Limited potentiation of blood pressure response to oral tyramine by brain-selective monoamine oxidase A-B inhibitor, TV-3326 in conscious rabbits

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Abstract

TV-3326 is a novel cholinesterase inhibitor that produces irreversible brain-selective inhibition of monoamine oxidase (MAO)-A and B and has antidepressant-like activity in rats after chronic oral administration. This study determined whether TV-3326 would cause less potentiation than other irreversible MAO-inhibitors of the blood pressure (BP) response to oral tyramine in conscious rabbits. Dose–response curves were established for the increase in BP induced by tyramine (5–200 mg/kg) administered orally via a naso–pharyngeal tube. From these, the dose that increased BP by 30 mmHg (ED30) was computed for each rabbit before and after oral administration of clorgyline, 1 mg/kg for one week, tranylcypromine 10 mg/kg, once, moclobemide, 20 mg/kg 3 times and TV-3326, 26 mg/kg for 2 weeks. Clorgyline, tranylcypromine and TV-3326 inhibited brain MAO-A by 90%; the former two inhibited intestinal MAO-A by 85–97% but TV-3326 had no effect. Tranylcypromine and clorgyline produced 6 and 20-fold increases in the pressor response to tyramine while TV-3326, like moclobemide, only potentiated it 2-fold. If TV-3326 is found to produce as little potentiation of the tyramine response in human subjects, it may be a potentially useful therapeutic agent for the treatment of Alzheimer’s disease with depression.

Keywords: Rabbits; Blood pressure; Oral tyramine; MAO-A and B inhibitors

1. Introduction

Both depression and normal aging may be accompanied by cognitive deficiencies. Subjects with Alzheimer’s disease (AD) suffer from severe memory impairment and have a high incidence of depressive symptoms (Levy et al., 1996; Newman, 1999). In addition to the forebrain cholinergic deficits, reductions occur in serotonergic and noradrenergic transmission in AD (Palmer et al., 1988). Many of the tricyclic antidepressant drugs are also cholinergic antagonists and therefore are contraindicated in such patients as they can exacerbate the cognitive deficits (Edwards, 1995). On the other hand, irreversible inhibitors of monoamine oxidase (MAO) A and B, like tranylcypromine and phenelzine or only of MAO-A, like clorgyline, do not block cholinergic transmission and are effective antidepressants (Paykel et al., 1982; Dowson, 1987). However, they are restricted in their use by the acute increase in blood pressure (BP) that can occur when patients receiving them ingest foodstuffs containing tyramine (Blackwell and Marley, 1966). The reversible MAO-A inhibitor, moclobemide is an effective antidepressant in elderly subjects and only induces an increase in BP when the tyramine-containing food is eaten at the time of maximal MAO inhibition (Amrein et al., 1988).

We have synthesized TV-3326, [(N-propargyl-(3R)-aminoindan-5-yl)-(ethyl methyl carbamate), a carbamate
derivative of the MAO-B selective inhibitor rasagiline (Fig. 1), which slows the progression of Parkinson’s disease (Rabey et al., 2000). TV-3326 inhibits acetyl-cholinesterase (AChE) and ameliorates cognitive deficits caused by cortical hypofunction (Weinstock et al., 2000), while retaining the neuroprotective actions of rasagiline (Huang et al., 1999; Weinstock et al., 2001; Youdim et al., 2001b). Although it proved to be a much weaker MAO-B inhibitor than rasagiline in vitro, or after acute administration, TV-3326 produced an irreversible inhibition of both brain MAO-A and B after chronic oral administration in rats at the same dose range that inhibited AChE (Weinstock et al., 2000). TV-3326 (26 mg/kg) administered by mouth once daily for two weeks inhibited rat brain MAO-A and B by about 70%, increased 5HT in several brain regions two-fold (Weinstock et al., 2002a) and produced antidepressant-like behaviour resembling that of moclobemide and amitriptyline in the forced swim test (Weinstock et al., 2002b). Since the MAO inhibition by TV-3326 was found to be brain-selective, with almost no effect on MAO in the intestine (Weinstock et al., 2000), it was important to demonstrate that TV-3326 would not cause significant potentiation of the cardiovascular response to oral tyramine.

We chose to perform these experiments in conscious rabbits because each animal can be used as its own control to determine the pressor responses to tyramine before and after chronic administration of the MAO inhibitor. This is particularly important, since rats and rabbits, like human subjects (Müller et al., 1988) vary in their responses to oral tyramine. In addition, both the MAO inhibitor and tyramine can be administered orally by a naso–pharyngeal tube, which remains in place throughout the experiment and obviates the necessity of handling the animals and thereby affecting their BP. We were also able to confirm the findings of Holzbauer and Youdim (1977) that the proportions of MAO-A and B in rabbit brain and intestine are similar to those in humans.

2. Methods

2.1. Animals

Male (30) pathogen-free New Zealand White rabbits weighing 2.75–3.25 kg (Harlan, Biotec, Jerusalem) were used for the experiments that were carried out according to the guidelines of the University Committee for Institutional Animal Care, based on those of the National Institutes of Health, USA. After their arrival from the breeding colony the rabbits were housed singly in a cage (49×45×50 cm) for one week in the Animal House at an ambient temperature of 22±1°C and a 12 h diurnal light cycle (lights on 0700 h) prior to testing. Food and water were provided ad libitum and the cages were changed twice weekly.

2.2. Measurement of the pressor response to tyramine after oral administration

Food was withheld for 3 h before the experiment. The rabbit was placed in a specially constructed hammock that supported its legs and abdomen. Catheters were introduced under local anaesthesia with lignocaine (2%) into the marginal ear vein and central ear artery. BP was measured on a Gould recorder by means of a pressure transducer attached to the arterial catheter. A naso–pharyngeal tube was introduced into the stomach for administration of tyramine. The rabbit was allowed to recover from these procedures for 30–45 min. Resting values of the mean arterial BP (MABP) were determined twice in each rabbit. Tyramine was given orally in doses starting at 10, 20, 50, 100 and occasionally 200 mg/kg, until a maximal increase in MABP of more than 30 mmHg was achieved. After the BP had returned to resting levels, noradrenaline (1 µg/kg) was injected intravenously to prevent the development of tachyphylaxis to the pressor effect of tyramine. At least 20 min elapsed from the time the MABP returned to baseline values and the next dose of tyramine was given. The maximum increase in BP for each dose of tyramine was recorded and dose–response curves were computed for each rabbit. From these we calculated the dose needed to increase MABP by 30 mmHg (ED₃₀) as described in human subjects by Müller et al. (1988).

The rabbits were then randomly allocated to one of 5 treatments. Five of the rabbits were administered water daily by stomach tube for 2 weeks, to determine the reproducibility of the measurement of their sensitivity to tyramine. The remaining 4 groups of rabbits were given: TV-3326, 26 mg/kg/day for 2 weeks (9 rabbits); tranylcyromine, 10 mg/kg once, 2 h before the experiment...
(5 rabbits), clorgyline; 1 mg/kg/day for 7 days (5 rabbits) or moclobemide; 20 mg/kg, 3 times during 24 h preceding the experiment (6 rabbits). The dosing regimens of the irreversible MAO inhibitors were selected to provide at least 85% inhibition of MAO-A in the brain. The dosing regimen of moclobemide was based on that given to human subjects (Müller et al., 1988) and calculated according to body surface area of the rabbit. This regimen produced a significant antidepressant effect in the forced swim test in rats (Weinstock et al., 2002b). The first dose of oral tyramine was administered 75–90 min after the last dose of MAO inhibitor and was 10 mg/kg in rabbits given TV-3326 or moclobemide and 2.5 mg/kg in those treated with tranylcypromine or clorgyline.

2.3. Measurement of MAO inhibition in the brain, intestine and liver of rabbits

Rabbits were treated with water (3) or the MAO inhibitors tranylcypromine, (3) clorgyline (4) or TV-3326 (4) as described above. Two–three hours after the last dose of drug they were deeply anaesthetized with pentobarbitone, 40 mg/kg i.v., and the brain, 5 cm of duodenum and one lobe of the liver were rapidly removed, washed in saline, frozen on dry ice and stored at −70°C until assayed for MAO activity. The activity of MAO-A and MAO-B was determined by the method adapted from Tipton and Youdim (1983). Briefly, rabbit tissues were homogenized in 0.3 M sucrose. The MAO assay was performed in 0.05 M phosphate buffer (pH 7.4) containing 0.1 µM l-deprenyl (for determination of MAO-A) or 0.1 µM clorgyline (for determination of MAO-B) in order to inactivate the appropriate enzyme. The homogenates were incubated for 60 minutes at 37°C before the addition of labelled substrates (14C-5-hydroxytryptamine creatinine disulphate, 100 µM for determination of MAO A or 14C-β phenylethylamine, 10 µM for determination of MAO-B). The incubation was continued for a further 30 and 20 min, respectively. The reaction was then stopped by the addition of citric acid, 2 M. Radioactive metabolites were extracted into toluene/ethyl acetate (1:1), a solution of 2,5-diphenyloxazole was added to a final concentration of 0.4% and the metabolite content estimated by liquid scintillation counting. The effect of drug treatment on enzyme activity in the three tissues was calculated by comparison of enzyme activity with that from the pooled values of the control rabbits. Protein was determined by means of Bradford reagent using bovine serum albumin as a standard and enzyme activity was determined per mg protein.

To determine the ratio of MAO A and B in rabbit brain, liver and intestine we employed the procedure developed by Johnston (1968) using clorgyline and 1-deprenyl as the inhibitors of MAO-A and B, respectively, and tyramine as a substrate.

2.4. Statistical analyses

Significance of the effect of drug treatment on the ED30 for the tyramine pressor response was analysed by paired t tests. Differences between the mean ED30 values for water and each treatment were analysed by one-way ANOVA and Duncan’s post hoc test.

2.5. Drugs

Clorgyline HCl, tranylcypromine HCl (Sigma, Ltd., St. Louis MO, USA); TV-3326 hemitartrate, (Teva Pharmaceuticals Ltd., Netanya, Israel); moclobemide HCl, (Hoffman–LaRoche Ltd., Basle, Switzerland).

3. Results

3.1. Relative proportions of MAO-A and B in tissues of rabbits

Inhibition curves with clorgyline in tissues containing both enzymes exhibited a double sigmoid curve joined by a plateau, since tyramine is a substrate for each of these enzymes (Fig. 2). The level of the plateau indicates
Fig. 3. Inhibition by l-deprenyl of the metabolism of 3 amines by MAO in three rabbit tissues in vitro. ○○ β-phenylethylamine (PEA); □□ p-tyramine (Tyr); ▲▲ 5HT.

The percentage of MAO-A and B. In the brain this was 35% and 65%; in intestine 90% and 10%, and in liver, 5% and 95%, respectively. Experiments performed with l-deprenyl gave similar values for the relative amounts of the two enzymes to those found with clorgyline (Fig. 3). The IC₅₀ values for the inhibition of MAO-A and B by clorgyline and l-deprenyl, using serotonin and β-phenylethylamine, respectively, as substrates are shown in Table 1. These values are close to those reported for these enzymes in the respective tissues of the rat and human (Waldmeier, 1987).

3.2. Inhibition of MAO by TV-3326, clorgyline, and tranylcypromine in the brain, liver and intestine of rabbits

The remaining activity of MAO-A and B in the brain, liver and intestine of rabbits treated with tranylcypromine, clorgyline and TV-3326 is shown in Fig. 4(a) and (b). MAO-A activity in the brain was reduced by more than 90% by all three drugs. MAO-A in the liver, which comprises less than 10% of all the MAO, was inhibited more than 85% by tranylcypromine and clorgyline, and by about 50–60% by TV-3326. While MAO-A activity in the intestine after clorgyline and tranylcypromine administration was reduced to about 10% of control, it was as much as 70% in one rabbit given TV-3326 and more than 100% in the remaining three. As expected, clorgyline caused much less inhibition of MAO-B than MAO-A in all the tissues. While tranylcypromine and TV-3326 reduced brain MAO-B by more than 85%, the inhibition of the enzyme in the liver and intestine by TV-3326 was considerably less (Fig. 4(b)).

3.3. Effect of MAO inhibitors on the BP response to tyramine

The resting MABP of the rabbits given water was 97.6±2.0 mmHg and this did not change significantly after any of the drug treatments. These were: 97.4±3.4 after TV-3326; 92.8±5.9 after moclobemide; 98.8±2.5 after clorgyline, and 101.6±2.5 mmHg after tranylcypromine. The average increase in MABP produced by tyramine (5–200 mg/kg) in 30 untreated rabbits (pooled untreated) is shown in Fig. 5. For the majority of these rabbits the dose of tyramine needed to increase MABP by 30 mmHg was between 50–120 mg/kg (mean 84.8±6.4 range 30–130 mg/kg). There were no signifi-

<table>
<thead>
<tr>
<th>Drug</th>
<th>Brain MAO-A</th>
<th>Brain MAO-B</th>
<th>Liver MAO-A</th>
<th>Liver MAO-B</th>
<th>Intestine MAO-A</th>
<th>Intestine MAO-B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clorgyline</td>
<td>4.3×10⁻⁸</td>
<td>2.6×10⁻⁵</td>
<td>2.1×10⁻⁷</td>
<td>5.1×10⁻⁵</td>
<td>2.2×10⁻⁸</td>
<td>2.4×10⁻⁵</td>
</tr>
<tr>
<td>l-Deprenyl</td>
<td>0.5×10⁻⁵</td>
<td>4.1×10⁻⁸</td>
<td>2.0×10⁻⁸</td>
<td>1.1×10⁻⁸</td>
<td>2.7×10⁻⁵</td>
<td>3.9×10⁻⁸</td>
</tr>
</tbody>
</table>

* IC₅₀ (concentration needed to inhibit the respective enzyme by 50%) were calculated graphically from inhibitor concentration ranging from 10⁻⁸–10⁻¹⁴ M, with at least 12 different concentration points. All studies were done on tissue homogenates in 3 separate experiments in duplicates. The values for each concentration did not differ by more than 10%.
significant differences in the average of the ED$_{30}$s for tyramine obtained before and after treatment of rabbits once daily for two weeks with water (1 ml/kg) by naso-pharyngeal tube (Table 2).

All the MAO inhibitors caused a leftward shift in the relationship between the dose of tyramine and the increase in MABP (Fig. 5). The mean of the ED$_{30}$s for the rabbits before and after each drug treatment and the ratio of the value for ED$_{30}$ after to that before drug treatment are shown in Table 2. The ratios for TV-3326 and moclobemide did not differ significantly from each other. The dose of tyramine that was needed to increase MABP by 30 mmHg in rabbits after treatment with these drugs was about half of that in controls. Clorgyline reduced the ED$_{30}$ to about 1/6th and tranylcypromine 1/20th of those in untreated rabbits.

4. Discussion

Tyramine is oxidatively metabolised equally well by MAO-A and B (Tipton et al., 1976). In untreated sub-
jects, ingested tyramine is deaminated by MAO to p-hydroxyphenylacetic acid in the intestinal mucosa, in which about 80% is of the A form (Tipton et al., 1976). Tyramine escaping enzymatic degradation in the intestinal wall is transported to the liver. Tyramine also undergoes sulpho-conjugation by phenol-sulphotransferase found in the intestine and liver (Wong, 1976). Irreversible, selective inhibition of peripheral MAO-A has been shown to be directly responsible for the potentiation of the pressor response to tyramine (Blackwell et al., 1967; Finberg and Tenne, 1982). By contrast, selective inhibition of MAO-B does not have this effect (Finberg et al., 1981).

In the present study in conscious rabbits, we compared the effect of TV-3326, a non-selective MAO-A and B inhibitor, with that of tranylcypromine and to those of two selective MAO-A inhibitors, clorgyline (irreversible) and moclobemide (reversible), on the pressor response to oral tyramine. The dosing regimens of these drugs and of TV-3326 were chosen to give a similar degree of MAO-A inhibition in the brain on the basis of previous data in other species and from unpublished findings in our laboratory in rabbits. The dose of moclobemide was one that had previously been shown to increase significantly brain 5-HT and noradrenaline levels and to reduce immobility of mice and rats in the forced swim test (Miura et al., 1996; Weinstock et al., 2002b) that predicts antidepressant activity in human subjects (Porsolt et al., 1979).

In rabbits, TV-3326, clorgyline and tranylcypromine inhibited brain MAO-A by more than 90%, while TV-3326 and tranylcypromine, but not clorgyline, inhibited MAO-B by more than 85%. Clorgyline inhibited MAO-
A in the liver by 85% and in the intestine by 90%, while tranylcypromine inhibited MAO-A in the liver and intestine by 97% and about 94%, respectively. TV-3326 caused much less inhibition of both MAO-A and B in the liver, but, as in the rat, it had virtually no effect on MAO-A in the intestine or even appeared to increase MAO-A activity in some animals. The reason for this is not clear, but could result from a faster rate of regeneration of MAO activity in the intestine than in neural tissue (Youdim et al., 2001a). Since our data confirm those of Holzbauer and Youdim (1977) that MAO in the rabbit liver is almost all of the B type, it is very unlikely that the 50–60% inhibition of MAO-A by TV-3326 would contribute appreciably to the tyramine potentiation.

Potentiation of the BP response to oral tyramine by the drugs was much more closely related to their effect on MAO-A in the intestine, as the pressor response was increased about 20-fold by tranylcypromine, 6-fold by clorgyline but only about 2-fold by TV-3326. Thus, in spite of the fact that TV-3326 is an irreversible inhibitor of MAO-A and B, the degree of tyramine potentiation was no greater than that seen with moclobemide, a drug that is currently used to treat depression in human subjects (Paykel, 1995). It is important to note that the doses of tyramine that are needed to induce a significant increase in BP after treatment with moclobemide or TV-3326 are much larger than those that would be present in most foods and beverages (Da Prada et al., 1988). Moreover, when tyramine is present in foodstuffs it causes a much smaller pressor response than that in an aqueous solution (Da Prada et al., 1988).

The reason for the selective effect of TV-3326 on brain MAO is not yet fully understood. It is possible that it results from a metabolite formed in the brain and liver but not in the intestine and that is a much more potent inhibitor than TV-3326 of MAO. This is currently being investigated. If TV-3326 is metabolised in a similar manner in humans and also shows such a low degree of MAO-A inhibition in the intestine, it should be much less likely to produce the “cheese reaction” previously reported for other irreversible MAO inhibitors.

In conclusion, we describe for the first time the limited potentiation of the BP response to oral tyramine by an irreversible MAO-A–B inhibitor. At a dose that inhibited both MAO-A and B in the brain by more than 90%, TV-3326 caused no greater potentiation of tyramine than the reversible MAO-A selective inhibitor, moclobemide. In the rat, TV-3326 and moclobemide show similar antidepressant-like activity that is associated with an increase in brain 5HT levels. The ability of TV-3326 to inhibit brain cholinesterase and to produce neuroprotective and antidepressant-like activity at the same dose without significant tyramine potentiation make this a potentially useful drug for the treatment of patients with AD and depressive symptoms.

References


Table 2

Effect of MAO inhibitors on the dose of tyramine needed to increase MABP by 30 mmHg (ED₃₀)

<table>
<thead>
<tr>
<th>Treatment (n)</th>
<th>Mean ED₃₀±SEM (mg/kg)</th>
<th>Range of ED₃₀</th>
<th>Ratio ED₃₀ Drug/no drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment* (5)</td>
<td>74±17.7</td>
<td>30–130</td>
<td>1.13±0.31</td>
</tr>
<tr>
<td>Water 1 ml/kg</td>
<td>68±8.8</td>
<td>35–88</td>
<td>0.47±0.10</td>
</tr>
<tr>
<td>Pretreatment (9)</td>
<td>85±10.1</td>
<td>40–130</td>
<td>0.39±0.09</td>
</tr>
<tr>
<td>TV-3326 26 mg/kg</td>
<td>37.3±6.3</td>
<td>12.5–74</td>
<td>0.17±0.04#</td>
</tr>
<tr>
<td>Pretreatment (6)</td>
<td>73.2±8.8</td>
<td>52–100</td>
<td>0.05±0.01##</td>
</tr>
<tr>
<td>Moclobemide 20 mg/kg</td>
<td>25.7±4.2</td>
<td>12–36</td>
<td>0.05±0.01##</td>
</tr>
<tr>
<td>Clorgyline 1 mg/kg</td>
<td>81.2±7.7</td>
<td>60–100</td>
<td>0.05±0.01##</td>
</tr>
<tr>
<td>Pretreatment (5)</td>
<td>12.9±2.2</td>
<td>6.8–19</td>
<td>0.05±0.01##</td>
</tr>
<tr>
<td>Tranylcypromine 10 mg/kg</td>
<td>87.2±12.2</td>
<td>52–110</td>
<td>0.05±0.01##</td>
</tr>
</tbody>
</table>

Significantly different from Pretreatment, ** P<0.01; significantly different from TV-3326, # P<0.05; ##, P<0.01.

* Pretreatment (water by gavage).


