In Vivo Characterization of T-794, a Novel Reversible Inhibitor of Monoamine Oxidase-A, as an Antidepressant with a Wide Safety Margin

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ABSTRACT

T-794 is a new reversible inhibitor of MAO type A. In order to predict its clinical utility as an antidepressant, we examined its pharmacological profile (i.e., MAO inhibitory activity, antidepressant-like activity and safety) in vivo in rodents. The p.o. administration of T-794 potentiated L-5-hydroxytryptophan-induced symptoms with ED_{50} = 1.01 mg/kg (mice) or 1.15 mg/kg (rats), and L-dopa-induced behavior with ED_{50} = 5.90 mg/kg (mice), whereas it did not alter the effect of \( \beta \)-phenylethylamine even at 100 mg/kg (mice). In the L-5-hydroxytryptophan test in rats, the activity of T-794 (at twice the dose of ED_{50}) disappeared by 8 h; the duration of action was similar to that of moclobemide. These results confirm the previous biochemical results that MAO-A inhibition by T-794 is highly selective and of short duration. T-794 was effective in three animal models of depression: reserpine reversal (mice, rats), behavioral despair test (mice) and learned helplessness (rats). In these tests, it had potency similar to or greater than moclobemide, tranylcypromine or imipramine. The p.o. administration of T-794 (30 mg/kg) did not affect the pressor effect of tyramine in anesthetized rats, whereas moclobemide (30 mg/kg) and tranylcypromine (6 mg/kg) potentiated the effect. Acute toxicity of T-794 proved to be very low (maximal tolerated dose > 2 g/kg p.o.) in contrast to brofaromine (maximal tolerated dose = 150 mg/kg p.o.). Unlike tricyclic antidepressants, T-794 did not prevent the oxotremorine-induced tremor even at 100 mg/kg p.o.; in this it demonstrated a lack of the anticholinergic activity. These results suggest that T-794 is an effective and particularly safe antidepressant and that it may make an important contribution in the treatment of depressive disorders.

Since their introduction into psychiatry in the late 1950s, it has been well established that MAOIs are clinically effective in the treatment of depressive disorders. MAOIs are reported to have some clinical advantages, such as superior therapeutic potential in atypical depression (Liebowitz et al., 1988; Liebowitz et al., 1984) and effectiveness in controlling anxiety disorders such as panic disorder and social phobia (Leibowitz et al., 1990; McDaniel, 1986). In addition, some patients with typical endogenous depression who do not respond well to tricyclic antidepressants may respond very favorably to MAOIs (Freeman, 1993). Despite those benefits, the use of early-generation MAOIs was greatly restricted because of their adverse side effects: hepatotoxicity of hydrazines (e.g., iproniazid) (Nelson et al., 1976) and life-threatening hypertensive crisis resulting from concurrent ingestion of a diet containing tyramine or other pressor amines (the so-called cheese effect) (Blackwell, 1963; Horwitz et al., 1964).

In recent years, reversible and selective inhibitors of MAO-A (so-called RIMAs) have been developed (Fitton et al., 1992; Waldmeier et al., 1993). These compounds appear to be safer than older MAOIs, particularly in terms of hypertensive crisis. It is probably because of its selectivity toward MAO-A that inhibitors leave the MAO-B intact and allow this isozyme to deaminate tyramine to a certain degree, and it is because of its reversibility that inhibitors could be displaced from the active site of MAO by high concentration of substrate, e.g., tyramine (Keller et al., 1987). Moreover, clinical studies have demonstrated that they are effective in the treatment of depressive disorders (Fitton et al., 1992; Paykel, 1995; Voz et al., 1996).

Considering the history of MAOIs, safety seems particularly important in their clinical use. In our aim to develop an effective and very safe MAOI as an antidepressant, we selected T-794 (fig. 1) in our laboratories as a potent, selective and reversible inhibitor of MAO-A (Iwata et al., 1994). Biochemical studies demonstrated the distinct characteristics of T-794: it inhibits MAO-A more selectively than moclobemide, at least in rodents (Iwata et al., 1994); in contrast to brofaro-
mine, it does not inhibit uptake of biogenic amines (Katayama et al., 1997). The purpose of the present study is to investigate the in vivo pharmacological profile of T-794 (MAO inhibitory activity, antidepressant-like activity and safety) in rodents, in order to estimate its potential utility as an antidepressant. RIMAs (moclobemide and brofaromine), an irreversible MAO-A inhibitor (clorglyline), an irreversible nonselective MAOI (tranylcypromine), an irreversible MAO-B inhibitor (deprenyl), tricyclic antidepressants (imipramine and amitriptyline) and a SSRI (fluoxetine) were used as reference compounds.

Materials and Methods

Animals. Male CD rats (7–8 weeks, Charles River Japan, Yokohama, Japan) or male CD-1 mice (5 weeks, Charles River Japan) were used, except the following cases: male Wistar rats (8 weeks, Japan SLC, Inc., Hamamatsu, Japan) for the tyramine interaction study and male ddY mice (5 weeks, SLC) for the behavioral despair test and the acute toxicity experiment. Food and water were available ad libitum. All animals were purchased at least 1 week before they were used in the experiment and were housed at constant room temperature (23 ± 1°C) and relative humidity (55–70%) under a 12-h light-dark cycle (lights on at 7:00 A.M.).

Potentiation of L-5-HTP-induced symptoms (mice and rats). This test was performed essentially as described by Koe et al. (1983). Test compounds were administered p.o. at several dose levels to groups of mice (n = 10/dose) or rats (n = 6/dose) 1 h before an i.p. injection of L-5-HTP (100 mg/kg). This dose of L-5-HTP produced no behavioral changes in control animals. Animals were observed for the presence of tremor, hindlimb abduction and head twitch from 30 to 40 min after L-5-HTP treatment. One score was assigned to each symptom observed per animal, giving a maximal obtainable score of 30 per group of 10 mice or 18 per group of 6 rats. The effect of a test compound was expressed as ED50, defined as the dose that provided 50% of the maximal obtainable score. The time course of the effect was determined in rats (n = 6/time-point), which received a p.o. administration of drug at a dose corresponding to twice the ED50 in this test. L-5-HTP (100 mg/kg i.p.) was injected 1, 2, 4, 8, or 24 h after the drug administration, and then, from 30 to 40 min later, rats were scored as described above.

Potentiation of PEA-induced symptoms (mice). This test was performed essentially as described by Worms et al. (1987). Test compounds were administered p.o. at several dose levels to groups of mice (n = 10/dose) 70 min before an i.p. injection of PEA (25 mg/kg). This dose of PEA produced no behavioral change in control mice. From 20 to 30 min after PEA treatment, mice were observed for the presence of stereotyped behaviors, using the following rating scale: 0, normal behavior; 1, sniffing without mouth movement; 2, mouth movement. Thus the maximal obtainable score was 20 per group of 10 mice. The effect of test compound was expressed as ED50, defined as the dose that provided 50% of the maximal obtainable score.

Potentiation of L-dopa-induced behavior (mice). This test was performed essentially as described by Jalfre et al. (1982). Test compounds were administered p.o. at several dose levels to groups of mice (n = 10/dose) 1 h before an i.p. injection of L-dopa (150 mg/kg). This dose of L-dopa produced no behavioral changes in control mice. Mice were observed for the presence of behavioral excitation (rapid running and jumping), on an all-or-none basis, from 30 to 50 min after L-dopa treatment. The effect of test compound was expressed as ED50, defined as the dose that caused behavioral excitation in 50% of mice.

Antagonism of reserpine-induced symptoms (mice and rats). This test was performed essentially as described by Worms et al. (1987).

Mice. Test compounds were administered p.o. to groups of mice (n = 10/dose) just before an i.p. injection of reserpine (5 mg/kg). Hypothermia was estimated by measuring the rectal temperature before and 4 h after the reserpine treatment. Ptosis and akinesia were evaluated 2 h after the reserpine treatment. The degree of ptosis was assessed according to the following rating scale: 0, eyes open; 1, eyes half closed; 2, eyes completely closed. For akinesia, mice were placed at the center of a circle (9.5 cm in diameter) on white paper and judged to be akinetic, on an all-or-none basis, if they remained within the circle for 15 s or more.

Rats. Test compounds were administered p.o. to groups of rats (n = 8/dose) 1 h before an i.p. injection of reserpine (6 mg/kg). This dose of reserpine did not induce significant hypothermia in rats. Ptosis and akinesia were evaluated 2 h after the reserpine treatment. The degree of ptosis was assessed according to the following rating scale: 0, eyes open; 1, eyes one-quarter closed; 2, eyes half closed; 3, eyes three-quarters closed; 4, eyes completely closed. For akinesia, rats were placed outside of the home cage and were judged to be akinetic, on an all-or-none basis, if they remained immobile (no change in their position) for 15 s or more.

The effect of test compound on ptosis and akinesia was expressed as T50, defined as the dose that antagonized the ptosis by 50% of the maximal obtainable score and as the dose that prevented the akinesia in 50% of animals, respectively. The effect on hypothermia was expressed as the lowest dose that produced a statistically significant prevention compared with reserpine-treated control (MED; P < .05, Dunnett’s method).

Behavioral despair test (mice). This test was performed essentially as described by Porsolt et al. (1977). Groups of mice (n = 10/dose) were individually introduced into a cylinder (13 cm in diameter) containing water (13 cm deep, 25°C) and left there for 15 min (habituation). Mice were then dried and returned to their home cage. Eighteen hours later, mice were replaced in the cylinder containing water (17 cm deep, 25°C) and left there for 6 min; the total duration of immobility in each mouse was measured during the last 4 min (test). Mice were judged to be immobile when they ceased struggling and remained floating motionless in the water, making only those movements necessary to keep their heads above water. Test compounds were administered i.p. 1 h before the test session, except imipramine, which was administered 30 min before the session. The effect of drug was expressed as the lowest dose that produced a statistically significant reduction in the duration of immobility as compared with control (MED; P < .05, Dunnett’s method).

Learned helplessness (rats). This test was performed essentially as described by Martin et al. (1986). To simplify the procedure and the interpretation of the results, particularly to avoid the possible interaction of unexpected drug effect (e.g., amnesic effect) and previous experience of the shuttle-box session, we evaluated the shuttle-box performance of rats only once, instead of conducting the repeated evaluation described in the original procedure.

On the first day (day 1), each rat (n = 15/dose) received 60 inescapable scrambled electric shocks (0.8 mA, duration 15 sec) with variable intertrial interval (mean 45 s) in a Plexiglas chamber (308 × 238 × 299 mm) from stainless-steel grid floor connected to a shock generator-scrambler (Neuroscience, Inc., Tokyo, Japan). Nonstressed control rats were placed in identical chambers for the same time without receiving electric shocks. Three days later (day 4), escape behavior of the rats was evaluated in automated two-way...
shuttle-boxes (450 × 200 × 191 cm; Neuroscience, Inc.). The rats were individually placed in shuttle-boxes and allowed to habituate to the environment for 5 min; then they were subjected to 30 trials (fixed intertrial interval of 30 s). In each trial, a tone signal (80–90 dB) was first presented for a maximum of 3 s. Rats were allowed to avoid the electric shock by moving to the other side of the box (escape response); the shock and the signal terminated on the response. If no avoidance response occurred, a scrambled electric shock (0.8 mA) was applied thorough the grid floor for a maximum of 3 s. Rats were allowed to escape the shock by moving to the other side of the box (escape response); the shock and the signal terminated on the response. If no avoidance response occurred, the behavior and escape failure were recorded. Test compounds were administered p.o. six times: the evening (17:30–18:30) on day 1; the morning (8:30–9:30) and the evening (twice a day) on days 2 and 3; 60 min before the shuttle-box session on day 4. According to the original procedure, the dose given in the first administration on day 1 was twice the amount of the dose on the consecutive days. Rats were assigned to groups before the inescapable shock treatment on day 1 so that the groups were roughly equal in mean body weight. Those subjects that either received mis-administration or showed toxic symptoms (observed in the group receiving the highest dose of fluoxetine) were omitted from the experiment.

**Interaction on the pressor effect of tyramine (rats).** Rats were implanted with a polyethylene catheter in the common carotid artery under urethane anesthesia. Test compounds were administered p.o. to rats (n = 6–9/dose) 1 h after the onset of anesthesia. Then tyramine 5 mg/kg was administered p.o. 1 h (T-794 and moclobemide) or 2 h (brofaromine and tranylcypromine) after the drug administration (pretreatment times were determined to produce a maximal inhibition of MAO activity according to our preliminary experiments). Arterial blood pressure was measured using a pressure transducer (MPU-0.5A, Nihon Koden, Tokyo, Japan) connected to a polygraph (RM-6000, Nihon Koden). The tyramine response was expressed as the maximal increase (%) in mean blood pressure (MBP, calculated using the pre-tyramine value) within 1 h after the administration of tyramine.

**Effect on oxotremorine-induced tremor (mice).** Test compounds were administered p.o. to groups of mice (n = 10/dose) 1 h before s.c. injection of oxotremorine (0.3 mg/kg). Mice were observed for the presence of tremor 15 min after the oxotremorine injection, using the following rating scale: 0, absence of tremor even when hanged by the tail; 1, absence of tremor when placed on a table but presence of tremor when hanged by the tail; 2, presence of tremor on a table. The effect of test compound was expressed as ED50, defined as the dose that antagonized tremor in 50% of the animals.

**Acute toxicity (mice).** Test compounds were administered p.o. to groups of mice (n = 5/dose). Mice were observed for their mortality and global behavior according to Irwin’s method (Irwin, 1968) at 10, 30, 60, 120, 180 and 300 min after the drug administration and once daily for the following 14 days.

**Statistics.** Comparisons of variables in different groups were performed by one-way ANOVA followed by multiple comparison (Dunnett’s method or Bonferroni’s method). Differences at a P value of less than .05 were considered statistically significant. ED50 values were calculated using log-probit analysis, except as otherwise described in the legend.

**Drugs.** The following drugs were synthesized at Tanabe Seiyaku: T-794, moclobemide, brofaromine and fluoxetine. All other drugs were purchased from commercial sources: clorgyline hydrochloride, tranylcypromine hydrochloride, imipramine hydrochloride, amitriptyline hydrochloride, L-5-HTP hydrodate, PEA hydrochloride, reserpine and oxotremorine sesquifumarate from Sigma (St. Louis, MO), deprenyl hydrochloride from Research Biochemicals Incorporated (RBI; Natick, MA) and tyramine hydrochloride from nacalai tesque INC. (Kyoto, Japan).

T-794 and moclobemide were suspended in 0.5% carboxymethylcellulose Na (CMC). Reserpine was dissolved in the aqueous solution containing 0.032% (v/v) phosphoric acid, 2.5% (w/v) propylene glycol, 1% (w/v) benzyl alcohol (5 mg/10 ml/kg solution for mice) or in the aqueous solution containing 0.049% (v/v) phosphoric acid, 10% (w/v) propylene glycol, 1% (w/v) glucose (6 mg/5 ml/kg solution for rats). All other drugs were dissolved in either distilled water (p.o. administration) or saline (i.p. injection). Doses of drugs were expressed as free base except in the behavioral despair test, acute toxicity experiments and tyramine interaction study, in which doses refer to the salts. Drugs were administered in a volume of 5 ml/kg (rats) or 10 ml/kg (mice).

**Results**

**Effect of T-794 in Behavioral Tests of MAO Inhibition**

**Potentiation of L-5-HTP-induced symptoms.** Oral administration of T-794 potentiated the effect of L-5-HTP at an ED50 value of 1.01 mg/kg in mice or 1.15 mg/kg in rats (table 1). In mice, T-794 was 2 to 11 times more potent than other MAO-A inhibitors (moclobemide, brofaromine and clorgyline) and was 2.7 times more potent than tranylcypromine. The MAO-B inhibitor deprenyl and the SSRI fluoxetine were active in higher doses. In rats, T-794, moclobemide and tranylcypromine had similar potency.

At the dose corresponding to twice the ED50, the activity of T-794 declined with time and disappeared by 8 h after the administration; the time-course of the effect was similar to that of moclobemide (fig. 2). By contrast, the activity of the

| TABLE 1 |
| Potentiation of L-5-HTP, PEA and L-dopa-induced symptoms |

L-5-HTP, PEA or L-dopa was injected p.o. 60, 70 or 60 min, respectively, after p.o. administration of test compounds. Then, 30 min (L-5-HTP and L-dopa) or 20 min (PEA) later, animals were assessed for their behavior. Data are expressed as ED50 with 95% CL. N = 10 (mice) or 6 (rats) per dose per compound.

<table>
<thead>
<tr>
<th>Drug</th>
<th>L-5-HTP</th>
<th>PEA</th>
<th>L-dopa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mice (mg/kg p.o.)</td>
<td>Rats (mg/kg p.o.)</td>
<td>Mice (mg/kg p.o.)</td>
</tr>
<tr>
<td>T-794</td>
<td>1.01 (0.89–1.15)</td>
<td>1.15 (0.91–1.48)</td>
<td>&gt;100 % at 100</td>
</tr>
<tr>
<td>Moclobemide</td>
<td>2.17 (1.69–2.88)</td>
<td>1.79 (1.27–2.64)</td>
<td>&gt;100 % at 100</td>
</tr>
<tr>
<td>Brefaromine</td>
<td>4.15 (3.22–5.43)</td>
<td>nd</td>
<td>85.0 (52.7–242)</td>
</tr>
<tr>
<td>Clorgyline</td>
<td>10.9 (8.50–13.9)</td>
<td>nd</td>
<td>9.99 (6.35–17.8)</td>
</tr>
<tr>
<td>Deprenyl</td>
<td>57.9 (51.3–65.9)</td>
<td>nd</td>
<td>0.89 (0.62–1.29)</td>
</tr>
<tr>
<td>Tranylcypromine</td>
<td>2.73 (2.46–3.04)</td>
<td>0.81 (0.66–0.99)</td>
<td>&gt;100 % at 100</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>26.8 (20.5–34.9)</td>
<td>nd</td>
<td>&gt;100 % at 100</td>
</tr>
<tr>
<td>Nisofen</td>
<td>&gt;100 % at 100</td>
<td>nd</td>
<td>&gt;100 % at 100</td>
</tr>
<tr>
<td>Imipramine</td>
<td>&gt;100 % at 100</td>
<td>nd</td>
<td>&gt;100 % at 100</td>
</tr>
</tbody>
</table>

nd, not determined.
Prevention of reserpine-induced ptosis, akinesia and hypothermia

**TABLE 2**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Ptosis ED50 (95% CL) (mg/kg p.o.)</th>
<th>Akinesia ED50 (95% CL) (mg/kg p.o.)</th>
<th>Hypothermia MED (mg/kg p.o.)</th>
<th>Ptosis ED50 (95% CL) (mg/kg p.o.)</th>
<th>Akinesia ED50 (mg/kg p.o.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-794</td>
<td>4.41 (3.23–6.21)</td>
<td>3.29 (2.04–5.37)</td>
<td>3</td>
<td>1.79 (1.32–2.43)</td>
<td>1.87*</td>
</tr>
<tr>
<td>Moclobemide</td>
<td>4.23 (2.82–6.93)</td>
<td>2.40 (1.49–3.87)</td>
<td>1</td>
<td>3.54 (2.52–5.30)</td>
<td>2.41*</td>
</tr>
<tr>
<td>Brofaromine</td>
<td>8.77 (7.12–11.3)</td>
<td>10.6 (7.99–16.7)</td>
<td>3</td>
<td>4.73 (3.40–6.52)</td>
<td>10.0*</td>
</tr>
<tr>
<td>Deprenyl</td>
<td>16.6 (6.67–27.5)</td>
<td>14.4 nc</td>
<td>10</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Tranylcyprome</td>
<td>3.85 (2.89–5.19)</td>
<td>4.48 (2.45–10.8)</td>
<td>3</td>
<td>0.72 (0.60–0.87)</td>
<td>0.77*</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>86.5 (nc)</td>
<td>&gt;100% at 100</td>
<td>&gt;100</td>
<td>54.8*</td>
<td>100*</td>
</tr>
<tr>
<td>Amitriptyline</td>
<td>21.9 (13.5–58.4)</td>
<td>30.9 nc</td>
<td>3</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Imipramine</td>
<td>60.3 (32.3–212)</td>
<td>24.5 (7.19–195)</td>
<td>3</td>
<td>16.8 (1.93–373)</td>
<td>&gt;100% at 100</td>
</tr>
</tbody>
</table>

nc, not calculated; nd, not determined; * graphically determined.

**Fig. 2.** Time course of the potentiation by T-794 and reference MAOIs of L-5-HTP-induced symptoms in rats. Rats received p.o. administration of drug at a dose corresponding to twice the ED50 in this test (see table 1). L-5-HTP was injected 1 to 24 h after the drug administration, and rats were scored 30 min after the injection of L-5-HTP. N = 6 per time-point.

Irreversible MAOI tranylcyprome remained (83% of maximal score) even 24 h after the administration.

**Potentiation of PEA-induced symptoms.** T-794 did not potentiate the effect of PEA even at 100 mg/kg p.o. (table 1). By contrast, p.o. administration of moclobemide and clorgyline potentiated the effect with ED50 values of 25.8 mg/kg and 85.0 mg/kg, respectively; the nonselective MAOI tranylcyprome and the MAO-B inhibitor deprenyl were active in lower doses (ED50 = 0.89 mg/kg and 9.99 mg/kg, respectively).

**Potentiation of L-dopa-induced behavior.** Oral administration of T-794 potentiated L-dopa-induced behavioral excitation at an ED50 value of 5.90 mg/kg (table 1). It was similar in potency to tranylcyprome and 2.9 to 4.5 times more potent than other MAO-A inhibitors (moclobemide and brofaromine). A MAO-B inhibitor, deprenyl, was inactive in this test.

**Effect of T-794 in the Animal Models of Depression**

**Antagonism of reserpine-induced symptoms.** The effects of T-794 and reference compounds on the reserpine-induced symptoms in mice and rats are presented in table 2 (the results of T-794 and imipramine in mice were cited from a previous report (Kato et al., 1997)). Oral administration of T-794 potently prevented reserpine-induced ptosis, akinesia and hypothermia in mice (ED50 = 4.41 mg/kg, ED50 = 3.29 mg/kg and MED = 3 mg/kg, respectively) and ptosis and akinesia in rats (ED50 = 1.79 mg/kg and ED50 = 1.87 mg/kg, respectively). Other MAOIs also prevented the symptoms of mice and rats in a dose range similar to, or at higher doses than, T-794; each MAOI showed comparable potencies on the three symptoms. By contrast, the tricyclic antidepressants imipramine and amitriptyline prevented hypothermia of mice at the same MED (3 mg/kg p.o.) as T-794 but prevented ptosis and akinesia at much (7.3 to 20-fold) higher doses. The SSRI fluoxetine showed no or very weak activity on the symptoms.

**Behavioral despair test.** T-794 significantly decreased the duration of immobility in the behavioral despair test at 30 mg/kg i.p. (table 3). Imipramine also reduced the duration at 10 mg/kg i.p., whereas moclobemide, brofaromine and clorgyline were inactive in this test even at 30 mg/kg i.p. The reduction in the duration at 30 mg/kg was 40.0% with T-794 and 26.8% with imipramine, when compared with the control group.

**Learned helplessness.** Stressed rats, those pretreated with inescapable shocks, exhibited significantly more escape failures than nonstressed controls. T-794 and imipramine reversed this stress-induced escape deficit at 50 mg/kg p.o. b.i.d. (fig. 3). Moclobemide and fluoxetine showed the tendency to reverse the deficit, although the effect was not significant up to 50 mg/kg p.o., b.i.d.

**Effect of T-794 in the Test to Evaluate Safety**

**Effect on the pressor response to tyramine.** The effects of T-794 and reference compounds on the pressor response to p.o. administration of tyramine are presented in figure 4. Pre-tyramine values (MBP just before the administration of tyramine) ranged between 78 and 120 mm Hg; mean values were roughly equal among the groups. Maximal increase of MBP after p.o. administration of tyramine (5 mg/kg) alone was 9.8 ± 1.9% compared with the pre-tyramine value (control group). Pretreatment with T-794 (30 mg/kg p.o.) or brofaromine (30 mg/kg p.o.) did not significantly affect the tyramine-induced change in MBP. By contrast, moclobemide (30 mg/kg p.o.) and tranylcyprome (6 mg/kg p.o.) significantly and markedly potentiated the pressor response to tyramine (24.2 ± 5.4% and 27.7 ± 3.3% increase, respectively, in MBP compared with pre-tyramine value).
Effect on oxotremorine-induced tremor. T-794 and moclobemide had essentially no effect on oxotremorine-induced tremor even at 100 mg/kg p.o. By contrast the tricyclic antidepressants imipramine and amitriptyline blocked the tremor at ED\textsubscript{50} values of 12.9 and 5.93 mg/kg p.o., respectively, and fluoxetine showed weak inhibition at high dose (40\% at 100 mg/kg p.o.) Atropine, an antimuscarinic agent, antagonized the effect of oxotremorine at a ED\textsubscript{50} value of 1.98 mg/kg p.o.

Acute toxicity. T-794 at 2000 mg/kg p.o. produced neither death nor marked behavioral changes (e.g., convulsion and stereotyped behavior) but caused piloerection in 3 of 5 mice and slight hypomotility in all five mice. Moclobemide at 500 mg/kg p.o. caused ataxia in one mouse and hypomotility in three mice; at a dose of 1000 mg/kg p.o., it produced convulsion in one mouse and hypomotility in the others but did not cause death. With brofaromine, at 150 mg/kg p.o., piloerection and sedation were observed in all five mice; at 300 mg/kg, 2 out of 5 mice died after the convulsion. Thus the MTD of T-794 (>2000 mg/kg p.o.) and of moclobemide (>1000 mg/kg p.o.) proved to be much higher than that of brofaromine (150 mg/kg p.o.).

Discussion

The results obtained in the present study demonstrate that T-794 is a selective and short-acting MAO-A inhibitor \textit{in vivo} and that it is active in a variety of rodent models predictive of antidepressant activity. They also suggest that T-794 not only is devoid of major problems of older antidepressants but also has an even wider safety margin than reference RIMAs.

Because the symptoms elicited by L-5-HTP (a precursor of 5-HT) are due to an increase in central 5-HT transmission (Ortmann \textit{et al.}, 1980) and 5-HT is the specific substrate for MAO-A, potentiation of the effect of L-5-HTP is assumed as behavioral evidence of MAO-A inhibition or 5-HT reuptake inhibition. By contrast, because PEA exerts an intrinsic behavioral stimulant effect and is catabolized by MAO-B (Sabeli \textit{et al.}, 1978), potentiation of PEA-induced behavior correlates with the ability of drug to inhibit MAO-B \textit{in vivo} (Ortmann \textit{et al.}, 1984). In the present experiments, T-794 potently potentiated L-5-HTP-induced symptoms, whereas even at 100-fold higher dose, it was virtually inactive in the PEA test (its ratio of potencies in the two tests was highest among the MAOIs tested). These results agree with the biochemical data (Iwata \textit{et al.}, 1994) demonstrating high selectivity for MAO-A of this compound. On the other hand, moclobemide inhibited MAO-B at a ED\textsubscript{50} value of 25.8 mg/kg, which shows that this compound is not as highly selective as T-794. This seems to be due in part to a MAO-B-inhibiting metabolite produced in rodents (Da Prada \textit{et al.}, 1989; Shoperlin and Da Prada, 1990). Although it is not clear whether T-794 inhibits 5-HT reuptake \textit{in vivo} (it has no such effect \textit{in vitro} (Katayama \textit{et al.}, 1997)), its similar potencies in the L-5-HTP test (ED\textsubscript{50} = 1.15 mg/kg, rats) and in the \textit{ex vivo} MAO-A inhibition experiment (ED\textsubscript{50} = 1.1 mg/kg, rats) (Iwata \textit{et al.}, 1994) imply that L-5-HTP potentiation by T-794 is due largely to MAO-A inhibition.

A major disadvantage of older MAOIs is the irreversibility of their action, which increases the risk of overdose and interaction with pressor amines. The irreversible activity of tranylcypromine was reflected in our finding that the potentiation of L-5-HTP was not alleviated over 24 h. In contrast, the action of T-794 declined steadily; the time course of its action was similar to that of moclobemide, which was shown to have reversible activity ( Keller \textit{et al.}, 1987). Thus the present result agrees well with the previous biochemical results suggesting T-794 is a reversible and short-acting MAOI (Iwata \textit{et al.}, 1994; Katayama \textit{et al.}, 1997).

The behavioral excitation induced by L-dopa is positively correlated with the increase in brain DA levels (Everett and Wiegand, 1962), and its potentiation has been regarded as an \textit{in vivo} index of MAO inhibition. Although previous studies suggested that this test does not discriminate MAO-A and B specificity well (Jalife \textit{et al.}, 1982), only those compounds with MAO-A inhibitory activity potentiated the effect of L-dopa, and a MAO-B inhibitor, deprenyl, was inactive in the present study. Species differences with respect to deamination of DA by MAO are well known. MAO-B is involved in the oxidative deamination of DA in human brain (Glover \textit{et al.}, 1977) and appears to be unimportant in rat brain (Waldmeier \textit{et al.}, 1976); there have been few reports on mice brain. Our result in the L-dopa experiment is consistent with the recent neurochemical study (Yu \textit{et al.}, 1994) that demonstrated that acute administration of a MAO-B inhibitor did not affect the levels of dopamine except at high, nonselective doses, and it may suggest that DA is deaminated mainly by MAO-A in mice brain. T-794 was also very potent in this test, probably because of the effective MAO-A inhibition by this compound.

In addition, this result suggests that T-794 has a facilitating effect on the brain DA transmission. T-794 was active in three animal models of depression: reserpine reversal, the behavioral despair test and learned helplessness. In these tests, T-794 had potencies similar to, or was more potent than, reference antidepressants, \textit{e.g.}, imipramine, moclobemide and tranylcypromine; the results suggest that T-794 may also be clinically effective as an antidepressant.

Among various animal models of depression, learned helplessness is accepted as one of most valid (Willner, 1984). Whereas most investigators have examined drug effects on learned helplessness by \textit{i.p.} injection (Drugan \textit{et al.}, 1987; Giral \textit{et al.}, 1988; Martin \textit{et al.}, 1986; Sherman \textit{et al.}, 1982), the present study demonstrated that T-794 and imipramine were effective by \textit{p.o.} administration. T-794 was active, however, at a dose much higher than its effective dose for inhibiting MAO-A or preventing the reserpine-induced symptoms. This may be because the period of administration was not sufficient for drugs to exert the effect at lower doses in this clinically relevant model. Antidepressants generally require several weeks to exhibit their efficacy in clinical use, but the
The effect was evaluated with only 3 days of administration in the present experiment. Other investigations suggest that the longer period of administration makes the effective dose lower in this model (Giral et al., 1988). Thus T-794 and other drugs may reverse the helpless behavior at lower doses in our test with the longer period of administration, although only further experiments can confirm this interpretation.

In prevention of the reserpine-induced symptoms, it was interesting to find that MAOIs and monoamine reuptake inhibitors showed different activity spectra: MAOIs antagonized the three symptoms (ptosis, akinesia and hypothermia) at similar doses; tricyclic antidepressants (imipramine and amitriptyline) required a much higher dose to prevent ptosis and akinesia than to prevent hypothermia; a SSRI, fluoxetine, had negligible effect on all three symptoms. In other studies, such a difference is generally not so evident, particularly in terms of ptosis. Many researchers, however, observed that tricyclic antidepressants were less effective in the prevention of akinesia than of hypothermia (Bourin et al., 1983; Worms et al., 1987). Bourin's study (Bourin et al., 1983) indicated that reserpine-induced ptosis is prevented by stimulation of \( \alpha \) adrenergic or serotonergic receptors, akinesia by stimulation of dopaminergic receptors and hypothermia by stimulation of \( \beta \) adrenergic receptors. On the other hand, imipramine and amitriptyline inhibit 5-HT or NE reuptake but have a very weak effect on DA reuptake (Baldessarini, 1983). Thus, although it is not clear why tricyclic antidepressants did not potently prevent ptosis, our results may confirm that imipramine and amitriptyline have facilitatory activity on NE but not on DA transmission, whereas MAOIs stimulate both NE and DA transmission in brain. The enhancement of DA transmission by MAOIs is also supported by our results in the L-dopa test.

The occurrence of hypertensive crises after ingestion of foodstuffs rich in tyramine was the major reason for the restricted clinical use of irreversible MAOIs (Blackwell and Marley, 1966). This phenomenon was reproduced in the present study; tranylcypromine, an irreversible and nonselective MAOI, markedly potentiated the pressor response of tyramine at 6 mg/kg, which corresponds to 7.4 times the ED\(_{50}\) values in the L-5-HTP test. In contrast, T-794 had almost no effect on the pressor response at 30 mg/kg, which corresponds to 26 times the ED\(_{50}\) value in the L-5-HTP test. T-794's weak interaction on the tyramine pressor effect is probably due to its highly selective inhibition of MAO-A (leaving MAO-B free to deaminate tyramine) and to the competitive nature of its inhibitory action (Iwata et al., 1994) (permitting displacement of the inhibitor from the active site of the enzyme by a high concentration of tyramine). Although many studies have shown the low likelihood that RIMAs will potentiate the effect of tyramine in the human (Berlin et al.,...
1989; Bieck and Antonin, 1989), moclobemide significantly potentiated the effect of tyramine in our experiment at 30 mg/kg, which corresponds to 17 times the ED$_{50}$ in L-5-HTP test. This appears to be due in part to an active metabolite produced in rodents that inhibits MAO-B (Da Prada et al., 1989; Shoerlin and Da Prada, 1990).

The tyramine pressor response is considerably stronger when tyramine is administered in an aqueous solution (as in our experiment) than when it is given mixed with foods (Fankhauser et al., 1994); thus the condition of our study is more severe than the real clinical situation wherein the patients ingest this amine contained in foodstuff. Moreover, a recent systematic analysis indicates that the tyramine content in most meals is less than 40 mg in total (Da Prada et al., 1988), whereas when moclobemide (600 mg/day) is given postprandially, tyramine of at least 150 mg is required to raise systolic blood pressure by 30 mm Hg (Zimmer et al., 1990). The present results imply that T-794 also has a sufficient safety margin between the doses that produce the potentiation of oral tyramine and those that show antidepressant activity. Consequently, this compound seems very unlikely to potentiate the pressor effect of tyramine in clinical use at therapeutic doses.

A number of antidepressants, particularly the tricyclics, possess anticholinergic activity, which is responsible for many of the side effects of these drugs. Similar adverse events were also—although less frequently—reported with the old MAOIs (Blackwell, 1981). In contrast to imipramine and amitriptyline, T-794 did not antagonize the effect of oxotremorine. Taking this together with our preliminary in vitro finding that the binding of $[^3H]$ quinuclidinyl benzylate (QNB) was not displaced by T-794 (data not shown), we conclude that T-794 does not exert anticholinergic activity in vivo.

The present study also demonstrated that the MTD of T-794 after a single p.o. administration is very high (>2 g/kg), particularly compared with those of monoamine reuptake inhibitors [e.g., LD$_{50}$ p.o. in mice: imipramine, 400 mg/kg; amitriptyline 350 mg/kg (Tobe et al., 1981)]. By contrast, the MTD of brofaromine was much lower than those of T-794 and moclobemide. In the human, the concurrent administration of an MAO inhibitor and a monoamine reuptake inhibitor leads to severe, but rare, adverse events, including severe CNS toxicity marked by hyperpyrexia, convulsions and coma (Baldessarini, 1995). The low MTD of brofaromine may be due to the fact that this compound inhibits not only MAO-A but also the reuptake of 5-HT (Waldmeier and Stocklin, 1989) and possibly that of NE (Katayama et al., 1997). The low acute toxicity of T-794 will offer a particular advantage in the case of overdose.

In conclusion, the present investigation demonstrated that T-794 is a potent, highly selective and short-acting MAO-A inhibitor in vivo. It also suggested the antidepressant activity and high safety of T-794: it was active in a variety of animal models of depression; its influence on the pressor effect of tyramine was very weak even compared with moclobemide; its acute toxicity was very low, particularly compared with brofaromine; it did not show antimuscarinic activity in vivo, unlike tricyclic antidepressants. It is reasonable to expect that T-794 may make an important contribution in the treatment of depressive disorders.

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