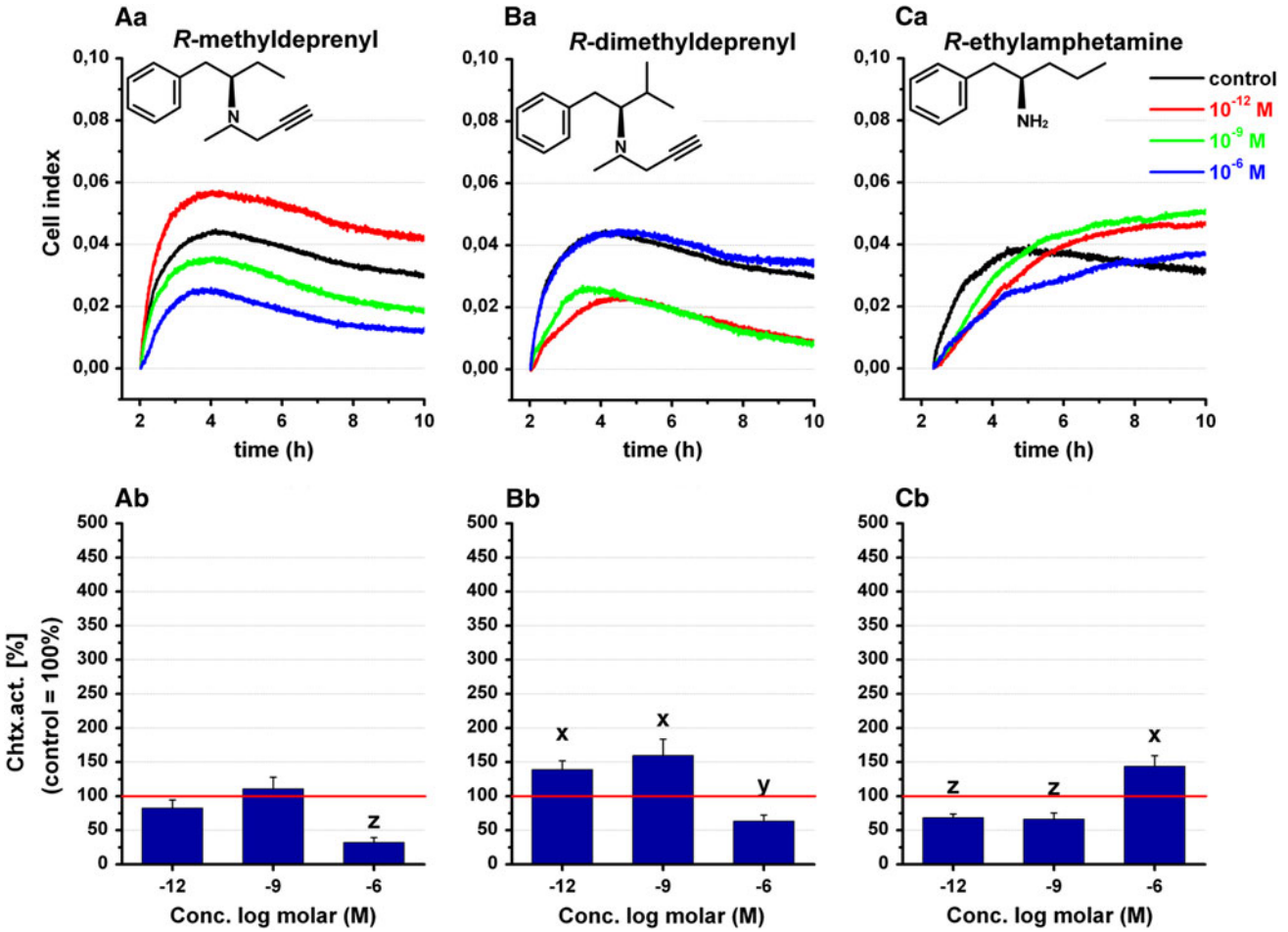

Fig. 4 Adhesion (**a**) and chemotaxis (**b**) induced by synthetic derivatives of *R*-deprenyl on MM6 human monocytic cell line. **a** Cell index was computed by the integrated software of xCELLigence system. Data represent the mean of 3 parallels. **b** Chemotactic activity (Chtx. act.) was expressed as a percentage of the control. Data represent the mean of 15 parallels ±SD. The level of significance is shown as follows: *x*: $P < 0.05$, *y*: $P < 0.01$, and *z*: $P < 0.001$

Synthetic derivatives of deprenyl

p-Fluoro substitution of the phenyl ring resulted not only in characteristic effects on the cell adhesion but could also reverse chemotactic effects of *R*- and *S*-deprenyl (Fig. 3Ab, Bb, Cb). This kind of modification has decreased the referent chemoattractant activity of *R*-deprenyl to extremely chemorepellent (10^{-12} M, 20.8%; 10^{-9} M, 54%; 10^{-6} M, 67.7%) (Fig. 3Ab). In contrast, the effect of *p*-fluoro-*S*-deprenyl proved to have a significantly increased, chemoattractant effect compared to *S*-deprenyl in 10^{-6} M (205.6%), while in lower concentrations could reduce the chemorepellent activity of the referent ligand (10^{-12} M, 80.2%; 10^{-9} M, 97.4%) (Fig. 3Bb). Chemotactic activity of the racemate showed that the *RS*-enantiomer has some advantages—see chemoattractant activity at 10^{-12} M (131.7%) and 10^{-9} M (157.5%) (Fig. 3Cb).

Substitution of deprenyl with methyl group on alkyl side chain of the C_1 position (*R*-methyl- and dimethyldeprenyl) resulted in a significant loss of chemotactic effect of the ligands (Fig. 4Ab, Bb). However, the concentration dependence of the chemotactic property resembles to activity elicited by selegiline ($10^{-9} > 10^{-12} > 10^{-6}$ M). The *R*-methyldeprenyl could elicit only significant repellent effects at 10^{-6} M (32.2%) (Fig. 4Ab), while the amplitude of activity of *R*-dimethyldeprenyl is wider, it was chemoattractant in 10^{-12} M (139%) and 10^{-9} M (159.7%), and worked as a strong chemorepellent at 10^{-6} M (63.3%) (Fig. 4Bb). The elongation of the alkyl chain of the *R*-amphetamine with an ethyl-group (*R*-ethylamphetamine) failed to modify the chemotactic character of the *R*-amphetamine (Fig. 4Cb).

Proliferation and cytotoxicity

Our results showed that the deprenyl derivatives have no cell proliferation inducer effects neither 48 nor 72 h time scale analysis; nevertheless, five derivatives had significant antiproliferative or cytotoxic effects in 48 or 72 h range of action (Table 1). In contrast, the 48-h treatment with 10^{-6} M selegiline could result significant (63.4%) cytotoxic effect while at 10^{-9} M selegiline possesses antiproliferative character (88%). Similar antiproliferative effects were observed in the case of the two enantiomers of 10^{-6} M *p*-fluoro-deprenyl (*R*-isomer 77.9% and *S*-isomer 85.9%), while 10^{-12}; 10^{-9} M *R*-methamphetamine (89.6 and 84.3%) and 10^{-9} M *R*-ethylamphetamine (80.0%) proved to be cytotoxic in monocytes.

Results of our time course experiment pointed to the reversible characteristic of the antiproliferative/cytotoxic effect described above as only *p*-fluoro-*S*-deprenyl could elicit a long-lasting (72 h) and significant cytotoxic effect (82.5%) even in a lower concentration (10^{-9} M) compared to the 48-h study. Cell cultures treated with the other derivatives (selegiline, *p*-fluoro-*R*-deprenyl, *R*-methamphetamine, and *R*-ethylamphetamine) could recover the antiproliferative/cytotoxic effects in 24 h.

Table 1 Antiproliferative or cytotoxic effect of *R*-deprenyl, its metabolites and derivatives on MM6 monocyte cells after 48 or 72 h treatment

	Viable cells (%) (Control = 100%), range: 12.5–25 μm	KS statistics	Dead cells (%) (control = 100%), range: 5–12.5 μm	KS statistics
48 h				
R-deprenyl (10^{-9} M)	63.4	*D*: 0.254	59.5	*D*: 0.605
		P: 0.000		*P*: 0.000
R-deprenyl (10^{-6} M)	88.0	*D*: 0.182	182.4	*D*: 0.500
		P: 0.026		*P*: 0.000
p-fluoro-*R*-deprenyl (10^{-6} M)	77.9	*D*: 0.230	59.4	*D*: 0.605
		P: 0.002		*P*: 0.000
p-fluoro-*S*-deprenyl (10^{-6} M)	85.9	*D*: 0.190	84.0	*D*: 0.368
		P: 0.018		*P*: 0.000
R-methamphetamine (10^{-12} M)	89.6	*D*: 0.174	187.8	*D*: 0.526
		P: 0.038		*P*: 0.000
R-methamphetamine (10^{-9} M)	84.3	*D*: 0.158	204.1	*D*: 0.592
		P: 0.075		*P*: 0.000
R-ethylamphetamine (10^{-9} M)	80.0	*D*: 0.198	216.1	*D*: 0.578
		P: 0.012		*P*: 0.000
72 h				
p-fluoro-*S*-deprenyl (10^{-9} M)	82.5	*D*: 0.182	113.4	*D*: 0.210
		P: 0.026		*P*: 0.059

Kolmogorov–Smirnov test (KS) was applied to estimate the minimum distances of source histograms

D values represent cumulative distribution, *P* values represent levels of significance

Discussion

Selegiline [(−)-phenyl-isopropyl-methyl-propargylamine] was discovered by Knoll et al. (1965). The most significant pharmacological effect of the drug is the selective, irreversible monoamine oxidase-B (MAO-B) inhibition (Knoll et al. 1965; Magyar et al. 1967; Knoll and Magyar 1972). The inhibitor rises the level of dopamine in the substantia nigra pars compacta and improves the clinical condition of parkinsonian patients (Birkmayer et al. 1975, 1977, 1983, 1985). Tatton (1994) published that selegiline in a concentration, which is too low to inhibit MAO-B, possessed antiapoptotic activity on cell culture. In addition to the antiapoptotic activity, low concentrations of the inhibitor could modulate cell to cell adhesion (Jenei et al. 2005), cell proliferation (Szende et al. 2010), apoptosis (Tatton et al. 1994, 1996; Szende et al. 2000) and gene-expression (Tatton et al. 1994, 1996; Tatton and Chalmers-Redman 1996) in neuronal and non-neuronal cells. These findings suggest that selegiline has biphasic effects; in concentrations higher than 10^{-9} M, there is a concentration-dependent MAO-B inhibition, but in lower concentrations 10^{-9} to 10^{-13} M it has an antiapoptotic activity.

Selegiline has a high 'first pass' metabolism after oral administration (Barrett et al. 1996b). Two groups of enzymes take part in the metabolic conversion of selegiline. The cytochrome P450-mediated desalkylations (depropargylation and demethylation) are the main metabolic pathways of selegiline metabolism, lead to the formation of *R*-methamphetamine *N*-desmethyl-*R*-deprenyl and *R*-amphetamine (Reynolds et al. 1978; Heinonen et al. 1989). Furthermore, the flavin-containing monooxygenase (FMO) enzymes are also capable to *N*-oxidize the drug, forming *R*-deprenyl-*N*-oxide (Wu and Ichikawa 1995; Szökő et al. 2004). The effects of the metabolites (e.g. *R*-amphetamines, *R*-deprenyl-*N*-oxide) have also characteristic effects on the basic cell biological mechanisms, which were also demonstrated (Jenei et al. 2005; Szende et al. 2010; Tekes et al. 1988; Szilágyi et al. 2009).

The well-known and well-documented therapeutic effects of selegiline support us to use it as a reference substance in our studies. Selegiline has been described to reduce both the incidence of spontaneous rat mammary tumors and the 9,10-dimethyl-1,2-benzanthracene-induced mammary tumors. The antitumor effect of the selegiline was assumed to achieve via the increased concentration of norepinephrine (NE) and IFN-gamma, as well as the increased percentages of CD8+ T lymphocytes and NK cells in the spleen (ThyagaRajan et al. 1995, 1999, 2000). The controlled modification of adhesion and chemotaxis of the tumor cells has high therapeutic significance in respect of the tumorigenesis and formation of metastasis. These data prompted us to examine whether the reported antitumor effect of selegiline is mediated through alteration of adhesion and chemotaxis, or via a direct anti-proliferative/cytotoxic effect.

Proliferation is considered to be one of the most basic cell physiological responses possessing high clinical significance (reparative mechanisms or tumor growth). In previous studies the basic pathological effects of selegiline were analyzed on A2058 human melanoma cell line. We have proved that this drug could decrease the apoptotic ratio and mitigated the oxidative injury resulting cell loss, without any promoter effect on cell proliferation (Szende et al. 2010).

As our present results gained on a leukemic tumor cell model to evaluate the hypothetical effect of selegiline on the tumorigenesis and formation of metastasis, beside characterization of the adhesion and chemotaxis inducer effects of selegiline, it was essential to clarify whether its effect on mitosis is a general character or a cell type-dependent one.

In the present work, our main purpose was to characterize the effects of the optical enantiomers (*R*- and *S*-deprenyls) and a set of their metabolites as well as novel synthetic derivatives in respect to their effects on the three essential cell physiological mechanisms: cell adhesion, chemotaxis, and proliferation.

Effects of selegiline in a concentration range required to inhibit MAO-B accompanied by increased cell adhesion, chemorepellent and cytotoxic activity. Lower concentration (10^{-9} M) of selegiline does not induce MAO-B inhibitory effect, but portrayed by decreased cell adhesion and strong chemoattractant and antiproliferative effects. The observed parallelism of increased cell adhesion and chemorepellent effects elicited by micromolar concentrations of selegiline could possess clinical significance as these effects are potentially accessible as selegiline is administered in high doses due to its short half lifetime. Jenei et al. (2005) demonstrated that the selegiline increases the cell–cell adhesion in NGF-treated PC12, which is model cells of central nervus system, and in NIH3T3 fibroblast cells modelling peripheral tissues. Our present results support that the selegiline has not only neuronal specific but also more general effect and it influences the adhesion and migratory behavior of non-neuronal cells and tumorigenic cells as well.

The role of the adhesion and migration in tumor progression and metastasis formation has been widely examined. The disrupted cell adhesion allows the tumor cells to detach and to escape from the primary tumor mass. The reduced cell adhesion contributes to gain a more motile phenotype of the malignant cell: these cells can leave the primary tumor, and eventually invade to distal organ. It is conventionally accepted that forced expression of different adhesion molecules (E-cadherin, integrin $\alpha 7$) in different

tumor cell lines could slow down the cell proliferation, and due to reduced migratory activity of the tumor cells could diminish the invasiveness of the tumor cells (Moh and Shen 2009). Based on the above findings, the cell adhesion inducer effect, the chemorepellent character, and the advantageous cytotoxic/antiproliferative effect of the selegiline might associate with the antitumorigenic activity of the molecule, and indicate its inhibitory effect in the metastasis formation of primary tumors.

Enantiomer of selegiline possesses different pharmacological activities. The *S*-isomer was proved to be less potent (1/150) enzyme inhibitor than its antipode (Magyar et al. 1967). It was also shown that *S*-deprenyl fails to prevent apoptosis induced by MPTP administration or serum withdrawal in PC12, M1 and A2058 cells (Magyar et al. 1998, 2004; Tatton et al. 1994).

The *S*-deprenyl had favorable effect on adhesion (enhanced) and chemotaxis (repellent) which suggests its preferable inhibitory effect on the development of metastasis. The reversed chemotactic effect of *S*-deprenyl compared to the *R*-derivative demonstrated a significant structure sensitive distinctiveness of the chemotactic response of the tumorous monocyte model. This antimetastatic effect of deprenyls is more underlined as it is expressed by both *R*- and *S*-enantiomer, but the cytotoxic effect was described only in case of selegiline.

Discussion of cell physiological effects of selegiline is more comprehensible in context of metabolic pathways and effects of main transformation products of selegiline.

In the routine clinical practice the drug is administrated in a dose 5–10 mg/day (peak serum level 1.2 ng/mL = 5×10^{-9} M; Heinonen et al. 1989; Barrett et al. 1996a); therefore, it is obvious that biological effects of its metabolites had also potential significance in our present research. On the basis of our experiments, we suppose that chemical conditions of the amino group (to be primary, secondary or tertiary amino group) have a potentially significant role to determine the biological activity of selegiline residues. In case *N*-desmethyl-*R*-deprenyl, the detected reciprocal concentration dependency on cell adhesion and chemotaxis are probably due to the more accessible secondary amino group in this metabolite. *R*-Amphetamine (possessing a primary amino group) is a metabolite of selegiline produced in the highest quantity in the human body besides *R*-methamphetamine (possessing secondary amino group) (Heinonen et al. 1989). As this molecule is present in the body even in relatively high doses it might be significant that a cell adhesion inducer effect and chemorepellent effect—similar to some deprenyl derivatives—were retained, which could be considered as a protective factor in metastasis formation of primary tumors.

Therefore, our results suggest the possibility that besides the parent drug, the formation of amphetamine metabolite also contributes to the effect of the selegiline. This is supported by the similar finding that the amphetamine-like metabolites could be responsible for the increase in cell–cell adhesion in NIH3T3 cells (Jenei et al. 2005). Furthermore, this assumption confers with data on the antiapoptotic function shown to be metabolism-dependent in melanoma cells (A2058), and PC12 cultures (Szende et al. 2001; Tatton et al. 1994). In our present experiments the cytotoxic effect of the methamphetamine was registered. This observation has a good correlation with the recent data of metabolism-dependent toxicity of selegiline which was demonstrated using metabolically competent mouse primary hepatocyte cultures by Mannerström et al. (2006).

The results of our experiments presented above show that relatively slight modifications of the basic drug deprenyl could result in significant changes in the cell physiological potency of the drug. The C1 position of the molecule proved to be very characteristic and sensible to modifications. The effect of the elongation of the side chain in the C1 position depends on the number of the substitution residues. Presence of one methyl group (*R*-methyldeprenyl) or two geminal methyl moieties (*R*-dimethyldeprenyl) have opposite effects on the adhesion and chemotactic response of the cells in low concentration (10^{-12} M): *R*-methyldeprenyl is adhesion inducer and neutral in chemotaxis versus *R*-dimethyldeprenyl is an adhesion blocker and chemotaxis inducer. The well-based molecular backgrounds of these effects are supported by the comparable results gained by closely related molecules *R*-amphetamine and *R*-ethylamphetamine. It is worth to be mentioned that elongation of the side chain in *R*-ethylamphetamine could result in less cell adhesion enhancer effect than the *R*-amphetamine; however in this derivative, unlike *R*-amphetamine, a cytotoxic effect was detected at on a concentration (10^{-9} M) significantly lower than the plasma concentration of selegiline-derived *R*-amphetamine (6.25 ng/mL–4.6×10^{-8} M) (Heinonen et al. 1989).

Based on the observations described above, we can assume that the elongation of the side chain in C1 position could reduce the adhesion enhancer capacity of the parent molecules (selegiline, *R*-amphetamine); however, this modification could influence the chemoattractant character only when the propargyl group is expressed in parallel in the molecules.

The *p*-fluoro-*R*-deprenyl, a halogenated derivative of selegiline, retains the irreversible and selective MAO-B enzyme inhibitory effect of its parent compound with similar potency (Terleckyj and Heikkila 1992; Erdö et al. 2000). The pharmacological profile of these drugs overlaps in some respect, for example in neuroprotection (Magyar 1997).

The presence of the *p*-fluoro group of the phenyl ring could reverse the chemotactic character of the deprenyl

enantiomers, and the adhesion of the cells was changed parallel with the chemotaxis (*p*-fluoro-*R*-deprenyl—chemorepellent and adhesion blocker, *p*-fluoro-*S*-deprenyl—chemoattractant or neutral, and adhesion enhancer). In respect of cytotoxicity this alteration proved to be highly efficient in both enantiomers, especially in the case of *p*-fluoro-*S*-deprenyl which had a long-lasting significant cytotoxic effect, while cell cultures could recover from antiproliferative effect elicited by other derivatives (selegiline, *p*-fluoro-*R*-deprenyl, etc.).

Since the selegiline induces adhesion and has chemotactic activity in doses that do not inhibit the MAO-B enzyme and its metabolites and derivatives are inactive to inhibit MAO-B enzyme or their blocker effect is developed only in higher concentrations than the parent molecule itself (10^{-6} M), we suppose that the adhesion modifying and the chemotactic effects elicited by deprenyl are mainly MAO-B-independent processes. This assumption is also confirmed by previous observations showing that selegiline increases cell–cell adhesion of neuronally differentiated PC12 cells, which is inactive in MAO-B expression, and of NIH3T3 fibroblast even at low (10^{-11} M) concentration (Jenei et al. 2005).

In conclusions, results of our other recent experiments (Kőhidai et al. 2010) which were carried out on two adenocarcinoma cell lines possessing diverse metastatic ability, also call attention that not only selegiline but also some members of its derivatives have the potency to prevent development of metastasis by their complex cell physiological activity expressed on enhancement of cell adhesion, induction of chemorepellent responsiveness of tumor cells and an advantageous antiproliferative effect.

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