

EFFECT OF COMPOUND 11g AS A NOVEL SELECTIVE COX-2 INHIBITOR ON MOVEMENT DISORDERS IN A RAT MODEL OF PARKINSON'S DISEASE

Mehdi Shafiee Ardestani¹, Hadi Fathi Moghaddam², Mostafa Saffari³

ABSTRACT

Objective: To test the effect of selective COX-2 inhibitors compound 11g on movement disorders of Parkinson's disease (PD).

Methodology: In the study the rat left substantia nigra pars compacta (SNc) has been destroyed using electrical lesion (10 Sec; 1mA DC) to generate PD model. Then 11g (2, 4mg/kg) and celecoxib a well known and standard COX-2 inhibitor (4, 8mg/kg) have been administrated orally to parkinsonian rats. Then the rigidity and locomotor activity of parkinsonian rats were evaluated.

Results: Both selective COX-2 inhibitors decreased the rigidity and improved the locomotor activity of parkinsonian rats $P < 0.05$ as compared to the control groups.

Conclusions: Based on the results of the Locomotor activity and rigidity tests using parkinsonian rats, we found that compound 11g had remarkable rigidity-improving effect.

KEY WORDS: Compound (11g), Celecoxib, Electrical lesion, Parkinson's disease, Inflammation, Cyclooxygenase -2.

Pak J Med Sci April - June 2008 (Part-II) Vol. 24 No. 3 436-441

How to cite this article:

Ardestani MS, Moghaddam HF, Saffari M. Effect of compound 11g as a novel selective cox-2 inhibitor on movement disorders in a rat model of Parkinson's disease. Pak J Med Sci 2008;24(3): 436-41.

1. Mehdi Shafiee Ardestani,
Department of Medicinal Chemistry
and Radiopharmacy,
Faculty of Pharmacy,
Tehran University of Medical Sciences,
Tehran, Iran.
2. Hadi Fathi Moghaddam,
Department of Physiology,
School of Medicine and Physiology Research Center,
Joundishapour University of Medical Sciences,
Ahwaz, Iran.
3. Mostafa Saffari,
Department of Pharmaceutics,
Faculty of Pharmacy,
Shahid Beheshti University of Medical Sciences,
Tehran, Iran.

Correspondence

Dr. Mehdi Shafiee Ardestani
Email: shafieeardestani@razi.tums.ac.ir

- * Received for Publication: October 3, 2007
- * Revision Received: March 25, 2008
- * Revision Accepted: April 1, 2008

INTRODUCTION

Parkinson's Disease (PD) is a degenerative neurodopaminergic disease in nigrostriatum pathway of human and the resultant loss of nerve terminals accompanied by dopamine deficiency in this pathway are responsible for most of the movement disorders such as rigidity, resting tremor or bradykinesia.^{1,2} Increasing evidence suggests that an inflammatory reaction accompanies the pathological processes seen in many neurodegenerative disorders, including PD.^{1,3}

Cyclooxygenase (COX) is the first enzyme in the prostaglandin/ prostacyclin/thromboxane pathway. Three COX isoforms, COX- 1, COX-2 and COX-3 have been identified; COX isoenzymes catalyze both the biooxygenation of arachidonic acid to form prostaglandin G₂ to

form prostaglandin H₂ in the biosynthesis of prostanoid.^{1,3}

COX-1 is the constitutive form of COX and performs a housekeeping function to synthesize prostaglandins, which are involved regulating normal cellular activities.⁴ In contrast, COX-2 is the inducible form of COX, as its expression can be induced by inflammatory stimuli or mutagens, tumor necrosis factor alpha (TNF- α) and the transcription factor CCAAT enhancer binding protein (c/EBP) beta. Both COX-1 and COX-2 isoforms have important roles in the biological functions of the body, but among the COX isoenzymes only COX-2 corresponds to inflammatory and degenerative brain disease. COX-2 appears to be expressed in dendrites and cell bodies of neurons in several areas of the brain such as nigrostriatal pathway, CA-1 hippocampus, amygdala nucleus.^{1,4} The differential tissue distribution of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) provides a rationale for the development of selective COX-2 inhibitors as anti-inflammatory-analgesic agents that lack the GI side effects exhibited by traditional non steroidal anti-inflammatory drugs NSAIDs. Previously we reported a new type of selective COX-2 inhibitor [compound 11g] with in vivo anti-inflammatory activity.⁵ In another previous study we showed the effectiveness of Non Steroidal Anti inflammatory Drugs (NSAIDs) on recovery of rigidity of PD in animal model.⁶

The objective of the research was based on the effects of COX-2 selective inhibitors on the PD affiliated rigidity besides further investigation and potency comparison between compound 11g and celecoxib. For this we decided to study the effects of 11g and celecoxib on movement disorders of parkinsonian rats.

METHODOLOGY

Animals: Forty eight male albino Wistar rats (200-250g) were included in the present study. The animals were purchased from Pasteur Institute of Iran and housed in groups of six animals in stainless steel cages. They were handled daily and provided with food and water

adlibitum. A 12h light/12-h dark cycle was maintained. The animals were tested during the light cycle. These animal experiments were carried out in accordance with the recommendations from the declaration of Helsinki and the internationally accepted principles in the use of experimental animals. In this study, each group contained six rats (6 animals/ group). *Drugs and Solvents:* Compound 11g was prepared as we previously described and celecoxib was purchased from Razak laboratory (Pharmaceutical Company, Iran), ketamine and Xylazin from Merck (Germany). 11g and celecoxib dissolved freely in dimethyl sulfoxide (DMSO), ketamine and Xylazin dissolved in distilled water.

Surgery: Each rat was anesthetized separately by injection of 75mg/kg ketamine combined with 8mg/kg Xylazin intraperitoneally. Then we prepared the rats for surgery and placed them in the stereotaxic instrument. The left SNc region of the nigrostriatum was targeted. Stereotaxic coordinators for the left SNc region were set at -4.8 mm posterior and -1.6mm lateral to bregma and 8.2mm ventral to the surface of the skull according to the atlas,⁷ and the left SNc was destroyed by lesion maker (1 mA, direct Current for 10 seconds). Lateral lesion of SNc in each rat caused PD. Then the rats were kept in individual cages for recovery for 7-10 days after the surgery. Estimation of violence and duration of lesion was accepted empirically in vitro by determination of clot-dimensions in electrocardiograph gel caused by electrical maker, and finally with animal examination and histological studies.⁶ Optimal lesion conditions were yielded.

Apparatus and behavioral procedure:

Rigidity Evaluation: At the time of study, all animals exhibited rigidity, a loss of vocalisation, diminished blinking, incoordination and a course action tremor. Rigidity evaluation method in this study was used to measure the rigidity of animals after orally administration of drugs or vehicles at the times: 0, 20, 40, 60, 90, 120, 180 and 240 minutes. The wood-platforms with the steps of 3 and 9cm were used in this study. The procedure of

behavior experiments was as follows: at the beginning of the test the animal was put on the bench, when it did not move by touch, it received the score of 0.5. Then the right hand of the animal was placed on the wood-platform with the height of 3cm, if the animal did not take its hand off the platform after at least 10 seconds, it received the score of 0.5. Rigidity evaluation was repeated as the same of the previous step for the left hand of the animal on the wood-platform with the height of 3cm and eventually when the animal did not take its hand off from the wood-platform after 10 seconds; it was given again score of 0.5.

In the next stage of the procedure the right hand of the animal was placed on the wood-platform with the height of 9cm, so that any other parts of the animal did not touch the platform, the animal was given one score if it did not take its hand off the platform after 10 seconds. Finally the test was repeated in the same way as the previous step for the left hand of the animal on the wood-platform with the height of 9cm, so that any other parts of the animal did not touch the platform. If the animal did not take its hand off the platform after at least 10 seconds, was given another one score.

It is pointed out that each of the animals that had full rigidity (PD) was given a total score of 3.5. Scores under 3.5 in this method indicated the recovery of the rigidity and the effectiveness of the treatment.

Locomotor activity test: To evaluate rigidity we measured the locomotor activity after the orally administration of drugs or vehicles at intervals of: 20, 40, 60, 90, 120, 180 and 240 minutes. Following the administration of drugs or vehicles, locomotor activity was measured in four metal cages (50×60×70cm) with perspex doors (50×70cm) equipped with eight horizontal infrared beams and photocells.

Two of the eight beams were above and parallel and two were above and perpendicular to each of the two perches. The number of light beam interruptions was accumulated in 10-m intervals and recorded for a total of six hour using an Intel-based computer running Windows 3.1 operating system.⁸ After the rigidity and locomotor activity tests, each animal was decapitated and the brain was removed and kept in a 10% formalin solution. Randomly selected brains were cut on a cryostat as 50µm thick coronal sections, mounted on glass slides, and stained with fuchsin. Sections were examined under a light-microscope to find the accuracy of lesion of the left SNc. Finally, for those animals whose lesion was shown not to be in the SNc, were excluded from the study.

Statistical Analysis: Non-parametrical Kruskal-Wallis, Wilcoxon and One and Two Way Variance Analysis (ANOVA) made comparison between groups and differences with p values <0.05 were considered significant.

Table-I: Illustration the mean of locomotor activity scores (number of beam breaks) of investigated rats and their standard errors.

Locomotor activity score	Time (Min)						
	20	40	60	90	120	180	240
Control	573 ±29	566 ±33	540 ±34	550 ±28	596 ±32	570 ±30	549 ±27
11g 2mg/kg	563 ±24	590 ±27	605 ±23	620 ±29	670 ±28	690 ±26	703 ±19
11g 4mg/kg	577 ±21	905 ±25	1013 ±18	1100 ±22	1209 ±27	1240 ±25	1243 ±26
Celecoxib 4mg/kg	575 ±17	596 ±15	610 ±21	619 ±19	664 ±23	702 ±25	700 ±22
Celecoxib 8mg/kg	582 ±24	890 ±27	998 ±16	1112 ±19	1222 ±28	1237 ±34	1250 ±33

RESULTS

Effects of Celecoxib on the rigidity of parkinsonian rats: The groups that received celecoxib (4 and 8mg/kg) had significant differences from sham, vehicles and positive control ($P<0.01$) elucidate 0, 20 and 240 minutes effect. Also the group that received celecoxib 8mg/kg had significant differences from those whose received celecoxib 4mg/kg ($P<0.05$). (Fig-1).

Effects of compound 11g on the rigidity of parkinsonian rats: The groups which received compound 11g (2, 4mg/kg) had significant differ-

ences from the sham, vehicles and positive control with ($P<0.05$), except at 0, 20 and 240 min as the well as celecoxib. Also the group that received compound 11g 4mg/kg had significant differences from those whose received compound 11g 2mg/kg ($P<0.05$), except at 0, 20 and 240 minutes.

Effects of vehicle, food and water on the rigidity: The parkinsonian rats have not shown any relaxation or rigidity recovery during within the study after receiving the vehicle or food and water.

Comparison between compound 11g and Celecoxib on the rigidity of parkinsonian rats: The group which received celecoxib (8mg/kg), in comparison with the group which received compound 11g (4mg/kg) had not any significant differences. Groups those received Celecoxib (4mg/kg) and compound 11g (2mg/kg) had the same potency in decrease the rigidity with $P<0.05$, except at 0, 180 and 240 min which no significant differences were observed. In addition, celecoxib (8mg/kg) had significant differences from compound 11g (2mg/kg) with

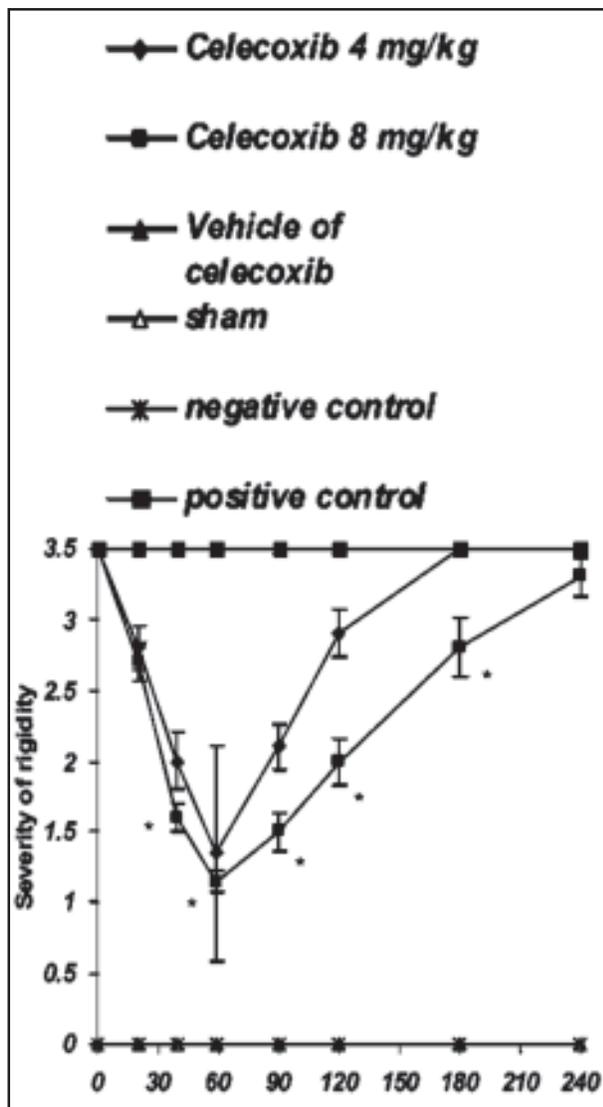


Figure-1: Comparison the mean of rigidity grade in groups that received Celecoxib with sham, negative and positive groups (* $P<0.05$).

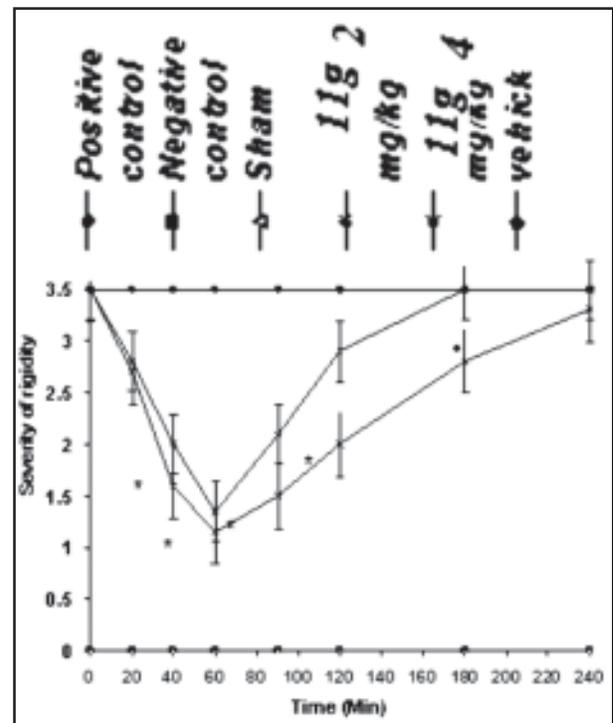


Figure-2: Comparison the mean of rigidity grade in groups that received Compound 11g with sham, negative and positive groups (* $P<0.05$).

$P < 0.05$, except at 0 and 240 minutes which had no significant differences. Our results suggest that compound 11g seems to be more potent COX-2 inhibitor than celecoxib because 11g decreased the rigidity with lower doses than celecoxib. Severity of rigidity in Figs-1-2 have been shown with the mean of rigidity grades of the parkinsonian rats and their standard error of means.

Effects of Celecoxib and compound 11g on locomotor activity: Prior to vehicle or drugs administration, all animals displayed marked bradykinesia showing mean locomotor counts over 30 minutes of between 500 and 1000 beam interruptions. A two-way ANOVA suggested a highly significant effect of COX-2- treatment Celecoxib (4, 8 mg/kg) and compound 11g (2, 4mg/kg) ($P < 0.05$) at all test times except at 20 min (Table-I). Interestingly, following the injection of Celecoxib (8mg/kg) and compound 11g (4mg/kg) produced a statistically significant ($P < 0.01$) increase in locomotor activity. Again our general results [total mean of locomotor activity] suggest that compound 11g acts as a more potent COX-2 inhibitor than celecoxib because compound 11g improved locomotor activity with lower doses than celecoxib (Fig-3).

DISCUSSION

Our observations in the present study have shown that the acute use of compound 11g caused to improve the rigidity and locomotor activity of PD as well as celecoxib in rat as animal model. Our results showed us that the effective times for recovery of the rigidity or locomotor activity occurred at 60-90 minutes. According to the results of the study recovery was more seen when the dose of compound 11g or celecoxib were increased. Furthermore the recovery of rigidity or locomotor activity in the Parkinsonian rats that received compound 11g (as a novel COX-2 selective inhibitor) was much effective than those receiving celecoxib. Our findings suggest a more important role for COX-2 inhibition in treatment of rigidity of PD. A previous study⁹ has also demonstrated that COX-2 and prostaglandin E2

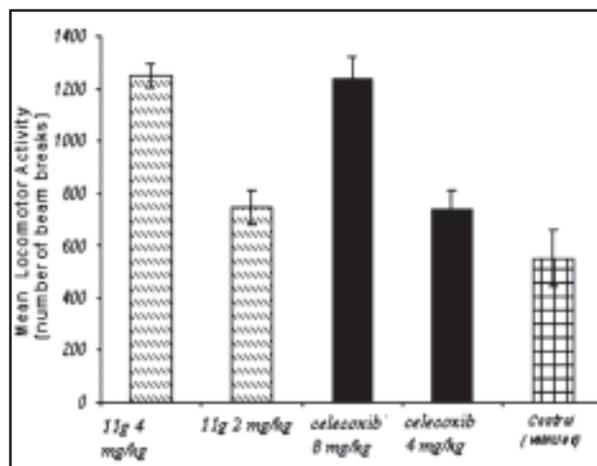


Figure-3: Effects of compound 11g and celecoxib on locomotor activity of investigated groups. [The data are shown as average scores of all test times]

level increased in PD which is in agreement with our study findings. The previous research¹⁰ that COX-2 caused to increase the level of acetylcholine in the brain to increase by producing of prostaglandin E2 and increasing the expression of cholinergic markers, such as choline acetyl transferase and vesicular acetylcholine transporter protein. It is worthwhile mentioning that prostaglandins have modulatory effects on adrenergic, noradrenergic and glutaminergic transmission.¹¹ In addition, some of the investigations have shown that COX-2 inhibitor impairs the spatial memory through the reduction of acetylcholine level in the brain, but COX-1 inhibitor has not any effect on spatial memory in rats.^{12,13} Free radicals and glutamate cause degeneration in SNc, but the inhibition of these agents by antioxidants or glutamate antagonists protects neurons from degeneration.^{14,15}

A previous study has also shown that aspirin and ibuprofen as non selective COX-2 inhibitors significantly attenuate decreases in dopamine uptake caused by glutamate, thus NSAIDs protects neurons against glutamate excitotoxicity in vitro.¹⁶ These observations suggest that it is probably the mode of celecoxib and compound 11g actions as selective COX-2 inhibitors to recover the rigidity, may contain the inhibition of the enzyme COX-2 and synthesize prostaglandin E2 and reduction in the

level of acetyl choline in the brain and. It may probably increase the release of dopamine from dopaminergic neurons in the brain and protects dopaminergic neurons from glutamate toxicity. Some other mechanisms of compound 11g or celecoxib action in the rigidity recovery interference to cellular calcium mediated events may be effective in releasing neurotransmitter and recovery of rigidity. However these suggestions including determination of the levels of glutamate, dopamine and acetyl choline after administration of NSAIDs in the striatum of parkinsonian rats and/ or can changes in striatum neurotransmitters to cause the improvement in the rigidity or not should be further investigated in the future experiments respectively.

CONCLUSION

It could be stated that selective COX-2 inhibitors compound 11g and celecoxib can be administered as alternative drugs in degenerative brain diseases such as PD.

ACKNOWLEDGMENTS

This research was supported by Shahid Beheshti and Jondishapour Universities of Medical Sciences. The authors would like to thank Dr. Rahmim, Department of Radiology, University of Johns Hopkins Baltimore, Maryland, USA for his guidance for statistical analysis and Dr. Navidpour for gift the chemicals used in the study.

REFERENCES

- McGeer PL, McGee EG. Innate immunity, local inflammation and degenerative disease. *Sci Aging Knowledge Environ* 2002;29 review 3.
- Fahn S, Przedborski S. Inhibition of cyclooxygenase-2 protects motor neurons in an organotypic model of amyotrophic lateral sclerosis. *Merritt's Neurology*. Williams & Wilkins, New York, 2000;679-93.
- McGeer PL, Schwab C, Parent A, Doudet D. Presence of reactive microglia in monkey substantia nigra years after 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine administration. *Ann Neurol* 2003;54:599-604.
- Herschman H.R. Prostaglandin synthase-2. *Biochem. Biophys. Acta* 1996;1299:153-79.
- Navidpour L, Shadnia H, Shafaroodi H, Amini M, Dehpour AR, Shafiee A. Design, synthesis, and biological evaluation of substituted 2-alkylthio-1, 5- diarylimidazoles as selective COX-2 inhibitors. *Bioorganic & Medicinal Chemistry* 2007;15:1976-82.
- Shafiee AM, Fathi MH, Hemmati AA, Nazari Z. Effects of Aspirin and Celecoxib on rigidity in a rat model of Parkinson's disease. *Pak J Biol Sci* 2007;10,3853-8.
- Paxinos G, Watson C. *The rat brain in stereotaxic coordinates*. Academic Press. San Diego. 1997.
- Jenner P, Costa S, Iravani MM, Pearce RKB. Glial cell line-derived neurotrophic factor concentration dependently improves disability and motor activity in MPTP-treated common marmosets. *Eur J Pharmacol* 2001;412:45-50.
- Buccafusco JJ, Lapp CA, Aronstam RS, Hays AC. Role of prostanoids in the regulation of central cholinergic receptor sensitivity. *J Pharmacol Exp Ther* 1993;266:314-22.
- Riechman W, Hokin LE. Acetylcholine releases prostaglandins from brain slices incubated in vitro. *J Neurochem* 1987;49:1261-21.
- Ito Y. Modulatory actions of prostaglandins in autonomic neuro-effector transmissions. *Nippon Yakurigako Zasshi* 1982;79:1-14.
- Rall JM, Mach SA, Dash PK. Intrahippocampal infusion cyclooxygenase-2 inhibitor attenuates memory acquisition in rats. *Brain Res* 2003;968:273-6.
- Sharifzadeh M, Tavasoli M, Naghdi N, Ghanbari A, Amini M, Roghani A. Post-training intrahippocampal infusion of nicotine prevents spatial memory retention deficits induced by the cyclo-oxygenase-2-specific inhibitor celecoxib in rats. *J Neurochem* 2005;95(4):1078-90.
- King D, Playfer JR, Roberts NB. Concentrations of vitamins A, C and E in elderly patients with Parkinson's disease. *Postgrad Med J* 1992;68:634-7.
- Etminan M, Gill SS, Samii A. Intake of vitamin E, vitamin C, and carotenoids and the risk of Parkinson's disease: A meta-analysis. *Lancet Neurol* 2002;4:362-5.
- Casper D, Yaparpalvi U, Rempel N, Wener P. Ibuprofen protects dopaminergic neurons against glutamate toxicity in vitro. *Neurosci Lett* 2000;289:201-4.