FK506 as effective adjunct to L-dopa in reserpine-induced catalepsy in rats

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Received 20 November 2002; revised 7 April 2003

Reserpine-induced catalepsy is a widely accepted animal model of Parkinson’s disease. In the present study reserpine (2.5 mg/kg, ip) 20 hr and alpha-methyl-para-tyrosine (AMPT; 200 mg/kg, ip), one hour before the experiment induced significant catalepsy in rats as assessed by bar test. There was a significant increase in the time spent on the bar in bar test as compared to the control untreated rats. L-dopa (100 mg/kg, ip) and carbidopa (10 mg/kg, ip) combination, a conventional therapy was less effective in reversing reserpine-induced catalepsy. Pretreatment with FK506, a neuroprotectant (0.5-2 mg/kg, po) not only dose dependently reduced the catalepsy in reserpine-treated rats but a lower dose (1 mg/kg) potentiated the motor stimulant actions of sub threshold dose of L-dopa (100 mg/kg, ip) and carbidopa (10 mg/kg, ip) combination. Anticataleptic effect of FK506 was blocked dose dependently by specific D2 receptor blocker sulpiride (25-100 mg/kg, ip). In conclusion, the findings of the present study suggest that FK506 has an indirect modulatory action on the dopamine D2 receptors. FK506 being a neuroprotectant; could be used as an effective adjunct to L-dopa for the treatment of neuroleptic-induced extrapyramidal side effects.

Keywords: Catalepsy, FK506 adjunct, L-dopa, Parkinson’s disease, Reserpine-induced catalepsy

Parkinson’s disease is one of the most common neurodegenerative disorders affecting the majority of the population who are older than 65 years of age. In Parkinsonism, an interplay of multifactorial components is envisaged, besides hereditary, ageing and environmental toxins. The degree of striatal dopamine depletion parallels the severity of the disease and for symptoms of Parkinsonism to appear, there should be at least 80-85% depletion of striatal dopamine. These dopamine-deficiency hypotheses led to the use of L-dopa, a dopamine precursor, the single most effective agent in Parkinson’s disease. In the brain, L-dopa is decarboxylated into dopamine which is responsible for the therapeutic effectiveness of the drug in Parkinson’s disease. In modern practice, L-dopa is always administered with peripheral decarboxylase inhibitor carbidopa or benserazide which prevents peripheral decarboxylation of L-dopa and allows maximum amounts of L-dopa to reach the CNS. This will not only reduce the dose but also the peripheral side effects of L-dopa per se treatment.

A principal limitation of long-term use of L-dopa therapy is that, with time there is a development of “wearing off phenomena” and the patient’s motor state may fluctuate dramatically. Increasing the dose of medication may help but this leads to development of dyskinesias. To avoid such problems, several agents have been tried as an adjunct therapy.

FK506 (tacrolimus), an FDA approved immunosuppressant drug, isolated from Streptomyces tsukubaensis, is used worldwide to prevent allograft rejection with relatively diminished side effects, notably nephrotoxicity. FK506 has shown neuroprotective effect against 6-hydroxydopamine (6-OHDA) toxicity in mice and also reduced the increased level of TNF-alpha level in the nigrostriatal dopaminergic region injured by 6-OHDA treatment in rats.

Recently, it was reported that pretreatment of tacrolimus significantly lowered the dose of quinpirole that produced the maximal effect on locomotor activity. The increase in the locomotor activity produced by a lower dose of quinpirole was significantly potentiated by tacrolimus in a dose-dependent manner. It was suggested that calcineurin may play an important role in modulation of locomotor activity produced by dopamine D2/D3 receptors but not by dopamine D1 receptors.

Against this backdrop, the present study has been designed to study the effect of FK506 as an adjunct to L-dopa against reserpine-induced catalepsy in rats.

Materials and Methods

Animals—Wistar rats of either sex (150-200 g) bred in Central Animal House of Panjab University,
Chandigarh, maintained on a 12:12-hr L:D cycle were used. Animals were housed under standard laboratory conditions, with free access to food and water. All behavioral experiments were carried out between 0900 and 1400 hrs. The experimental protocol was approved by Institutional Animals Ethics Committee.

Assessment of catalepsy — Using the following test procedure, assessment of catalepsy was done in animals.

Bar test — In the bar test, front paws of the rat were gently placed on a horizontal metal bar with 5-6 mm diameter and placed 10 cm above ground level and the length of time the rats maintained in this abnormal posture with at least one paw was measured. The test was terminated when the paw of animal touched the ground or 180 sec had passed. If the animal did not hold on to the bar after three attempts, it received the score of 0 seconds.

Drug treatment and schedule — Reserpine (Searle India) was dissolved with aid of diluted acetic acid and pH was adjusted to neutral and volume made up with distilled water. FK506 (Archer Chemical, Mumbai, India), L-dopa (Hi media, Mumbai, India), carbidopa (Sun Pharmaceuticals, Mumbai, India), were suspended in 0.5% carboxy methyl cellulose solution. Sulpiride hydrochloride (Research Biochemical Inc., Natick, MA) and α-methyl-p-tyrosine (AMPT) HCl (Sigma, St.-Louis, MO, USA) were dissolved in saline.

All drugs were administered in a constant volume of 0.5 ml/100 g body weight of rat. Reserpine was administered 20 hr and AMPT 1 hr before FK506 administration and catalepsy was scored for next 4 hr. FK506 was administered 30 min prior to administration of carbidopa. Carbidopa was administered 15 min prior to L-dopa administration. Sulpiride was administered 10 min before FK506 administration.

Statistical analysis — The fall-off time in rota-rod test, the time spent in bar test was expressed as mean±SE. The data were analyzed using analysis of variance (ANOVA) followed by Dunnett's test by a statistical package STAT. In the test, the criterion for statistical significance was $P<0.05$.

Results

Effect of FK506 on reserpine-induced catalepsy — FK506 dose dependently (0.5–2 mg/kg) reversed catalepsy in reserpine-treated animals, showing a maximum effect at 2 mg/kg dose. The effect was seen even after 4 hr after FK506 treatment (Fig. 1).

![Figure 1](image1.png)

**Fig. 1** — Effect of FK506 (0.5–2 mg/kg po) treatment on reserpine-induced catalepsy in mice. Values expressed as mean± S.E. $P<0.05$ *as compared to reserpine-treated group. (ANOVA followed by Dunnett’s test), $^a$ as compared to vehicle-treated control group. (ANOVA followed by Dunnett’s test).

Effect of L-dopa and carbidopa combination on reserpine-induced catalepsy and its modification by FK506 — L-dopa (100 mg/kg, po) plus carbidopa (10 mg/kg, po) did not offer significant protection against reserpine-induced catalepsy FK506 (1 mg/kg, po) when combined with ineffective dose of L-dopa (100 mg/kg, po) plus carbidopa (10 mg/kg, po). Potentiated the motor stimulating effect of threshold dose of L-dopa as indicated by complete reversal of catalepsy in the bar test (Fig. 2).

![Figure 2](image2.png)

**Fig. 2** — Modification of FK506 reversal of reserpine-induced catalepsy by sulpiride — Sulpiride (50–100 mg/kg, ip)
not only dose dependently and significantly reversed the effect of FK506 on reserpine-induced catalepsy; there was a significant increase in the time spent on the bar in the bar test (Fig. 3).

Modification of FK506 potentiation of L-dopa in reserpine-induced catalepsy by sulpiride — Sulpiride (50-100 mg/kg, ip) dose dependently and significantly reversed the FK 506 motor stimulating effect of threshold dose of L-dopa in reserpine-induced catalepsy; there was a significant increase in the time spent on the bar in the bar test (Fig. 4).

Discussion
Besides phenothiazines, reserpine is another drug well known to produce Parkinson's-like condition in animals. Reserpine acted by depleting presynaptic catecholamines particularly dopamine through the process of degranulation of storage vesicles. Accordingly, when reserpine along with a tyrosine hydroxylase inhibitor, AMPT was administered 20 and 1 hr respectively before the experiment, animals developed severe catalepsy, as there was a considerable decrease in the fall off time in rotarod test and increase in the time spent on the bar in bar test. When an ineffective dose of L-dopa (100 mg/kg, ip) plus carbidopa (10 mg/kg, ip) was administered to reserpinized rats there was a reversal of catatonia but to a little extent, with only significant effect seen after 2 hr of L-dopa administration. L-dopa, when administered along with a peripheral dopa-decarboxylase inhibitor carbidopa, is actively transported across the blood brain barrier where it is decarboxylated and the so formed dopamine acts on D1 and D2 receptors, controlling movement. L-dopa when administered as monotherapy has poor bioavailability and a short half-life. Less than 1% of orally administered L-dopa penetrates into the brain because of rapid peripheral metabolism by the enzymes dopa decarboxylase and catechol-O-methyl transferase (COMT). To improve its bioavailability, L-dopa is formulated with a decarboxylase inhibitor like carbidopa or benserazide. Nevertheless, much of the oral dose of L-dopa is still wasted despite co-administration with carbidopa. L-dopa is associated with a number of problems. L-dopa therapy benefits patients for only 5-7 years of the treatment and the long term use results in of complications such as motor fluctuations ("wearing off" and "on-off" phenomena) and dyskinesias. Therefore, the present study is aimed at finding a suitable adjunct therapy to L-dopa.

FK506 was reported to be a powerful neuroprotective agent in focal ischemia in animals, which showed a wide spectrum of therapeutic potential in various stroke models including permanent, transient, and thrombotic middle cerebral artery (MCA) occlusion models in rats. FK506 has neuroprotective potential in animals. Many in vitro findings of neurite outgrowth potentiation underline this stimulative effect. FK506 is reported to protect dopaminergic and...
GABAergic neurons followed by cerebral ischemia. FK506 also reduces the microglial activation in the substantia nigra. So far the exact mechanism of neuroprotection offered by this compound is not well known, although various putative mechanisms have been speculated. Calcineurin inhibition may still be a valid target for FK506-mediated neuroprotection in the light of more recent data implicating the involvement of calcineurin in the apoptotic cascade by its interaction with Bcl-2 family proteins. FK506 prevents apoptosis by inhibiting BAD (Bcl-2 family) dephosphorylation and translocation or via Bcl-2-sensitive pathways, supports this hypothesis.

Recently it has been reported that pretreatment of tacrolimus (2 mg/kg) significantly lowered the dose of quinpirole that produced the maximal effect on the locomotor activity. The increase in the locomotor activity by 0.5 mg/kg of quinpirole was significantly potentiated by 0.5, 1, 2 or 5 mg/kg of tacrolimus as compared to the vehicle-treated group. The dose related increase in the locomotor activity produced by administration of SKF 82958, (a dopamine D1 receptor agonist) was not significantly altered by the administration of FK506 at any dose. Thus they suggested that calcineurin may play a role in the alteration of locomotor activity produced by dopamine D2/D3 receptors but not dopamine D1 receptors.

In the present study, FK506 not only reversed the reserpine-induced catalepsy but also potentiated the ineffective dose of L-dopa in both reserpine-induced catalepsy in animals. The protective action of FK506 and its potentiating actions of lower threshold dose of L-dopa were blocked by sulpiride, a specific dopamine D2 receptor blocker in reserpine-induced catalepsy. These results suggest that the modulation of dopamine D2 may be responsible for anti cataleptic and L-dopa potentiating actions of FK506 observed.

Dose employed in the present study (1 mg/kg) has been reported to be insufficient to prevent rejection of neuronal transplants in the rat and hence lacks immunosuppressive effects. Even a lower dose, which is 1% of the immunosuppressive dose of FK506, has shown neuroprotection in animal models of neurodegeneration.

FK506 has been reported to be neuroprotectant in lesioning models of Parkinson’s disease. FK506 has some serious side effects. Thus, a lower non-immunosuppressive dose, with minimal side effects could be combined with L-dopa in drug therapy of Parkinson’s disease.

Acknowledgement
The Senior Research Fellowships to A. Singh (Himalaya Drug Co.) and P.S. Naidu (CSIR, New Delhi) are gratefully acknowledged.

References
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