13/0168/42

เอ็มพีทีพี และสารยับยั้งโมโนเอมีนออกซิเดส บี

MPTP and Monoamine Oxidase B Inhibitors

ลีณา ลีลาศวัฒนกิจ

ภาควิชาเภสัชเคมี คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล

ABSTRACT

Parkinson's disease is caused by the imbalance of dopaminergic and cholinergic neurotransmitters in the central nervous system. Damages of substantia nigra, by endogenous (e.g., formations of oxygen free radical and hydrogen peroxide) and exogenous (e.g., viral infection and environmental toxins) factors, decrease dopamine level resulting in Parkinsonism syndrome. N-Methyl-4-phenyl-tetrahydropyridine (MPTP), an exogenous cause of the disease, is metabolized by monoamine oxidase B (MAO B) to the toxic species, MPP^+. Design of mechanism-based MAO B inhibitors provides a novel protection pathway against this neurotoxin. Five major classes of MAO B inhibitors are described in this paper: acetylenic, arylhydrazine, benzyl-dimethyl-silyl-methanamine, (aminoalkyl)trimethylsilanes and novel MPTP analogs including 1-substituted-4-phenyl-tetrahydropyridine and 1-methyl-4-substituted-tetrahydropuridine derivatives.

Key words: Parkinson's disease, MPTP, monoamine oxidase B inhibitors

บทคัดย่อ

โรคพาร์กินสันเกิดจากความไม่สมดุลของระดับโดพามีนและอะเซตทิลโคลีนในระบบประสาทส่วนกลาง สาเหตุการเกิดโรคมาจากปัจจัยภายใน เช่น การเกิดอนุมูลอิสระออกซิเจน และไฮโดรเจนเปอร์ออกไซด์ในร่างกาย และจากปัจจัยภายนอกซึ่งได้แก่ การติดเชื้อไวรัส และการได้รับสารพิษจากสิ่งแวดล้อม ปัจจัยเหล่านี้มีผลทำลายเนื้อเยื่อ substantia nigra ซึ่งทำหน้าที่ผลิตโดพามีน จึงทำให้ระดับโดพามีนลดลงและมีผลทำให้เกิดอาการพาร์กินสัน เอ็มพีทีพี เป็นสารอันตรายจากภายนอกซึ่งเมื่อร่างกายได้รับเข้าไปจะถูกเปลี่ยนเป็นสารพิษ เอ็มพีพี ประจุบวก โดยเอนไซม์โมโนเอมีนออกซิเดส บี และจะไปทำลายเนื้อเยื่อ substantia nigra การออกแบบสารที่มีฤทธิ์ทำลายโมโนเอมีนออกซิเดส บี (mechanism-based MAO B inhibitors) เป็นวิธีหนึ่งในการป้องกันเนื้อเยื่อ substantia nigra จาก เอ็มพีทีพี บทความนี้อธิบายสารที่มีฤทธิ์ทำลายโมโนเอมีนออกซิเดส บี 5 ประเภท ได้แก่ acetylenic, arylhydrazine, benzyl-dimethyl-silyl-methanamine, (aminoalkyl)trimethylsilanes และ novel MPTP analogs รวมทั้ง 1-substituted-4-phenyl-tetrahydropyridine และอนุพันธ์ของ 1-methyl-4-substituted-tetrahydropuridine

INTRODUCTION

Parkinson's disease is characterized by the imbalance of dopaminergic and cholinergic neurotransmitters. Decreasing of dopamine in substantia nigra and basal ganglia region produces Parkinsonism (Nutt *et al.*, 1992; Jankovic and Tolosa, 1993). This depletion is caused by the destruction of nigral dopaminergic neurons, however, the origin of the disease is still unknown in some cases (idiopathic Parkinsonism). Factors that damage the neurons are classified as followings (Calne *et al.*, 1989).

A. Endogenous Factors

Examples of endogenous factors, which cause damage of nigral dopaminergic neurons are 1) formation of free (oxygen) radical from dopamine 2) formation of hydrogen peroxide from the auto-oxidation of dopamine to melanin or the oxidative deamination of dopamine by monoamine oxidase, and 3) subnormal functioning or lack of biochemical mechanism including lacking enzyme to remove the destructive oxygen radical (e.g. catalase, glutathione reductase, glutathione peroxidase and superoxide dismutase) (Cohen, 1983; Cohen, 1984; Halliwell, 1987; Sounthorn and Powis, 1988).

B. Exogenous Factors

Exogenous factors, which cause damage of nigral dopaminergic neurons include 1) viral infection, and 2) environmental toxins (e.g., herbicides, pesticides, carbon disulfide, manganese and MPTP).

MPTP inducing dopaminergic neuron damage is one of the important causes of Parkinson's disease. The pharmacological effects and toxicity of MPTP and treatments of Parkinson's disease using monoamine oxidase B inhibitors are reviewed in this paper.

NEUROTOXIN: MPTP

A. General Feature

1. Route of Administration

MPTP is the side product in the synthesis of N-methyl-4-propionoxy-4-phenylpiperidine (MPPP) known as synthetic heroine (Brossi *et al.*, 1986). It can get into human body by several routes such as absorption through the skin, inhalation, ingestion, subcutaneous

injection, and intravenous injection. After absorption, MPTP is distributed to many tissues including the brain where it acts at the sona compacta of substantia nigra and causes irreversible Parkinson syndrome (Marti-Masso, 1997; Langston, 1996).

2. Parkinson Symptoms Induced by MPTP

Parkinsonism caused by MPTP and other factors are not similar in all aspects. They are different in topological pattern, nature of cytological alteration and selectivity. Tremor, bradykinesia, rigidity and postural defect are the general symptoms found in patients. MPTP dose produce these conditions with some unique features (Parky *et al.*, 1986).

Classification	Symptoms
Motor Features	Hypophonia, Hypomimia, Microphagia, Flexed posture of extremities and trunk
Non Motor Features	Seborrhea, Subtle congenital defect
Rare Features	Blepharospasm, Kinesia paradoxia

B. Toxicity

1. MPTP Metabolism and Neurotoxicity

MPTP has been known as a Parkinson disease inducer in primates including human (Marti-Masso, 1997; Langston, 1996). However, it is not the direct neurotoxin. MPTP is extraneuronally converted to the dihydropyridinium analog ($MPDP^+$) by monoamine oxidase B and nonenzymatically auto-oxidized to N-methyl-4-phenylpyridinium (MPP^+). The pyridinium is taken up by dopaminergic neurons and slowly released to cause nigrostiatal cell death. Structures of MPPP, MPTP, $MPGP^+$ and MPP^+ are depicted in Figure 1 and the conversion and re-uptake process are shown in Figure 2.

In primate, MPTP shows delayed toxicity which is different from the rodent (Muralikrishnan and Mohanakumar, 1998; Marti-Masso, 1997; Langston, 1996). MPP^+ has long half-life in primate and accumulates in the substantia nigra if the toxic dose of MPTP is continued. Thus, toxicity still progresses after stopping the administration up to ten days (Parky *et al.*, 1986).

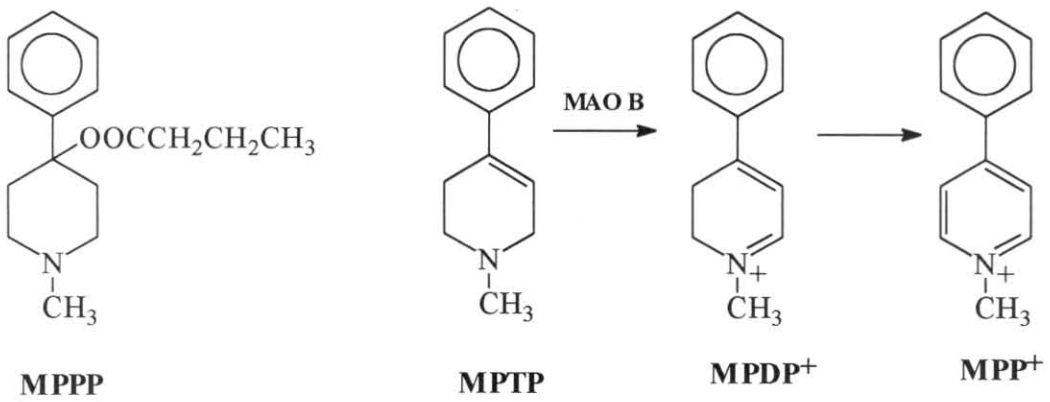


Figure 1 Structure of MPPP, MPTP, $MPDP^+$ and MPP^+.

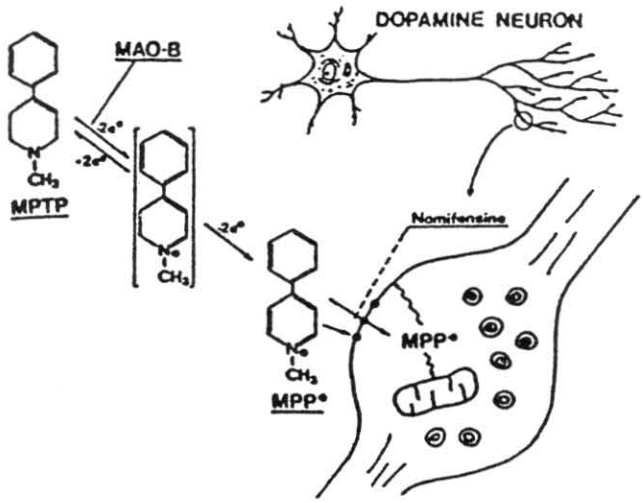
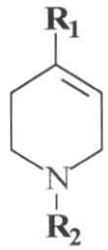


Figure 2 Conversion of MPTP by MAO B and re-uptake by dopaminergic neuron (Parky *et al.*, 1986).

2. MPTP and Monoamine Oxidase B

Monoamine oxidase (MAO) is an important enzyme in the metabolic pathway of a number of neurotransmitters in the central nervous system. Figure 3 shows the metabolism of monoamine neurotransmitters. There are two classes of this enzyme, A and B, both can oxidize biogenic amines (e.g. 5-hydroxytryptamine, norepinephrine and tyramine) and xenobiotics which can detoxify or toxify the substrate. MAO B oxidizes certain compounds which have specific structures shown in Table 1. MPTP has a structure that fits these requirements and serves as a good substrate for MAO B. It is not toxic until selectively oxidized by MAO B, and generates MPP^+ as described above.

Table 1 Substrates specificity of human MAO B.



1. R_1 : -H = $-CH_3$ >> alkyl
2. R_2 : phenyl >>> 4-chlorophenyl
3. 1-Methyl analog >> 1,3-dimethyl- = 1,5-dimethyl analogs
4. 4-Phenyl >>> 2-phenyl- = 5-phenyl-isomers
5. Tetrahydropyridinium derivative >>> dihydropyridinim derivative

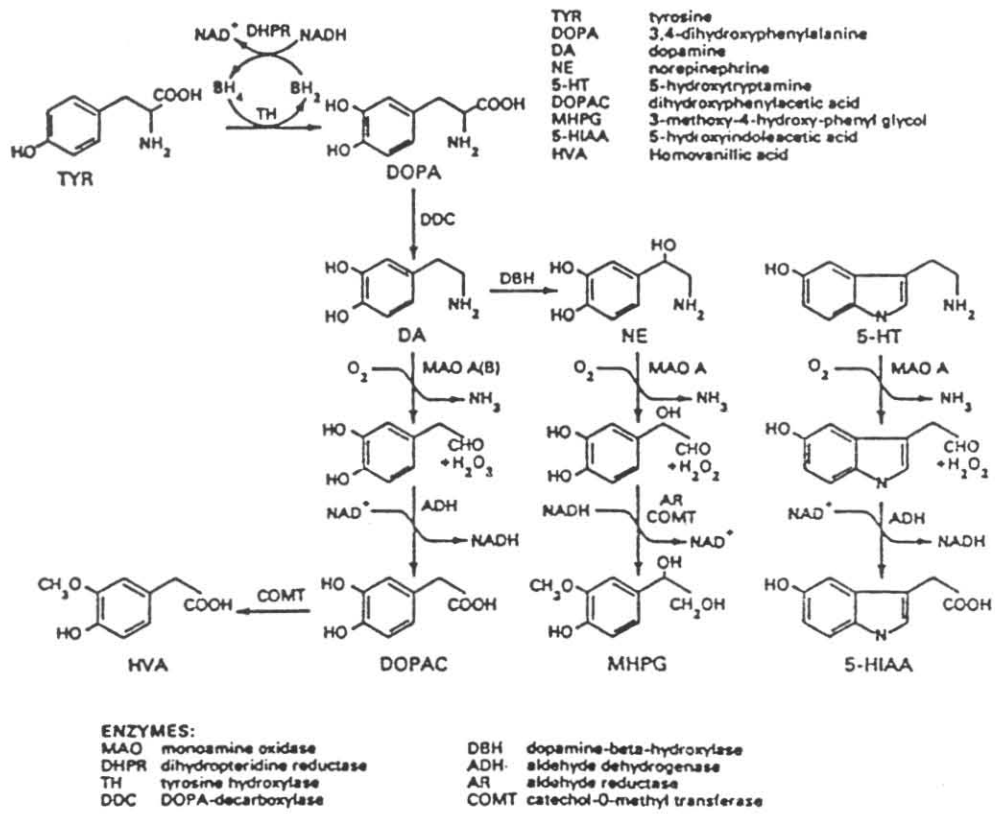


Figure 3 Metabolism of monoamine neurotransmitters (Parky *et al.*, 1986).

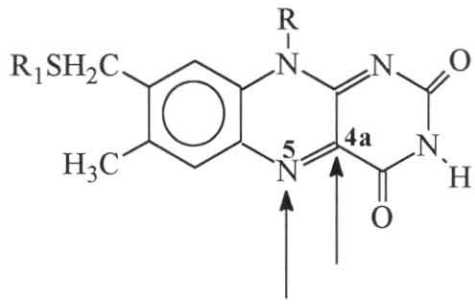


Figure 4 Potential nucleophilic sites in the 8-alpha-S-cystenyl FAD molecules.

MONOAMINE OXIDASE B INHIBITORS

Dopamine is metabolized by MAO as shown in Figure 3. Therefore, an attempt to design MAO inhibitors would be another approach for anti-parkinsonism (Lieberman, 1994). Five major classes of MAO B inhibitors are discussed; all are mechanism-based inhibitors that will react with MAO B and inactivate the enzyme (suicide substrates).

Compounds in groups A and B are electrophilic and attacked by the nucleophilic group of the active site of MAO as a result of processing by the enzyme as the -SH group at the substrate at the N(5) and C(4a) in the reduced form of 8-alpha-S-cystenyl FAD prosthetic group (Figure 4).

A. Acetylenic Inhibitors

The acetylenic substrates are bound stoichiometrically and covalently to MAO and dehydrogenation of the acetylenic amine to imminium (Figure 5) (Parky *et al.*, 1986; Helerman and Erwin, 1968; Maycock *et al.*, 1976; Singer, 1979). Pargyline, clorgyline and deprenyl are examples of this category; they form flavacyanine analogs with MAO A and B (Figure 5). Deprenyl (N-alpha-dimethyl-N-2-propynyl benzene-ethanamine) is selective for MAO B, potentiates the pharmacological properties of L-dopa and benefits by decreasing the dose of L-dopa. It reduces the on-off phenomena for long time L-dopa users and prolongs life expectancy of patients. Deprenyl can prevent neurotoxicity of MPTP by blocking the generation of MPP^+ which will prevent behavioral changes and the loss of endogenous dopamine.

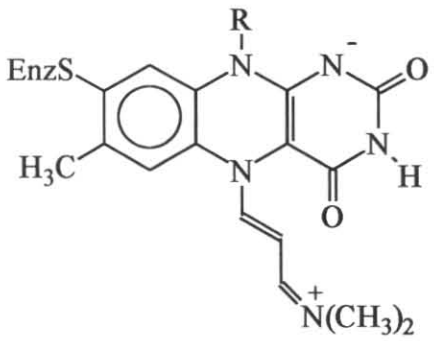


Figure 5 Structure of the flavocyanine isolated from MAO B after inactivation with 3-dimethylpropynylamine.

B. Arylhydrazines

Arylhydrazines have common ring structures as acetylenic inhibitors. This group of compounds acts as suicide substrates, in which MAO B oxidizes phenylhydrazine to phenyldiazine that irreversibly combines to the enzyme. The adduct inactivated MAO B is shown in Figure 6.

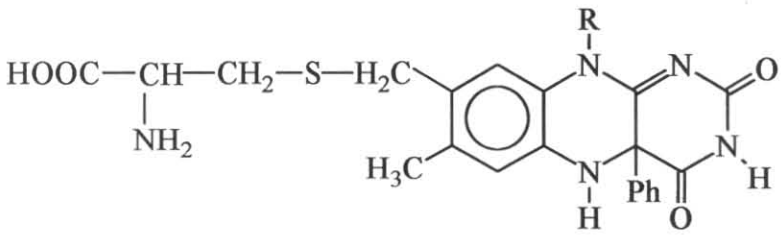


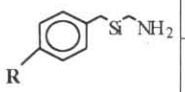
Figure 6 Structure of the adduct isolated from MAO B following inactivation by phenylhydrazine.

C. Benzyl-dimethyl-silyl-methanamine

Although deprenyl is a selective MAO B inhibitor, it produces amphetamine-like effects (Fozard *et al.*, 1985) and the selectivity for MAO B versus MAO A is limited (Sandler, 1981). The compounds of benzyl-dimethyl-silyl-methanamine category based on organosilicon are found to be more selective for MAO B providing less hypertensive response due to inhibition of MAO A (Danzin *et al.*, 1989). Aromatic silicon amines in Table 2 show high potency in MAO inhibition while compound 2 is the most potent and selective for MAO B. This indicates that the nature of the substitution at *para*-position of the aromatic ring is important. The powerful electron withdrawing property of fluorine gave the best affinity for MAO B and the selectivity is 100 fold higher than that of L-deprenyl (Palfreyman *et al.*, 1988; McDonal *et al.*, 1985). However, their

activities are lower than that of (aminoalkyl)trimethylsilanes.

Table 2 Influence of ring substitution on MAO A and MAO B inactivation (Danzin *et al.*, 1989).

	MAO B			MAO A	Selectivity MAO B/ MAO A
	K_I (μM)	$t_{1/2}$ (min) at 1 μM	$t_{1/2}$ (min) at 10 μM	$t_{1/2}$ (min) at 10 mM	
R = H	80	> 100	19	> 100	$> 5 \times 10^3$
R = F	11	25	4.6	38	1.5×10^4
R = Cl	90	> 100	20	65	3×10^3

D. (Aminoalkyl)trimethylsilanes

(Aminoalkyl)trimethylsilanes ($Me_3Si(CH)_nNH_2$) is a new class of MAO B inhibitors where n = 1, 2 and 3. The mechanism of inactivation of these three derivatives is produced by a radical mechanism for MAO catalyzed amine oxidation generating the inactivated enzyme adducts (Silverman and Banik, 1987).

E. Novel MPTP Analogs

The specific structure of N-methyl-4-phenyl tetrahydropyridine (MPTP) makes it a very good substrate for MAO B. This suggested the design of tetrahydropyridine analogs that may interact with MAO B to produce nontoxic derivatives and protect against the toxicity of MPTP. The postulated pathway of MAO B catalysis of these compounds is shown in Figure 7.

1. 1-Substituted-4-phenyl-tetrahydropyridine

The 1-ally, 1-propargyl and 1-cyclobutyl analogs are oxidizes by MAO B giving iminium species shown in Figure 8. The intermediates could form a covalent linkage with the flavin cofactor leading to the inactivation of MAO B (Kalgutkar and Castagnoli, 1992; Gartner *et al.*, 1976; Krantz and Lipkowitz, 1977).

2. 1-Methyl-4-substituted tetrahydropyridine

4-Ethynyl-substituted tetrahydropyridine is an example of this group. The corresponding dihydropyridinium in Figure 9 from the oxidation by MAO B gives the stabilized resonance protein adduct.

Compound 1-3 in Figure 8 and compound 4 in Figure 9 showed weak activity; they are lacking in inhibitory properties. Therefore, the tetrahydropyridine system is unlikely to yield pharmacologically useful inactivators of MAO B (Kalgutkar and Castagnoli, 1992).

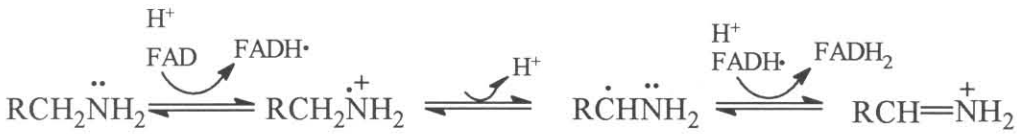


Figure 7 Postulated pathway for MAO B catalysis: the novel MPTP analogs.

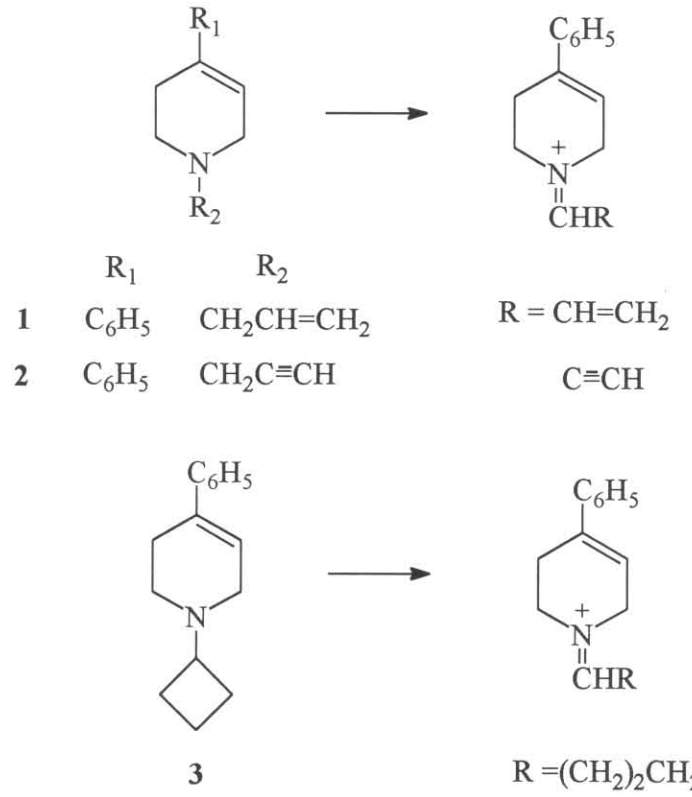


Figure 8 Oxidation of 1-substitution-4-phenyl tetranydropyridine by MAO B.

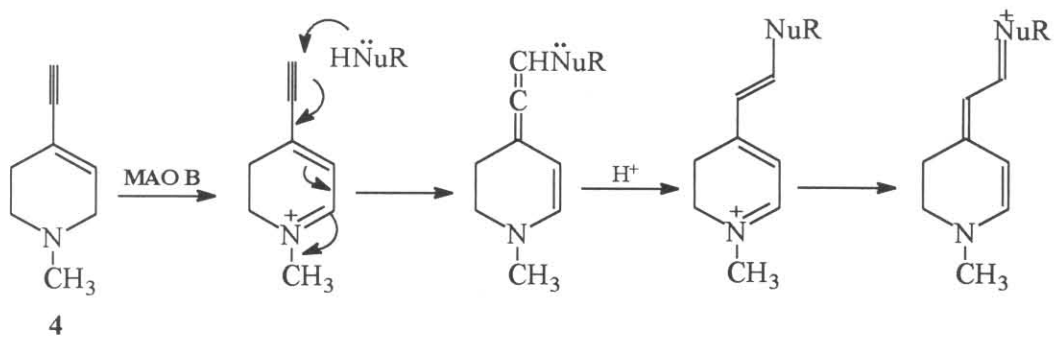


Figure 9 Oxidation of 4-Substitution-1-methyl-tetrahydropyridine.

CONCLUSION

The mechanism of the toxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is now well understood. Searching to develop novel mechanism-based inactivators of MAO B may lead to the protection against this neurotoxin. From the five major classes of MAO B inhibitors discussed in this paper, only L-deprenyl is now clinically useful for human. It can prevent MPTP induced Parkinsonism; however some amphetamine-like effect should be warned to the patients and the selectivity of MAO B versus MAO A is still limited. The benzene-dimethyl-silyl-methanamines and (aminoalkyl) trimethyl silanes are potent irreversible inhibitors of beef MAO B and rat brain MAO B, respectively. They may present a new family of anti-parkinson agents. The MPTP analogs are not likely inhibitors, these compounds lack of inhibitory properties to MAO B enzyme.

REFERENCES

1. Brossi A, Gessner W, Fritz RR, Bembenek ME and Abell CW. 1986. Interaction of monoamine oxidase B with analogs of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine derived from prodine-type analgesics. J Med Chem 29: 444-445.
2. Calne DB, Comi G, Crippa D, Horowski R and Trabucchi, M. 1989. Parkinsonism and Aging. In: Aging Series volume 36, pp. 57-58. New York : Raven Press.
3. Cohen G. 1983. The pathobiology of Parkinson's disease: Biochemical aspect of dopamine neuron senescence. J Neural Trans 19 (suppl): 89-103.
4. Cohen G. 1984. Oxy-radical toxicity in catecholamine neuron. Neurotoxicology 5: 77-82.
5. Danzin C, Collard JN, March P and Schirlin S. 1989. Selective inactivation of MAO B by benzyl-dimethyl-silyl-methanimes in vitro. Biochem Biophys Res Commun 160: 540-544.
6. Fozard JR, Zreika M, Robin M. and Palfreyman MG. 1985. Naunyn-S Chmiedeberg's. Arch Pharmacol 331: 186-193.
7. Gartner B, Hemmerich P, Zeller EA. 1976. Structure of flavin adducts with acetylenic substrates: Chemistry of monoamine oxidase and lactate oxidase inhibition. Eur J Biochem 63: 211.
8. Halliwell B, 1987. Oxidants and human disease : some new concepts. FASBE J 1: 359-364.
9. Helerman L and Erwin VG. 1968. Mitochodrial monoamine oxidase, II. Action of various inhibitors on the bovine kidney enzyme. J Biol Chem 243: 5234-5343.
10. Kalgutkar AS and Castagnoli N Jr. 1992. Synthesis of novel MPTP analogous as potential monoamine oxidase B (MAO B) inhibitors. J Med Chem 35: 4165-4174.

11. Krantz A, Lipkowitz G S. 1977. Studies of mitochondrial monoamine oxidase inactivation of the enzyme by isomeric actylenic and allenic amines yielding mutually exclusive products. J Am Chem Soc 99: 4156-4159.

12. Langston JW. 1996. The etiology of Parkinson's disease with emphasis on the MPTP story. Neurology 47 (6 suppl 3): 5153-5160.

13. Lieberman, A. 1994. Monoamine oxidase inhibitors in neurological disease. New York : Marcel Dekker.

14. Marti-Masso JF. 1997. Drug-related motor disorders in aged people. Revista de Neurologia 25 (suppl 1): 537-543.

15. Maycock AL, Abeles RH, Salach JI. and Singer TP. 1976. The structure of the covalent adduct formed by the interaction of 3-dimethylamino-1-propyne and the flavin of mitochondrial amine oxidase. Biochemistry 15: 114-125.

16. McDonald IA, Lacoste J-M, Bey P, Palfreyman M. and Zreika M. 1985. Enzyme-activated irreversible inhibitors of monoamine oxidase : phenylallylamine structure-activity relationships. J Med Chem 28: 186-193.

17. Muralikrishnan D. and Mohnanakumar KP. 1998. Neuroprotection by bromocriptine against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced neurotoxicity in mice. FASEB J 12(10): 905-912.

18. Palfreyman MG, McDonald IA, Bey P, Schechter PI and Sjoerdsma A. 1988. Prog Neuro-Psychopharmacol Biol Psychiat 12: 967-987.

19. Parkey SP, Castagnoli N, Trevor AJ and Kopin IJ. 1986. MPTP: A Neurotoxin Producing a Parkinsonian Syndrome. New York : Academic Press.

20. Sandler M. 1981. In MHB Youdim and ES Paykel (eds.), Monoamine oxidase inhibitors: The state of Art, pp. 207-212. Chichester : John Wiley and Sons Ltd.

21. Silverman RB and Banik GM. 1987. (Aminoalkyl)trimethylsilane: A New Class of Monoamineoxidase Inactivator. J Am Chem Soc 109: 2219-2220.

22. Singer TP. 1979. In TP Singer, RW Von Korff and DL Murphy (eds.), Active site-directed, irreversible inhibitors of monoamine oxidase : In Monoamine Oxidase: Structure, Function and Altered Functions, pp. 7-24. New York : Academic Press.

23. Southorn PA and Powis G. 1988. Free radical in medicine. I. Chemical nature and biological reactions. Mayo Clin Proc 63: 381-389.