Possible Therapeutic Effect of T-794, a Novel Reversible Inhibitor of Monoamine Oxidase-A, on Post-Stroke Emotional Disturbances, Assessed in Animal Models of Depression

Masaya Kato,* Hiroshi Iwata, Taiichi Katayama, Hidetoshi Asai, Hiroshi Narita, and Toshio Endo

Pharmaceutical Development Research Laboratory, Tanabe Seiyaku Co., Ltd., 2–2–50 Kawagishi, Toda-shi, Saitama 335, Japan. Received October 28, 1996; accepted January 6, 1997

Emotional disturbances, such as lack of motivation or depression, are common after stroke. The drugs mainly used to treat these syndromes in Japan are the cerebral metabolic enhancers whose biochemical and pharmacological profiles are similar to those of antidepressant drugs. In order to examine the possible therapeutic effect of T-794 [(5R)-3-(6-cyclopropylmethoxy) 2-naphthalenyl]-5-(methoxymethyl) 2-oxazolidone], a new reversible inhibitor of monoamine oxidase (MAO) type A, on those emotional disturbances, its antidepressant activity was compared with those of major cerebral metabolic enhancers in rodents with or without treatment of cerebral ischemia. Oral administration of T-794 potently prevented reserpine-induced ptosis (ED$_{50} = 4.41$ mg/kg), akinesia (ED$_{50} = 3.29$ mg/kg), and hyperthermia (minimum effective dose = 3 mg/kg) in mice. It was at least 3.7, 13.0, and 3.3 times more potent than cerebral metabolic enhancers tested (indoxylazine, bifemelane, amantadine and idabenone) in antagonism of the ptosis, the akinesia, and the hyperthermia, respectively. Effect of T-794 was also examined in the behavioral despair test in rats subjected to forebrain ischemia. The ischemia was induced by a combination of bilateral common carotid artery occlusion (15 min) and systemic hypotension (sodium nitroprusside 5 mg/kg, s.c.). From 13 d after the surgery, drugs were orally administered twice daily 7 times, and following the last administration rats were assessed for their behavior. T-794 reduced the duration of immobility in the behavioral despair test at 30 mg/kg without affecting spontaneous motor activity, whereas indoxylazine showed no significant effect. Antidepressant-like activity of T-794 was suggested in rodents with as well as those without cerebral ischemia. The results suggest that T-794 may make an important contribution to the treatment of emotional disturbances following stroke.

Key words T-794; monoamine oxidase type A (MAO-A) reversible inhibitor; stroke; depression; ischemia; antidepressant

Emotional disturbances, including lack of motivation (loss of drive) or depression, are frequently observed after stroke; for instance, post-stroke depression has been reported in 10–60% of patients.¹ Those syndromes appear to adversely affect both participation in rehabilitation and long-term outcomes such as recovery in activities of daily living,² functional status and cognitive performance.³ As medication for those emotional disturbances, the cerebral metabolic enhancers, e.g., indoxylazine (IDX), bifemelane (BFL), and amantadine (AMD), are primarily selected in Japan.⁴ These drugs have a similar mechanism of action as antidepressants on the monoaminergic system. For instance, IDX inhibits uptake of norepinephrine, serotonin, and dopamine into synaptosomes,⁵ and BFL inhibits monoamine oxidase (MAO)⁶ and uptake of norepinephrine into synaptosomes.⁷ AMD is likely to enhance dopamine and nor- epinephrine transmission by activating synthesis and release⁸–¹⁰ and inhibiting uptake.¹¹ Moreover, these drugs were reported to exhibit antidepressant activity in animal models¹²–¹⁴ and in the clinic.¹⁵

In recent years, reversible and selective inhibitors of MAO type A (MAO-A) have been developed.¹⁶¹⁷ These compounds offer an advantage over the older irreversible MAO inhibitors in that they cause only minimal potentiation of the pressor response of dietary tyramine (so-called cheese effect); clinical studies have further suggested that they are effective in the treatment of depressive disorders.¹⁸ T-794, (5R)-3-(6-cyclopropylmethoxy) 2-naphthalenyl]-5-(methoxymethyl) 2-oxazolidone (Fig. 1), is a reversible inhibitor of MAO-A found in our laboratory. It was shown that T-794 inhibits MAO-A more selectively and more potently, and that it shows lower liability to potentiate tyramine-induced pressor effect in rat, than moclobemide (a reversible inhibitor of MAO-A in clinical use).¹⁹ It was also demonstrated that T-794 is, in contrast to tricyclic antidepressants, devoid of anticholinergic activity and that it was effective in various rodent models of depression: it antagonized reserpine-induced hyperthermia, reduced immobility in behavioral despair test, and reversed learned helplessness.²⁰²¹ In addition, MAO inhibitors are reported to exhibit superior drive-enhancing effect in comparison with other types of antidepressants,²² suggesting beneficial effect on reduced motivation. These lines of evidence prompted us to examine the possibility that T-794 may be useful in the treatment of post-stroke emotional disturbances. This was done here by comparing its antidepressant activity with those of cerebral metabolic enhancers in rodents treated with or without cerebral ischemia.

Fig. 1. Chemical Structure of T-794

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* To whom correspondence should be addressed.
ischemia.

MATERIALS AND METHODS

Animals Male CD-1 mice (4 weeks) or male CD rats (6 weeks) were purchased from Charles River Japan (Yokohama, Japan) at least 1 week before being used in the experiment. Food and water were available ad libitum. All animals were housed at constant room temperature (23 ± 1°C) and relative humidity (55–70%) under 12 h light–dark cycle (lights on at 7:00 a.m.). Rats were subjected to handling twice before use in experiments. The experimental protocols used in the present study followed the guiding principles for the care and use of laboratory animals (approved by the Japanese Pharmacological Society) and were approved by the Ethical Committee of Tanabe Seiyaku Co., Ltd.

Antagonism of Reserpine-Induced Symptoms in Mice This test was performed essentially as described by Worms et al.23) Test drugs were orally administered to groups of 10 mice simultaneously with reserpine (5 mg/kg, i.p.). Ptoosis and akinesis were evaluated 2 h after the reserpine treatment. The degree of ptoosis was assessed according to the following rating scale: 0, eyes open; 1, half closed; 2, completely closed. For the evaluation of akinesis, animals were placed at the center of a white paper circle (9.5 cm in diameter) and were judged, on an all-or-none basis, to be akineic if they remained within the circle for 15 s or more. Hypothermia was assessed by measuring the difference of rectal temperature just before and 4 h after the reserpine and drug administration.

For ptoosis and akinesis, the effects of drugs were expressed as ED_{50} values, defined as the dose which antagonized the ptoosis by 50% of the maximum obtainable score and the dose which prevented the akinesis in 50% of the animals, respectively. For hypothermia, drug effects were expressed as the lowest dose producing a statistically significant prevention of reserpine-induced hypothermia as compared to the control group (minimum effective doses, M EDs, p<0.05).

Behavioral Despair Test and Measurement of Spontaneous Motor Activity (SMA) in Rats Subjected to Forebrain Ischemia Reversible forebrain ischemia was performed in rats essentially as described by Borzeix.24) Under halothane anesthesia, both common carotid arteries (CCA) were isolated. Bilateral CCA occlusion was performed by ligation, and sodium nitroprusside (5 mg/kg, s.c.) was simultaneously injected; halothane was then discontinued. Fifteen minutes later, ligations of the bilateral CCA were released under halothane anesthesia. Our preliminary experiment showed that this dose (5 mg/kg, s.c.) of sodium nitroprusside was sufficient to keep the mean blood pressure under the accepted level of 50 mmHg for more than 15 min. The day of surgery was defined as day 0.

Behavioral despair test was performed as described by Porsoi et al.25) with some modification. Twelve days after the treatment of ischemia (day 12), rats were individually placed in plexiglass chambers (45 × 45 × 35 cm), in which their SMA was measured for 10 min using a moving analysis system (SCANET®, Toyo Sangyo Co., Ltd., Toyama, Japan). This system traces and counts the animal’s movement by scanning 144 pairs of photocells on the side walls every 0.1 s. Then, rats were returned to the home-cage, and after a 10 min interval, they were forced to swim in a cylinder (40 cm height, 17.4 cm diameter) containing water (18 cm depth, 25°C) for 15 min. The duration of immobility of rats was measured during the first 5 min (pre-drug session); they were judged to be immobile when they ceased struggling and remained floating in the water, with minimal movements necessary to keep their head above water. Rats were then assigned to 5 groups (n = 5–6) according to their SMA and duration of immobility. Subsequently, they were orally administered T-794, IDX or vehicle (carboxymethyl cellulose Na, CMC) 7 times, i.e. twice daily from the morning of day 13 to the morning of day 16. Fifty minutes after the last drug administration (day 16), SMA was measured for 10 min by SCANET®. Then, after a 10 min interval in their home-cage, i.e. 70 min after the last drug administration, rats were placed in the cylinder and the total duration of immobility was measured during 5 min of forced swimming (post-drug session).

Statistics Drug effects in reserpine-induced hypothermia or on behavioral parameters (duration of immobility and SMA) were analyzed using one-way ANOVA followed by post-hoc multiple comparison. Differences at a p value of less than 0.05 were considered to be statistically significant. ED_{50} values were calculated using log-probit analysis, except as otherwise described in the legend.

Drugs T-794, IDX hydrochloride, BFL hydrochloride, AMD hydrochloride and idebenone were synthesized at Tanabe Seiyaku. Imipramine hydrochloride and reserpine were purchased from commercial sources (Sigma). T-794 and idebenone were suspended in 0.5% CMC. Imipramine, IDX, BFL and idebenone were dissolved in distilled water. Doses of drugs were expressed as free base in the reserpine test and as salt in the behavioral despair test. Test drugs were administered at a volume of 5 ml/kg to rats and 10 ml/kg to mice.

RESULTS

Antagonism of Reserpine-Induced Symptoms in Mice Mean (±S.E.M.) ptoosis score, incidence of akinesia, and mean (±S.E.M.) decrease in rectal temperature of reserpine-treated control animals were 1.9 ± 0.04, 93%, and −8.4 ± 0.3°C, respectively. Oral administration of T-794 potently prevented reserpine-induced ptoosis (ED_{50} = 4.41 mg/kg), akinesia (ED_{50} = 3.29 mg/kg), and hypothermia (MED = 3 mg/kg) (Table 1). It was at least 3.7, 13.0, and 3.3 times more potent than cerebral metabolic enhancers tested in the antagonism of ptoosis, akinesia, and hypothermia, respectively. Imipramine, a tricyclic antidepressant, prevented hypothermia with MED of 3 mg/kg, while higher doses were required in the antagonism of ptoosis and akinesia.

Among cerebral metabolic enhancers, each drug exhibited a different activity spectrum on the three symptoms (Table 1). For prevention of ptoosis and hypothermia, IDX was the most potent among these enhancers (ED_{50} = 16.4 mg/kg on ptoosis and MED = 10 mg/kg on
Table 1. Antagonism of Reserpine-Induced Symptoms in Mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Ptois ED_{50} (95% CL) mg/kg, p.o.</th>
<th>Akinesia ED_{50} (95% CL) mg/kg, p.o.</th>
<th>Hypothermia MED (Effect at MED) 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 (47)</td>
</tr>
<tr>
<td>Imipramine</td>
<td>60.3 (32.3–212)</td>
<td>24.5 (7.19–195)</td>
<td>3 (30)</td>
</tr>
<tr>
<td>IDX</td>
<td>16.4 (10.6–25.1)</td>
<td>&gt;100 40% at 200</td>
<td>10 (33)</td>
</tr>
<tr>
<td>BFL</td>
<td>100* 50% at 100</td>
<td>&gt;100 40% at 200</td>
<td>30 (31)</td>
</tr>
<tr>
<td>AMD</td>
<td>67.6 (36.9–334)</td>
<td>42.9 (14.7–702)</td>
<td>30 (42)</td>
</tr>
<tr>
<td>Idebenone</td>
<td>&gt;100 10% at 100</td>
<td>&gt;100 20% at 100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

Test drugs were orally administered simultaneously with reserpine (5 mg/kg, i.p.). Ptois and akinesia were evaluated 2h after reserpine injection. Hypothermia was assessed 4h after reserpine injection. N = 10 per dose per drug. a) Graphically determined. b) % prevention at MED compared with control group.

Fig. 2. SMA (Upper) and the Duration of Immobility in Forced Swimming (Lower) in Groups of Rats Subjected to Forebrain Ischemia in Pre-drug Session (12d after Surgery)

Data are means±S.E.M. (n = 5–6). There was no significant difference among groups.

hypothermia), while for prevention of akinesia, AMD was the most potent (ED_{50} = 42.9 mg/kg), and the effects of other enhancers on this symptom were negligible.

Behavioral Despair Test and Measurement of SMA in Rats Subjected to Forebrain Ischemia Behavioral parameters of groups in pre-drug and post-drug sessions are demonstrated in Figs. 2 and 3, respectively. Mean SMAS and mean durations of immobility of control group were 1160 and 134 s, respectively, in the pre-drug session, and 712 and 189 s in the post-drug session. These parameters were roughly equal among the groups in the pre-drug session. After repeated oral administration, T-794 dose-dependently reduced the duration of immobility in the swim session and the effect was statistically significant at 30 mg/kg, whereas SMA was not affected by T-794. IDX did not exhibit significant effect on the duration of immobility at the doses tested, while it caused a dose-dependent but non-significant reduction of SMA. Neurological symptoms, which might be caused following ischemia, were not observed at the time of the behavioral sessions.

Fig. 3. Effects of T-794 and IDX on the SMA (Upper) and the Duration of Immobility in Forced Swimming (Lower) in Rats Subjected to Forebrain Ischemia in Post-drug Session (16d after Surgery)

Groups A–E correspond to those groups in Fig. 2. Drugs were administered 7 times between pre-drug and post-drug sessions. Doses represent the amount of a single administration. Data are means±S.E.M. (n = 5–6). *p < 0.05 vs. CMC-treated control group, comparison by ANOVA, followed by multiple comparison (Fisher’s PLSD method).

DISCUSSION

In treatment of post-stroke emotional disturbances, the cerebral metabolic enhancers, e.g., IDX, BFL and AMD, are mainly prescribed in Japan. As previously described, these drugs possess similar biochemical and pharmacological profiles as antidepressant drugs. In the present study, the antidepressant activity of T-794 was compared with those of cerebral metabolic enhancers. Firstly, the effects of T-794 on reserpine-induced symptoms were assessed. As demonstrated in Table 1, T-794 was more potent than cerebral metabolic enhancers tested in antagonism of any of three symptoms (ptosis, akinesia, and hypothermia). These results support the potential benefits of T-794 for post-stroke emotional disturbance.

T-794 not only showed a potent effect on reserpine-induced symptoms, but also prevented the three symptoms
with similar potency; some of the cerebral metabolic enhancers, in contrast, exhibited rather different potencies on the three symptoms. For instance, IDX antagonized ptosis and hypothermia at potencies 1/4—1/3 those of T-794, whereas its effect was negligible on akinesia. Such activity spectra of the drugs on the reserpine-induced symptoms may be attributed to their different effect on each brain monoaminergic system, as it is reported that ptosis antagonism is obtained by stimulation of α-adrenergic or serotonergic receptors, akinesia antagonism by stimulation of dopaminergic receptors, and hypothermia antagonism by stimulation of β-adrenergic receptors. The present results that AMD was relatively potent and IDX was very weak in prevention of akinesia support the notion that antagonism of akinesia is mediated by stimulation of dopaminergic receptors, as AMD possesses indirect dopaminomimetic action, while no such activity has been reported for IDX. The result that T-794 showed similar potencies on the three symptoms suggests that it possesses a broad spectrum of action on monoaminergic systems, including adrenergic and dopaminergic system. In addition, since a number of investigations suggest involvement of the brain dopaminergic system in neurobiology of motivation, and since imipramine was weak in antagonism of akinesia despite being as potent as T-794 in the prevention of hypothermia, the difference in activity spectrum in reserpine antagonism may have some relevance to the clinical activity profile of antidepressant drugs as reported by Kielholz (e.g., MAO inhibitors have a relatively strong drive (motivation) enhancing effect in contrast to other antidepressant drugs).

Secondly, the antidepressant activity of T-794 was examined in rats subjected to forebrain ischemia, which was used as an animal model of cerebrovascular disorder. In the present study, forebrain ischemia was induced by a combination of bilateral CCA occlusion with systemic hypotension sufficient to reduce the cerebral blood flow markedly. This procedure was first proposed more than 20 years ago. The disadvantages of this model include the need for anesthesia and some interanimal inconsistency with respect to the decrement in cerebral blood flow as well as the pathologic outcome. Our procedure caused passive avoidance deficits in rats and neuronal cell injury in the CA1 region of the hippocampus and in the neocortex, although the degree was rather mild and there was some variation among subjects (data not shown). This procedure does offer advantages, however, i.e., its simplicity (one-stage surgical preparation), suitability for chronic survival studies, and low experimental failure rate. These advantages prompted us to select this model. In order to equalize the interanimal variation between the groups, animals were assigned to groups so that the behavioral parameters of the groups, namely SMA and duration of immobility in forced swimming, were roughly equal.

Behavioral sessions to evaluate drug effect started 12 d after ischemia, when acute biological reaction to the cerebral ischemia is considered to be completed. Antidepressant activity was assessed in the behavioral despair test essentially by the procedure of Porsolt et al. except that the interval between the first (pre-drug) and the second (post-drug) swim session was changed to 4 d instead of 1 d in the original procedure. As reported, the mean duration of immobility of control rats showed a tendency to increase in the post-drug session compared with that in the pre-drug session. This may be caused by acclimatization to the novel environment or may reflect lowered mood or hopelessness in the rats as discussed. Additionally, the mean SMA of control rats tended to decrease in the post-drug session, probably due to acclimatization to the apparatus. According to Porsolt et al., the antidepressant drugs reduce the duration of immobility in the post-drug session without increasing SMA. T-794 significantly reduced the duration of immobility in the post-drug session, and the effect was specific because SMA was not affected. Thus the antidepressant activity of T-794 was also suggested in the rats subjected to forebrain ischemia. It seems unlikely that the reduction of immobility by T-794 was due to the drug-induced impairment of memory, or prevention of acclimatization, since drugs which are thought to impair memory were not effective in reducing immobility; additionally, in the present study T-794 did not affect SMA which tended to decrease in the post-drug session in comparison with the pre-drug session.

IDX did not exhibit significant effect on the duration of immobility at doses tested. It was reported that IDX reduced the immobility in mice with a single oral administration of 50 mg/kg. Moreover, the present results showed that IDX was at most 30% as potent as T-794 in antagonizing reserpine-induced symptoms. These lines of evidence imply that IDX may be effective at higher doses than used in the present study.

In conclusion, T-794 was effective in the test predictive of antidepressant activity, in rodents treated with or without cerebral ischemia. It was more potent in these tests than the cerebral metabolic enhancers which have a similar biochemical and pharmacological profile as antidepressant drugs and are frequently prescribed to treat patients with post-stroke emotional disturbances. These results suggest and support the potential benefits of T-794 in the treatment of such patients.

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REFERENCES