Iranian Journal of Pharmaceutical Research (2003) 167-171
Received: May 2003
Accepted: August 2003

Original Article

The Effect of Oral Administration of L-Tyrosine, Folic acid and Pyridoxine on Perphenazine Induced Catatonia in Rat

Ardeshir Arzi*, Mohsen Rezaei

Department of Pharmacology, School of Pharmacy, Ahvaz University of Medical Sciences, Ahvaz, Iran.

Abstract

L-tyrosine, B6 and folic acid are involved in biosynthesis of DOPA and consequently dopamine. The aim of this study was to investigate the antiparkinsonian effect of these agents in perphenazine-induced catatonia in rats. Murprogo method or scored muscular rigidity, which is induced by a phenothiazine, was used to evaluate the antiparkinsonian effect of these agents. A significant decrease in muscular rigidity was observed in groups that received L-tyrosine. However, groups which had received vitamin(s) only showed no significant decrease in muscular rigidity as compared with the control group. On the other hand, the group receiving folic acid plus L-tyrosine showed a lower degree of muscular rigidity in comparison with the other groups.

In conclusion, L-tyrosine has been found to be effective in improving perphenazine-induced muscular rigidity. Furthermore, when used in combination with folic acid, L-tyrosine could be found advantageous in the early stages of Parkinson's disease.

Keywords: L-tyrosine; Folic acid; Pyridoxine; Parkinson's disease; Rat.

Introduction

Parkinson's disease (PD) is a progressive disorder of movement that occurs mainly in the elderly. The chief symptoms of this disease are tremor at rest, usually starting in the hand, which tends to diminish during voluntary activity, muscle rigidity, and suppression of voluntary movements (hypokinesia) due partly to muscle rigidity and partly to an inherent inertia of the motor system, which means that motor activity is difficult to stop as well as to initiate (1).

A variety of medications and medical strategies have been proposed as means of slowing the progression of PD. Effective pharmacotherapy generally improves movement disturbances. Never-the-less, other problems could arise as side effects (2).

Levodopa therapy can have a dramatic effect on all of the signs and symptoms of PD, but principal limitations of the use of Levodopa therapy are its unwanted effects such as diskinesia, rapid fluctuations (on/off effect) and eventually reduction of its efficiency (3, 4).

Thus, investigations for finding new and more specific dopamine agonists are in progress and on the other hand, and regarding the advantages of Levodopa therapy, new but less harmful strategies in Levodopa therapy must be established. Hence, in the present study the use of L-tyrosine (LTN), a precursor of dopamine, in combination with folic acid (FA) and /or vitamin B6, which are involved in dopamine (DA) biosynthesis, has been investigated.

DA is a catecholamine which is produced in dopaminergic terminals from LTN. It is transported across the blood-brain barrier by an active process. The rate-limiting step in biosynthesis of DA is the conversion of LTN to L-DOPA (L-dihydroxy phenylalanine), catalyzed by tyrosin hydroxylase which is present within catecholaminergic neurones. In the next step, aromatic L-aminoacid

* Corresponding author:
E-mail: ardeshirarzi@yahoo.com

decarboxylase converts L-DOPA to DA. Tetrahydrobiopterin (BH4) and B6 (pyridoxal) are the coenzymes of these synthesis pathways, respectively (3, 4, 5).

Experimental

Catatonia and muscular rigidity which are induced by a phenothiazine (perphenazine 5 mg/kg) somehow can mimic parkinsonian symptoms and therefore standardization of the severity of rigidity can be useful in evaluation of antiparkinsonism effects of new strategies, as described by Murprogo (6, 7). This method was used for the determination of LTN dose-response and time-response characteristics. Eventually, a dose of 1500 mg/kg of LTN (oral) was found to be optimal, after 2 weeks of treatment. This dose of LTN was then used with or without FA and/or B6, and the antiparkinsonism effect of each treatment in rats was determined as follows: 7 h after administration of the last oral dose of LTN in each group of animals, perphenazine (5 mg/kg) was administered intraperitoneally and the relative muscular rigidity was determined 20, 40, 60, 90, 120, 180 and 240 min after the injection. Three steps were used for grading muscular rigidity:

(1) The rat was placed on the table. If it remained motionless and hand-touching made the animal to move, grade 0.5 was given.
(2) Right front paw of the rat was placed on platform with an altitude of 3 cm. A grade of O.5 was awarded if the animal remained in this position for l0 sec. This step was also repeated on the left front paw. Thus, 1 grade was awarded at the most.
(3) The right front paw of the rat was placed on a platform with an altitudo of 9 cm. If the animal remained in this position for period of 10 s, 1 grade was awarded. In addition to left front paw testing, 2 grades could be given in this stage.

The effects of treatment were statistically analyzed using Kruskal-Wallis nonparametric test and Wilcoxon matched-pairs test.

Results and Discussion

The means and standard errors of the scoring for all groups under investigation are summarized in table 1.

The comparison of muscular rigidity between groups receiving 500, 1000, 1500 and 2000 mg/kg LTN, revealed a dose dependent improvement in rigidity. However, a dose of 2000 mg/kg in all the studies carried out was found to be significantly different ($p<0.05$) from doses of 500 and 1000 mg/kg, but not with a dose of 1500 mg/kg in any specified times (Figure 1).

The FA treated group (250 µg/kg) in comparison with the control group (treated with distilled water in the same period), showed no significant difference in any time period, except 20 min after perphenazine injection (Table 1).

The B6 treated group (2.5 mg/kg), also showed no significant difference, compared with the control group, except at 20, 60, 180 and 240 min time intervals ($p<0.05$)(Figure 2).

With the LTN treated group (1500 mg/kg), a significant difference ($p<0.05$) was noted in all the specified time intervals, compared to the control group, except for 20 and 40 min time periods (Figure 3).

The group which received FA (250 µg/kg) and B6 (2.5 mg/kg) was not significantly different in any time intervals, compared with the control group (Table 1).

FA (250 µg/kg) plus LTN (1500 mg/kg) treated group had a lower muscular rigidity,

Table 1. Perphenazine-induced muscle rigidity at various intervals in different groups (Mean ± SEM). Perphenazine (Per) 5 mg/kg, Distilled Water (DW), Folic Acid(FA) 250 µg/kg, Pyridoxine(B6) 2.5 mg/kg, L-tyrosine(LTN) 500-2000mg/kg.

Groups \ Time(min)	20	40	60	90	120	180	240
DW+Per	0.14±.0.09	0.93±0.17	2.64± 0.44	2.78±0.36	3.07±0.20	3.5 ±0	3.5 ±0
FA+Per	0.67±0.21	1.42±0.55	2.25±0.47	2.25±0.47	2.5 ±0.36	2.67±0.40	3.25±0.25
B6+Per	0.60±0.24	0.80±0.20	1.2 ±0.37	1.7 ±0.49	2.2 ±0.50	2.7 ±0.37	2.7 ±0.37
LTN(500)+Per	0.80±0.58	1 ±0.58	2.5 ±0.29	3.5 ±0	3.5 ±0	3.5 ±0	3.5 ±0
LTN(1000)+Per	0.70±0.69	1 ±0.69	2.12±0.55	2.5 ±0.58	3 ±0.29	3.5 ±0	3.5 ±0
LTN(1500)+Per	0.67±0.38	1 ±0.48	1.1 ±0.45	1.2 ±0.40	1.25±0.40	1.75±0.50	1.80±0.50
LTN(2000)+Per	0.50±0.30	0.80±0.30	0.90±0.20	1.1 ±0.40	1.5 ±0.27	2 ±0.30	2.1 ±0.20
FA+B6+Per	0.25±0.11	1.25±0.30	2.67±0.47	2.92±0.40	3.25±0.25	3.25±0.25	3.5 ±0
FA+LTN(1500)+Per	0.10±0.10	0.30±0.12	0.40±0.10	0.70±0.20	1 ±0.20	1.40±0.45	1.80±0.30
B6+LTN(1500)+Per	0.10±0.10	0.20±0.12	0.90±0.48	1.5 ±0.60	1.6 ±0.60	2 ±0.60	2.1 ±0.60
FA+B6+LTN(1500)+Per	0±0	0.33±0.24	0.67±0.36	1.33±0.60	1.67±0.60	2.17±0.50	2.17±0.50

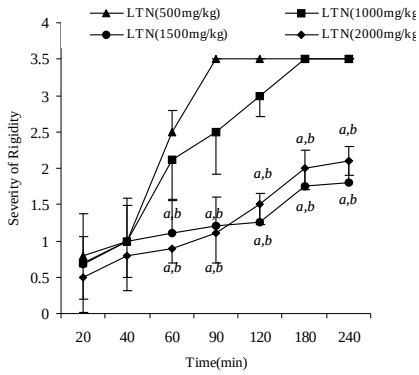

Figure 1. Comparison of Perphenazine-induced muscular rigidity in L-tyrosine received groups. (*a*) Significantly different (P<0.05) from LTN (500mg/kg) group. (*b*) Significantly different (P<0.05) from LTN (1000mg/kg) group.

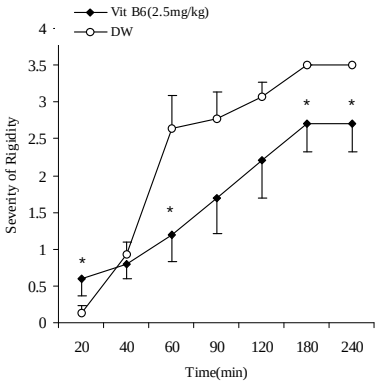

Figure 2. Comparison of Perphenazine-induced muscular rigidity between B6 treated (2.5mg/kg) and control group. (*) Significant difference(P<0.05).

which was significantly different from the control group except after a period of 20 min (p<0.05) (Figure 4). There was also no significant difference between LTN treated group (part D) and LTN plus FA treated group (Table 1).

The B6 (2.5 mg/kg) plus LTN (1500 mg/kg) treated group, except after 20 min, was found to be significantly different from the control group (p<0.05) (Figure 5). There was no significant difference between the LTN treated group and the B6 plus LTN treated group (Table 1). On the other hand, no difference was also seen between the FA plus LTN treated group and the B6 plus LTN treated group (Table 1).

The FA, B6 and LTN treated groups had lower muscle rigidities (p<0.05) in comparison with the control group, except after a period of 20 min (Figure 6). This comparison was significant only at 20 min time interval, between the LTN treated group and the FA, B6 and LTN treated groups (Table 1). On the other hand, the FA plus LTN and the B6 plus LTN treated groups had no significant decreasing effect on muscular rigidity, in comparison with the groups receiving FA, B6 and LTN (p<0.05) (Table 1).

Onset of Levodopa therapy may be accompanied with suffering from unwanted effects such as nausea, vomiting and anorexia. The metabolite of Levodopa, dopamine, is known as the main responsible factor for causing side effects (1, 3). It seems that the influence of LTN on the onset of therapy could be more advantageous, compared with the use of Levodopa. This is because LTN is the rate limiting step in the biosynthesis of DA and a gradual occurrence of DA, could help to reduce suffering from adverse reactions. However, following the endurance occurring, Levodopa therapy could be started. In this study weight loss, which could be due to anorexia, was not seen in animals (data not shown). This finding is in agreement with the notion mentioned above.

LTN is produced by phenylalanine

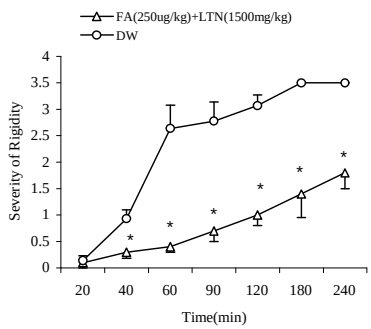

Figure 3. Comparison of Perphenazine-induced muscular rigidity between L-tyrosine treated(1500mg/kg) and control group.
(*) Significant difference (P<0.05).

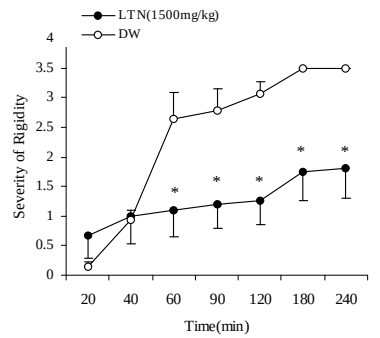

Figure 4. Comparison of Perphenazine-induced muscular rigidity between Folic acid (250µg/kg) + L-tyrosine (1500mg/kg) and control group.
(*) Significant difference (P<0.05).

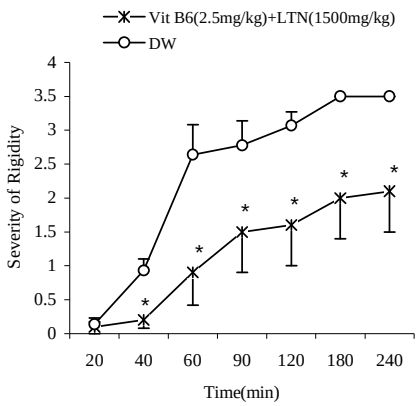

Figure 5. Comparison of Perphenazine-induced muscular rigidity between B6 (2.5mg/kg) + L-tyrosine (1500mg/kg) treated and control group. (*) Significant difference (P<0.05).

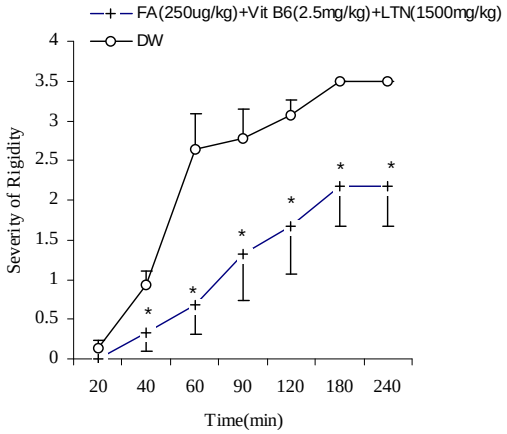

Figure 6. Comparison of Perphenazine-induced muscular rigidity between Folic acid (250µg/kg) + B6 (2.5mg/kg) + L-tyrosine (1500 mg/kg) treated and control group. (*) Significant difference (P<0.05).

hydroxylation. Thus, it is nutritionally non-essential (3). Phenylalanine is found in abundance in some food stuff such as sesame. Therefore, it could be useful in the early stages of treatment (899).

B6 and BH4, which are produced in the body by enzymatic pathways in which FA has a coenzymatic role, are involved in the biosynthesis of DA as stated previously (10). Therefore, in the present study, the effect of coenzymes accompanying LTN was investigated on perphenazine-induced rigidity in rat. According to the data obtained, groups which received vitamins alone (FA and /or B6) had no significant decrease in muscular rigidity, except for time intervals mainly belonging to B6 group. However, the coenzymatic role of B6 in DA production may partly be the reason for this decrease in muscular rigidity. On the other hand, cooperation of the two coenzymes, B6 and FA, may lead to excess depletion of DA precursors and their inability to decrease muscular rigidity. The groups receiving vitamins (separately or together) showed no significant decrease in muscle rigidity among themselves.

All four groups having LTN in their received regimen (LTN, LTN plus FA, LTN plus B6 and LTN plus FA plus B6) at all time intervals, except for 20 min, had muscular rigidities lower than the control group ($p<0.05$). This finding indicates the desirable effect of LTN**.** Furthermore the four groups studied, when compared to each other, indicated no significant decrease in muscle rigidity and therefore no significant effect was noted with respect to the addition of vitamins ($p<0.05$). However, the state of muscle rigidity in these four groups was better in the LTN plus FA treated group, and the group that received all three agents together had a higher degree of rigidity, compared to the other three groups. This may be a result of excess peripheral metabolism of LTN in the presence of both coenzymes.

In our study, FA has been more efficient than B6. FA has some role in GTP production and eventually BH4 production and BH4 has a coenzymatic role in DA biosynthesis. In fact the superior effect of LTN plus FA treated group in improving muscular rigidity could be explained by the fact that hydroxylation of LTN to L-DOPA is the rate-limiting step in DA production.

In conclusion, LTN has been found to be effective in preventing muscle rigidity which is induced by perphenazine, and the group which received FA plus LTN appeared to have a lower degree of muscular rigidity in comparison with the other groups.

References

(1) Hirch EC. Mechanism and consequences of nerve cell death in parkinson's disease. *J. Neural. Trans.* (1999) 56: 127-137

(2) Ahlskog JE. Treatment of early Parkinson's disease: are complicated strategies justified? *Mayo Clin. Proc.* (1996) 71: 659-670

(3) Standaert DG and Young AB. Treatment of central nervous system degenerative disorders. In: Hardman

JG and Limberd LE. (Eds) *Goodman ond Gilman's, The Pharmacological Basis of Therapeutics*. 10th ed. McGraw & Hill, NewYork (2001) 549-568

(4) Maxwell SRG. Prospects for use of antioxidant therapies. *Drugs* (1998) 49: 345-361

(5) Kolb B and Whishaw IQ. *An Introduction to Brain and Behavior*. 1st ed. Worth Publishers, New York (2001)

(6) Morpurgo C. Effect of antiparkinson drugs on a phenothiazine induced catatonia reaction. *Arch. Int. pharmacodyn*. (1962) 137: 84-90

(7) Bala S, Chawla N and Garg KN. Drug induced catalepsy as influenced by Apomorphine, Methamphetamine, Atropine, Imipramine, Cyproheptadine and Promethazine. *Ind. J. Pharm.* (1978) 10: 271-275

(8) Bernstein JG. *Handbook of Drug Therapy in Psychiatry*. 3rd ed. Mosby, St Louis (1995) 19: 320-321

(9) During MJ, Acworth IN and Wurtman RJ. Dopamine release in rat striatum; physiological coupling to tyrosine. *Supply J. Neurochem.* (1989) 52: 1449-1454

(10) Braunwald E, Hauser SL, Fouci AS at el. *Harrison's Principles of Internal Medicine*. 15th ed. Vol. 2, McGraw-Hill, New York (2001) 2305