IJPSR (2013), Vol. 4, Issue 7 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 March, 2013; received in revised form, 07 April, 2013; accepted, 23 June, 2013

EVALUATION OF EFFECT OF PIRACETAM IN EXPERIMENTAL MODELS OF DEPRESSION

V.S. Gokhale*, S.S. Bhide, S.V. Jalgaonkar, P.A. Marathe, Y. Mane, F.M. Khan, N.N. Rege

Department of Pharmacology and Therapeutics, Seth G.S. Medical College and King Edward VII Memorial Hospital, Mumbai, Maharashtra, India

Keywords:

AMPA receptors, BDNF, Neurogenesis, Neuronal plasticity

Correspondence to Author:

Dr. V.S. Gokhale

Department of Pharmacology and Therapeutics, Seth G.S. Medical College and King Edward VII Memorial Hospital, Mumbai, Maharashtra, India

E-mail: dr.vs.gokhale@gmail.com

ABSTRACT:

Aims: To study effect of piracetam in experimental models of depression.

Methods: Mice (n = 6/group) pretreated with distilled water, fluoxetine (28 mg/kg) and piracetam - 100, 200, 300, 400, 500, 750 and 1000 mg/kg for 7 days were subjected to tail suspension test (TST) on day 7. Rats (n=6/group) were pretreated for 7 days with distilled water, fluoxetine (20 mg/kg) and piracetam (300, 400, 500, 750 and 1000 mg/kg) and on day 7, forced swim test (FST) was conducted. Immobility time in seconds was noted in both the models and analysed using one-way ANOVA (level of significance $p<0.05$).

Results: In TST, immobility time was reduced significantly ($p<0.01$) and in a dose-dependent manner with all but one dose of piracetam compared to vehicle. In FST, significant difference from control group was seen with higher doses of piracetam (500, 750 and 1000 mg/kg) with a dose-dependent trend. The mean immobility durations in the groups with significant improvement were found to be comparable to that of the respective fluoxetine groups in both models. No signifiant difference was seen in the general motor activity in all the treatment groups as assessed by the open field test.

Conclusion: Piracetam shows anti-depressant activity in experimental models of depression over a wide dose range and in a dose-dependent manner.

INTRODUCTION: Clinical depression is a serious illness that involves the body, mood, and thoughts, affecting a person's general health, work and ability to enjoy life [1]. The global burden of disease report by the WHO that was released in October 2008 identifies depression as the leading global cause of "years of health lost to disease" in both men and women [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.4(7).2667-72

Article can be accessed online on: www.ijpsr.com

Currently used antidepressants [selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs)] have a number of limitations which include unsatisfactory response to treatment and low remission rates, delayed onset of action, poor tolerability, persistent adverse effects and the potential for clinically significant pharmacokinetic drug interactions [3].

Therefore, discovery of newer targets and agents for anti-depressant action is the need of the hour. Research in neurobiology for understanding role of various neurotransmitters involved in pathogenesis of depression and their modulators is on-going.

In this regard, disturbances in neuroplasticity and neuronal loss have been identified as key factors in the pathogenesis of major depression. One of the targets recently identified in this area of research is the glutamatergic AMPA receptor [4]. Positive modulation of AMPA receptors has shown to increase brain derived neurotropic factor (BDNF) and enhance neurogenesis [5].

Piracetam is a commonly used nootropic agent which enhances memory particularly in patients of dementia. Although mechanism of action of piracetam is poorly understood, it is proposed that it is a positive allosteric modulator at AMPA receptors [6]. Studies of AMPA receptor (AMPAR) potentiators in animal models of depression are few.

A study by Farley *et al*., showed that AMPAR potentiator LY392098, when administered alone, acted like classic antidepressants by reducing weight loss, fur deterioration and immobility in the tail suspension test [7].

In view of the above finding, it was of interest to study piracetam for its potential antidepressant effect.

Methods: The study was commenced after obtaining approval of the Institutional Animal Ethics Committee. The study was conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA) guidelines, Government of India.

Experimental animals: Six to eight week old Wistar rats (n=56) of either sex weighing 150-200 g and five to six weeks old Swiss albino mice of either sex (n=72) weighing 25- 30 g were used. The study animals were bred in house in the institute's animal house facility.

Animals were housed in cages (four per cage) with a stainless steel top grill having facilities for food and drinking water in polypropylene bottles with stainless steel sipper tube. The animal house facility was air conditioned with 12 to 15 filtered fresh air changes per 24 hrs, temperature 22 ± 3° C, relative humidity maintained between 30% and 70% and with a 12 hours light-dark cycle . Rodent feed in form of pellets was provided *ad libitum.* Filtered and ultraviolet radiation treated pure drinking water was supplied *ad libitum*.

Drugs and Chemicals: Seven doses of piracetam were used for the tail suspension test viz. 100, 200, 300, 400, 500, 750 and 1000 mg/kg. The acute FST was done using the higher five doses only. Piracetam was purchased from UCB Pharma, India. Fluoxetine (gift sample from Sun Pharma, India) was used in dose of 20 mg/kg in rats as a positive control to compare the effects of the study drug and its dose was decided based on an earlier experimental study [8]. The dose used in mice was 28 mg/kg which was calculated from rat dose [9].

Both the drugs were procured in the form of dry powders and reconstituted in distilled water every day prior to feeding and the solution was administered by oral gavage. Control group received appropriate doses of distilled water by the oral route. The feeding was done in the experimentation room of the animal house facility every day between 11 a.m. and 12 noon followed half hour later by the pretest, wherever applicable.

Study Procedures: The study was divided into 3 parts.

Part 1- Tail Suspension Test in mice (**Table 1**) [10]: In this part of the study, piracetam was tested in 7 different doses in the model of tail suspension test (TST) in mice. Test drug and vehicle were administered orally to the respective groups for 7 consecutive days. For TST, mice were suspended individually from a horizontal bar 50 cm above the floor using adhesive tape. The point of attachment on the tail was one centimetre from the tip. On seventh day, test was conducted 1 hour after the last dose of study drugs. The duration for which the mice remained immobile i.e. without making any physical attempts at escape was counted in seconds over a period of 6 minutes by a blind observer.

Part 2- Forced Swim Test in rats (Table 2) [11]: In this part of the study, the most effective doses of piracetam identified in the previous part were selected. The study drugs were administered orally to respective groups for 7 consecutive days. The rats were subjected to Forced Swim Test (FST) 1 hour after the last dose of the study drugs on seventh day. FST was done in 2 sessions. On day 6 of the study drug administration, each rat was exposed to the pre-test session of 15 minutes. For the pre-test session, the rats were forced to swim in a 45 cm tall, 18 cm diameter Plexiglas cylinder filled to a height of 25

cm with water (temperature 25°C ± 3°C). Twenty four hours later, on the seventh day each animal was reintroduced in the same cylinder for a period of 5 minutes. During this test session, the duration of immobility of rats was counted in seconds by an observer who was blind to the treatment scheme.

Open field test (OFT) was carried out to measure motor activity of all the animals; 5 minutes before they were subjected to the swim test session. The apparatus used for OFT was a wooden square box, 60 cm x 60 cm with 25-cm high walls. Its floor was divided into nine smaller squares of equal dimensions (20cm x 20cm) (marked with lines drawn using white paint). For open field observations, each rat was individually placed in the centre of the floor of the box and was allowed to explore the box for 5 minutes.

The behavioural parameters, *viz.* line crossings (number of time a rat crossed any line with its hind limbs), rearing frequencies **(**number of times rat stood on its hind limbs) and number of faecal boli by each rat were counted for a period of 5 minutes. The open field apparatus was washed with a detergent solution every time before testing a rat to eliminate possible odour clues left by previous rats.

Statistical Analysis: After ensuring that the data is normally distributed using the method of Kolmogorov and Smirnov, the results were assessed for statistical significance using one-way ANOVA. Wherever statistically significant difference was found *posthoc* Tukey-Kramer multiple comparison test was applied to make intergroup comparisons. The level of significance was set at $p<0.05$. The statistical analysis was performed using the GraphPadInStat (version 3.06, Sept. 2003) software for Windows.

RESULTS:

Part 1 – Tail suspension test in mice (Table 1): Immobility time was found to be reduced significantly ($p<0.001$) in the group which received piracetam in the dose of 100, 300, 400, 500, 750 and 1000 mg/kg as compared to the control. Further, the duration of immobility remained comparable between all doses of piracetam and fluoxetine group. From the dose of 300 mg/kg onwards the duration of mobility was seen to be progressively higher with higher doses.

TABLE 1: DURATION OF IMMOBILITY IN DIFFERENT GROUPS OF MICE FOR TST

Group	Immobility Time Mean±SD
Control (Distilled water)	240.83 ± 31.02
Fluoxetine 28 mg/kg	154.5* ± 38.68
Piracetam 100 mg/kg	187.17* ± 32.76
Piracetam 200 mg/kg	200.33 ± 36.61
Piracetam 300 mg/kg	131.33* ± 25.39
Piracetam 400 mg/kg	123.33* ± 16.69
Piracetam 500 mg/kg	121.83* ± 18.94
Piracetam 750 mg/kg	118* ± 16.94
Piracetam 1000 mg/kg	111* ± 12.02

p<0.001 compared to control using ANOVA with post hoc Tukey test

Part 2 –Forced swim test in rats (Table 2): The mean duration of immobility in the forced swim test was reduced significantly ($p<0.001$) in the groups receiving piracetam at doses of 500, 750 and 1000 mg/kg as compared to the control group. The mean durations of immobility of these 3 groups were comparable to that of the fluoxetine group.

Also there was a dose dependent trend observed with minimum reduction in immobility time in the group receiving 300mg/kg and maximum reduction in the group receiving the highest dose i.e. 1000 mg/kg. This difference in immobility time between the groups receiving 300 mg/kg and 1000 mg/kg of piracetam was also found to be statistically significant (($p< 0.05$).

In the OFT, it was seen that all treatment groups were similar to control group with respect to mean number of line crossings, instances of rearing and number of *fecal boli* voided.

TABLE 2: DURATION OF IMMOBILITY IN DIFFERENT GROUPS OF RATS FOR FST

Group	Immobility Time Mean±SD
Control (Distilled water)	208.5 ± 48.46
Fluoxetine 20 mg/kg	139.5* ± 26.48
Piracetam 300 mg/kg	177.66 ± 23.78
Piracetam 400 mg/kg	159.5 ± 21.8
Piracetam 500 mg/kg	149.17* ± 19.38
Piracetam 750 mg/kg	127.33*$ ± 17.66
Piracetam 1000 mg/kg	119.66*$ ± 18.80

*****p<0.001 compared to control, ANOVA with post hoc Tukey test

$ p< 0.05 compared to Piracetam 300 mg/kg, ANOVA with post hoc Tukey test

DISCUSSION: Current evidence suggests that major depression is associated with neuronal loss in the hippocampus and prefrontal cortex, and that antidepressant therapies of different kinds act by inhibiting or actually reversing this loss by stimulating neurogenesis [12]. The patients of depression have been found to have elevated blood glutamate levels. Literature reports that increased hippocampal expression of the GluR1 subunit of the AMPA receptor was observed in animals chronically treated with imipramine [13]. AMPA receptor potentiators (ARPs) have been shown to produce antidepressant-like activity in preclinical studies [4].

Piracetam has been a subject of research for many years. It is the principal member of a class of drugs called "Nootropics". It has been shown to enhance memory function and cognitive abilities in both animals and humans [14, 15, 16]. It is currently approved for clinical use in cases of myoclonus of cortical origin [17], however, it is widely prescribed (off-label) to cases of dementia and Alzheimer's disease [18], as a general "memory tonic" and is also undergoing clinical trials for use as a neuro-protective agent in cases of cerebral vascular strokes [19]. Piracetam has been reported to have very low toxicity and is devoid of serious adverse effects [20].

Piracetam is a positive allosteric modulator of AMPA type of glutamate receptors. The downstream effects of this are manifold. Firstly it increases neurotropin secretion e.g. that of brain derived neurotropic factor, by neural and glial cells and regulates neurite growth. Secondly, it serves to rectify dysfunction in glutamatergic transmission that occurs in certain cognitive disorders. Finally, it increases excitatory neurotransmission and in effect promotes the induction of long-term potentiation.

The effect on excitatory neurotransmission is subsequently augmented by the changes brought about in synaptic plasticity by the neurotropin releasing action. These effects of piracetam target areas of neural dysfunction that are implicated in the pathogenesis of depressive and mood disorders [21].

Considering that piracetam is an AMPA potentiator which is a novel target for development of anti-depressants, its low toxicity and paucity of data with respect to its effects in animal models of depression, we decided to explore its anti-depressant potential.

For the present study we decided to use doses that were used previously in animals both as anti-depressant and as neuro-protective agent [22, 23]. A study by Schmidt done in 1984 found that piracetam caused reduction in immobility in FST in mice [24]. The authors labelled these results as false positive. This was followed up by a study in 1987 by Cavoy, et al, wherein piracetam was found to show activity in the model of learned helplessness in rats. The researchers proposed a number of theories for explaining this phenomenon such as an amphetamine-like excitatory action of piracetam and existence of a cognitive theory of depression [22].

At the time of these studies, the possible role of AMPA receptors in depression and their modulation by piracetam was not known. Further studies in this area were not done until recently. In 2002, a group of researchers postulated about an association between depression and cognitive dysfunction based on the observation of impaired learning and memory in depressed animals. The validity of this association was explored by studying the effects of piracetam and other drugs (including ampakines), that are typically used to treat cognitive disorders, for anti-depressant effects using the model of "Reduction in Submissive Behavior" in rats [25].

The study found that piracetam and aniracetam exhibited promising results. Recently a study has shown that the AMPAR potentiator, LY 392098 augments the reduction in immobility of animals brought about by SSRIs, TCAs and nisoxetine, a noradrenaline reuptake inhibitor in model of FST [4]. In another study, LY 392098 when administered *alone* was found to be functioning like classic antidepressants by reducing weight loss, fur deterioration and immobility in the tail suspension test [7]. Considering these previous studies indicating efficacy of piracetam in the models of depression and the new hypothesis regarding promotion of neurogenesis by piracetam through AMPA receptor pathway, we conducted the present study to consolidate the evidence regarding of antidepresant effect of piracetam.

The findings of our study corroborate those of the above stated studies. Piracetam showed a dose dependent effect in decreasing immobility in models of behavioural despair and the effect was analogous to that of fluoxetine.

The antidepressant effect was maximum at 1000 mg/kg which was the highest dose tested. Piracetam did not have any effect on general locomotor activity at the doses tested as shown by results of OFT. Although the effect of piracetam was similar to fluoxetine, the mechanisms by which these two drugs act may be entirely different. This is evident from a study reported by Li et al., [4] in which AMPA receptor potentiators did not elevate extracellular levels of biogenic amines (e.g., 5HT, NE) in prefrontal cortex.

The difference in the mechanisms of action of piracetam and fluoxetine may be therapeutically exploited to use them in combination in refractory cases of depression and in those who show intolerance to therapeutic doses of SSRIs. It would be worthwhile to study whether piracetam potentiates antidepressant like effect of fluoxetine and other classical antidepressants.

It is documented in literature that the models used here, though sensitive for drugs with anti-depressant activity, are purely behavioural without presuppositions concerning the mechanism of action of potential anti-depressants. A more selective model of depression such as hypermotility in olfactory bulbectomised rats [26], model of chronic mild stress may be chosen in future studies to validate the antidepressant effect of piracetam.

It would be ideal to use AMPA receptor antagonist in combination with piracetam in models of depression to provide evidence about the involvement of AMPA receptors in mediating effects of piracetam.

Further, potential for anti-depressant activity of congeners of piracetam that are also known to act on AMPA receptors like oxiracetam, aniracetam, levetiracetam has not been evaluated so far and presents an avenue for future studies.

Our study was not planned to address the above stated aspects.

CONCLUSION: In the present study, piracetam showed dose dependent effect in decreasing immobility in two conceptually similar models of behavioural despair in rodents. Its potential antidepressant effect should be evaluated in further studies.

REFERENCES:

1. World Health Organisation, 2010. Depression. [Online]. Available at: http://www.who.int/about/copyright/en/ [Accessed on 16 October 2010].
2. Daly R. Depression Biggest Contributor to Global Disease Burden. Psychiatric News. 2009; 44(1):7.
3. Mathew SJ, Manji HK, Charney DS. Novel Drugs and Therapeutic Targets for Severe Mood Disorders. Neuropsychopharmacol 2008; 33:2080-92.
4. Li X, Witkin JM, Need AB, Skolnick P. Enhancement of antidepressant potency by a potentiator of AMPA receptors. Cell Mol Neurobiol. 2003; 23(3):419-30.
5. Alt A, Witkin JM, Bleakman D. AMPA receptor potentiators as novel antidepressants. Current Pharmaceutical Design 2005;11(12):1511-27.
6. Ahmed AH, Oswald RE. Piracetam defines a new binding site for allosteric modulators of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors. J Med Chem 2010;53(5):2197-203.
7. Farley S, Apazoglou K, Witkin JM, Giros B, Tzavara ET. Antidepressant-like effects of an AMPA receptor potentiator under a chronic mild stress paradigm. The International Journal of Neuropsychopharmacol 2010; 13:1207-1218.
8. Renshaw PF, Parsegian A, Yang CK, et al. Lovastatin potentiates the antidepressant efficacy of fluoxetine in rats. Pharmacol Biochem Behav 2009; 92(1):88-92.
9. Paget GE, Barnes JM. In: Lawrence DR and Bacharach AL, (editors) Evaluation of drug activities pharmacometrics., vol 1, Academic Press, New York, 1964.
10. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. Neuroscience and Biobehavioral Reviews 2005; 29: 571–625
11. Porsolt RD, Anton G, Blavet N, Jalfre M. Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol. 1978;47(4):379-91.
12. Hasler G, Drevets WC, Manji HK, Charney DS. Discovering endophenotypes. Neuropsychopharmacol 2004;29:1765-81
13. Schmidt MV, Dietrich T, Weber P, et al. Individual Stress Vulnerability Is Predicted by Short-Term Memory and AMPA Receptor Subunit Ratio in the Hippocampus. The Journal of Neuroscience 2010 Dec; 30(50):16949 –50.
14. Grossman L, Stewart A, Gaikwad S, Utterback E, Wu N, Dileo J, et al. Effects of piracetam on behavior and memory in adult zebrafish. Brain Res Bull 2011;85(1-2):58-63
15. Fresenko UA. Piracetam improves children's memory after general anaesthesia. Anestezjol Intens Ter 2009; 41(1):16-21
16. Neznamov GG, Teleshova ES. Comparative studies of Noopept and piracetam in the treatment of patients with mild cognitivedisorders in organic brain diseases of vascular and traumatic origin. Neurosci Behav Physiol 2009; 39(3):311-21
17. Drugs information online. http://www.drugs.com/uk/nootropil-800mg-1200mgtablets-and-solution-spc-6733.html accessed on 20/9/2011.
18. Medew J. Call for testing on 'smart drugs' by watoday.com.au http://www.watoday.com.au/national/call-for-testing-on-smart-drugs-20091002-gf8t.html#ixzz1YW18r6Io accessed on 20/9/2011.
19. Hankey GJ, Ricci S, Celani MG, Cantisani TA, Righetti E. Piracetam for Acute Ischemic Stroke Stroke 2006;37:2191-2192
20. Gouliaev AH, Senning A. Piracetam and other structurally related nootropics. Brain Res Rev 1994 May;19(2):180-222

21. Zarate CA, Manji HK. The Role of AMPA Receptor Modulation in the Treatment of Neuropsychiatric Diseases. Exp Neurol 2008 May; 211(1):7–10
22. Cavoy A, Ennaceur A, Delacour J. Effects of piracetam on learned helplessness in rats. Physiology and Behaviour 1987; 42: 545-9
23. Kurz C, Ungerer I, Lipka U, et al. The metabolic enhancer piracetam ameliorates the impairment of mitochondrial function and neurite outgrowth induced by ß-amyloid peptide. British J Pharmacol 2010; 160(2):246-57.
24. Schmidt J. Nootropic drugs reduce immobility in behavioural despair test in mice. Biomed Biochim Acta 1984; 43(11):1295-9.
25. Knapp JR, Goldenberg R, Shuck C, et al. Antidepressant activity of memory-enhancing drugs in the reduction of submissive behavior model. Eur J Pharmacol 2002;440:27-35
26. Cairncross KD, Cox B, Forster C, Wren AF. A new model for the detection of anti-depressant drugs: olfactory bulbectomy in rat compared with existing models. J Pharmacol Meth 1978; 1:131-143.

How to cite this article:
Gokhale VS, Bhide SS, Jalgaonkar SV, Marathe PA, Mane Y, Khan FM and Rege NN: Evaluation of effect of Piracetam in experimental models of Depression. *Int J Pharm Sci Res* 2013: 4(7); 2667-2672. doi: 10.13040/IJPSR. 0975-8232.4(7).2667-72