Abstract

Rationale: A high incidence of depression is found in subjects with Alzheimer’s disease (AD), in whom many antidepressants are contraindicated because they have anticholinergic activity. We have designed a new cholinesterase inhibitor TV3326 [(N-propargyl-(3R)-aminoindan-5-yl)-ethyl methyl carbamate] for the treatment of AD, which has neuroprotective activities and also blocks monoamine oxidase (MAO) A and B in the brain but not in the intestine after chronic administration.

Objectives: To examine the antidepressant and anxiolytic potential of TV3326 in rats and compare them with those of its R isomer TV3279, which lacks MAO-inhibitory activity, and of amitriptyline and moclobemide.

Methods: Each of the drugs was administered orally, acutely or once daily for 2 weeks, and its effect was evaluated on the behavior of rats in the forced swim test (FST) and plus maze (EPM) test.

Results: Immobility in the FST was reduced by 56% after acute and chronic administration of amitriptyline (10 mg/kg) and by 42% after acute administration of moclobemide (20 mg/kg) and by 63% when this drug was given chronically. TV3326 (26 mg/kg) only reduced immobility (by 44%) when given chronically and inhibited brain MAO-A and -B by more than 66%. TV3279 had no significant effect in the FST. All the drugs except TV3326 increased anxiogenic activity in rats in EPM, as indicated by a more than 50% decrease in the time in open arms after chronic administration.

Conclusions: TV3326 has potential antidepressant-like activity when given in a dose regimen that causes significant inhibition of brain MAO-A and -B. Together with its neuroprotective properties, this action could make TV3326 a potentially valuable drug for the treatment of dementia in patients with depression.

Keywords: Depression · Forced swim test · Anxiety · Plus maze test · Monoamine oxidase inhibitor · TV3326

Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder with characteristic cognitive deficits that are associated with a loss of cholinergic transmission in the basal forebrain (Whitehouse et al. 1982). Significant reductions also occur in serotonergic and noradrenergic transmission (Palmer et al. 1988), which could explain the relatively high incidence of depression found in AD patients (Newman 1999). Many effective antidepressants which are also cholinergic antagonists are contraindicated in such patients as they can exacerbate the cognitive deficits (Edwards 1995).

We have recently designed a new drug, TV3326 [(N-propargyl-(3R)-aminoindan-5-yl)-ethyl methyl carbamate], to combine several pharmacological actions including neuroprotection against oxidative stress, cholinesterase (ChE) inhibition to enable it to ameliorate cognitive deficits, and monoamine oxidase (MAO) inhibition to combat depression (Weinstock et al. 2000a, 2000b, 2001b). Like other propargylamines, TV3326 prevented apoptosis, cell death, and the fall in the mitochondrial action potential induced by ischemia and oxidative free radicals in cultured neuronal systems (Weinstock et al. 2001a; Youdim et al. 2001). After chronic but not acute oral administration, TV3326 also produced a brain-selective, irreversible inhibition of MAO-A and -B (Weinstock et al. 2000b). The virtual absence of MAO inhibition in the intestine and liver suggested that the drug should not cause significant potentiation of the cardiovascular response to oral tyramine.

The aim of the current study was to determine whether TV3326 showed similar activity to that of known antidepressants, moclobemide and amitriptyline,
in animal tests that predict clinical efficacy. One of these is the forced swim test (FST) or “Porsolt test”, in which a form of “learned helplessness” indicated by immobility is reduced by several different groups of antidepressant drugs including MAO-A inhibitors (Porsolt et al. 1979; Borsini and Meli 1988; Kelly and Leonard 1994; Lucki 1997). In order to substantiate the suggestion that any antidepressant activity obtained with TV3326 resulted from brain MAO inhibition, we compared the effects in rats of acute and repeated administration to those of its S-enantiomer TV3279, which also blocks AChE but not MAO, even after chronic administration. AChE inhibitors decrease mobility by increasing cholinergic stimulation in the brain (Karczmar 1978; Sweeney et al. 1989; Riekkinen et al. 1991). This could counteract the usual effect of antidepressants to decrease immobility in the forced swim test. It was therefore important to determine whether TV3326 and TV3279 had a general depressant effect on movement at the doses used in this test. This was accomplished by assessing their effect on exploratory activity in the open field test.

Antidepressants have been shown to possess anxiolytic activity after chronic but not acute treatment in rats (Bodnoff et al. 1988) and humans (Feighner 1999). Since the present study involved the repeated administration of TV3326 and other antidepressants, it was of interest to determine whether they displayed anxiolytic activity in rats in the elevated plus maze (EPM), a test that has been validated for the detection of both anti-anxiety and anxiogenic drugs (Pellow et al. 1985; Cruz et al. 1994).

Materials and methods

Animals

Male Sprague-Dawley rats (Harlan, Ltd. Jerusalem) weighing 230–280 g were housed four per cage (36×26×22 cm) at an ambient temperature of 22±1°C and a 12-h diurnal light cycle (lights on at 0700 hours). Standard food pellets and water were provided ad libitum, and the cages were changed once weekly. Experiments were carried out between 1400 hours and 1800 hours in a room adjacent to that in which the rats were housed under the same conditions of temperature, humidity, and light cycle, and to which the animals had been brought at least 30 min prior to the experiment to avoid alterations in their behavior due to their transfer from one room to another (Morato and Brandao 1996). All the experiments were carried out according to the guidelines of the University Committee for Institutional Animal Care, based on the “Principles of laboratory animal care” of the National Institutes of Health, USA.

Experimental procedures

Different groups of rats, 7–10 per group, were used for acute and chronic drug treatment and for each behavioral test. For the FST, the rats were placed individually in a Perspex cylinder, 50-cm high, 19-cm diameter filled 30-cm high with water at a temperature of 25°C for 15 min. They were then towel dried and returned to their home cages in which they received the first dose of drug given as acute treatment. A second dose was given 5 h before and a third 1 h before the rats were re-exposed to the cylinder of water for 5 min on the following day. This timing and number of drug administrations was reported to be the most effective for detecting potential antidepressant drugs (Borsini and Meli 1988). The amount of time spent by each rat in swimming, struggling, comparing attempts to climb out of the cylinder, and diving, floating or immobility during a 5-min re-exposure were recorded.

The EPM comprised two open and two enclosed arms (40-cm high walls), each 50 cm long and 10 cm wide, with a central area of 10 cm². The whole maze was elevated 50 cm above the ground. The rats were placed in the center of the maze and allowed to explore for 5 min. Assessments were made of the amount of time spent in open arms, and of the number of entries and into any arms. An open arm entry was recorded if four paws were in it. Diazepam was used as a positive control for this test (Pellow et al. 1985).

The open field consisted of a circular arena, 1 m in diameter with walls 40 cm high. Rats were placed singly into its center for 4 min, and assessments were made using a computer program of the amount of time spent in locomotion, rearing with front paws against the wall, and immobility (defined as interruption of any activity). This apparatus and the EPM were cleaned with a detergent and dried after occupancy by each rat. The order in which the rats were assessed in each of the three tests was randomized for the different treatments and performed by an observer that was unaware of the treatment each rat had received.

Measurement of enzyme activity

For measurement of MAO-A and -B activity, groups of eight rats given water, TV3326, or TV3279, acutely or once daily for 14 days, were sacrificed immediately after exposure to the FST and their brains were rapidly removed and frozen at –20°C. After homogenization in 0.3 M sucrose, the activities of MAO-A and -B, respectively, were determined using the radioassay method of Tipton and Youdim (1976) with 14C-serotonin creatinine sulfate (100 µM) and 14C phenylethylamine (10 µM). MAO inhibition by the drugs was calculated from a comparison of the activities of drug-treated and control rats.

For measurement of ChE activity after acute drug administration, groups of five rats were given water, TV3326, or TV3279 acutely and sacrificed 2 h later. Other groups of five rats were given water or one of these drugs once daily for 2 weeks and then sacrificed 2 h after the last administration. The frontal cortex was rapidly removed, weighed, and homogenized in 0.1 M phosphate buffer, pH 8.0, containing 1% Triton (33 mg/ml). Total ChE activity (acetyl and butyryl) was measured in 25-µl aliquots of enzyme homogenates using the method of Ellman et al. (1961). ChE inhibition by the drugs was calculated from a comparison of the enzyme activities of drug-treated rats with those of their respective controls.

Drugs and doses

TV3326 hemitartrate (26 mg/kg), TV3279 mesylate (24.5 mg/kg), and diazepam (2 mg/kg) from ampoules of injectable material (10 mg/2 ml) were obtained from Teva Pharmaceuticals Ltd (Netanya, Israel); amitriptyline HCl (10 mg/kg) was from Sigma Ltd (Mo., USA); and moclobemide HCl (20 mg/kg) was from Hoffman-LaRoche Ltd. (Basle, Switzerland). All drugs and water or saline were administered in a volume of 1 ml/kg. For oral administration by gastric gavage, drugs were dissolved in distilled water. For intraperitoneal administration, diazepam was diluted to the required concentration in 0.9% saline. All doses are expressed as milligrams per kilogram body weight of the respective salt.

To study the effect of acute administration on behavior in the open field and EPM, the drugs were given orally 2 h before the test. Diazepam and saline (eight rats per group) were injected i.p. 15 min before the experiment. In the FST, the same dose of drug was given three times during the 24 h prior to the second exposure. For chronic administration, the drugs were administered once daily via oral gavage for 2 weeks prior to testing. The doses of TV3326 and TV3279 (26 mg/kg and 25 mg/kg, respectively) were chosen because they were able to antagonize the memory im-