RESEARCH PAPER

Accepted 21 February 2004
Revised 3 December 2003
Received 8 August 2002
Indian J. Pharm. Sci., 2004, 66(2): 212-218

Effect of Combination of Amantadine and Clomipramine on Various CNS Activities

A. G. JAGTAP* AND SONAL V. SHAH
Department of Pharmacology Bombay College of Pharmacy,
Kalina, Santacruz (East), Mumbai-400098.

Our aim is to study various CNS activities of clomipramine and amantadine. The antidepressant activity of clomipramine and amantadine and also the synergistic antidepressant effect of a combination of amantadine and clomipramine was studied using forced swim test and tail suspension test. Tolerance studies were carried out using the forced swim test model in mice. Cognition studies with clomipramine, amantadine and combination of both drugs were carried out by using Morris water maze test. Antidepressant inhibitory effect of verapamil on clomipramine was assessed by forced swim test model. It was decided to check whether amantadine could inhibit the antidepressant inhibitory effect of verapamil on clomipramine.

Depression is now the most common mental illness diagnosed by psychiatrists. According to the survey conducted by National Institute of Mental Illness, depression ranks number 10 in diseases that cause death[1]. Depression is a mood disorder caused by a functional deficit of monoamine neurotransmitters at certain sites in brain. Antidepressant drugs increase the level of these neurotransmitters in brain[2].

It has been known that currently used antidepressants show therapeutic efficacy in 60-70% patients. Tolerance is the most common response to repetitive use of the same drug and can be defined as the reduction in response to the drug after repeated administrations. Medical literature documented case reports of possible tolerance development to the therapeutic effects of tricyclic antidepressants after prolonged use[3]. The problem of therapy resistant depressive patients has been studied for a long time but with no significant success. Generally drug-tolerant depression was treated with combinations of various antidepressant drugs[4].

Amantadine is an adamantane derivative. It basically activates the dopaminergic system in brain to cause increased release of dopamine. It is also reported to be a non-competitive NMDA receptor antagonist[5]. Certain NMDA antagonists are successfully tried as antidepressants[6]. Reports have suggested that dopamine and glutamate may also be involved in pathophysiology of depression[7,8]. This formed the basis of selecting amantadine for use as an antidepressant.

Clomipramine is a tricyclic antidepressant with antiobsessional properties. It is a selective serotonin reuptake inhibitor. Clomipramine is used in the treatment of depression[9]. Clomipramine is also reported to inhibit mesolimbic and striatal dopamine reuptake in central nerve terminals[10]. Hence a dopaminergic mechanism in its antidepressant action has been suggested. Amantadine also activates dopaminergic system in brain. Thus it was decided to try out synergistic antidepressant activity of clomipramine and amantadine.

Since tolerance to tricyclic antidepressant treatment is reported, it was decided to check for development of tolerance to clomipramine treatment and if developed whether it can be overcome by amantadine treatment. Drug resistant depression is generally treated by using combination of antidepressant drugs. When two drugs with different mechanism of action are combined, more patients may respond and the chance of tolerance development could

*For correspondence
E-mail: sonnaline@yahoo.com

be reduced[4]. This formed the basis for selecting amantadine to overcome tolerance developed to clomipramine.

LTP (long term potentiation) is a long-lasting (hours *in vitro*, days or weeks *in vivo*) enhancement of synaptic transmission induced by brief high frequency tetanus. LTP of excitatory synaptic transmission is a candidate for the neural mechanisms underlying learning and memory formation. NMDA receptor activated by glutamate is an essential element for the induction of LTP. NMDA antagonists have been reported to completely block this process and hence lead to memory impairment[11]. Clomipramine and amantadine both have been reported to be NMDA receptor antagonist[5,12]. Hence it was proposed to evaluate their effect on learning and memory formation on prolonged treatment with amantadine and clomipramine.

Recent reports have indicated that a calcium channel blocker, verapamil facilitates depression. Verapamil blocks the antidepressant effect of clomipramine[13]. Hence it was decided to assess whether amantadine is able to unblock the effect of verapamil on clomipramine by using forced swim test in mice model.

MATERIALS AND METHODS

Swiss mice of either sex weighing between 20-30 g were used in the study. Mice were fed with commercially available Gold Mohur Brand feed manufactured by M/S Lipton Ltd. Mumbai. Water supplied by the Municipal Corporation of Greater Mumbai was provided to all animals *ad libitum*. Clomipramine, amantadine and imipramine were obtained from Sun Pharmaceuticals, Cipla Ltd., Mumbai and Torrent Pharmaceuticals, Ahmedabad, respectively. Clomipramine, amantadine, imipramine and verapamil were dissolved in distilled water. The drug solutions of required concentration were used in the studies. All animal experimental protocols have been approved by the Institutional Animal Ethics Committee.

Synergistic antidepressant activity of amantadine and clomipramine:

Forced swim test model[14]:

Swiss mice of either sex weighing between 20-25 g were randomly divided into 15 groups of 5 animals each. The study was carried out using single and multiple doses. Single dose study was carried out to find the active and the inactive dose of clomipramine and amantadine and also both the active and inactive doses of amantadine and clomipramine were used in combination to assess for synergistic antidepressant effect. Active dose was the one, which decreased the immobility time and showed antidepressant activity. Inactive dose increased the immobility time and was unable to show antidepressant activity. The active dose was amantadine 20 mg/kg and clomipramine 40 mg/kg and the inactive dose was amantadine 10 mg/kg and clomipramine 20 mg/kg. Several reports have suggested that injections 1, 5, and 24 h prior the test gave the reproducible results in reducing immobility time and hence these multiple dose treatment was chosen[14,15]. The treatment group consisted of single dose and multiple doses. The single dose group received the drug 30 min prior to the test and the multiple dose group received the drug 24, 5, and 1 h prior to the test.

For single dose studies the control group received vehicle. Two drug treated groups received amantadine 20 mg/kg i.p. and clomipramine 40 mg/kg i.p., respectively. The third drug treated group received a combination of amantadine 20 mg/kg i.p. and clomipramine 40 mg/kg i.p. simultaneously. The remaining groups received amantadine 10 mg/kg i.p., clomipramine 20 mg/kg i.p. and a combination of amantadine 10 mg/kg i.p. and clomipramine 20 mg/kg i.p. simultaneously. Standard group received imipramine 5 mg/kg i.p.

For mutiple dose studies[14,15] the control group received vehicle. Two drug treated groups received amantadine 20 mg/kg i.p. and clomipramine 40 mg/kg i.p., respectively. The other drug treated group received a combination of amantadine 20 mg/kg i.p. and clomipramine 40 mg/kg i.p. simultaneously. The remaining groups received amantadine 10 mg/kg i.p., clomipramine 20 mg/kg i.p. and a combination of amantadine 10 mg/kg i.p. and clomipramine 20 mg/kg i.p., simultaneously. Standard group received imipramine 5 mg/kg i.p.

All animals used in the study were acclimatized to the test conditions by subjecting them to a trial swim period of 15 min in the experimental model 24 h prior to the test. During the test, mice were individually placed and forced to swim in a plastic container (25 cm height x15 cm breadthx25 cm length). The diameter of wheel was 15 cm. The height of the water was always kept constant at 15 cm of water. The animals first showed a bout of vigorous activity followed by immobility. A mouse was considered immobile when it floated in the water with an upright position and made only small movements to keep its head above water. The total duration of immobility was measured during a 6 min test[14].

Tail suspension test:

The study was carried out using the same single and multiple doses as explained previously in the forced swim test. The tail suspension is found to be an easy method to test potential antidepressant compounds. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair, which in turn may reflect depressive disorders in humans. Hence this model was chosen.During the test the mice were suspended on the edge of shelf 58 cm above a tabletop by adhesive tape placed approximately 1 cm from the tip of tail. Duration of immobility was recorded for a period of 5 min. Mice are considered immobile when they hang passively and completely motionless[15].

Development of tolerance to clomipramine in mice by using forced swim test model:

Swiss mice (n=5) of either sex in the weight range of 20-25 g were taken in each group. Forced swim model was used for this study. The drug treated group received clomipramine 40 mg/kg/d i.p. till the day tolerance developed. An increase in immobility time indicates development of tolerance. Once the tolerance was developed, next day clomipramine 40 mg/kg i.p. was given in combination with amantadine 40 mg/kg i.p. All the drugs were administered half an hour prior to the test. Control group received vehicle for the same number of days.

All animals used in the study were accustomed to the test conditions by subjecting them to a trial swim period of 15 min in the experimental model 24 h prior to the test. The total duration of immobility was measured during a 6 min test as explained earlier[14].

Each day 30 min after the drug administration immobility time was recorded. Antidepressant drugs decrease the immobility time in forced swim test model. Over a period of time if tolerance starts developing to the antidepressant drug, the immobility time then recorded will start increasing. This increase in immobility time is to be observed in order to confirm that tolerance is developed.

Effect of amantadine, clomipramine and combination of clomipramine and amantadine treatment on memory in Morris water maze test[15]:

Swiss albino mice (n=5) of either sex in the weight range of 20-25 g were taken in each group. The drug treated groups received clomipramine 40 mg/kg i.p. for 7 d, amantadine 20 mg/kg i.p. for 7 d and combination of clomipramine 40 mg/kg i.p. and amantadine 20 mg/kg i.p.for 7 d simultaneously. Control group received vehicle for same number of days. All the above compounds were administered 30 min prior to the test. Clinically antidepressant drugs are taken for a prolonged period and hence to check the effect of these drugs on memory 7 d treatment was chosen.

In this test mice were placed in a pool filled with milky water. Submerged just below the surface of water in one location was a small platform that allowed the mice to escape. Morris water maze was a circular tank of diameter 49 cm and the platform was located at a height of 11cm. A naive mouse when placed in the milky water will swim around until it bumps into the hidden platform, and then it will climb onto it[16]. Initially the mice were trained to find the platform and then test compounds were administered daily, 30 min prior to the test for 7 d and time required to find the platform was recorded. Mice with memory impairment took more time to find the platform.

Antidepressant inhibiting effect of verapamil on clomipramine:

Swiss mice (n=5) of either sex in the weight range of 20-25 g were taken in each group. The drug treated groups received clomipramine 40 mg/kg i.p., amantadine 20 mg/kg i.p. and verapamil 40 mg/kg i.p. The other group received verapamil 40 mg/kg i.p. and then 15 min later clomipramine 40 mg/kg i.p. was administered. Another group received verapamil 40 mg/kg i.p. and then 15 min later clomipramine 40 mg/kg i.p. and amantadine 20 mg/kg i.p. Clomipramine and amantadine were jointly administered. All the drugs were administered 30 min prior to test.

Antidepressant activity was observed by using forced swim test model as explained earlier[14]. Antidepressants decrease the immobility time in this test. The observation to be made is that if verapamil tends to block the antidepressant effect of clomipramine then it would increase the immobility time and if amantadine is able to reverse this effect then it would decrease the immobility time.

Data analysis:

The data observed from forced swim test and tail suspension test were analyzed using students 't' test. The immobility time of single drug treated animals was compared with control. Combination group was compared with clomipramine/amantadine treated group. In the Morris water maze test, the data were analyzed using students 't' test by comparison of drug treated group with control. Combination

group was compared with clomipramine treated group. In the other studies the data were analyzed using students 't' test by comparison of the drug treated group with control.

RESULTS AND DISCUSSION

Synergistic antidepressant effect of amantadine and clomipramine were evaluated using forced swim test model and tail suspension model. Similar results were observed in both the tests. Amantadine showed significant antidepressant effect at the dose of 20 mg/kg and clomipramine showed significant antidepressant effect at 40 mg/kg dose. Combined administration of amantadine 20 mg/kg and clomipramine 40 mg/kg reduced the immobility time more significantly as compared to treatment with amantadine and clomipramine alone (Table1, 2 and Table 3, 4).The synergistic antidepressant effect was also observed when amanatdine 10 mg/kg and clomipramine 20 mg/kg were used. The doses were found to be inactive when administered alone.

Depression is caused by a functional deficit of monoamine neurotransmitter in certain sites in brain. Although there is no single neurotransmitter theory of depression, the hypothesis maintains both serotonergic and noradrenergic systems need to be functional for an antidepressant effect to be exerted[2]. Reports have indicated that mesolimbic/mesocortical systems are engaged in regulation of pathology of depression. These areas receive dopaminergic innervations[17]. Clomipramine apart from being a selective serotonin reuptake inhibitor is reported to inhibit dopamine reuptake in mesolimbic neurons[10]. Dopaminergic systems have been shown to be involved in antidepressant effects of tricyclic antidepressants[18].

Amantadine a dopamine-releasing agent, is also reported to be a non-competitive NMDA receptor antagonist. It showed significant antidepressant activity in our studies. It has been suggested that amantadine as a non-competitive NMDA receptor antagonist indirectly activates the dopaminergic system by blockade of glutamatergic system (which leads to disinhibition of dopaminergic systems)[14].

Amantadine showed significant antidepressant effect. The combined treatment with clomipramine and amantadine greatly reduced the immobility time in comparison with the administration of clomipramine/amantadine alone. That effect was particularly strong in the group treated with amantadine 20 mg/kg and clomipramine 40 mg/kg. Even when inactive doses of amantadine and clomipramine were combined, a synergistic antidepressant effect was observed and this may

TABLE 1: EFFECT OF DRUG TREATMENT ON IMMOBILITY TIME IN THE FORCED SWIM TEST IN MICE

Treatment	Dose mg/kg i.p.	Mean Immobility time (s)
Control	Vehicle	123±1.9
Imipramine	5	82±4.8*
Clomipramine	40	80±3.2*
Amantadine	20	84±1.5*
Clomipramine + Amantidine	40 + 20	68±8.8#
Clomipramine	20	120±2.7
Amantadine	10	115±4.8
Clomipramine + Amantadine	20 + 10	99±2.8*#

Each treatment was given 30 min prior to test. Each value is represented as mean±s.e.m. (n=5) of immobility time using forced swim test model. *P<0.05 is considered to be statistically significant as compared to control by students 't' test. #P<0.05 is considered to be statistically significant as compared to amantadine/clomipramine by students 't' test.

TABLE 2: EFFECT OF DRUG TREATMENT ON IMMOBILITY TIME IN THE FORCED SWIM TEST IN MICE

Treatment	Dose mg/kg i.p.	Mean Immobility time (s)
Control	Vehicle	120±2.6
Clomipramine	40	74±6.1*
Amantadine	20	78±6.4*
Clomipramine + Amantidine	40 + 20	60±4.2#
Clomipramine	20	105±5.4*
Amantadine	10	104±6.6*
Clomipramine + Amantadine	20 + 10	77±2.7*#

Treatment was given 24, 5 and 1 h prior to test. Each value is represented as mean±s.e.m. (n=5) of immobility time using forced swim test model. *P<0.05 is considered to be statistically significant as compared to control by students 't' test. #P<0.05 is considered to be statistically significant as compared to amantadine/clomipramine by students 't' test.

TABLE 3: EFFECT OF DRUG TREATMENT ON IMMOBILITY TIME IN THE TAIL SUSPENSION TEST IN MICE

Treatment	Dose mg/kg i.p.	Mean Immobility time (s)
Control	Vehicle	114±3.2
Imipramine	5	69±2.9*
Clomipramine	40	71±2.3*
Amantadine	20	68±3.5*
Clomipramine + Amantidine	40 + 20	60±2.0*
Clomipramine	20	107±4.6
Amantadine	10	110±2.6
Clomipramine + Amantadine	20 + 10	77±2.8#

Treatment was given 30 min prior to test. Each value is represented as mean±s.e.m. (n=5) of immobility time using tail suspension model. *P<0.05 is considered to be statistically significant as compared to control by students 't' test. #P<0.05 is considered to be statistically significant as compared to amantadine/clomipramine by students 't' test.

be helpful in optimizing the therapy by reducing the side effects.

The above results support the hypothesis that joint administration of amantadine a non-competitive NMDA receptor antagonist, which indirectly activates the dopaminergic system by blockade of glutamatergic system and leads to disinhibition of dopaminergic systems[14] and clomipramine may evoke a more effective antidepressant activity than treatment with typical antidepressants alone. Amantadine and clomipramine act as antidepressant via dopaminergic mechanisms. This could explain the synergistic antidepressant activity obtained. The combination of amantadine and clomipramine might hold promising value in treatment of depression.

Tolerance studies were carried out using forced swim test model. It was observed that tolerance to clomipramine treatment appeared to develop on d 6. An increase in period of immobility or the immobility time observed is similar to control animals indicates development of tolerance. On d 6 of clomipramine treatment the immobility time recorded was 120±8.3 s while on the day of clomipramine treatment it was

TABLE 4: EFFECT OF DRUG TREATMENT (24,5 AND 1 H PRIOR TO TEST) ON IMMOBILITY TIME IN THE TAIL SUSPENSION TEST IN MICE

Treatment	Dose mg/kg i.p.	Mean Immobility time (s)
Control	Vehicle	116±1.9
Clomipramine	40	64±2.9*
Amantadine	20	61±1.4*
Clomipramine + Amantidine	40 + 20	49±5.2#
Clomipramine	20	93±2.9*
Amantadine	10	93±2.5*
Clomipramine + Amantadine	20 + 10	70±2.9#

Treatment was given 24, 5 and 1 h prior to test. Each value is represented as mean±s.e.m. (n=5) of immobility time using tail suspension model. *P<0.05 is considered to be statistically significant as compared to control by students 't' test. #P<0.05 is considered to be statistically significant as compared to amantadine/clomipramine by students 't' test.

TABLE 5: EFFECT OF DRUG TREATMENT ON IMMOBILITY TIME (S) IN THE FORCED SWIM TEST IN MICE

Day	Mean Immobility time (s)	
	Control	Drug
1	129±5.7	77±6.8*
2	128±6.0	65±6.0*
3	120±3.1	68±8.2*
4	131±4.8	92±9.7*
5	124±7.3	96±10.3
6	121±7.2	120±8.3
7	121±6.1	80±8.1*

Drug treatment: clomipramine 40 mg/kg i.p. and on d 7 combination of clomipramine 40 mg/kg i.p. and amantadine 20 mg/kg i.p. Each value is represented as immobility time mean±s.e.m. (n=5) of immobility time using forced swim test model. *P<0.05 is considered to be statistically significant as compared to control by students 't' test.

TABLE 6: EFFECT OF DRUG TREATMENT ON ESCAPE TIME IN MORRIS WATER MAZE TEST

Day	Escape time (s)			
	Control (Vehicle)	Clomipramine 40 mg/kg i.p.	Amantadine 20 mg/kg i.p.	Clomipramine 40 mg/kg i.p. + Amantadine 20 mg/kg i.p.
1	5.4±0.81	10.0±0.31*	5.0±1.12	14.6±0.92
2	4.6±0.51	14.0±1.41*	3.2±0.19*	8.0±1.09#
3	5.0±0.44	11.2±1.24*	3.2±0.48*	7.0±1.13#
4	4.2±0.58	13.0±1.43*	3.4±0.67	4.6±0.39#
5	5.4±0.49	7.2±0.96	4.0±0.31*	4.6±0.51#
6	4.6±1.12	8.8±1.35*	3.6±0.24	4.0±0.19#
7	5.4±0.39	9.4±1.32*	3.4±0.58*	4.0±0.19#

Each value is represented as mean±s.e.m. (n=5) of escape time in morris water maze. *P<0.05 is considered to be statistically significant as compared to control of respective day by students 't' test. #P<0.05 is considered to be statistically significant as compared to clomipramine treated group by students 't' test.

77±6.8 s (Table 5). The immobility time on d 7 of clomipramine treatment was same as day 6, so a 6 day study with clomipramine was planned. On the d 7 amantadine was administered to mice along with clomipramine. The interesting fact observed was that the immobility time significantly reduced to 80±8.1 s, which indicates that amantadine might have reversed the tolerance so developed to long term treatment with tricyclic antidepressant. Amantadine was also able to combat the tolerance developed to long-term clomipramine treatment. This finding may be of particular importance in case of drug-resistant depressive patients.

Cognition studies were carried out using Morris water maze test. Amantadine at the given dose (20 mg/kg) showed significant memory retention activity. Whereas, clomipramine tends to show memory impairment. But the combination of amantadine and clomipramine showed memory improvement (Table 6). Clomipramine 40 mg/kg, administered daily for 7 d showed memory hampering activity. But combination of amantadine 20 mg/kg and clomipramine 40 mg/kg showed memory improvement activity. Depression is often associated with memory loss. The combination of amantadine and clomipramine at the same dose shows synergistic antidepressant effect. This combination could be useful in management of depressive patients with memory loss.

Antidepressant inhibiting effect of verapamil on clomipramine was evaluated by forced swim test. Verapamil, a calcium channel blocker is reported to facilitate depression. It was observed that verapamil 40 mg/kg i.p. showed immobility time similar to control (Table 7). Clomipramine

TABLE 7: EFFECT OF DRUG TREATMENT ON IMMOBILITY TIME (S) IN THE FORCED SWIM TEST IN MICE

Treatment	Dose mg/kg i.p.	Mean Immobility time (s)
Control	Vehicle	120±2.6
Clomipramine	40	80±2.8*
Verapamil	40	136±5.8*
Verapamil + Clomipramine	40 + 40	149±9.1
Amantadine	20	83±1.9*
Verapamil + Clomipramine + Amantadine	40 + 40 + 20	76±5.4#
Control	Vehicle	120±2.6

Each value is represented as mean±s.e.m. (n=5) of immobility time using forced swim test model. *P<0.05 is considered to be statistically significant as compared to control by students 't' test. #P<0.05 is considered to be statistically significant as compared to combination verapamil and clomipramine treated group by students 't' test.

showed immobility time of 79±2.8 s while the combination of clomipramine and verapamil increased the immobility time to 149±9.1 s indicating that verapamil has blocked the antidepressant effect of clomipramine (Table 7). It was further observed that when a combination of verapamil, clomipramine and amantadine was administered the immobility time significantly decreased. Verapamil inhibited the antidepressant effect of clomipramine. But when combination of clomipramine and amantadine is administered along with verapamil, the antidepressant effect of clomipramine is retained. This could suggest that calcium may be playing a vital role in mechanism of antidepressant effect of clomipramine.

REFERENCES

1. Horwell, D.C., Ed., In; Antidepressants, Marcel Dekker Inc, New York, 1985, 71.
2. Rang, H.P., Dale, M.M., Ritter, J.M., Eds., In; Pharmacology 14th Edn., Churchill Livingstone, Edinburg, 1999, 550.
3. Cohen, B.M and Baldessarini, R.J., **Amer. J. Psychiatry,** 1985, 142, 489.
4. Greene, R.J. and Harris, D., Eds., In; Pathology and Therapeutics for Pharmacists. 2nd Edn., The Pharmaceutical press, London, U.K., 1997, 338.
5. Parfitt, K., Eds., In; Martindale: The Complete Drug Reference, 32nd Edn., The Pharmaceutical press, London, 1999, Part I, 1130.
6. Chaturvedi, H.K. and Dinesh Chandra, J.S., **Indian J. Pharmacol.,** 1999, 31, 104.
7. Brown, A.S. and Gershon, S., **J. Neural. Transm. Gen. Sect.,** 1993, 91, 75.
8. Levine, J. and Rapoport, A., **Biol. Psychiatry,** 2000, 47, 586.
9. Parfitt, K., Ed., In; Martindale: The Complete Drug Reference, 32nd Edn., The Pharmaceutical press, London, 1999, Part I, 281.
10. Friedman, E., Fung, F., and Gershon, S., **Eur. J. Pharmacol.,** 1977, 42, 47.
11. Kulkarni, S.K. and George, B., **Indian J. Pharmacol.,** 1999, 31, 14.
12. Reynolds, J. and Miller, R.J., **Pol. J. Pharmacol.,** 1988, 95, 95.
13. Srivastava, S.K. and Nath, C., **Indian J. Pharmacol.,** 1999, 31, 107.
14. Maj, J. and Rogoz, Z., **Pol. J. Pharmacol.,** 2000 , 52, 111.
15. Vogel, H.G. and Vogel, W.H., Eds., In; Drug Discovery and Evaluation-Pharmacological Assays, Springer-Verlag, Berlin, 1997, 303.
16. Bear, M.F., Connors, B.W. and Paradiso, M.A., Eds., In; Neuroscience: Exploring the brain, 1st Edn., Williams and Wilkins, Baltimore, U.S.A., 1996, 548.
17. Wedzomy, K. and Czyrak, A., **Pol. J. Pharmacol.,** 1997, 49, 277.
18. Chiodo, L.A. and Antelman, S.M., **Nature,** 1980, 287, 451.