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# Neuroprotective effect of rasagiline, a selective monoamine oxidase-B inhibitor, against closed head injury in the mouse

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**Abstract**

The potential neuroprotective effects of rasagiline, *N*-propargyl-1*R*-aminoindan, a selective monoamine oxidase-B inhibitor and its inactive enantiomer TVP1022, *N*-propargyl-1*S*-aminoindan were assessed against the sequelae of closed head injury in the mouse. Injury was induced in the left hemisphere under ether anaesthesia. Rasagiline (0.2 and 1 mg/kg) or TVP1022 (1 and 2 mg/kg) injected 5 min after injury accelerated the recovery of motor function and spatial memory and reduced the cerebral oedema by about 40–50%, ($P < 0.01$). The neuroprotective effects on motor function and spatial memory, but not on cerebral oedema, were prevented by scopolamine (0.2 mg/kg). Daily injection of rasagiline (1 mg/kg) from day 3 after injury accelerated the recovery of spatial memory but not motor function. Conclusions: Early administration of rasagiline or TVP1022 can reduce the immediate sequelae of brain injury. The mechanism of action does not appear to involve monoamine oxidase-B inhibition but could be mediated by the maintenance of cholinergic transmission in brain neurons. 

*Keywords:* Brain oedema; Cholinergic mediation; Monoamine oxidase-B inhibition; Motor function; Neuroprotection; Spatial memory

## 1. Introduction

Traumatic brain injury induces persistent neurological deficits which include motor and memory impairments (Smith et al., 1991; Capruso and Levin, 1992; Hamm et al., 1996). Cerebral oedema is an additional acute complication of brain injury and results from excess accumulation of water in the intra- and extracellular space (Lobato et al., 1988). The mechanisms underlying the production of these deficits are still unclear, but a role for glutamate excitotoxicity has been suggested (Shohami et al., 1995; Zauner and Bullock, 1995). Reactive oxygen radicals are also triggered by the injury and could contribute to its patho-physiology (Smith et al., 1994). These radicals can be generated from oxidation of catecholamines, and from an increase in free iron. The latter could occur from extravasated haemoglobin through rupture of cerebral vessels or from the release of iron from stores by the injury.

Similar mechanisms are involved in the brain damage resulting from ischemia. The selective monoamine oxidase-B inhibitor, selegiline, has been shown to improve neuronal survival in gerbils following transient global ischaemia (Lahtinen et al., 1997) and in rats subjected to unilateral hypoxia ischaemia (Knollema et al., 1995; Paterson et al., 1997). Selegiline also rescued immature rat axotomised facial neurons from death (Ansari et al., 1993). Its action has been attributed to a reduction in the formation of free radicals by stimulating catalase and superoxide dismutase (Knollema et al., 1995), an increase in the expression of nerve growth factor (Semkova et al., 1996) and a reduction of apoptosis (Paterson et al., 1997). Selegiline is metabolised to amphetamine and methamphetamine by liver enzymes (Heinonen et al., 1994). These metabolites lack the neuroprotective effect of the parent drug and may even cause cell damage in some preparations (Oh et al., 1994). This could interfere with the potential beneficial effects of selegiline in vivo, particularly after oral administration.

Rasagiline (TVP1012; *N*-propargyl-1*R*-aminoindan) is an irreversible monoamine oxidase inhibitor with selectivity towards the B form similar to that of selegiline (Ster-

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ling et al., 1998). Unlike selegiline, rasagiline is not metabolised to amphetamine and methamphetamine, and thus should be devoid of the undesirable effects of these substances. Rasagiline has recently been shown to reduce glutamate toxicity in cultured hippocampal neurons (Finberg et al., 1998a) and to prolong survival of cultured, serum-deprived rat fetal mesencephalic cells (Finberg et al., 1998b). Chronic prophylactic treatment of the nursing rats with rasagiline also reduced memory impairments in their offspring in adulthood, resulting from prolonged hypoxia during the neonatal period (Speizer et al., 1998).

The aim of the current study was to see whether rasagiline could protect mice against the brain oedema, impairments in motor function and memory that occur after closed head injury. In order to establish whether or not any neuroprotective effects of the drug resulted from inhibition of monoamine oxidase-B, we compared its effects with those of its enantiomer, *N*-propargyl-1*S*-aminoindan, TVP1022. This compound is at least 100-fold less active as an inhibitor of this enzyme in rat brain (Finberg, personal communication). A reduction in cholinergic indices occurs after brain trauma (Leonard et al., 1994; Gorman et al., 1996), which may be responsible for the motor and memory deficits. Therefore, we also determined whether any neuroprotective effects of rasagiline or its enantiomer were associated with prevention of the loss in cholinergic activity by testing their susceptibility to blockade by scopolamine.

## 2. Materials and methods

### 2.1. Animals

Male Sabra mice (Hebrew University strain) weighing 35–40 g were used in this study which was performed according to the guidelines of the National Institutes of Health USA and Israeli Law for the correct use of laboratory animals and obtained approval from the Institutional Committee for Animal Care.

### 2.2. Head trauma

Severe head injury was induced in the mice under ether anaesthesia by a weight-drop device as previously described (Chen et al., 1996). This consisted of an impacting rod, 3 mm in diameter, weighing 333 g, which was dropped from a height of 3 cm on the left hemisphere. Rasagiline (1 mg/kg), TVP1022 (2 mg/kg) or saline (1 ml/kg) was injected s.c., alone or with scopolamine (0.2 mg/kg) 5 min after head injury for all the following measurements. The effect of rasagiline 0.2 mg/kg ($n = 10$) and of TVP1022 1 mg/kg ($n = 10$) was also assessed on motor function and spatial memory.

### 2.3. Evaluation of monoamine oxidase activity in mouse brain

The effect of acute administration of rasagiline 0.2 and 1 mg/kg and of TV1022 1 and 2 mg/kg, 5 min after closed head injury, on the activity of monoamine oxidase-A and -B was measured in the brain. The mice were killed by cervical dislocation and the brains were rapidly removed and stored at −70°C until assay. For the measurement of the effect of chronic administration of rasagiline (1 mg/kg) on brain monoamine oxidase-A and -B, the mice were killed 6 days after daily administration of rasagiline (1 mg/kg) or saline (1 ml/kg), commencing 3 days after injury as described below in 2.8. The brains were removed 2 h after the last injection. The activity of monoamine oxidase-A and -B were measured according to a modification of the methods of Otsuka and Kobayashi (1964) and O'Carrol et al. (1983). The brains were weighed and 5% homogenates prepared in 0.3 M sucrose; 50 μl portions of brain homogenate were incubated for 1 h at 37°C with 0.15 μM clorgyline in phosphate buffer 0.1 M, pH 7.4, to inhibit monoamine oxidase-A. [$^{14}$C]phenylethylamine (final concentration of 13 μM) was then added and the incubation continued for a further 30 min. Deprenyl (0.15 μM) was added to other tubes containing brain homogenate to inhibit monoamine oxidase-B and after 1 h incubation, [$^{14}$C]5-hydroxytryptamine, (final concentration 100 μM) was added as substrate for monoamine oxidase-A and the incubation was continued for 20 min. Blanks for each enzyme contained 10 μl of tranylcypromine (0.01 M) and the respective substrates as above. The reaction was stopped with 250 μl of 2 M citric acid and the radioactive product was extracted into an toluene:ethyl acetate 1:1 and measured in a scintillation counter after the addition of 2,5-diphenyl-oxazole.

### 2.4. Evaluation of cerebral oedema

The degree of cerebral oedema was determined 24 h after head injury in groups of 9–10 mice, injected with rasagiline (1 mg/kg) or TVP1022 (2 mg/kg) alone or with scopolamine, by measuring the tissue water content in the injured brain, as previously described (Chen et al., 1996). Oedema had been found to reach its peak at this time in the left hemisphere after injury (Chen et al., 1996).

### 2.5. Integrity of the blood brain barrier

The effect of rasagiline (1 mg/kg) ($n = 6$) and saline (1 ml/kg) ($n = 6$) on the integrity of the blood brain barrier was assessed in mice after the i.v. injection of Evan's blue dye (2% w/v in saline) as described by Uyama et al. (1988). This method can be applied to the model of closed head injury, since the dye was injected 3 h after injury

when the initial haemorrhage had ceased. The dye was allowed to circulate for at least 60 min and measurements were made 4 h later, when disruption of the blood brain barrier had reached its peak (Chen et al., 1996).

### 2.6. Neurological disability score

Neurological and motor function were assessed at 1 h, 1, 7 and 14 days after injury by an uninformed observer according to a modification of the score previously described (Chen et al., 1996). A point is given for the absence of reflexes and for the failure to perform a number of tasks shown in Table 1.

### 2.7. Evaluation of spatial memory

The Morris water maze consisted of a circular aluminium pool, 1 m in diameter and 60 cm depth, filled to a depth of 17.5 cm with water maintained at 22–24°C. A glass vessel (15 cm diameter × 16.5 cm high) was placed upside down at a fixed location in the pool and served as the hidden goal platform. All the mice were given three trials per day for 5 consecutive days to establish baseline performance. They were released from the same starting point in the maze in all the trials and given a maximum of 120 s to find the hidden platform. Those that failed to do so in that time were placed on the platform for 30 s between trials. The location of the platform remained the same for all tests. Latency, i.e., the time taken to reach the platform, was recorded for each trial. On the 8th day, the mice were subjected to closed head injury and 5 min later, injected either with saline or rasagiline, 0.2 and 1 mg/kg, TV1022, 1 or 2 mg/kg. Other groups of mice were injected with rasagiline, 1 mg/kg or TV1022, 2 mg/kg together with scopolamine, 0.2 mg/kg. Post-traumatic testing commenced 24 h after head injury and consisted of a session of three trials per day for 5 consecutive days, a rest of one day followed by a further three trials per day for another 5 consecutive days. Swimming speed was assessed in control and injured mice by measuring the time taken to swim a fixed distance in the pool.

Table 1
Modified neurological and motor disability score in mice

| Behaviour | At 1 h | At other times |
|---|---|---|
| Inability to exit circle (30 cm in diameter) when left in its centre for: | | |
| 30 min | 1 | |
| 60 min | 1 | |
| > 60 min | 1 | 1 |
| Inability to walk straight on floor | 1 | 1 |
| Absence of startle reflex | 1 | 1 |
| Failure in beam balance task (0.5 cm wide) | | |
| For 20 s | 1 | 1 |
| For 40 s | 1 | 1 |
| For 60 s | 1 | 1 |
| Failure in beam-walking task | | |
| 3 cm wide | 1 | 1 |
| 2 cm wide | 1 | 1 |
| 1 cm wide | 1 | 1 |
| Failure in round stick grasping task for 10 s | 1 | 1 |
| Absence of exploration on flat surface | 1 | 1 |

A mouse that is unable to exit the circle for 30 min is given a score of 1. An additional point is given for failure to do so within 60 min, up to a maximum of 3 points if this is greater than 60 min. This scale also applies to the beam balance and beam walking tasks. One point is given for the failure to perform other tasks or the absence of reflexes. The highest possible score at 1 h is 13 and at 24 h or later, 11.

### 2.8. Chronic post-trauma drug administration

The potential ability of rasagiline to improve memory and motor impairments when given several days after injury was also assessed. The drug or saline was injected daily from the third day after head injury and its effect on spatial memory in the Morris water maze and on motor function was determined as described above. Forty mice were used for the experiment. All were anaesthetised, two groups of 10 were uninjured, and served as sham controls. The remaining 20 were subjected to closed head injury. Neurological and motor function were assessed 1 and 24 h later. Three days after injury or sham injury, the mice were injected s.c. daily for 11 days with saline, (1 ml/kg) or rasagiline, (1 mg/kg). Spatial memory was assessed daily for 6 days, 2 h after drug injection. Neurological and motor function were assessed 7 and 14 days after injury.

### 2.9. Histological examinations

Eleven days after head injury or sham injury mice injected once with saline or rasagiline, 1 mg/kg, with and without scopolamine, 0.2 mg/kg (8 per group) were anaesthetized with ether and then immediately perfused transcardially with 10% phosphate buffered formalin. The brains were fixed in the same solution for 6 days, then serial coronal sections (10 μm) were prepared and stained with hematoxylin and eosin to quantify cell loss in the $CA_1$, $CA_2$ and $CA_3$ areas of the hippocampus. Other sections at the site of injury were stained with Perl's stain for hemosiderin, as previously described (Chen et al., 1996). Gross cortical damage at the site of impact was scored by an uninformed observer according to the following scale: 0 = no discernible damage; 1 = very mild damage, 2 = mild–moderate damage, 3 = moderate damage; 4 = severe damage involving loss of most of the cortical area on the left side.

### 2.10. Statistical analysis

Differences between saline and drug treatments regarding the degree of cerebral oedema were analysed by one

way analysis of variance (ANOVA) followed by Duncan's post-hoc test where appropriate. Morris water maze latencies and neurological scores were analysed by two-way ANOVA with days as one variable and treatment as another, followed by a post-hoc Student's *t*-test with a Bonferroni correction. Differences between mean values for percentage of dead cells in the hippocampus and number of siderocytes were analysed with a non-parametric Mann–Whitney test. All data are expressed as the mean values for the group ± S.E.M. A $P < 0.05$ was considered statistically significant.

### 2.11. Drugs

Rasagiline mesylate, (TVP1012) and TVP1022 mesylate, Teva Pharmaceuticals Netanya Israel; (−)-scopolamine HCl, Sigma, Holon, Israel. All doses are expressed in mg/kg of the respective salt.

## 3. Results

### 3.1. Monoamine oxidase inhibition

Two hours after an acute injection of 0.2 mg/kg rasagiline brain monoamine oxidase-B was inhibited by almost 92% while monoamine oxidase-A was only inhibited by 10% (Table 2). A higher dose, 1 mg/kg, did not produce appreciably greater inhibition of either enzyme. In contrast, TVP1022 had negligible inhibitory effects on either forms of the enzyme at 1 and 2 mg/kg. Chronic daily administration of rasagiline (1 mg/kg) for 6 days produced complete inhibition of brain monoamine oxidase-B and 50% inhibition of the A form (Table 2).

### 3.2. Oedema formation

Head injury increased cerebral water content in the left, contused hemisphere from 78.8 ± 0.1 to 84.1 ± 0.2% ($P < 0.001$). Rasagiline (1 mg/kg) and TV1022 (2 mg/kg) significantly reduced the cerebral oedema in the left, contused hemisphere by 43% and 51%, respectively. Scopolamine did not affect cerebral oedema when given alone, nor did it antagonise the effect of either of the two isomers. The data are shown in Fig. 1.

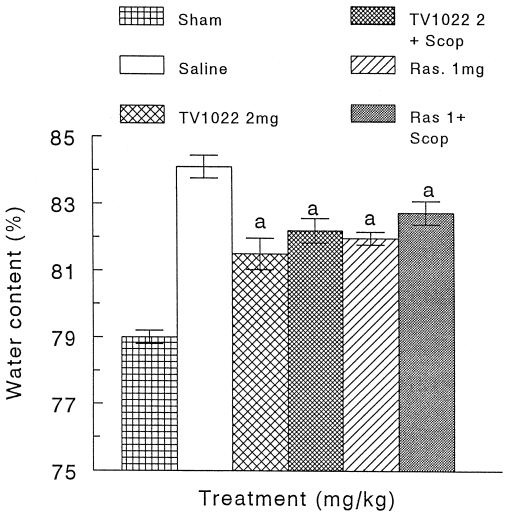


Fig. 1. Effect of rasagiline and TVP1022 on the increase in cerebral water content in the contused hemisphere, 24 h after closed head injury. Significantly different from saline, [a] $P < 0.05$.

### 3.3. Disruption of the blood brain barrier

Closed head injury caused a 2-fold increase in the amount of Evan's blue dye extracted from the injured hemisphere in saline-treated mice. Rasagiline did not affect the disruption of the blood brain barrier caused by injury. The amount of Evan's blue in the injured and control hemispheres in mice injected with saline or rasagiline is shown in Table 3.

### 3.4. Neurological and motor function

Sham controls injected with saline had a score of 1.1 ± 0.2, at 1 h and 0 ± 0, at 24 h. After head injury the

Table 2
Effect of acute and chronic administration of rasagiline and TV1022 on monoamine oxidase activity in mouse brain after closed head injury

| Treatment (mg/kg) | Activity MAO-A[a] | % Inhibition | Activity MAO-B[a] | % Inhibition |
|---|---|---|---|---|
| *Acute injection* | | | | |
| Saline (1 ml) | 20.75 ± 0.97 | | 1.12 ± 0.04 | |
| Rasagiline (0.2 mg/kg) | | 10.2 ± 2.9 | | 91.6 ± 1.6 |
| Rasagiline (1 mg/kg) | | 18.0 ± 1.7 | | 96.7 ± 0.3 |
| TVP1022 (1 mg/kg) | | 0 | | 6.2 ± 2.1 |
| TVP1022 (2 mg/kg) | | 5.1 ± 2.7 | | 18.9 ± 7.0 |
| *Chronic injection* | | | | |
| Saline (1 ml) | 19.30 ± 0.97 | | 0.93 ± 0.05 | |
| Rasagiline (1 mg/kg) | | 50.2 ± 1.3 | | 98 ± 0.4 |

[a] nmol substrate/(h mg tissue).

Table 3
Effect of rasagiline on Evan's blue extravasation in the right and left cerebral hemispheres 4 h after closed head injury in mice

| Treatment | Right hemisphere (ng/g) | Left hemisphere (ng/g) |
|---|---|---|
| Sham | 181 ± 20 | 180 ± 23 |
| Saline | 219 ± 35 | 406 ± 56[a] |
| Rasagiline (1 mg/kg) | 205 ± 41 | 423 ± 34[a] |

[a]Significantly different from sham-treated mice and from value in right, uninjured hemisphere, $P < 0.01$.

disability score at 1 h of saline-treated mice increased to more than 12 (Fig. 2). Rasagiline (0.2 mg/kg) and TVP1022 (1 mg/kg) only produced a significant reduction in the disability score 14 days after injury. At the higher doses, rasagiline significantly reduced the disability score 24 h, and TVP1022 7 days, after injury (Fig. 2). Scopolamine (0.2 mg/kg) prevented the effects of rasagiline and TVP1022 on motor disability.

### 3.5. Spatial memory (Morris water maze)

After 5 days of training all the mice reached a stable performance with latencies ranging between 20 and 30 s.

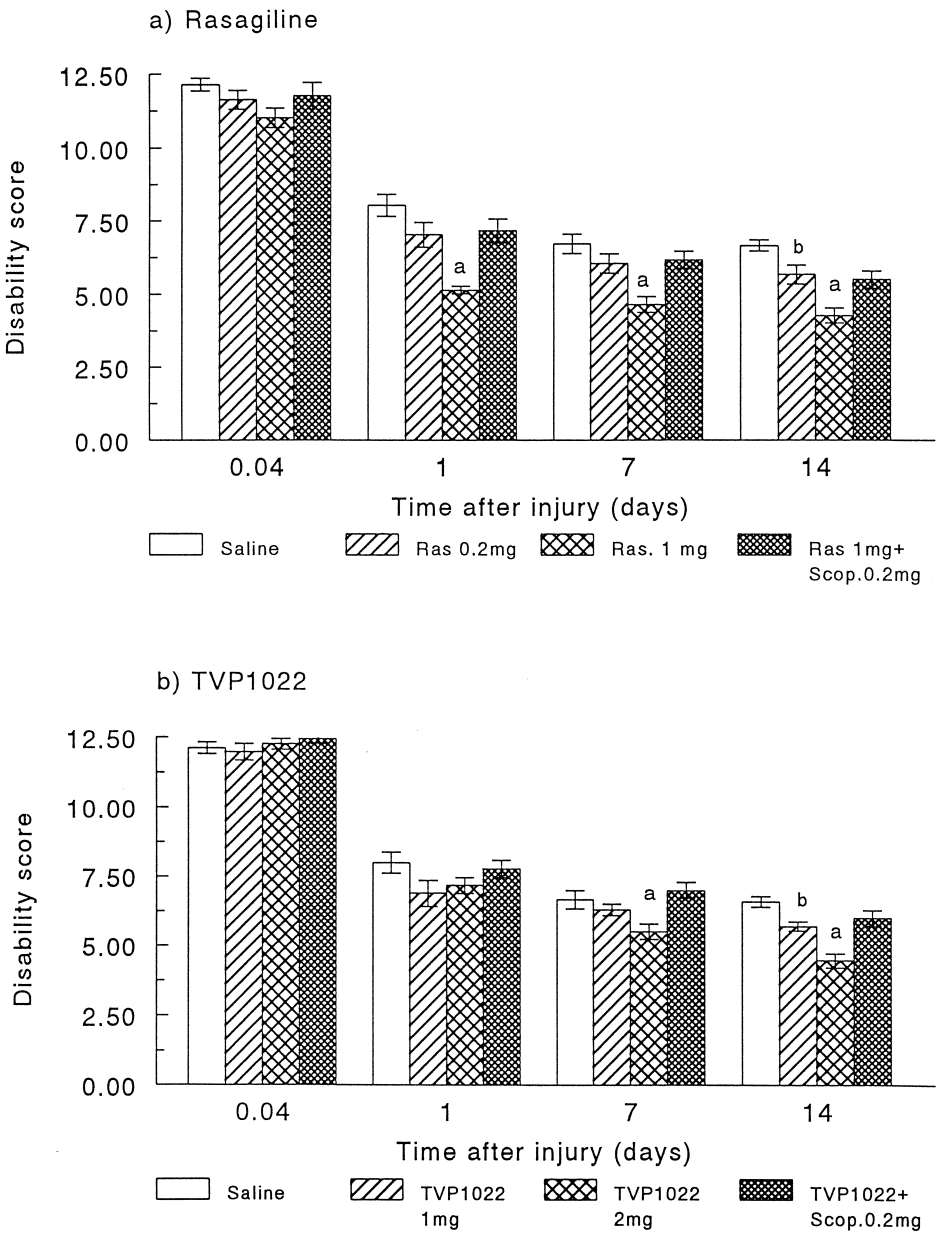


Fig. 2. Effect of acute injection of (a) rasagiline and (b) TVP1022 on the impairment of motor function (disability score) in mice after closed head injury. [a]Significantly different from saline and rasagiline + scopolamine, or TVP1022 + scopolamine, respectively, $P < 0.05$. [b]Significantly different from saline, $P < 0.05$. (a) Rasagiline. ANOVA $F(3, 157) = 30.25$, $P < 0.0001$ for treatment and $F(3, 157) = 293.0$, $P < 0.00001$ for day. (b) TVP1022 ANOVA $F(3, 176) = 17.91$, $P < 0.0001$ for treatment and $F(3, 176) = 372.0$, $P < 0.00001$ for day.

This is shown in Figs. 3 and 4, together with their performance after head injury when given saline, rasagiline, 0.2 or 1 mg/kg or TVP1022, 1 or 2 mg/kg. The majority of the saline-treated mice failed to relearn the position of the platform within the 11-day testing period. Rasagiline (1 mg/kg) enabled the mice to regain their pre-injury latencies by the 3rd day after injury. A dose of 0.2 mg/kg significantly reduced escape latencies compared with those in the controls from the 5th day (Fig. 3a). Mice injected with TVP1022 (1 mg/kg) only showed significantly lower escape latencies than those of the saline controls on days 10 and 11. At a dose of 2 mg/kg, escape latencies were significantly shorter from day 4 after injury (Fig. 3b). Scopolamine (0.2 mg/kg) did not affect the escape latencies of the injured mice when given alone (Fig. 4a) but

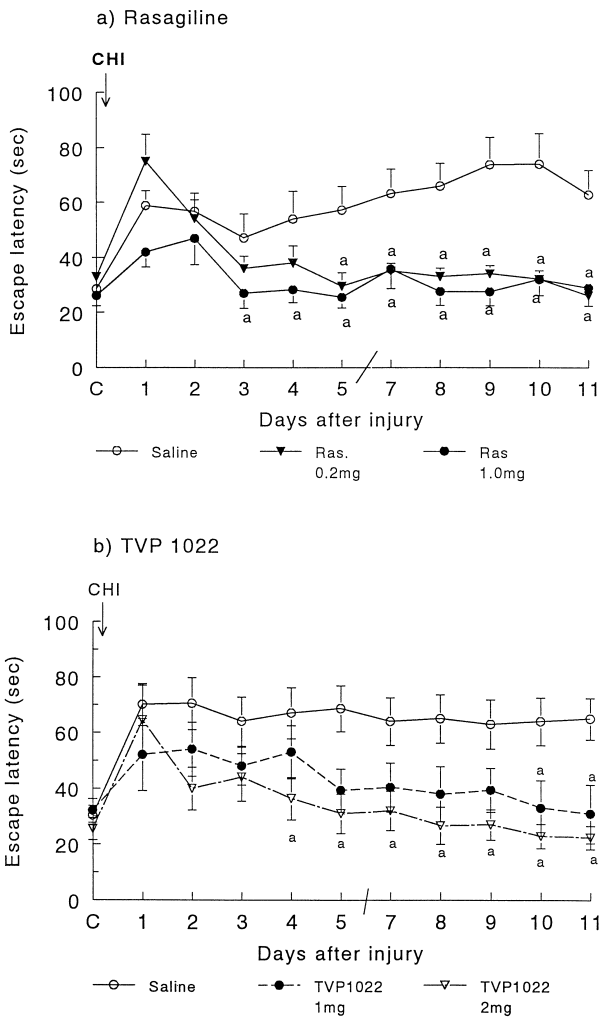


Fig. 3. Effect of acute injection of (a) rasagiline and (b) TVP1022 on the impairment of spatial memory after closed head injury. (a) Significantly different from saline, $P < 0.05$. Rasagiline: from day 1, ANOVA $F(2, 299) = 41.2$, $P < 0.0001$ for treatment and $F(9, 299) = 123.0$, $P < 0.00001$ for day. Treatment × day interaction, $F(18, 399) = 4.08$, $P < 0.01$. TVP1022: from day 1, ANOVA, $F(2, 299) = 18.1$, $P < 0.001$ for treatment and $F(9, 299) = 41.0$, $P < 0.0001$ for day. Treatment × day interaction, $F(18, 299) = 3.11$, $P < 0.02$.

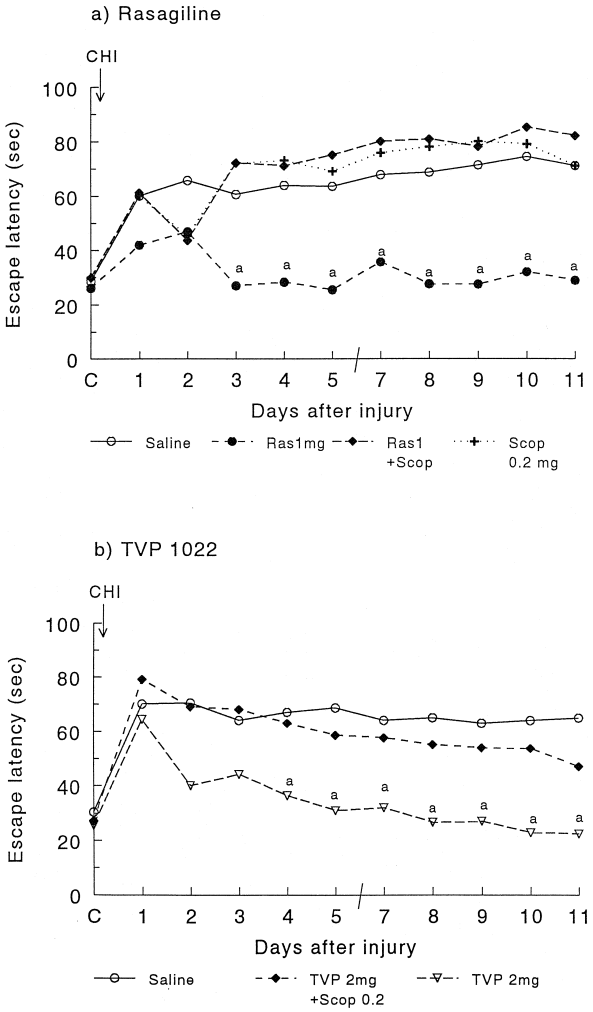


Fig. 4. Effect of scopolamine on the amelioration of spatial memory impairment after closed head injury induced by (a) rasagiline, (b) TVP1022. [a]Significantly different from saline and rasagiline or TVP1022 + scopolamine, $P < 0.05$. Rasagiline: from day 1 ANOVA, $F(3, 401) = 59.4$, $P < 0.0001$ for treatment and $F(9, 401) = 144.0$, $P < 0.00001$ for day. Treatment × day interaction, $F(27, 401) = 8.02$, $P < 0.001$. TVP1022: from day 1 ANOVA, $F(2, 299) = 8.25$, $P < 0.001$ for treatment and $F(9, 299) = 18.2$, $P < 0.0001$ for day.

prevented the improvement in performance induced by both rasagiline and TVP1022 (2 mg/kg) (Fig. 4a and b). Failure of injured mice to find the platform in the water maze was not due to impairment of their swimming ability, as the swimming speed of saline-treated, injured mice was $26.3 \pm 2.5$ cm/s compared to $23.9 \pm 2.2$ cm/s for uninjured mice.

### *3.6. Chronic treatment with rasagiline on reference memory and motor disability*

The effect of daily treatment with rasagiline (1 mg/kg), from the third day after injury, on spatial memory in the Morris water maze test is shown in Fig. 5. Injured mice given saline showed significantly higher escape latencies

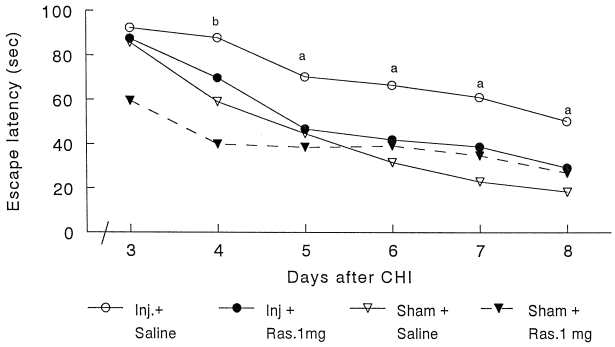


Fig. 5. Effect of chronic daily treatment with rasagiline on spatial memory after closed head injury or after sham injury. [a]Significantly different from all other groups, $P < 0.05$. ANOVA, $F(3, 241) = 28.4$, $P < 0.0001$ for treatment and $F(5, 241) = 25.3$, $P < 0.0001$ for day.

than sham-treated mice from the 4th day after injury. Rasagiline reduced the escape latencies of the injured mice to those of the sham controls from the second day of treatment, i.e., the 5th day after injury. Although rasagiline caused a small reduction in the disability score of motor function on day 14 after injury, this did not reach statistical significance. The scores for sham controls were 0; saline-treated injured mice were $6.6 \pm 0.2$ and $6.0 \pm 0.3$ on days 7 and 14, respectively and for rasagiline-treated mice they were $6.0 \pm 0.4$ and $5.1 \pm 0.4$.

### 3.7. Histology

The score for the area of cortical damage at the site of impact and the number of siderocytes (hemosiderin containing macrophages) in the area adjacent to it are shown in Table 4, together with the latencies to find the escape platform in the Morris water maze that were measured in these mice before killing. There were no significant differences in the scores for damage or in the number of siderocytes per field from mice given saline, rasagiline or rasagiline plus scopolamine, although the escape latencies for the rasagiline-treated mice were significantly lower than those of the other groups. Injured mice given rasagiline (1 mg/kg) had fewer dead cells in the $CA_2$ and $CA_3$ areas of the hippocampus than mice given saline (Table 5). Concomitant treatment with scopolamine did not alter the number of dead cells in any area of the hippocampus.

## 4. Discussion

The major new finding in this study was that two *N*-propargyl-aminoindan derivatives rasagiline (1 mg/kg) and TVP1022 (2 mg/kg), significantly protected mice against the consequences of neuronal damage induced by closed head injury. A single injection of either drug given 5 min after closed head injury, reduced by 40–50%, the oedema in the contused cerebral hemisphere 24 h later, restored spatial memory in the water maze test by the third day and accelerated the recovery of motor deficits.

Previous studies have shown that the impairments in motor function and memory resulting from brain injury are associated with a prolonged deficit in cholinergic transmission (Leonard et al., 1994; Gorman et al., 1996). These impairments can be corrected by a number of drugs that are able to increase acetylcholine levels (Pike and Hamm, 1995; Chen et al., 1998). The effect of one of these, rivastigmine, an acetylcholinesterase inhibitor, was completely prevented by the concomitant administration of scopolamine, when both drugs were given once, immediately after closed head injury (Chen et al., 1998). The neuroprotective effects of each drug against the impair-

Table 4
Effect of rasagiline on area of cortical damage and siderocytes in left cortical area 11 days after injury

| Treatment (mg/kg) | Area of cortical damage (score) | No. of siderocytes in area of damage | Escape latency on day 11 (s) |
|---|---|---|---|
| Saline (1 ml) | $1.52 \pm 0.18$ | $34.7 \pm 4.5$ | $65.2 \pm 7.4$ |
| Rasagiline (1) | $1.59 \pm 0.38$ | $42.5 \pm 11.0$ | $25.9 \pm 6.4$[a] |
| Rasagiline + scopolamine (0.2) | $2.12 \pm 0.31$ | $67.9 \pm 14.9$ | $71.5 \pm 6.9$ |

Score for area of cortical damage: 0 = no discernible damage; 1 = very mild damage, 2 = mild–moderate damage, 3 = moderate damage; 4 = severe damage involving loss of most of the cortical area on the left side.
[a]Significantly different from saline and rasagiline + scopolamine-treated mice, $P < 0.05$.

Table 5
Percent of dead cells in three areas of the hippocampus in the left and right hemispheres of injured mice

| Treatment | $CA_1$ left | $CA_2$ left | $CA_3$ left | $CA_1$ right | $CA_2$ right | $CA_3$ right |
|---|---|---|---|---|---|---|
| Saline | $5.47 \pm 3.09$ | $7.67 \pm 1.59$ | $22.2 \pm 6.7$ | $1.89 \pm 0.50$ | $2.94 \pm 1.04$[a] | $4.64 \pm 1.82$[a] |
| Rasagiline (1 mg/kg) | $3.03 \pm 0.42$ | $3.42 \pm 0.28$[b] | $7.85 \pm 3.39$[b] | $2.20 \pm 0.38$ | $2.18 \pm 0.45$ | $2.61 \pm 0.87$ |
| Rasagiline + scopolamine (0.2 mg/kg) | $16.4 \pm 8.5$ | $3.38 \pm 1.21$ | $4.43 \pm 1.83$ | $4.8 \pm 1.41$[a] | $3.19 \pm 1.57$ | $2.63 \pm 1.38$ |

[a]Significantly different from value in left hemisphere, $P < 0.05$.
[b]Significantly different from saline, $P < 0.05$.

ments in spatial memory and motor function after head trauma were antagonised by the concomitant administration of scopolamine. This suggested that brain cholinergic systems mediated these effects of the two enantiomers. The finding is consistent with that of Speizer et al. (1998), who showed that chronic treatment with rasagiline prevents the reduction in the activity of hippocampal choline acetyltransferase by neonatal hypoxia in rats. However, its mechanism of action is unclear. Neither rasagiline nor TVP1022 inhibits acetylcholinesterase or stimulates muscarinic receptors at concentrations below $10^{-3}$ M (Weinstock, unpublished observations).

Acute administration of rasagiline (0.2 or 1 mg/kg) resulted in an almost complete inhibition of monoamine oxidase-B in the brain of mice with less than 20% inhibition of the A form. There is a relatively high density of dopaminergic synapses on cholinergic cells in the nucleus basalis (Martinez-Murillo et al., 1988; Smiley and Mesulam, 1996) and dopamine $D_1$ and $D_2$ receptor agonists are able to increase the release of acetylcholine in the hippocampus in mice (Imperato et al., 1996). Therefore, it is possible that rasagiline promotes acetylcholine release by increasing dopamine levels in the basal forebrain as a result of monoamine oxidase inhibition (Nilsson et al., 1992). On the other hand, the *S*-enantiomer, TVP1022, (1 and 2 mg/kg) only blocked monoamine oxidase-B by 6 or 19% and -A by 0–5%. Since the accelerated recovery of memory and motor impairments by both isomers showed essentially the same temporal characteristics and since the effects of both drugs were antagonised by scopolamine, it is unlikely that they resulted from blockade of monoamine oxidase. Rasagiline and TVP1022 are metabolised to 1-(*R*)-aminoindan or 1-(*S*)-aminoindan, respectively. These metabolites may also have some neuroprotective action. Chronic treatment of rats (4 days) with 1-(*R*)-aminoindan, like rasagiline, prevented the memory impairment induced by hypoxia (Speizer et al., 1998). Further studies both in vivo and in vitro are currently in progress to substantiate this finding.

The drugs could also act by protecting cholinergic neurones from the damage incurred soon after injury by interfering with the release of destructive mediators such as excitatory amino acids, cytokines and oxidative free radicals. The tissue content of oxidative free radicals after injury correlates well with the degree of motor impairment, and their neutralisation by endogenous or exogenous radical scavengers, correlates with the level of neuroprotection against the sequelae of injury (Shohami et al., 1997).

Brain trauma causes cerebral oedema which results from a combination of a loss of the integrity of the blood brain barrier (Klatzo, 1967) and excessive accumulation of ions and water within the cells (Zauner and Bullock, 1995). Although rasagiline significantly reduced the oedema in the injured hemisphere it did not prevent the increase in the permeability of the blood brain barrier, suggesting that it probably acted by decreasing intracellular fluid accumulation. Rasagiline did not reduce the area of cortical damage or the number of hemosiderin-containing macrophages that invade the site of injury. The hemosiderin probably comes from extravasated haemoglobin due to blood vessel damage and disruption of the blood brain barrier by the injury, which was not affected by rasagiline. However, rasagiline did reduce the number of dead cells in the $CA_2$ and $CA_3$ areas of the hippocampus 11 days after injury, indicating that it may be able to interfere with the release or action of cell-destructive mediators, as suggested above. This effect was not antagonised by scopolamine, showing that it does not result from changes in cholinergic activity.

HU-211 (dexanabinol), a non-competitive antagonist of NMDA receptors and a potent scavenger of per-oxy and hydroxy radicals (Biegon and Bar Joseph, 1995) and ENA713 (rivastigmine), an acetylcholinesterase inhibitor (Chen et al., 1998) both have effects similar to those induced by rasagiline on cerebral oedema in the mouse brain trauma model. Although rasagiline reduces the toxic effect of glutamate on cultured hippocampal neurons (Finberg et al., 1998a), it does not bind to NMDA receptors (Rehavi, unpublished observations) and therefore acts at another step in the cascade of events resulting in neuronal death. Rasagiline does not increase cholinergic activity directly by inhibiting acetylcholinesterase or stimulating muscarinic receptors. In contrast to the effect of rivastigmine, that of rasagiline on cerebral oedema was not prevented by scopolamine. This suggests that the reduction in oedema and in hippocampal cell death by rasagiline did not involve activation of the cholinergic system. It remains to be determined whether rasagiline and TVP1022, can inhibit the formation of free radicals and accumulation of intracellular $Ca^{2+}$ as does HU-211 (Biegon and Bar Joseph, 1995).

Rasagiline was also able to restore the spatial learning ability when given chronically, starting from the third day after injury. This action could have resulted from restoration of the levels of acetylcholine towards normal. A similar beneficial effect on memory occurred after administration of different agents several days after injury, all of which can elevate brain acetylcholine by different mechanisms (Pike and Hamm, 1995; Yamaguchi et al., 1996). The finding that rasagiline could prevent and reverse several of the sequelae of head injury supports the use of this drug for the treatment of this condition in human subjects.

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