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Gas-chromatographic study on the stereoselectivity of deprenyl metabolism

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Abstract

The metabolism and urinary elimination of both (−)-deprenyl and (+)-deprenyl have been studied. Gas-chromatographic analysis with mass specific detection indicated that the metabolism of (−)-deprenyl results in a large excess of methamphetamine compared to amphetamine, while the metabolism of (+)-deprenyl gave nearly equal amounts of amphetamine and methamphetamine. A novel deprenyl metabolite, phenylacetone, was also identified in our studies.

Keywords: (−)-Deprenyl metabolism; (+)-Deprenyl metabolism; Efferent metabolites; Phenylacetone

1. Introduction

(−)-Deprenyl (also called Selegiline, Movergan®, Jumex® or Eldepryl®) was developed as a psychoenergizer with a new spectrum of pharmacological activity. Its therapeutic carrier is based on the selective inhibition of monoamine oxidase B (MAO-B). (−)-Deprenyl has been widely used alone or in combination in the treatment of Parkinson's disease [1,2].

For the development of (−)-deprenyl much effort has been directed to studying its pharmacokinetic behaviour and metabolism. The highest blood level of mice was found at 30 min after subcutaneous administration [3] and within 1 h after oral treatment. Michaelis et al. [4] performed a single-dose pilot study on four healthy male volunteers, each of whom took a single 10 mg oral dose of (−)-deprenyl. In the plasma, they found the maximal concentration of (−)-deprenyl within 1 h (t_{max} was 0.69 ± 0.38 h; the t_{max} of nordeprenyl was 0.81 ± 0.24 h), while the half life ($t_{1/2}$) of (−)-deprenyl was estimated to be 1.88 h. In consequence of its lipophilicity [5,6], (−)-deprenyl penetrates into tissues, giving an apparent volume of distribution as high as 130 l after intravenous administration [6]. Moreover, (−)-deprenyl binds to serum proteins, particu-

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larly to macroglobulins, as shown by size-exclusion chromatography [7,8].

The distribution of ^{11}C-labelled deprenyl in the human brain was studied by positron emission tomography (PET); this technique also demonstrated the very fast penetration of (−)-deprenyl into the brain tissue. (−)-Deprenyl entered the brain within seconds, and the radioactivity was found to be constant during 90 min of PET examination. At the same time (+)-deprenyl (not having a comparable level of MAO-B activity) was rapidly washed out from the brain [9,10].

The basic metabolic conversions of (−)-deprenyl are depropynylation and demethylation, resulting in methamphetamine and nordeprenyl as well as amphetamine [11–14].

In this study, the ratio of amphetamine to methamphetamine in human urine was determined after a single dose was taken of either (−)- or (+)-deprenyl. We also looked for some oxo metabolites of (−)-deprenyl and (+)-deprenyl.

2. Experimental data

2.1. Materials

Standard compounds, including (−)-deprenyl [(*R*)-(−)-*N*-methyl-*N*-(1-phenyl-2-propyl)-*N*-propynylamine], (+)-deprenyl [(*S*)-(+)-*N*-methyl-*N*-(1-phenyl-2-propyl)-*N*-propynylamine], nordeprenyl, methamphetamine, amphetamine, *p*-hydroxy-methamphetamine, phenylacetone, ephedrine and norephedrine, were the kind gift of Chinoin Pharmaceutical Works (Budapest, Hungary). Fine grade solvents and other chemicals were purchased from commercial sources.

2.2. Samples and sample preparation

Healthy male volunteers (80–90 kg body weight) took a single 10 mg dose of either (−)-deprenyl or (+)-deprenyl, after which urine was collected and stored at −40°C until analysis. Urine samples were acidified with 10% (w/v) hydrochloric acid and extracted with chloroform–ethyl acetate (3:1, v/v) to remove the acidic components. The pH was adjusted to 10 with 2.5 M sodium hydroxide, and the neutral and basic components were extracted with chloroform–ethyl acetate (3:1, v/v); the organic layer was evaporated in vacuum. Metabolites were identified directly without any derivatization.

2.3. Gas chromatography–mass spectrometry

Analyses were performed using a Hewlett-Packard GC–MSD system consisting of an HP 5890 Series gas chromatograph equipped with an HP 5971 mass selective detector. Samples were introduced by splitless injection (3 μl, delay time 1 min) from an HP 7673 autosampler onto a fused silica HP-2 capillary column (25 m × 0.2 mm; 0.11 μm film of a cross-linked 5% phenylmethyl silicon as stationary phase). The temperature programme consisted of an initial oven temperature of 100°C, followed by further heating up to 280°C with 5°C min^{-1}, then held at 280°C for 5 min. The injector port and detector temperatures were 280°C. The carrier gas was high-purity helium (Ultra plus 6.0) at a column head pressure of 10 psi. The mass selective detector was operated in SCAN acquisition mode. Solvent delay was 3 min and EM Voltage operated at 2400 V.

Standards were dissolved in toluene–methanol (2:1, v/v), and 1 μl of the solution was injected. Retention times of standard compounds and those of metabolites were in good agreement. Moreover, identification of the peaks with those of the standards were also verified by the mass spectra. Monitored mass fragments are given in Table 1. Standard curves of peak areas for deprenyl, nordeprenyl, methamphetamine, amphetamine and phenylacetone were uniformly linear ($r = 0.999+$) across the entire range of determinations with negligible intercepts. Sensitivity was <10 ng ml^{-1}. Each concentration value in Table 2 represents the average of three parallel, independent determinations; reproducibility was within 7%, calculated from the peak area data.

Table 1
GC–MS characteristics of tentative deprenyl metabolites (the occurrence of the specific m/z fragments is indicated by +)

Tentative metabolite	96	82	58	44	43	91	105	107
Deprenyl	+					+		
Nordeprenyl		+				+		
Methamphetamine			+			+		
Amphetamine				+		+		
Phenylacetone					+	+		
p-Hydroxy-methamphetamine			+					+
Ephedrine			+				+	
Norephedrine				+			+	

3. Results

Deprenyl and some of its tentative metabolites do not give molecular ions but fragmentation takes place. Characteristics of the mass spectra of deprenyl and its tentative metabolites are given in Table 1. Thus, the monitored ions for deprenyl, nordeprenyl, methamphetamine and amphetamine should be 96, 82, 58 and 44, respectively, which are the characteristic ions for the side-chains of the compounds after the benzyl part is split to form a cycloheptatrienium cation with m/z of 91. These ions were extracted when the ratios of amphetamine to methamphetamine were determined in human urine collected for 6 h after a single 10 mg oral dose of either (−)-deprenyl or (+)-deprenyl was taken.

Total ion current gas chromatogram of urine collected for 6 h after taking 10 mg of (−)-deprenyl is given in Fig. 1, while Fig. 2 shows that of human urine collected for 6 h after taking 10 mg of (+)-deprenyl. These chromatograms indicate striking differences in the ratios of amphetamine and methampethamine peaks (at about 3.6 and 4.1 min).

On the basis of the area under the most specific ion (m/z), a quantitative evaluation was done. Numerical results are given in Table 2.

4. Discussion

(−)-Deprenyl is an irreversible inhibitor of MAO-B [15–17], which facilitates dopaminergic transmission in the brain [18,19].

It is generally accepted that dopamine concentration is lower in the brain of parkinsonian patients than in brains of healthy human beings. Dopaminergic neurones are degenerated in parkinsonian patients and glial cells possessing

Table 2
Results of GC-MS analyses of urine samples

Compound	Monitored ion (m/z)	$t_{r,average}$ (min)	Concentration (μg ml^{-1}) after taking:	
			(−)-Deprenyl	(+)-Deprenyl
Deprenyl	96	7.4	2.3	1.0
Nordeprenyl	82	6.2	17.3	15.3
Methamphetamine	58	4.1	51.3	94.3
Amphetamine	44	3.6	1.3	123
Phenylacetone	43	3.7	5.7	8.2

Samples of urine taken after (−)-deprenyl and (+)-deprenyl ingestion were extracted, evaporated and taken up in 1.0 ml of toluene–methanol (2:1, v/v). For identification of deprenyl, nordeprenyl, methamphetamine, amphetamine and phenylacetone, fragment ions 96 and 91, 82 and 91, 58 and 91, 44 and 91, 43 and 91 (m/z), respectively, were found at the corresponding retention times. Quantitative evaluations of deprenyl, nordeprenyl, methamphetamine, amphetamine and phenylacetone were performed and monitored at 96, 82, 58, 44 and 43 (m/z), respectively.

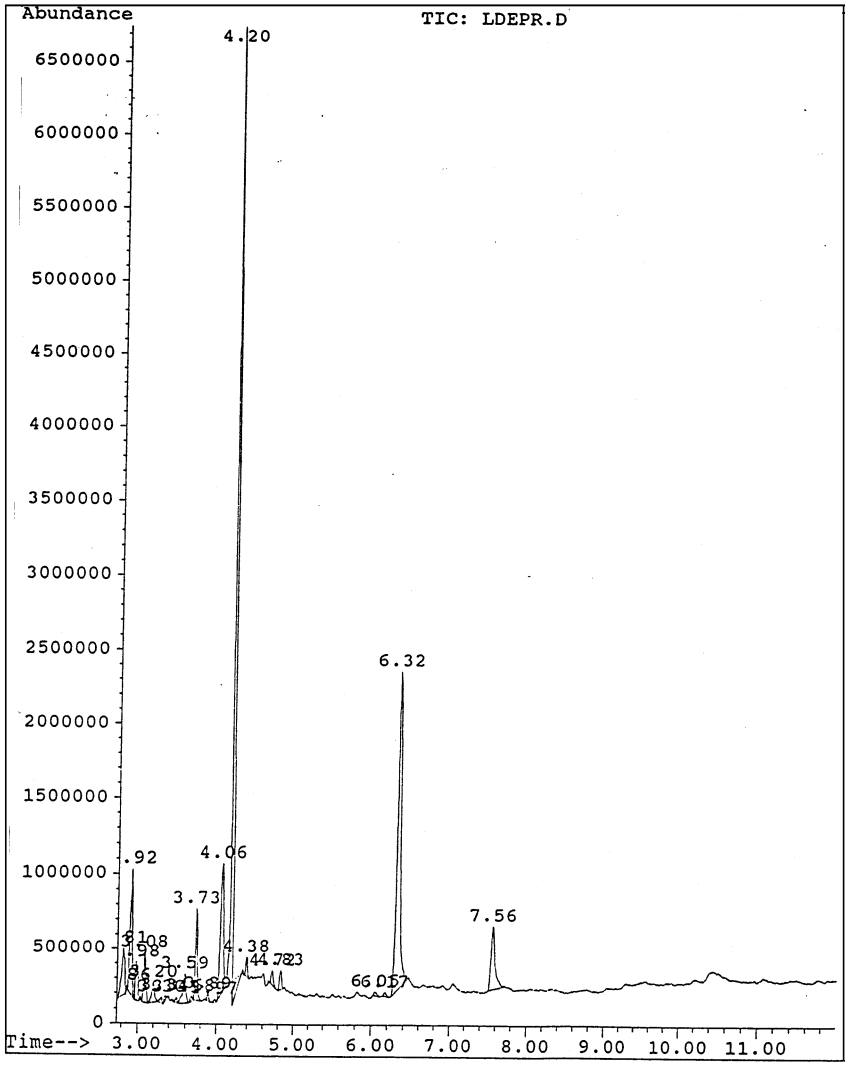

Fig. 1. Gas chromatogram of human urine collected after a single dose of (−)-deprenyl was taken.

MAO-B activity fill the space of the degenerated neurones. Consequently, dopaminergic modulation in the brain, especially in the striatum and hypothalamic area, declines in senescence, and increased MAO-B activity develops. Deprenyl is free of the 'cheese effect' and is generally accepted in the treatment of Parkinson's disease (for review, see [17]). It has been demonstrated that he beneficial effects of (−)-deprenyl in parkinsonian patients are the consequence of its MAO-B inhibitory effect and also of its psychoenergizer effect. (−)-Deprenyl treatment is followed by a definite increase in dopamine content and release in animal experiments.

Analysis of the therapeutic role of (−)-deprenyl (marketed under various names, including Jumex®, Eldepryl®, Movergan®) has emphasized the role of the specific inhibition of MAO-B. The irreversible inhibition of MAO-B is induced by the formation of a covalent bond between the flavine group of the enzyme and (−)-deprenyl. The active site of the enzyme was located and the adduct has also been isolated [20].

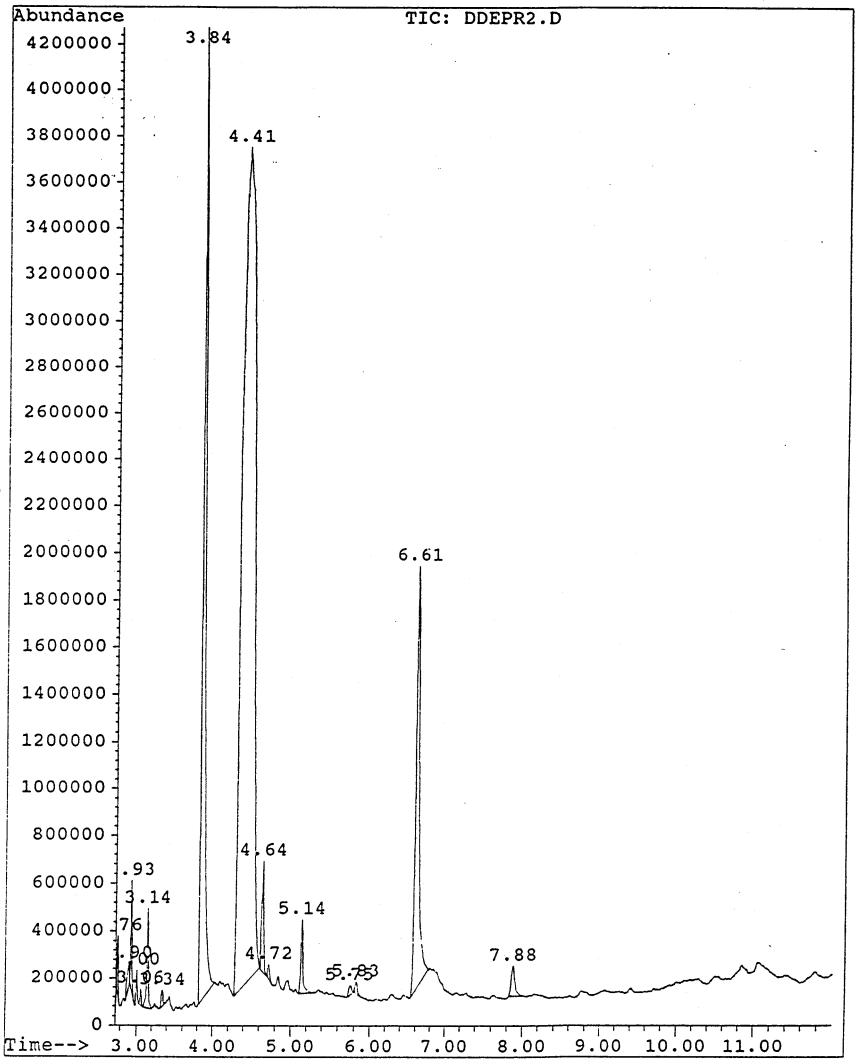

Fig. 2. Gas chromatogram of human urine collected after a single dose of (+)-deprenyl was taken.

Published findings provide evidence for the stereoselectivity of deprenyl metabolism. *R*-(−)-Methamphetamine and *R*-(−)-amphetamine were identified in the urine of rats treated with *R*-(−)-deprenyl. No racemization was found during the metabolism of the inhibitor [21,22]. Szökö and Magyar [22] used cyclodextrin-modified capillary zone electrophoresis (the buffer contained 12 mM 2,6-di-*O*-methyl)-*β*-cyclodextrin) as chiral selector.

A recent publication [23] has indicated that the intact deprenyl molecule stays far away from the substantia nigra, because no radioactivity was detected at that site if the radiolabelling was carried in the propynyl group of deprenyl. At the same time, several publications connected the effect of deprenyl to the substantia nigra. This contradiction was resolved as a similar enhancing effect on catecholaminergic and serotoninergic regulation can be due to (−)-methamphetamine as well as the parent compound [18].

Our aim was also the identification of other possible metabolites of (−)-deprenyl. Rat liver microsomal preparations metabolize xenobiotics

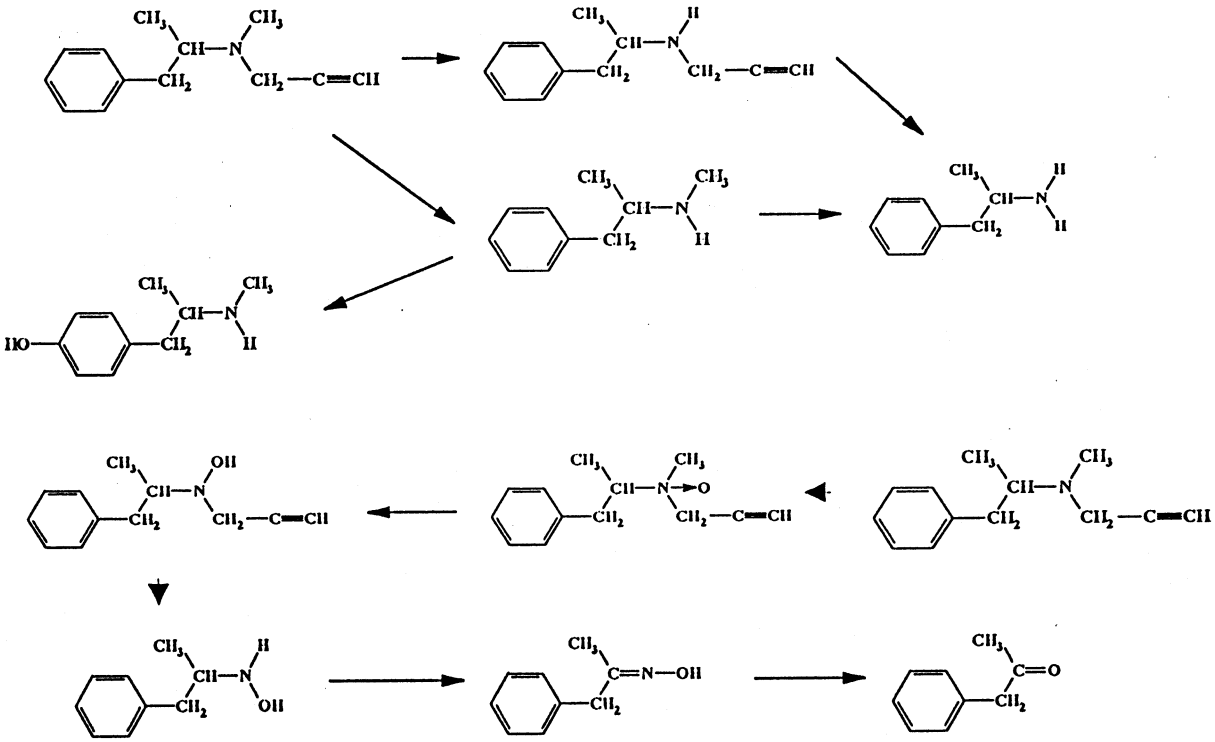

Fig. 3. Suggested human metabolism of deprenyl.

in a similar way to that taking place in the rat in vivo [24]. Experiments with components structurally similar to deprenyl [25] definitely suggest the existence of each of the three types of dealkylation reactions of the tertiary amino compounds. Through an instable *N*-oxide, the reaction can be terminated by the formation of the keto compounds. One example is the dealkylation of the 1-phenyl-2-propyl substituent, which results in phenylacetone, the compound that has been identified from urine (Table 2). Using this information, the metabolic pathway of deprenyl can be completed as given in Fig. 3.

The other two substituents on the nitrogen of deprenyl may also be eliminated in the formation of oxo compounds (formaldehyde and propynylaldehide). These efferent metabolites may play a role in situ, where they are generated. Efferent metabolites may substitute the effect of superoxide dismutase and catalase, which are supposed to scavenge free radicals, thereby reducing tissue damage caused by ageing [26].

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