Slow Recovery of Human Brain MAO B After L-Deprenyl (Selegeline) Withdrawal

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ABSTRACT L-Deprenyl (Selegeline) is an enzyme-activated irreversible inhibitor of monoamine oxidase B (MAO B; EC 1.4.3.4). It is used to treat Parkinson's disease at a dose of 5 mg twice a day. Since enzyme inhibition is irreversible, the recovery of functional enzyme activity after withdrawal from L-deprenyl requires the synthesis of new enzyme. We have measured a 40 day half-time for brain MAO B synthesis in Parkinson's disease and in normal subjects after withdrawal from L-deprenyl. This is the first measurement of the synthesis rate of a specific protein in the living human brain. L-Deprenyl is currently used by 50,000 patients with Parkinson's disease in the United States and its use is expected to increase with reports that it may be beneficial in Alzheimer's disease. The slow turnover of brain MAO B suggests that the current clinical dose of L-deprenyl may be excessive and that the clinical efficacy of reduced dosing should be evaluated. Such an evaluation may have mechanistic importance as well as an impact on reducing the side effects and the costs arising from excessive drug use.

INTRODUCTION Parkinson's disease is a progressively debilitating neurodegenerative disease affecting 1% of the population over the age of 60. Though it is known that the motor symptoms are associated with a loss of striatal dopamine due to degeneration of the dopaminergic neurons of the substantia nigra pars compacta, the fundamental mechanism(s) involved in the neurodegenerative process remain a mystery. The classical approach in treating the disease has been to restore brain dopamine by providing the precursor levodopa (Calne, 1993; Kopin, 1993). While this provides symptomatic relief, speculation that it may be possible to retard or to prevent neuronal loss was reinforced in 1989 with the first reports that L-deprenyl (Selegeline), an enzyme-activated irreversible inhibitor of MAO B, may retard the progression of Parkinson's disease as indicated by significantly increasing the time period between first symptoms and the need for levodopa therapy (Tetrud and Langston, 1989; Parkinson's Study Group, 1989a,b). This observation suggested a neuroprotective effect and was heralded as a major new milestone in the treatment of neurodegenerative disease. It was consistent with reports of increased lifespan in Parkinson's patients (Birkmayer et al., 1985) and in animals (Kitani, 1993; Knoll, 1988) and of protection afforded by L-deprenyl against neurotoxicity produced by 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP) (Heikkila et al., 1984). Accordingly, L-deprenyl is now the recommended therapy in the treatment of patients with early neurological symptoms of Parkinson's disease (Olanow, 1993). Additionally, new MAO B inhibitor drugs with different properties are under clinical investigation (Parkinson's Study Group, 1993) and the National Institutes on Aging has recently funded a clinical study evaluating L-deprenyl in the treatment of Alzheimer's disease (Growdon, 1992).

Though L-deprenyl treatment delays the rate of progression of symptoms in patients with early Parkinson's disease, the suggestion that it may be due to a decrease in neuronal loss, rather than a symptomatic effect, is controversial (Langston, 1990). In evaluating the efficacy of L-deprenyl treatment (Parkinson's Study Group, 1989a,b; Tetrud and Langston, 1989), a time interval of 1 month between the last dose of L-deprenyl and the evaluation of symptoms was chosen, the assumption being that at that time the residual effects

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of L-deprenyl would be absent. Since L-deprenyl is an enzyme-activated irreversible inhibitor of brain MAO B causing enzyme inhibition by covalent modification (Maycock et al., 1976), the recovery of enzyme after drug withdrawal requires the synthesis of new enzyme. Thus, a knowledge of the rate of synthesis of this specific brain protein is of relevance in choosing an appropriate washout time prior to the assessment of symptoms in clinical trials in patients with neurodegenerative disease. It is also of intrinsic importance in basic studies of the functional significance of brain MAO B and its association with normal aging and neuronal degeneration (Strolin Benedetti and Dostert, 1989).

We report here the measurement of the synthesis rate for human brain MAO B in 4 elderly normal volunteers and in 4 patients with early Parkinson’s disease who were treated with L-deprenyl to irreversibly inhibit MAO B and then withdrawn from the drug. Brain MAO B was measured using positron emission tomography and [11C]L-deprenyl, a tracer which tracks the concentration of brain MAO B (Fowler et al., 1987, 1988) and which has been applied to the measurement of MAO B synthesis rates (Arnett et al., 1987; Brust et al., 1991; Fowler et al., 1993; Oreland et al., 1993).

SUBJECTS AND METHODS

Study subjects

Four elderly normal subjects (males; ages: 62–69) were recruited for this study. These normal subjects were non-smokers and were free of neurologic, psychiatric, and cardiovascular disease. An MRI was obtained on each subject to confirm the absence of structural brain pathology. Subjects were free of medication.

Four patients (2 males and 2 females; ages 62–70) were recruited for this study. All of these patients had a diagnosis of early idiopathic Parkinson’s disease. Excluded were patients with atypical Parkinsonism, or with neurological or psychiatric diseases apart from Parkinson’s, or who had received dopamine antagonists in the preceding 6 months. Also excluded were those treated with the following drugs: L-DOPA, dopamine agonists, and/or MAO B inhibitors (within the past 3 months). Patients with evidence of structural brain pathology as assessed from the NMR scan were also excluded.

PET studies and L-deprenyl dosing

Informed consent was obtained after the nature and consequences of the study had been explained. Each subject had 4 PET scans with [11C]L-deprenyl (6–7.5 mCi each; 50 µg) to measure baseline MAO B activity and to follow the recovery of enzyme activity after the last dose of L-deprenyl. Following the baseline PET scan, subjects were given a Medication Event Monitoring System (MEMS) vial containing 14 tablets of L-deprenyl and instructed to follow a 12-hour dosing regimen for 1 week. The MEMS system provided a computerized record of the date and time at which the vial was opened. Compliance was verified prior to the second PET scan. Timing for the four PET scans was as follows: the first (baseline) was carried out before L-deprenyl, the second at 12 hours after the last dose, the third at 1–2 weeks, and the fourth 3–6 weeks after the last dose of L-deprenyl. In one of the normal subjects, a fifth PET study one year and 46 days after the last dose of L-deprenyl was carried out. For each PET study, a complete arterial input function for [11C]L-deprenyl was measured as described previously (Fowler et al., 1993). Tracer doses and administration, performance of the PET studies, and region of interest selection were carried out as described in a previous study (Fowler et al., 1993).

Data analysis

Time-activity data from PET and the arterial plasma were analyzed using a three-compartment model to calculate $k_3E$, the kinetic term related to the concentration of MAO B as described previously (Fowler et al., 1993). Briefly, since PET measures the total radioactivity in a given region of interest and cannot measure the concentrations of the intermediate species, all of the species in the box labeled PET ROI (region of interest) are indistinguishable to the tomograph. The simple model shown below is used because it is not possible to uniquely determine all constants involved in the complex process of suicide inactivation (Walsh, 1982).

$$S_p \overset{k_3}{\rightarrow} S_b + E \rightarrow S_{tr}$$

PET ROI

$S_p$ is the concentration of [11C]L-deprenyl in plasma, $S_b$ is the concentration of [11C]L-deprenyl in brain which has not reacted with the enzyme (E), and $S_{tr}$ is the concentration of labeled L-deprenyl bound to enzyme and $k_3$ is a kinetic term related to the processes involved in the MAO B catalyzed trapping of carbon-11 in tissue. The differential equations corresponding to this model are given by the following:

$$\frac{dS_b}{dt} = k_3 S_p(t) - k_3 S_b - (k_3 E) S_{tr}$$

$$\frac{dS_{tr}}{dt} = (k_3 E) S_b$$

The model equations were solved using for the input function the arterial plasma radioactivity corrected for the fraction of [11C]L-deprenyl at different time points. Model constants were optimized to obtain the best fit to the data as described previously (Fowler et al., 1993).

The half-time for recovery of brain MAO B after withdrawal from L-deprenyl was determined from the equa-
kinetic term containing the thalamus and the basal ganglia), basal ganglia, thalamus, occipital cortex, frontal cortex, prefrontal cortex, and cerebellum.

The synthesis rate of brain MAO B measured in human brain is similar to that found in baboon brain (half-time: 30 days [Arnett et al., 1987]) and predicted in human brain, and longer than that reported in rodents (8-11 days [Goridis and Neff, 1971]) and in pig (6.5 days [8-11 days [Goridis and Neff, 1971]).
Fig. 2. PET scans of an elderly normal human subject at the level of the thalamus after the injection of [14C]L-deprenyl. The top left image is at baseline, the top right image is 12 hours after 1 week treatment with L-deprenyl (5 mg twice a day), the bottom left image is at 1 week after the last dose of L-deprenyl, and the bottom right image is at 3 weeks after the last dose. A rainbow color scale is shown to the right of the images.
TABLE I. Tabulation of half-times (days) for the global region and different brain regions for normal volunteers (N) and patients (P)

<table>
<thead>
<tr>
<th>Study</th>
<th>GL</th>
<th>BG</th>
<th>TH</th>
<th>OC</th>
<th>FR</th>
<th>PRE</th>
<th>CB</th>
</tr>
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<tbody>
<tr>
<td>N-1^2</td>
<td>42</td>
<td>43</td>
<td>53</td>
<td>45</td>
<td>41</td>
<td>47</td>
<td>44</td>
</tr>
<tr>
<td>N-2^3</td>
<td>42</td>
<td>—</td>
<td>42</td>
<td>34</td>
<td>40</td>
<td>40</td>
<td>34</td>
</tr>
<tr>
<td>N-3^3</td>
<td>35</td>
<td>34</td>
<td>36</td>
<td>32</td>
<td>37</td>
<td>37</td>
<td>27</td>
</tr>
<tr>
<td>N-4^4</td>
<td>33</td>
<td>32</td>
<td>32</td>
<td>32</td>
<td>37</td>
<td>37</td>
<td>32</td>
</tr>
<tr>
<td>ave ± SD</td>
<td>38 ± 4.7</td>
<td>37 ± 5.2</td>
<td>40.8 ± 9.1</td>
<td>37 ± 5.7</td>
<td>38.8 ± 2.1</td>
<td>29.5 ± 5.9</td>
<td>34.3 ± 7.1</td>
</tr>
<tr>
<td>P-1^5</td>
<td>46</td>
<td>46</td>
<td>51</td>
<td>45</td>
<td>50</td>
<td>45</td>
<td>41</td>
</tr>
<tr>
<td>P-2^6</td>
<td>48</td>
<td>50</td>
<td>52</td>
<td>30</td>
<td>43</td>
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<td>40</td>
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<td>33</td>
<td>45</td>
<td>47</td>
<td>34</td>
</tr>
<tr>
<td>ave ± SD</td>
<td>42.8 ± 4.8</td>
<td>43 ± 7.7</td>
<td>45.8 ± 6.7</td>
<td>35.6 ± 6.5</td>
<td>44.3 ± 4.6</td>
<td>47.3 ± 2.6</td>
<td>37.5 ± 3.5</td>
</tr>
</tbody>
</table>

^1Values are determined from 4 PET studies on each subject. The first two studies were performed at baseline and 12 hours after the last dose of L-deprenyl and the last two studies were performed at the times indicated in the footnotes. The calculation of t_{1/2} depends critically on the baseline value of k_{E} which is generally associated with the largest standard error, due to lower sensitivity at higher enzyme concentration. The effect of variations in the baseline value of k_{E} on the t_{1/2} calculations was tested by calculating the t_{1/2} using baseline values 10% above and below the determined value. This leads to a spread of ±5 days in t_{1/2} (e.g., for the global region for 1 of the subjects, t_{1/2} was found to be 33 days, with a range of 27 to 38 days when the baseline k_{E} was varied by ±10%). Regions are (GL) basal ganglia, (BG) thalamus, (TH) occipital (OC), frontal cortex (FR), prefrontal cortex (PRE), cerebellum (CB).

^27 and 22 days.

^320 and 42 days.

^414 and 29 days.

^519 and 29 days.

^615 and 43 days.

^732 and 46 days.

(Dantrolene, 1990)) after irreversible inhibition with L-deprenyl.

In addition to providing information on the duration of brain MAO B inhibition after L-deprenyl is withdrawn, this study also demonstrates the feasibility of measuring the rate of enzyme protein turnover. MAO B is located in the outer mitochondrial membrane. Its recovery after irreversible inactivation requires the removal of inactivated MAO B from the outer mitochondrial membrane, synthesis of the MAO B protein on cytosolic ribosomes (a process encoded on the nuclear gene), and insertion of the protein into the outer mitochondrial membrane (Zhuang et al., 1988). With PET, it is feasible to study factors such as normal aging, hormones, drugs, diet, and disease on the rate of turnover of this specific brain protein directly in different regions of the human brain. The influence of diet and other factors on MAO B synthesis in different regions has been described (Sourkes, 1984).

Though MAO B inhibition is the principal pharmacological action of L-deprenyl, as well as its presumed therapeutic mechanism, its pharmacological profile is complex and a number of mechanisms accounting for its therapeutic properties have been discussed (Gerlach et al., 1992). The symptomatic effects of L-deprenyl are generally attributed to MAO B inhibition which reduces hydrogen peroxide formation. In addition, L-deprenyl has been reported to inhibit dopamine reuptake which could enhance dopaminergic effects (Knoll, 1987), although relevant concentrations are probably not achieved with clinical doses of the drug (Gerlach et al., 1992). Up-regulation of the dopamine carrier has been reported to occur with chronic treatment (Wiener et al., 1989). There is also evidence that some of the pharmacological effects of L-deprenyl may be mediated by L-methamphetamine and L-amphetamine metabolites (Engberg, 1991; Okuda et al., 1992; Reynolds et al., 1978) and by phenethylamine, which is found in striatum post mortem in patients with Parkinson's disease (Paterson et al., 1990). A recent report has presented evidence that the antiparkinsonian effects of L-deprenyl may result from its targeting of nuclear receptors stimulating aromatic L-amino acid decarboxylase (AADC; EC 4.1.1.1.) (Li et al., 1992). The neuroprotective effects of L-deprenyl are generally attributed to the reduction of oxidative stress either through MAO B inhibition, which reduces hydrogen peroxide formation (and subsequent hydroxyl radical formation), or through other mechanisms, including a direct anti-oxidant effect (Chiueh et al., 1993; Cohen and Spina, 1989; Olanow, 1990; Tipton and Singer, 1993). For example,
chronic treatment with L-deprenyl has been reported to increase the activities of superoxide dismutase and catalase (Carrillo et al., 1991, 1992, 1993). Additionally, a new action of L-deprenyl, the rescue of injured neurons, was recently reported. Interestingly, the L-deprenyl-promoted enhancement of the survival of injured neurons occurs by a stereoselective, non-MAO B mechanism at significantly lower doses than those required for MAO B inhibition, suggesting the involvement of a high-affinity stereospecific interaction with a specific molecular target (Ansari et al., 1993a,b; Salo and Tatton, 1992; Tatton and Greenwood, 1991).

One key issue in characterizing the molecular mechanisms contributing to the pharmacotherapeutic effects of L-deprenyl is to distinguish MAO B inhibition from other mechanisms. From the results of the present studies, it is clear that the MAO B inhibitory effects of L-deprenyl persist long after the last dose of the drug. Thus, with a half-time of 40 days for MAO B synthesis, a drug-free interval of several months would be required for recovery of brain MAO B to >90% of control values. Assessment of symptoms after this drug-free interval would assure the absence of long term symptomatic effects reducing ambiguity in the distinction of symptomatic vs. neuroprotective effects.

The results of this study are of special relevance in the increasing use of L-deprenyl to treat Parkinson’s disease and other neurodegenerative diseases like Alzheimer’s disease because it raises questions on the frequency and amount of L-deprenyl which is actually needed to effectively inhibit brain MAO B, and the relationship between this dose and disease progression. It is estimated that at least 50,000 individuals in the United States alone are being treated with L-deprenyl at a dose of 5 mg twice a day at an annual cost of about $1,500 per patient. The use of this drug is expected to increase with reports that L-deprenyl may be beneficial in Alzheimer’s disease (Palmer and DeKosky, 1993; Schneider et al., 1991; Tariot et al., 1987) and with the clinical trials evaluating its efficacy in Alzheimer’s disease. If MAO B inhibition is the mechanism mediating the therapeutic effect of L-deprenyl, our studies indicate that the current dose of 5 mg bid is excessive and that MAO B could be inhibited with a fraction of this dose. The minimum dose required to achieve and maintain this degree of MAO B inhibition could be determined in a dose ranging study with [11C]L-deprenyl and PET. Clinical trials at this reduced dose would be needed to determine whether this dose was effective in delaying the progression of the disease. Though it could be argued that mechanisms other than MAO B inhibition are responsible for L-deprenyl’s therapeutic effects and that the currently used amount and frequency of L-deprenyl administration is needed for the observed benefits of the drug, to our knowledge no studies investigating the effect of dose or dose rate on response to L-deprenyl in humans have been reported. An evaluation of the efficacy of L-deprenyl at lower or less frequent doses would also contribute to the clarification of the mechanism(s) of action of L-deprenyl. For example, if the minimum effective dose of L-deprenyl required to inhibit brain MAO B to the same degree as the current clinical dose does not provide the same benefit as the current dose, then it is likely that mechanisms other than MAO B inhibition are responsible for the therapeutic effect.

Though L-deprenyl is a very safe drug with few side effects, the use of lower doses may actually improve efficacy by allowing its use in patients who cannot tolerate the current dose (Vezina et al., 1992), by reducing the need for dietary restrictions, and by reducing the concentration of amphetamine metabolites which may be damaging to injured neurons (Ansari et al., 1993b). Interestingly, a recent study investigating the L-deprenyl-promoted increases in antioxidant enzymes as it impacts on increased life span in rodents has reported that an overdosage of L-deprenyl decreases rather than increases these enzymes, and that age effects may come into play because of changes in metabolizing enzymes (Carrillo et al., 1993). The use of a lower dose would also reduce the costs of treatment, making the use of L-deprenyl more accessible to individuals with limited economic resources. Clearly, there is a need to determine the mechanisms involved and to determine the optimal dose and timing for L-deprenyl and to use this information in designing clinical trials to optimally apply this and other drugs in the treatment of neurodegenerative diseases.

This study demonstrates how PET can be used to determine the duration of drug action. We have recently reported studies in patients with Parkinson’s disease using PET and [11C]L-deprenyl to directly determine the minimum effective dose and reversibility of R0 19 6327, a new MAO B inhibitor drug under clinical investigation for the treatment of Parkinson’s disease (Fowler et al., 1993). Health care costs have escalated dramatically over the past ten years. PET when used in conjunction with appropriate radiotracers can be expected to help to reduce some of these costs by providing objective measures for choosing an appropriate drug dose to minimize side effects and decrease medication costs.

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