Anti-apoptotic action of anti-Alzheimer drug, TV3326 [(N-propargyl)-(3R)-aminoindan-5-yl]-ethyl methyl carbamate, a novel cholinesterase-monoamine oxidase inhibitor

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Abstract

The anti Parkinson drug, rasagiline [R-(+)-N-propargyl-1-aminoindan], an inhibitor of type B monoamine oxidase, has been shown to suppress apoptosis induced by neurotoxins and oxidative stress. A series of novel propargylaminoindans with a carbamate moiety to inhibit cholinesterase were developed from pharmacophore of rasagiline to protect or rescue deteriorated neurons in Alzheimer’s and Lewy Body disease and provide a beneficial effect on the cognitive deficits. Rasagiline analogues were found to protect dopaminergic SH-SY5Y cells against apoptosis induced by peroxynitrite donor. SIN-1. TV3326, [(N-propargyl)-(3R)-aminoindan-5-yl]-ethyl methyl carbamate, was as effective as rasagiline in preventing apoptosis, followed by its \textit{S}-enantiomer, TV3279. The anti-apoptotic-neuroprotective activity was shown to reside in the propargylamine and not the carbamate moiety. This resulted in stabilization of the mitochondrial membrane potential, the collapse of which initiates the apoptotic cascade.

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Neurodegenerative disorders, such as Parkinson’s (PD) and Alzheimer’s (AD), are characterized by progressive cell death of selective neurons in the brain. Apoptosis is considered to be a common type of neuronal cell death in neurodegenerative diseases that may be induced by various environmental and genetic factors. The apoptotic cascade is activated by tightly controlled step-wise processes and has been proposed to be a target of neurorescue or neuroprotective strategies [15]. The anti-Parkinson drug, rasagiline [N-propargyl-(1R)-aminoindan] [5], a selective irreversible monoamine oxidase (MAO)-B inhibitor [19] has neuroprotective activity against death in cultured hippocampal, PC-12 and SH-SY5Y cells [4,7,11,20] and in animal models of head trauma [6], and MPTP induced neurotoxicity [14]. The suppression by rasagiline of the apoptotic process induced by an endogenous neurotoxin, \textit{N}-methyl(\textit{R})saltsolinol and a donor, SIN-1 was confirmed in human dopaminergic neuroblastoma SH-SY5Y cells [8,9]. Structure-activity studies have indicated that the propargyl moiety is essential for the anti-apoptotic function of cyclic benzyl-(rasagiline) [8] and aliphatic-[\textit{N}-(2-heptyl)-\textit{N}-propargylamine] propargylamines [9]. In addition, the anti-apoptotic activity of rasagiline is independent of inhibition of MAO-B, since its \textit{S}-isomer, TVP1022, lacking in MAO inhibitory activity still protects SH-SY5Y and PC12 cells which do not express MAO-B [7,11,18].

A series of analogues were synthesized with a carbamate cholinesterase inhibitory moiety in the aminoindan structure of rasagiline with the purpose of preserving its neuroprotective activity [6,15,17,18] and also to inhibit acetylcholinesterase [acetylcholine acetylhydrolase, EC 3.1.1.7, ChE] to increase cholinergic transmission. The deficits in cholinergic activities are closely related to clinical symptoms in AD and Lewy Body disease and cholinesterase inhibitors, such as rivastigmine, have been shown to have

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beneficial effects in such subjects [2]. TV3326, [\(N\)-propargyl-(3R)aminoidan-5-y]-ethyl methyl carbamate] (Fig. 1), inhibits acetylcholinesterase (ChE) and is brain selective type A and B MAO inhibitor and improves memory impairments in scopolamine treated rats. Its S-isomer, TV3279, which is also ChE inhibitor (Fig. 1) has no MAO inhibitory activity [16,17] but also has similar action in scopolamine impairment test. These compounds retained the neuroprotective activities of rasagiline in partially differentiated PC-12 cells deprived of serum and NGF and in vivo [16,18,20]. Furthermore, recently they have been shown to process amyloid precursor protein in vitro and in vivo by a PKC and MAP kinase dependent reaction and release the neuroprotective anti-apoptotic soluble amyloid precursor protein alpha [18].

In this paper, neuroprotective activities of TV3326, its S-isomer, TV3279, and related compounds were examined for their potential protection against apoptosis and the fall in mitochondrial membrane potential (\(\Delta \Psi\)m) associated with apoptosis induced by the peroxynitrite-generating agent, SIN-1 [N-morpholino sydnonimine] in dopaminergic neuroblastoma SH-SY5Y cells.

Rasagiline and derivatives (Fig. 1) were kindly donated by Teva Pharmaceutical (Netanya, Israel). Hoechst 33342 and Rhodamin 123 were purchased from Molecular Probes (Eugene, OR, USA); SIN-1 from Dojindo (Kumamoto, Japan); propidium iodide (PI) from Sigma (St. Louis, MO, USA); other reagents were from Nacalai Tesque (Kyoto, Japan).

SH-SY5Y cells were incubated with 0.01–10 \(\mu\)M of propargylamine derivatives for 20 min, then cultured for 18 h in the presence of 250 \(\mu\)M SIN-1, and the morphological changes in the cells were observed by phase-contrast and fluorescence microscopy after staining with PI and Hoechst 33342 [8]. PI (at the final concentration of 10 \(\mu\)M) and Hoechst 33342 (10 \(\mu\)M) was added to the medium and the cells were incubated for 30 min at 37 °C. Cells stained with PI were observed fluorometrically (emission, above 580 nm; excitation, 520–550 nm) and positively stained cells were assumed to be dead. After staining with Hoechst 33342, cells with condensed and fragmented nuclei were assessed to be apoptotic by fluorescence microscopy (emission, above 420 nm; excitation, 330–385 nm). In four microscopic fields containing 200 cells, the number of dead cells and apoptotic cells were counted.

The effects of SIN-1 with and without propargylamines were examined on mitochondrial permeability transition pore by measurement of \(\Delta \Psi\)m, as reported previously [13]. Changes in \(\Delta \Psi\)m were quantitatively assessed from the reduction in fluorescence of Rhodamin 123 pre-trapped in SH-SY5Y cells. The cells cultured in a 6-well poly-l-lysine-coated tissue flask were washed with phosphate-buffered saline (PBS), then incubated with 5 \(\mu\)M Rhodamin 123 in Dulbecco’s modified Eagle minimum essential medium (MEM) for 1 h at 37 °C. After washing twice with PBS, the cells were treated with propargylamines (0.1–10 \(\mu\)M) for 20 min, then with 250 \(\mu\)M SIN-1 in MEM for 1 h, washed twice with PBS and collected by treatment with trypsin and centrifugation. The cells were suspended in PBS and the fluorescence intensity measured using a Shimadzu spectrofluorometer, RF5000 at 535 nm with excitation at 505 nm. The data were analyzed by ANOVA and probability (P) values less than 0.05 were considered to be statistically significant.

The chemical structures of rasagiline and its derivatives, TV3326, TV3279, TV3218 a (3S)-aminoindan-5-y]-ethyl methyl carbamate, without the propargylamine, TV3294 (6-hydroxy-rasagiline), a propargylaminoindan metabolite of TV3326 without a carbamate moiety are shown in Fig. 1.

Table 1 summarizes the results of the antiapoptotic activity of rasagiline, TV3326 and related compounds. Rasagiline and TV3326 were the most potent in suppressing apoptosis induced by 250 \(\mu\)M SIN-1. This concentration of SIN-1 was chosen because it causes roughly 35% apoptosis of the cells rather than necrosis [8]. TV3326 (0.1 \(\mu\)M) reduced from 32.9 to 5.9% the number of dead cells resulting from SIN-1 treatment. The (S)-enantiomer was also effective, but less potent. The carbamate derivative without propargylamine, TV3218, did not suppress apoptosis, whereas TV3294, a propargyl derivative metabolite of TV3326, without a carbamate moiety had a significant protective effect, almost similar to TV3326 and rasagiline. These results indicate that \(N\)-propargyl-1-aminoindan structure is necessary for the anti-apoptotic activity.

The effects of TV3326 and rasagiline on \(\Delta \Psi\)m were examined by measurement of the reduction in Rhodamin 123 fluorescence. SIN-1 (250 \(\mu\)M) reduced the fluorescence to 28.3% of control, and the pre-treatment with TV3326 or rasagiline (0.1–10 \(\mu\)M) prevented the fluorescence reduction, as summarized in Table 2. TV3326 was slightly less potent in this action than rasagiline. TV3218, the carbamate metabolite of TV3326, had no effects at the
The effect of TV3326 and related compounds on apoptosis induced by SIN-1 in SH-SY5Y neuroblastoma cells

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (μM)</th>
<th>Apoptotic Activity (% of apoptotic cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.18 ± 0.42</td>
</tr>
<tr>
<td>SIN-1</td>
<td>250</td>
<td>32.90 ± 6.02</td>
</tr>
<tr>
<td>TV 3326</td>
<td>0.1</td>
<td>5.88 ± 4.49**</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>10.58 ± 4.45**</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>13.78 ± 6.47*</td>
</tr>
<tr>
<td>TV 3279</td>
<td>0.1</td>
<td>10.62 ± 2.37**</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>11.62 ± 1.59**</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>22.17 ± 6.73</td>
</tr>
<tr>
<td>TV 3218</td>
<td>1.0</td>
<td>45.65 ± 7.87</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>37.99 ± 8.04</td>
</tr>
<tr>
<td>TV 3294</td>
<td>1.0</td>
<td>7.26 ± 1.73**</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.63 ± 2.67**</td>
</tr>
<tr>
<td>Rasagiline</td>
<td>1.0</td>
<td>4.13 ± 0.47**</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.45 ± 2.67**</td>
</tr>
</tbody>
</table>

SH-SY5Y cells were treated with the various propargylamines for 20 min and then with 250 μM SIN-1 for 18 h. Necrotic dead cells were less than 2% of the total cells. The Values represent the mean ± SD of four microscopic fields experiments containing about 200 cells in triplicate. *P < 0.05, **P < 0.01, compared to SIN-1.

The present study on the structure-activity relationship among propargylamines studied shows that the propargyl moiety is responsible for the anti-apoptotic activity. The presence of the carbamate moiety in TV3326 and TV3279, did not affect the antiapoptotic function associated with rasagiline [20]. Whereas the metabolite TV3218, devoid of propargyl moiety, was devoid of anti apoptotic activity. By contrast the hydroxylpropargyl major metabolite of TV3326, TV3294, had anti apoptotic activity similar to rasagiline and TV3326 and TV3279 (Fig. 1). We are presently investigating at which site these propargylamine interact with mitochondria to exert their antiapoptotic activity. NeverthelessTV3326 and TV3279 with a carbamate moiety inhibit butryl-ChE and thus increase acetylcholine in the brain, and improve the cognition and memory impairment [16,17]. In combination with the anti-apoptotic-neuroprotective function derived from the propargylaminoidan structure of rasagiline, these carbamate-containing rasagiline analogues may ameliorate cognitive deficits caused by a loss of acetylcholine in AD [16,17]. They may also be of potential neuroprotective therapeutic benefit for the treatments of not only AD [18], but also Lewy Body disease that is an extrapyramidal disorder co-morbid with dementia.

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