THE PHARMACOLOGY OF SELEGILINE

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Abstract

Selegiline, the *R*-optical enantiomer of deprenyl (phenyl-isopropyl-methyl-propargylamine), was almost exclusively used MAO-B inhibitor during the past decades to treat Parkinson's disease. Oral treatment prolongs the need of levodopa administration. Selegiline is rapidly metabolized by the microsomal enzymes to amphetamine, methamphetamine, and desmethyl-deprenyl. In addition, the flavin-containing monooxigenase is synthesizing deprenyl-*N*-oxide. Selegiline in rather low concentrations (10^{-9}–10^{-13} M), does not influence MAO-B, but it has an antiapoptotic activity in tissue culture. The neuroprotective effect of selegiline has a biphasic character. In higher concentrations than 10^{-7} M increases the rate of apoptosis (proapoptotic activity). The metabolites are also taking part in the complex pharmacological activity of selegiline. The simultaneous presence of the pro- and antiapoptotic effects of selegiline and its metabolites frequently hindered its clinical usage. During the past years rasagiline has been introduced to replace

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selegiline in clinical application. MAO-B inhibitors beside their effect on the enzyme MAO-B could hold different spectrum of pharmacological activities. Selegiline is administered orally and it possesses an intensive "first pass" metabolism. To circumvent the "first pass" metabolism, parenteral administration of the drug might lead to different distribution and pharmacological activity of selegiline.

ABBREVIATIONS

A	amphetamine
CNS	central nervous system
DNO	deprenyl-*N*-oxide
DD	desmethyl-deprenyl
DA	dopamine
FMO	flavin-containing monooxigenase
GIT	gastrointestinal tract
i.p.	intraperitoneally
i.v.	intravenously
MA	methamphetamine
MAO	monoamine oxidase
NA	noradrenaline
p.o.	orally
PD	Parkinson's disease
5-HT	serotonin
s.c.	subcutaneously

I. Historical Aspects of Selegiline

Early observations in the 1950s, namely, that the tuberculostatic iproniazid elevated the mood of patients infected with tuberculosis, made a major impact on the development of modern biological psychiatry. Zeller discovered the monoamine oxidase (MAO) inhibitory effect of iproniazid that is, as a consequence of enzyme inhibition, the concentration of the transmitter amines was elevated in the central nervous system (CNS), which regulates the mood of the patients (Zeller and Barsky, 1952). Due to this concept, a lot of MAO inhibitors have been synthesized (hydrazines, cyclopropylamines, and *N*-propargyl-derivates) to treat human depression, the most frequent psychiatric disorder. The hope to discover a clinically

applicable antidepressant gave the impetus to the Chinoin Pharmaceutical Company in 1962 to synthesize a "me-too" drug on the name of E-250. This number indicated that deprenyl was the 250th in the series of compounds. The patent of deprenyl was issued in Hungary in 1962. It is worse to mention that neither in the patent nor in the first paper, in which the pharmacological properties of deprenyl were described by Knoll *et al.* (1965), a word was found about the Parkinson's disease, whereas that is the only accepted therapeutic indication of the drug nowadays (Parnham, 1993). The deprenyl story is rather colorful, as the important discoveries usually are; it contains subjective elements from the part of the contemporary participants. Nevertheless, deprenyl is an original Hungarian drug, synthesized by Z. Ecsery in Budapest and developed by Knoll and his group. It was synthesized as an antidepressant, psychostimulant agent, but its therapeutic application had been realized in totally different field, as it was originally planned.

Deprenyl is a propargylamine derivate; the propargyl moiety is simply attached to the amino group of methamphetamine (MA). It is closely similar to pargyline, which is also an *N*-propargyl derivate of benzylamine, synthesized earlier.

It was generally agreed that the MAO inhibitors elicit antidepressive activity, but the ingestion of foods, containing high concentration of tyramine (cheese, red vine, salted herrings), induce hypertensive crises, sometimes causing hemorrhage in the CNS (Blackwell *et al.*, 1967; Natoff, 1964). Because of the serious side effects of the nonselective (first generation) MAO inhibitors—named "cheese effect"—they were not the drugs of choice to treat depression (Jarrott and Vajda, 1987; Youdim and Finberg, 1987). Deprenyl, similarly to the other MAO inhibitors was neglected. In spite of the fact that Knoll and his group in rat vas-deferens preparation and on the blood pressure of cats gave experimental evidence, that deprenyl does not potentiate tyramine effect, neither *in vitro*, nor *in vivo* studies (Knoll *et al.*, 1968). In 1967 it was published that the levorotatory enantiomer of deprenyl is more potent inhibitor of MAO than the dextrorotatory form, and after 1967, all of the studies, including the clinical application of the drug, were conducted with the (−)-optical antipode of deprenyl, named selegiline (Magyar *et al.*, 1967).

Due to the discovery of the heterogenic nature of MAO (Johnston, 1968) and the selective enzyme inhibitory properties of selegiline, it became the only MAO inhibitor, which survived the shock of "cheese effect" (Magyar *et al.*, 2010).

II. The Multiplicity of Monoamine Oxidase

In 1968, on the basis of substrate specificity and inhibitor sensitivity, Johnston discovered that the MAO enzyme exists in multiple forms, being sensitive or not to the effect of clorgyline, using the substrate of 5-HT. The sensitive part of the enzyme was named MAO-A, while the other one MAO-B. We used ^{14}C-labeled tyramine as a

substrate in our studies, and the enzyme inhibition curve obtained with selegiline has shown a double sigmoid character. After the discovery of Johnston, we immediately realized that the strange inhibition curve is due to the existence of MAO-A and MAO-B izoenzymes, when a mixed type of substrate is used, and the enzyme activity is inhibited with a selective inhibitor which cannot be anything else in our case, just selegiline (Knoll and Magyar, 1972). The enzyme inhibition curve unanimously showed that selegiline, on the contrary to clorgyline, is a potent, selective inhibitor of MAO-B (Magyar, 1993). The ratio of selectivity (IC_{50} of MAO-A/MAO-B) was ~500. Similar inhibition curve was obtained, when the metabolism of a mixed substrate was inhibited with clorgyline. Table I shows the substrate specificity and inhibitor sensitivity of the subtypes of MAO, in case of the most frequently used inhibitors and substrates. Our results were presented in the conference organized in Sardinia in 1971, devoted to the 70 years anniversary of H. Blaschko, who discovered, together with Hare, the MAO enzyme itself (Blaschko, 1974; Hare, 1928). The results were published a year later in "Advances in Biochemical Psychopharmacology" (Knoll and Magyar, 1972). The paper obtained international attention and became a "Science Citation Classic" in 1982. It was declared in the paper that selegiline is a potent B-type selective irreversible MAO inhibitor without "cheese effect." Initially, it forms a noncovalent complex with the flavin containing MAO enzyme, but its subsequent oxidation leads to the formation of a covalent bound with the enzyme complex.

The existence of MAO-A and MAO-B has received extensive attention from the biochemical and pharmaceutical communities to facilitate the development of more and more selective and effective drugs. The subtypes of MAO were differentiated not only on the basis of substrate specificity and inhibitor sensitivity but also with immunohystochemical methods. Molecular genetics was rarely used for this purpose (Shih and Chen, 2004).

Nevertheless, the localization of the genes was determined on the X-chromosomes. Initial studies on purified enzymes utilized liver mitochondria to have large

Table I
Substrates and Inhibitors of MAO.

	MAO-A	MAO-A and -B	MAO-B
Substrate specificity	Serotonin (5-HT)	dopamine (D) noradrenaline (NA) tyramine (T)	β-Phenylethylamine (PEA)
Inhibitor sensitivity	Clorgyline[a]	Iproniazide Phenelsine Tranylcypromine	Selegiline[a]

[a] Selective inhibitors.

quantity of MAO-B, while human placental mitochondria served as a source for MAO-A.

Thanks to the excellent work of Edmondson and his group, the structure of MAO-A and MAO-B has been determined (Edmondson *et al.*, 2004). His studies on MAO will bring our understanding of both the structure and catalytic mechanism of this enzyme to a new level. Cloning and sequencing the respective genes, convincingly demonstrated that MAO-A and MAO-B are two separate enzymes that share many similar properties, for example, 70% sequence identity. It was also discovered on knock out rats of MAO-A that the animals become aggressive. It is rather interesting that the MAO-A can be inhibited totally by clorgyline, but the aggressive behavior of the animals did not appear (Chen *et al.*, 2007).

Interesting findings have been published by Oreland and his group, detecting association between the platelet MAO-B activity and personality traits such as sensation seeking, behavior, and impulsiveness. In human platelet, only the MAO-B is expressed which is readily accessible in venous blood. Recent results suggest an association between the platelet MAO-B activity and personality as well as vulnerability in the second type of alcoholism (Oreland *et al.*, 2004).

The existence of the subtypes of MAO offers acceptable explanation for the lack of the ''cheese reaction'' in case of selegiline treatment; mainly if we take into consideration the uneven distribution of the isoforms of MAO. High quantity of MAO-A can be found in the gastrointestinal tract (GIT), while more MAO-B are located in the CNS, compared to other organs. The food-derived amines are metabolized by the MAO-A. Due to the ''first pass'' metabolism, food-derived amines are inactivated in the GIT. MAO-A inhibitors protect the ''first pass'' metabolism of amines and increases the chance of the ''cheese effect.'' On the contrary, the quantity of MAO-B is high in the CNS and plays an essential role in the metabolism of dopamine (DA). Nevertheless, DA and tyramine are substrates for both MAO subtypes. Consequently, the selective MAO-B inhibitors preferentially raise the concentration of DA in the CNS. The selectivity of the inhibitors is relative and concentration-dependent. Both selegiline and clorgyline inhibit the reversed subtypes of the enzyme in high molar concentrations. We are aware of the chemical structure of the purified MAO-A and MAO-B. The question is how these achievements can serve to find new better inhibitors which can over-exceed the clinical value of the presently available drugs.

It was firmly established that, in the serotonergic nerve endings, the MAO-B activity is high, while in dopaminergic nerves, the concentration of MAO-A is elevated. High MAO-B activity in human blood is localized in platelets, which can be determined without any ethical hindrance in the rest of the blood, which is normally taken during clinical examination of the patients. It is interesting that the generally used laboratory animals (rats, mice, cats, and rabbits) do not contain platelet MAO-B, only the pig contains in a certain but measurable amount (10–15%) of MAO-B activity compared to human platelets.

MAO activity is localized in the outer membrane of the mitochondria; therefore, drugs which inhibit MAO should reach the mitochondrial surface.

III. The Pharmacology of MAO-B Inhibition

The following reaction is catalyzed by MAO:

$$R - CH_2 - NH_2 \xrightarrow[H_2O+O_2]{MAO} R - COH + H_2O_2 + NH_3$$

The following pharmacological consequences are elicited by the treatment with selegiline, the selective inhibitor:

1. Selegiline treatment increases the concentration of DA in the CNS, the lack of which neurotransmitter plays the primary role in the pathogenesis of Parkinson's disease (PD) (Hornykiewicz, 2001, 2002).
2. Selegiline treatment decreases both the oxidative and nutritive stress by lowering the H_2O_2 overproduction as well as diminishes the amount of reactive oxygen and nitrogen species (ROS and RNS). Superoxide anion radicals can react with nitric oxide (NO) and form peroxynitrate, which spontaneously decomposes to $^{\circ}OH^{-}$ and NO_2 (Tipton *et al.*, 2004).
3. Selegiline inhibits the age-related increase of MAO-B activity; consequently, it can slow down the oxidative damage of the CNS, presumably prolonging life expectancy (Knoll, 1988).
4. Long-term treatment with selegiline increases the capacity of the protective mechanisms against oxidative damage. In addition, the long-term treatment also increases the activity of *superoxide dismutase* (SOD), mainly the soluble form of that (CuZn SOD). The catalase activity is also enhanced (Carrillo *et al.*, 1991).
5. MAO is localized partly on the outer membrane of the mitochondria inside the nerve endings and in glial cells. Synaptic transmission is increased by the inhibition of mitochondrial MAO, but MAO-B inhibition elevates the concentration of DA in the glia as well, which takes part in the extrasynaptic DA transmission.

IV. Structure–Activity Relationship Studies

Compounds from which selegiline has been selected gave us the possibility to analyze the structure–activity relationship. It became clear that even small alterations of the amphetaminergic structure—such as alkylation of the side chain (–methyl–isopropyl groups), saturation, or halogenation of the ring—resulted in a

decrease of the MAO inhibitory potency. Nevertheless, replacing the phenyl ring with a furan or an indenyl group resulted in potent inhibitors of MAO-B (Magyar *et al.*, 1980). The indenyl derivate J-508 exceeded the MAO-B inhibitory potency of selegiline by one order of magnitude. In contrast to selegiline, the (+)-enantiomer of J-508 is a more potent inhibitor than the (−)-variant on MAO-B. In spite of some favorable properties of J-508, we did not continue the study on this compound, as we were highly satisfied with selegiline, on the other hand, since it became the "gold standard" of MAO inhibitors for decades. Selegiline was the drug of choice of MAO-B inhibitors to treat Parkinson's disease, without any important competitor on the market.

Presently, rasagiline—under the trade-name of Azilect—appeared on the market, which is structurally a nor-J-508 (Youdim *et al.*, 2001). Azilect dominates the market nowadays. Its privilege can partly be due to the novelty of the drug. Nevertheless, the complexity of the multifunctional effects of rasagiline might lead to its success. The studies of MAO-B inhibitors during the past decades have shown us that concentrating only the enzyme inhibitory potency led us to a myopic vision.

Among the new compounds, promising MAO-B inhibitors were found, although their further analysis had been stopped.

It is difficult to maintain the selective irreversible inhibition of selegiline *in vivo* during prolonged administration. Nevertheless, in a dose of 0.05–0.25 mg/day injected subcutaneously to rats, the selective spectrum can be maintained, but in a dose of 1 mg/kg, it was lost (Ekstedt *et al.*, 1979).

V. Effects of Selegiline Not Relating to MAO-B Inhibition

It is generally accepted that a selective transport mechanism exists in the synapse, localized on the membrane of the nerve endings. The physiological role of the transport (uptake) is to carry the released transmitter amines from the synaptic gap back to the nerve endings. This process preserves transmitter amines released by nerve impulses from metabolic inactivation by catechol-O-methyltransferase (COMT). Moreover the transport is the most effective terminating mechanism of transmitter action. The (+)-optical isomer of deprenyl is more effective to inhibit transport proteins and facilitates the transmission than selegiline.

Our studies on selegiline and its metabolites unequivocally proved that the MAO-B enzyme inhibitory potency and the inhibition of transport are independent mechanisms. Some of the metabolites of deprenyl are strong inhibitors of uptake, without possessing enzyme inhibitory effect in structure–activity relationship studies, we found metabolites capable to inhibit DA, NA, and 5-HT uptake selectively with or without MAO inhibitory potency. The IC_{50} of MAO inhibition

in μmol/l and the relative potency on the transport were presented in 2000 (Tekes *et al.*, 1988). The inhibitory potency of the compounds on DA uptake was measured in striatal synaptosomes of rats, while on NA and 5-HT uptake, that was determined in the hypothalamus and hippocampus, respectively.

The transporter protein is able to take up selective toxins into the nerve endings. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine), a petidine analog, is the most extensively studied selective neurotoxin, which damages dopamin neurons in the substantia nigra pars compacta. The MPTP story strengthened the role of the neurotoxin hypothesis in the pathogenesis of PD. MPTP is a pretoxin, which is converted to MPDP by MAO-B, and without enzymatic interference, it leads to the formation of MPP^+ (1-methyl-4-phenyl-piperidine), a toxin, which is selectively taken up by the nigrostriatal system of the CNS. The reason of the high affinity of MPP^+ to the dopaminergic system in the CNS, but not in the periphery, are not known. The conversion of MPTP to MPDP can be inhibited by selegiline pretreatment, which prevents the toxicity. A comprehensive paper about the mechanism of MPTP toxicity was published by Glover *et al.* (1986). The protective effect of selegiline on MPTP toxicity, described by Langston, greatly enhanced the respect of selegiline and simultaneously strengthened the concept of the existence of neuroprotection (Langston, 1990).

There are other toxins, such as DSP-4, selective to noradrenergic- (Haberle *et al.*, 2002), 5,6-dihydroxy-triptamine to serotonergic- (Fowler and Tipton, 1982), and AF64A to cholinergic (Ricci *et al.*, 1992) nerves. Their effect can be prevented by selegiline administration before the insult caused by the toxins. It is interesting to note that selegiline seemed to be effective, when it was administered a few hours later following DSP-4 treatment, indicating some neuro-restoring property of selegiline.

Among the insecticides, there are selective dopaminergic toxins, such as rotenone and paraquate, which might play a certain role in the etiology of Parkinson's disease (Choi *et al.*, 2010). Against all of these toxins, selegiline shows up neuroprotective effect, which is mostly independent of its MAO-B inhibitory property.

It was published in several studies that selegiline has an antitumor effects (Thyaga Rajan and Felten, 2002; Thyaga Rajan and Quadri, 1999; Thyaga Rajan *et al.*, 1999, 2000). A novel effect of selegiline is to increase cell-to-cell adhesion (Jenei *et al.*, 2005) and the inhibition of hyperpermeability of vascular endothelial cells (Tharakan *et al.*, 2010; Whaley *et al.*, 2009).

VI. Antiapoptotic Effect of Selegiline

In 1994, Tatton and his group published an important discovery, namely, that selegiline reduces the rate of apoptosis in cell culture by inducing new protein synthesis, in a concentration range (10^{-9}–10^{-12} M) which is too low to inhibit the

MAO-B enzyme (Tatton *et al.*, 1994). They used PC12 cells of neuroectodermal origin, where the apoptosis was elicited by serum and/or NGF deprivation of the cell culture. It was postulated that the apoptotic mechanism is likely to be responsible, at least partly, for cell death in neurodegenerative diseases, such as the Parkinson's and the Alzheimer's diseases as well as in the amyotrophic lateral sclerosis. Drugs which are able to inhibit apoptosis could be promising candidates for neuroprotective therapy in the future. It was demonstrated that selegiline can prevent or diminish the apoptosis, induced by serum deprivation, glutation depletion and toxins, such as okadaic acid, nitric oxide, peroxynitrate, cytosine arabinoside, as well as peripheral nerve crush and axotomy (Tatton *et al.*, 1996). In our studies, we used the A2058 human melanoma cell line of neuroectodermal origin (Magyar and Szende, 2000, 2004; Magyar *et al.*, 1996, 1998; Szende, 2004; Szende *et al.*, 2001); however, our culture was not differentiated by nerve growth factor (NGF) deprivation, which might explain the differences of the results between the two research groups. Serum withdrawal for 5 days resulted in the appearance of a high number of apoptotic cells. The opposite antipode of (−)-deprenyl did not influence apoptosis.

High concentration of selegiline (10^{-3}–10^{-4} M) induces proapoptotic cell damage. Tatton described that SKF-525A (Proadiphene) pretreatment might prevent the antiapoptotic activity of selegiline. SKF-525A is an inhibitor of the microsomal drug metabolizing enzymes. Neither Tatton and Chalmers-Redman (1996) in PC12 cells nor our group in A2058 melanoma cell culture observed antiapoptotic effects in trophycally withdrawn culture, where the metabolites of selegiline, namely MA (methamphetamine) and A (amphetamine), were in low concentrations (10^{-9}–10^{-13} M) (Szende *et al.*, 2001; Tatton *et al.*, 1994).

DD ((−)-desmethyl-deprenyl) showed a dose-dependent inhibition of tumor growth *in vivo* on A2058 human melanoma stenographs (Szende *et al.*, 2000). These studies convincingly have shown that species variations of the cell cultures (origin of cells) differentiated or not differentiated cells, further, the neuronal or nonneuronal cultures behaved differently regarding the proapoptotic and antiapoptotic effects of selegiline. We proved that selegiline decreased the apoptotic effect of ischemia-reperfusion in rats, in a dose usually applied in human therapy (Toronyi *et al.*, 2002). Quin and his group reported an important finding, namely, that selegiline has antiapoptotic effect on nonneural cells, such as cardiac tissue (Qin *et al.*, 2003).

The mechanism of the established antiapoptotic effect of selegiline is most likely achieved through the modulation of gene expression, interfering with the apoptotic cascades, especially the mitochondrial pathway. Apoptosis is mediated by the loss of mitochondrial membrane potential and the opening of the mitochondrial membrane permeability transition pores, through which the proapoptotic factors are released, such as cytochrome *C*. Increased quantity of BCL2 helps to minimize the loss of mitochondrial membrane potential and closes the transition pores (Tatton and Chalmers-Redman, 1996; Wadia *et al.*, 1998). Selegiline, in a low concentration, might modulate the regulation of antiapoptotic BCL2 or the proapoptotic BAX concentrations.

The cytoprotective activity of selegiline, DD, and DNO (deprenyl-*N*-oxide) was studied against L-buthionine-(*S,R*)-sulfoximine (BSO) toxicity in serum deprived A-2058 melanoma cell culture. DD was the most effective compound in decreasing apoptotic activity, while DNO stabilized the cell number on the control level and increased the ratio of mitotic cells above the level measured in serum deprived control (Szende *et al.*, 2010).

VII. Pharmacokinetics of Selegiline

Selegiline is a highly lipid-soluble substance. Its distribution between hexane and phosphate buffer (pH 7.4) is very high in favor of hexane (82%) compared to the distribution of NA and A (0.01% and 0.32%, respectively). The fate of selegiline has been studied in many laboratories, including ours (Reynolds *et al.*, 1978; Riederer and Youdim, 1986; Rohatagi *et al.*, 1997a,b). In our early studies, we used ^{14}C-labeled selegiline; the label was positioned in the side chain or in the *N*-methyl or *N*-propargyl groups. We proved with others that selegiline is absorbed completely and rapidly from every routes of administration, but the bioavailability ($AUC_{0-\infty}$) of the drug highly depends on the type of administration (Barrett *et al.*, 1996a; Heinonen *et al.*, 1989, 1994; Magyar, 1994; Magyar *et al.*, 2004; Magyar and Tóthfalusi, 1984; Mahmood, 1997; Szebeni *et al.*, 1995). The $AUC_{0-\infty}$ values of selegiline, together with those of MA, DD, and A, were examined in our laboratory after oral (p.o.), subcutaneous (s.c.), intraperitoneal (i.p.), and intravenous (i.v.) administrations, and the results were presented in the paper of Magyar *et al.* (2004, 2010). Surprisingly, the $AUC_{0-\infty}$ values detected after p.o. administration of selegiline were rather low (25%) compared to those in the i.v. studies (100%). Azzaro and his coworkers registered only 4% $AUC_{0-\infty}$ in humans (Azzaro *et al.*, 2007). The highest plasma concentration of 0.5 μg/l was reached 1.5 h after p.o. administration (Heinonen *et al.*, 1994; Magyar and Tóthfalusi, 1984; Szebeni *et al.*, 1995). Food consumption surprisingly increased the efficiency of p.o. absorption of selegiline (Barrett *et al.*, 1996a). The low p.o. $AUC_{0-\infty}$ values compared to parenteral administration indicate an intensive "first pass" metabolism, thus a smaller amount of the parent drug reaches the systemic circulation (Barrett *et al.*, 1996a; Heinonen *et al.*, 1989; Magyar *et al.*, 2004, 2010; Mahmood *et al.*, 1994, 1995). In contrast to oral route, parenteral administration of selegiline shows much less "first pass" metabolism of the drug ($\sim$10%).

Parenteral treatment with selegiline results in relatively high inhibitor concentration in the brain, compared to other organs. It is due to the high concentration of selegiline in the arterial blood, the high perfusion rate of the brain, and the high lipid solubility of the inhibitor. After a usual daily dose of selegiline (10 mg/day in man), these factors can result in so high selegiline concentration in the brain, that

is able to inhibit MAO-A, the enzyme known to play an important role in human depression. At the same time, MAO-A is not so efficiently inhibited in the GIT, mainly because of its lower perfusion rate. Table II shows this experimental finding demonstrated by an animal model. The special distribution of selegiline might explain the lack of "cheese effect." This concept is supported by the distribution of MAO, namely, that 80% of the total MAO activity localized in human brain (Tolosa and Katzenschlager, 2007).

This can explain why MAO-B inhibitors lack "cheese effect" (Barrett *et al.*, 1996b; Feiger *et al.*, 2006; Wecker *et al.*, 2003). It is worth to mention that during the preclinical studies of selegiline, the animals were treated s.c., while in human therapy; selegiline was almost exclusively administered orally. Because of the different distribution pattern as well as the different routes of administration in the former cases, it is difficult to draw valid conclusions from animal experiments to human studies.

The whole body autoradiography studies on mice have shown a rapid penetration of ^{14}C-selegiline into the brain and fatty tissues, such as brown-fat. The rapid rise of selegiline concentration was followed by a fast decrease after i.v. administration of the drug (Magyar, 1994). In some of these studies, doubly and alternatively labeled radio isomers of selegiline were used, when the ^{3}H-labeling

Table II
The Effect of Selegiline in Different Organs of MAO-A and -B After Chronic Treatments of Rats.

MAO	Selegiline			
	Orally		Subcutaneously	
	0.5 mg/kg	5 mg/kg	0.5 mg/kg	5 mg/kg
Brain MAO inhibition in %				
MAO-B	45.47	92.08	93.75	98.95
MAO-A	5.05	17.99	0.01	84.87
Liver MAO inhibition in %				
MAO-B	45.52	92.04	43.06	91.23
MAO-A	24.48	31.59	19.32	33.83
Bowel MAO inhibition in %				
MAO-B	68.55	84.92	68.29	80.31
MAO-A	36.49	84.13	24.67	61.49

Rats were treated orally or subcutaneous during 5 days.
The daily dose was 0.5 or 5.0 mg/kg.
Animals were decapitated 2 h after last injection.
Substrates: MAO-A, 5-HT; MAO-B, PEA.

in the ring indicated the total quantity of the parent compound and its metabolites, while ^{14}C-labeling in the propargyl group has shown the concentration of intact molecules. The evaluations of these results are complicated, but it has been shown that repeated administration of selegiline can lead to a stabilized long-lasting steady-state concentration of the inhibitor. That can be explained by the irreversible binding of selegiline to MAO-B.

From the pharmacokinetic studies of selegiline in dogs, the following important parameters were obtained: the absorption is fast (t_{max} 25 ± 5.8 min), the distribution volume is high (6.56 ± 0.56 l/kg), the elimination half-life is short ($t_{½}^{\beta}$ 60.24 ± 9.56 min), and the bioavailability depends on the route of administration (8.51 ± 3.31%). Parameters of the metabolism are published in the paper of Magyar *et al.* (2007). The metabolite concentrations of selegiline are rather low in the blood, because of the rapid tissue penetration and the intensive "first pass" metabolism. To determine the concentration of metabolites in tissues, a sensitive GC/MS analysis was performed.

VIII. Metabolism of Selegiline

Selegiline is readily metabolized in experimental animals and in humans, when mainly MA, DD, and A are formed and excreted into the urine. The metabolism of selegiline is stereospecific. Schacter was the first, who proved, that there is no racemization during metabolism of the selegiline (Schachter *et al.*, 1980). The metabolites are pharmacologically active. The *S*-enantiomers are more potent to inhibit the transporter proteins than the *R*-antipades. A chiral capillary electrophoresis method was developed in our laboratory for the separation of the enantiomers (Szökő and Magyar, 1995). CYP isoforms, which dealkylate selegiline, are the CYP206 (Grace *et al.*, 1994; Mahmood, 1997), the CYP3A4 (Dragoni *et al.*, 2003; Taavitsainen *et al.*, 2000), and the CYP2E1 (Valoti *et al.*, 2000). The flavin-containing monooxigenase (FMO) enzymes play a role in the formation of N-oxide (Szökő *et al.*, 2004). In addition to dealkylation, the *para*-hydroxylation can also be observed (Dragoni *et al.*, 2003; Kalász *et al.*, 1990; Lengyel *et al.*, 1997) in rats or in humans (Shin, 1997; Tarjányi *et al.*, 1998). Conjugates of the *para*-hydroxy-metabolites can be found in the urine. Ephedrine-type urinary metabolites were also produced by β-OH-formation in low amounts and excreted into the urine (Rohatagi *et al.*, 1997a). The formation of a new metabolite, selegiline-N-oxide (DNO), in a quantity less than 1% of the dose, was suggested by Japanese authors (Katagi *et al.*, 2001). In earlier studies with gas chromatography (GC), this metabolite was not detected because of its heat degradation. In the very early period, using thin-layer chromatography, we detected this metabolite although its chemical structure remained to be undetermined (Magyar and Szüts, 1982). The HPLC/MS method is suitable to

the quantitative determination of DNO, which has quaternary nitrogen with an additional new chiral center. Consequently in contrast to selegiline, DNO has four stereoisomers.

DNO is excreted into the urine; its quantity during 24 h was about 1–5% of the dose administered, comparable to the quantity of MA and A (Katagi *et al.*, 2002). Para-hydroxy metabolites are more intensively formed. Their total urinary excretion can reach 50–60%, mostly conjugates. It worth to mention, that some publications described a retro-reduction of DNO (Sugiura and Kato, 1977). DNO is the ultimate metabolite having propargyl group, which is a pro-requisite of neuroprotection. Currently, an intensive study is going on in our lab to define the function of DNO. As a quaternary compound, its MAO inhibitory potency is lost.

Katagi and his group published in 2002 that DNO can be used as a reliable indicator of selegiline administration. They developed a sensitive method which is suitable to detect DNO, in cases of clinical and forensic toxicology, when selegiline administration should be distinguished from MA abuse (Katagi *et al.*, 2002).

IX. The Role of Birkmayer and His Group in the Introduction of Selegiline in the Therapy of Parkinson's Disease

The symposium organized in Sardinia in 1971 played an important role in the therapeutic application of selegiline. It was reported on the symposium by Knoll and Magyar, that (−)-deprenyl selectively inhibits the clorgyline insensitive part of MAO and (−)-deprenyl does not potentiate the hypertensive effect of exogenous catecholamines, neither *in vitro* nor *in vivo* studies. The lack of tyramine potentiation in clinical studies was also published by Varga and Tringer, however, Hungarian psychiatrists did not take part in selegiline research any further (Varga and Tringer, 1967). Being aware of the knowledge of the inhibitory spectrum of selegiline, P. Riederer and M. Youdim recognized the relevance of its potential benefit in the management of Parkinson's disease and suggested to W. Birkmayer in Vienna, the application of selegiline as an antiparkinson drug. Selegiline was found to be effective in parkinsonian patients in the clinical practice, and their results were published in the literature (Birkmayer *et al.*, 1975, 1977, 1985).

X. Future Perspectives

After three decades of unlimited use of selegiline as a gold standard in the treatment of PD, the question is arised whether it is reasonable or not to speak about the future perspectives of selegiline or, in a broader sense, the future

perspectives of the MAO-B inhibitors. The answer is: yes! The introduction of rasagiline (*N*-propargylamine-inden, Azilect), recently, gives positive answer for the question. Rasagiline has 10-times higher potency to inhibit MAO-B, compared to selegiline. Nevertheless, this is not the main reason for its introduction in human therapy. The complexity of the spectrum of its activity should be considered, which makes it possible to choose the most suitable inhibitor. Recent findings firmly suggest that all of the biochemical and pharmacological properties of MAO-B inhibitors experienced in preclinical and clinical studies are not characteristic of all substances equally, but some characters are unique to a certain compound. Selegiline and rasagiline are more than simple MAO-B inhibitors.

The physicochemical properties of the inhibitors can be different which can modify the fate of drugs in the body (absorption, distribution, metabolism, elimination; ADME). The route of administration is an important factor, as it can alter the blood and the brain concentrations of the substances. It would be advisable to find the most suitable way of parenteral administration with a good efficiency and tolerability, mainly in the case of chronic administration.

To recite all of the possibilities would be impossible and useless, but it should be mentioned that the antiapoptotic effects of the MAO-B inhibitors offer a large scale of neuroprotective activity possessed by the generally used MAO-B inhibitors, and those that will be synthesized in the future.

XI. Overall Conclusions

Selegiline is an original Hungarian drug, which has been discovered by Knoll and his coworkers, and during the past three decades, it was almost exclusively used inhibitor of MAO-B to treat PD. PD is a motor and cognitive disorder, characterized by the progressive loss of dopaminergic neurons of the substantia nigra pars compacta and with the presence of Lewy bodies in the cytoplasm. Besides dopaminergic neurons, the noradrenergic neurons in the locus coeruleus are also degenerated. Selegiline, in a dose of 10 mg/day, delays the need of levodopa administration and decreases the dose applied. The neuroprotective effect of selegiline treatment was proved in the controlled, randomized, double blind DATATOP studies conducted on 800 parkinsonian patients. Nevertheless, because of the irreversible inhibition of MAO-B it is difficult to distinguish between the neuroprotective and symptomatic effects of the drug. The clinical effects of selegiline are summarized in the brilliant paper of Riederer *et al.* (2004).

In vitro studies on tissue cultures and *in vivo* observations in experimental animals proved that selegiline and some of its metabolites accelerate the degeneration of neurons in high concentrations, especially in supersensitivity of the treated subjects; while in a low concentrations, insufficient to inhibit MAO-B activity, the drug and some of the metabolites were found to be neuroprotective. Due to the proapoptotic

effect of the high dose of selegiline, its administration is just like walking on the razor's edge. The debate between neurologists on the administration of MAO-B inhibitors or DA-agonists in the initial treatment of parkinsonian patients has not been settled yet, but if we stay on the ground of preclinical studies, MAO-B inhibitors, applied in a suitable dose, should be administered as early as the clinical signs appear. The therapeutic application of MAO-B inhibitors does not interfere with the effect of DA-agonists. The effectiveness of DA-agonists is not limited in time, in contrast to MAO-B inhibitors, which are effective as long as functioning neurons exist. The efficacy of DA-agonists is maintained even after the neurons had been totally lost. Large portion of MAO-B activity are localized in glial cells and its putative role is similar to DA-agonists.

Selegiline is readily metabolized by the microsomal enzymes (mixed function oxidases), which convert selegiline to A, MA, DD, *para*-hydroxy-metabolites, and their conjugates, all of which are excreted into the urine. In addition to the microsomal enzymes, the FMO synthesizes DNO. As a result of its quaternary character, DNO cannot penetrate passively through the membrane. The N-oxides DNO can also be reduced back to the parent compound, selegiline. DNO has two chiral centers and four optical enantiomers. We published recently that DNO increases the mitotic rate of melanoma cells in culture. Both selegiline and its metabolites can elicit diverse pharmacological activities, which all play a role in the complex effects of selegiline. In all concentration ranges, A and MA induced proapoptotic effect as well as damaged neurons. It is generally accepted that the metabolites possessing *N*-propargyl moiety can be neuroprotective (Youdim and Weinstock, 2002). Both DD and the DNO are metabolites suspected to possess neuroprotective property similarly to the parent compound, selegiline.

The dose–response curve of selegiline in neuroprotection is bell-shaped. High concentrations ($> 10^{-7}$ M) of the drug induce proapoptotic effect, while in small concentrations neuroprotection can be observed. It would be advisable to find a compound (metabolites), which would be neuroprotective in any concentration range, and would not have apoptosis inducing property. Currently, further studies are carried out in our laboratory to clear up the nature of neuroprotective activity of DNO. It is time to understand more precisely the complex molecular and pharmacological mechanism of neuroprotection induced by MAO-B inhibitors after three decades of their usage in the treatment of Parkinson's disease. In spite of the tremendous efforts presently there are only questions and no answers.

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