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Publication

The journal is published six times in a year in the months of February, April, June, August, October and December.

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The journal is printed on acid free paper

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Published by

Medknow Publications
A-109, Kanara Business Centre, Off Link Road, Ghatkopar (E), Mumbai - 400075, India. Phone: 91-22-6649 1818/1816, Fax: 91-22-6649 1817, Web: www.medknow.com

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Effects of dexamethasone and betamethasone as COX-2 gene expression inhibitors on rigidity in a rat model of Parkinson's disease

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Received: 11.05.2007

Revised: 10.08.2007

Accepted: 12.10.2007

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disease in the nigrostriatal pathway of animals and humans and is responsible for most of the movement disorders, including the rigidity. Increasing evidence suggests that an inflammatory reaction accompanies the pathological processes caused by cyclooxygenase-2 (COX-2) seen in many neurodegenerative disorders, including PD. In this study oral betamethasone and dexamethasone were administered to parkinsonian rats chronically and their effect on rigidity was evaluated. As the results of this study show, both the molecules were able to decrease rigidity.

KEY WORDS: Betamethasone, dexamethasone, inflammation, Parkinson's disease

Cyclooxygenase (COX) is the first enzyme in the prostaglandin-prostacyclin-thromboxane pathway. It converts arachidonic acid to prostaglandins and thromboxanes, which are collectively known as its metabolites.^[1] Three COX isoforms—COX-1, COX-2 and COX-3—have been identified. COX-1 is the constitutive form of COX and performs a housekeeping function to synthesize prostaglandins, which are involved in regulating normal cellular activities. In contrast, COX-2 is the inducible form of COX, as its expression can be induced by inflammatory stimuli or mutagens, tumor necrosis factor- α (TNF- α) and the transcription factor CCAAT enhancer binding protein (c/EBP) β . The brain possesses both COX-1 and COX-2 isoforms. There is COX-2 upregulation during stressful conditions such as cerebral ischemia; it is also upregulated by neuronal apoptosis and neurobehavioral defects.^[2]

In addition, steroidal anti-inflammatory drugs such as dexamethasone can inhibit COX-2 gene expression. The glucocorticoids have widespread effects because they influence the function of most cells in the body. Glucocorticoids dramatically reduce the manifestations of inflammation. This is due to their profound effects on the concentration, distribution and function of peripheral leukocytes and their suppressive effects on the inflammatory cytokines, such as TNF- α or interleukin-6 (IL-6) and chemokines or other lipid and glucolipid mediators of inflammation. In addition to these effects, glucocorticoids influence the inflammatory response by reducing the prostaglandin synthesis that results from activation of phospholipase A₂.^[3]

COX-2 appears to be expressed in dendrites and cell bodies of neurons in several areas of the brain, including the nigrostriatal pathway, CA-1 hippocampus and amygdala nucleus.^[4]

Among the COX isoenzymes, only COX-2 corresponds to inflammatory and degenerative brain disease.^[5] Parkinson's disease (PD) is a degenerative neurodopaminergic disease in the nigrostriatal pathway of humans. The loss of nerve terminals, accompanied by dopamine deficiency, in this pathway are responsible for most of the movement disorders.^[6] Increasing evidence suggests that an inflammatory reaction accompanies the pathological processes seen in many neurodegenerative disorders, including PD.^[7,8] Glial activation is part of a defense mechanism to remove debris and pathogens and promote tissue repair. However, inflammatory activation of microglial cells may contribute to the neurodegenerative process through structural invasion and the release of proinflammatory cytokines, reactive oxygen species (ROS), nitric oxide (NO) and excitatory amino acids at synapses and cell bodies.^[9] In cell culture and animal models, inflammation contributes to neuronal damage and nonsteroidal anti-inflammatory drugs (NSAIDs) have been shown to provide some neuroprotection in different paradigms,^[10] including PD models.^[11] Reactive microglia inhibits neuronal cell respiration via NO and causes neuronal cell death *in vitro* and *in vivo*.^[12] Investigators have reported an uncertain relationship between COX-2 and its inhibition by NSAIDs and PD. They suggest that chronic use of

NSAIDs can decrease the risk of PD.^[13] They also suggest that there is a role for COX-2 in degenerative diseases such as PD.^[14] In addition, there is the evidence that steroidal compounds like dexamethasone can protect dopaminergic neurons against the lesion.^[15] These studies have not examined the effect of COX-2 gene expression inhibitors, such as the steroidal anti-inflammatory drugs dexamethasone and betamethasone, on the rigidity of PD. In the present study, we have investigated the effect of the COX-2 gene expression inhibitors on the rigidity seen in PD.

Materials and Methods

Animals

Ninety male albino Wistar rats (200-250 gm) were the subjects in the present study. The animals were purchased from Pasteur Institute of Iran and then housed in groups of ten in stainless steel cages, handled daily and provided food and water *ad libitum*. A 12-h light/12-h dark cycle was maintained; the animals were tested during the light cycle. These animal experiments were carried out in accordance with recommendations of the declaration of Helsinki and the internationally accepted principles in the use of experimental animals. In this study, we divided the animals into nine groups as shown in Tables 1 and 2. Each group contained ten rats.

Drugs and solvents

Dexamethasone and betamethasone were purchased from Razak and Abidi Laboratories (Iran) and ketamine and xylazine were purchased from Merck (Germany). Dexamethasone was dissolved freely in glycerin 70% and dimethyl sulfoxide (DMSO) 30% and betamethasone was dissolved freely in glycerin 70% and acetone 30%; similarly, ketamine and xylazine were dissolved in distilled water.

Surgery

Each rat was anesthetized separately by intraperitoneal injection of 75 mg/kg of ketamine combined with 8 mg/kg xylazine. We prepared the rats for surgery and placed them in the stereotaxic instrument. The left substantia nigra pars compacta (SNc) region of the nigrostriatum was targeted.

Table 1

Description of the control and vehicle groups

Lesion of SNc (Positive control)	n = 10	Received saline
Negative control	n = 10	Received saline
Sham	n = 10	Received saline
Vehicle of dexamethasone	n = 10	Received DMSO and glycerin
Vehicle of betamethasone	n = 10	Received acetone and glycerin

Table 2

Description of the test groups

Test group 1	n = 10	Received dexamethasone 0.075 mg/kg
Test group 2	n = 10	Received dexamethasone 0.15 mg/kg
Test group 3	n = 10	Received betamethasone 0.12 mg/kg
Test group 4	n = 10	Received betamethasone 0.24 mg/kg

Stereotaxic coordinators for the left SNc region were set at –4.8 mm posterior and –1.6 mm lateral to bregma and 8.2 mm ventral to the surface of the skull, according to the atlas of Paxinos and Watson.^[16]

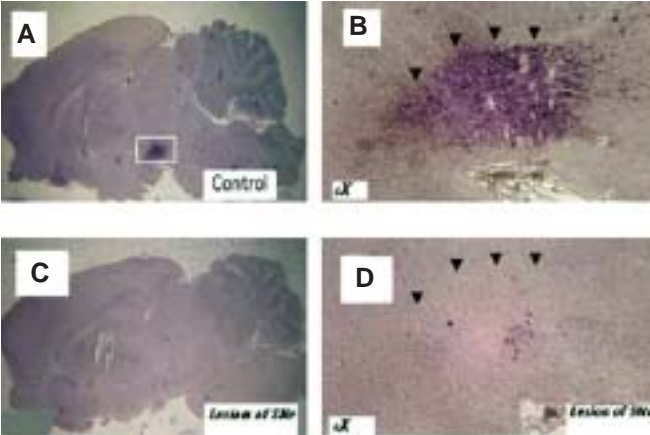
Stainless steel electrode was then placed in the left SNc and it was destroyed by the electrical lesion maker (Siemens, Germany), using an electrical current (1 mA, 8 s). A lateral lesion of the SNc in each rat caused PD and its associated disorders, such as rigidity, due to the decrease in the inhibitory dopaminergic effects on the caudate nucleus and putamen, which are the main rigidity-inducing neurotransmitter releasing areas in the striatum.^[3] Because of these changes in the brains of the lesioned rats, there was rigidity of the limbs on both sides. Following surgery, they were kept in individual cages for 7-10 days and allowed to recover.

Estimation of violence and duration of lesion was estimated empirically *in vitro* by determination of clot dimensions in electrocardiograph gel caused by the electrical lesion maker and, finally, with animal examination and histological studies (Illustration 1) the actual lesion conditions were obtained. [Figure 1A-D] shows the accuracy and the precision of the lesion.

Experimental procedure

After recovery from surgery (i.e., after 7-10 days) all animals received daily oral COX-2 gene expression inhibitors for four weeks—either dexamethasone (0.075 or 0.15 mg/kg) or betamethasone (0.12 or 0.24 mg/kg)—depending on the test group to which the animal had been assigned. The same procedure was repeated in the control or vehicle groups as shown in Table 1. Murprogo’s Method^[17] was then used to measure the rigidity of the animals 12 h after the last oral administration of drug or vehicle; the measurements were made at the following time points: 0, 20, 40, 60, 90, 120, 180 and 240 min. Wood platforms with steps of 3 and 9 cm were used

Figure 1: Histological study about accuracy the lesion of SNc in comparison with control groups (negative control and sham) Image A: SNc without microscopic magnification in control and sham groups Image B: SNc with microscopic magnification (4X) in control and sham groups Image C: Destroyed SNc in Parkinsonian rats without microscopic magnification Image D: Destroyed SNc in Parkinsonian rats with microscopic magnification (4X). Note: Chronic treatment of Parkinsonian rats did not improve lesion of the left SNc



in this study. The procedure for the behavior experiments was as follows: At the beginning of the test, the animal was placed on the bench. If it did not move when touched, it received a score of 0.5. Next, the right paw of the animal was placed on the wood platform with the height of 3 cm; if the animal did not take its paw off the platform after at least 10 s, it received a score of 0.5. The rigidity evaluation was repeated for the left paw and, as before, a score of 0.5 was given if the animal did not take its paw off the wooden platform after 10 s. In the next stage of the procedure the right paw of the animal was placed on the 9 cm high wood platform, such that no other part of the animal touched the platform; the animal was given a score of 1 if it did not take its paw off the platform after 10 s. This same procedure was repeated for the left paw.

It should be pointed out that when an animal had full rigidity (PD) it was given a total score of 3.5. Scores under 3.5 by Murprogo's Method indicate recovery from rigidity and the effectiveness of the treatment. After the Murprogo's test, each animal was decapitated and the brain was removed and kept in a 10% formalin solution. Randomly selected brains were cut on a cryostat; 50 μ m thick coronal sections were obtained, mounted on glass slides and stained with H & E. Sections were examined under a light microscope to find out the accuracy of lesion of the left SNC. If the lesion was shown not to be in the SNC, any collected data on that particular animal were discarded.

Results

Statistical analysis

Nonparametric Kruskal-Wallis test, Wilcoxon test and one way analysis of variance (ANOVA) was used to compare the differences between groups; differences with P values <0.05 were considered significant.

Effect of betamethasone on rigidity in parkinsonian rats

In those test groups which received betamethasone 0.12 and 0.24 mg/kg, the rigidity in the PD model decreased and showed significant differences ($P < 0.01$) from sham, vehicle and positive control at all of the test times. Also, the test group that received betamethasone 0.24 mg/kg showed significantly greater reduction in rigidity than that which received betamethasone 0.12 mg/kg ($P < 0.01$ at 0 min and 90 min; $P < 0.05$ at 20, 60 and 240 min; and $P > 0.05$ at the other time points) [Figure 2].

Effect of dexamethasone on rigidity in parkinsonian rats

Our results showed that dexamethasone (0.075 and 0.15 mg/kg) was able to decrease rigidity in the PD model, with significant differences ($P < 0.01$) from sham, vehicle and positive control at all of the test times. We found that dexamethasone 0.15 mg/kg had significantly greater effect than dexamethasone 0.075 mg/kg, with $P < 0.01$ at 0, 20 and 60 min and $P < 0.05$ at the other time points [Figure 3].

Comparison of the effect of the two COX-2 gene expression inhibitors on rigidity in parkinsonian rats

The test group that received dexamethasone (0.075 mg/kg) showed more reduction in rigidity than that which received betamethasone (0.24 mg/kg); the difference was statistically significant, with $P < 0.01$ at 0, 20 and 40 min and $P < 0.05$ at

Figure 2: Comparison of the mean rigidity grade in the groups that received betamethasone with that in the sham, negative and positive control groups. (* $P < 0.05$) and (** $P < 0.01$)

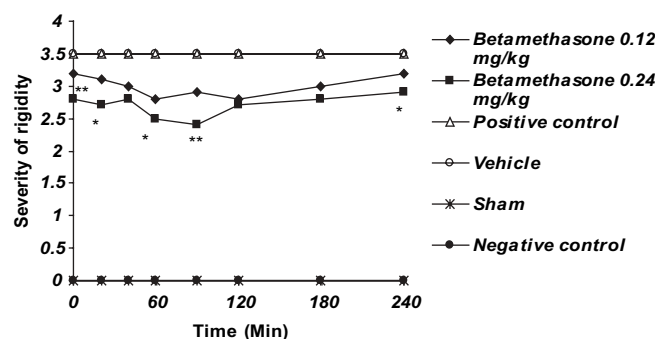
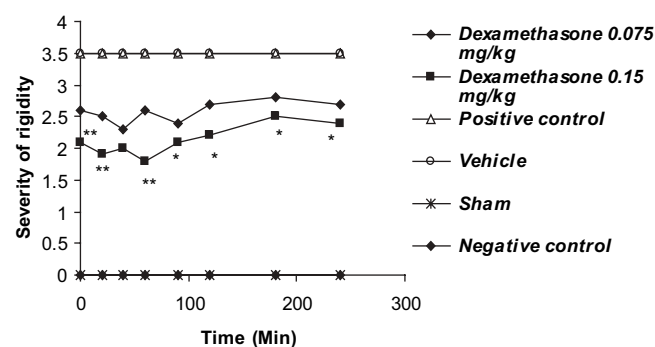


Figure 3: Comparison of the mean rigidity grade in the groups that received dexamethasone with that in the sham, negative and positive control groups. (* $P < 0.05$) and (** $P < 0.01$)



240 min. At the other time points there was no difference between the two in their ability to decrease rigidity in the PD model.

The test group that received dexamethasone 0.075 mg/kg showed significantly greater decrease in rigidity than the test group that received betamethasone 0.12 mg/kg, with $P < 0.01$ at 0, 20, 40, 90 and 240 min and $P < 0.05$ at 90 and 180 min; at 120 min the two test groups showed the same degree of decrease in rigidity. In addition, the test group that received dexamethasone 0.15 mg/kg showed significantly ($P < 0.01$) greater decrease in rigidity than the group that received betamethasone 0.24 mg/kg at all the evaluation time points; at 90 and 180 min the difference was statistically significant, with $P < 0.05$.

Finally, we saw significant differences between the groups that received dexamethasone 0.15 mg/kg and that which received betamethasone 0.12 mg/kg, with $P < 0.001$ for the differences at 0, 20, 40 and 60 min and $P < 0.01$ at the other time points.

Discussion

Our observations show that the chronic use of COX-2 gene expression inhibitors, such as steroidal anti-inflammatory agents, caused an improvement in the rigidity of PD in the animal (rat) model.

Reduction in rigidity in Parkinsonian rats was more with dexamethasone than with betamethasone and both the drugs were effective in reducing rigidity at all the evaluation time points during the study. Our findings also suggest that there is an important role for COX-2 gene expression in the management of the rigidity of PD. In agreement with our work, a previous study,^[18] using postmortem analysis, has shown that COX-2 and prostaglandin E_2 level are increased in the brains of persons who suffer from PD.

In another study, Riechman and Hokin^[19] suggested that COX-2 causes an increase in the level of acetylcholine in the brain through the production of prostaglandin E_2 and by an increase in the expression of cholinergic markers, such as choline acetyltransferase and vesicular acetylcholine transporter protein.

It is worth mentioning that prostaglandins, especially prostaglandin E_2 , have modulatory effects on adrenergic, noradrenergic and glutaminergic transmission and prostaglandin synthesis inhibitors increase the blood pressure by increasing catecholamine release; for example, using large doses of glucocorticoids in humans may cause insomnia, euphoria and increase in intracranial pressure.^[3] In addition, some investigations have shown that COX-2 inhibitors impaired spatial memory by reducing acetylcholine levels in the brain but COX-1 inhibitors do not have any effect on spatial memory in rats.^[20] Free radicals and glutamate cause degeneration in SNc and inhibition of these agents by antioxidants or glutamate antagonists protects neurons from degeneration.^[21] Other anti-inflammatory effects of steroidal anti-inflammatory drugs possibly include decreasing the production of free radicals and interference with calcium-mediated intracellular events.^[3] Neuronal COX-2 overexpression may kill neurons in a cell-autonomous manner and lead to the pathogenesis of PD;^[22] In support of this hypothesis is the fact that COX-2 cell-autonomous toxicity may arise from the formation of ROS generated during COX peroxidase catalysis of prostaglandin G_2 conversion to prostaglandin H_2 . That COX-2 cell-autonomous toxicity may arise from the formation of reactive oxygen species generated during COX peroxidase catalysis of prostaglandin G_2 conversion to prostaglandin H_2 and also electrons donation to COX, co-substrate such as dopamine oxidized to dopamine-quinone and thus neuronal death is happe. In PD, there is evidence of an increase in the oxidative and inflammatory nigral environment, which includes the presence of (COX)-immunoreactive activated microglial cells in the substantia nigra. Microglial cells can also produce and release pro-inflammatory cytokines, in particular TNF- α and cytotoxic molecules, including ROS and NO.^[23] Although such responses are nonspecific to lesion type, after 6-hydroxydopamine intrastratial infusion there is an acute increase in TNF- α in the striatum.^[24] In this study we suggest that one of the possible mechanisms for the reduction in rigidity by administration of dexamethasone and betamethasone may probably be a decrease in microglial activation or/and the level of TNF- α , components of the inflammation pathway and free radicals in the striatum region. This hypothesis needs to be further investigated in future studies.

In another report scientists have suggested that aspirin and ibuprofen, as nonselective COX-2 inhibitors, significantly attenuate the decreases in dopamine uptake caused by

glutamate and thus protect neurons against glutamate excitotoxicity *in vitro*.^[25] These observations suggest that other possible mechanisms exist by which dexamethasone and betamethasone caused reduction in rigidity in the present study. Inhibition of the enzyme COX-2 with inhibition of synthesis of prostaglandin E_2 , reduction in the level of acetylcholine in the brain and, probably, increase in the release of dopamine from dopaminergic neurons in the brain protects dopaminergic neurons from glutamate toxicity similarly to NSAIDs. In support of this, COX-2 inhibition and interference with cellular calcium-mediated events may be effective in achieving neurotransmitter release and recovery from rigidity.

Acknowledgments

The authors would like to thank Dr. Amin Gravand and Dr. Mostafa Saffari for their kind collaboration in the preparation of this article.

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