RESEARCH ARTICLE

Quantitative Analysis of Behavior in Rotenone-Induced Parkinsonism in Rats Using Force Plate Actimeter

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Abstract

Rotenone is a chemical that has been used to induce Parkinsonism-like behavioral symptoms such as tremor, bradykinesia, rigidity and postural instability in rats. In this study, we used rotenone to induce parkinsonism-like symptoms in rats. Eighteen Wistar rats were randomly divided into three groups: control group, 2.5 mg/kg, rotenone-treated group and 2.5 mg/kg rotenone+10 mg/kg Sinemet® group. Rotenone was dissolved in soybean oil and injected subcutaneously every day excluding weekends. After 9 doses of soybean oil or rotenone, a series of oral doses of 3% (w/v) acacia or 10 mg/kg Sinemet® was administered daily for 5 days, followed by a dose of acacia+soybean oil, acacia+rotenone, or Sinemet®+rotenone. Animal behavior was assessed by using force plate actimeter. The results showed a significant decrease in motor ability of rats injected with rotenone compared to control group. Oral treatment of 10 mg/kg Sinemet® improved the movement of rotenone-treated rats. Power spectra revealed that the parkinsonian features induced by rotenone possessed the frequency between 0.5-2.5 Hz and 4-12 Hz. This is the first model that applies the behavioral assessment using force plate actimeter in rotenone-induced parkinsonism in rats and thus may be used for evaluating new anti-parkinsonian drugs.

Keywords: Rotenone, frequency, force plate actimeter

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การวิเคราะห์พฤติกรรมเชิงปริมาณของหนูขาวที่ได้รับโรติโนนในการเหนี่ยวนำให้เกิดอาการพาร์กินสันด้วยเครื่อง Force Plate Actimeter

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บทคัดย่อ

โรติโนนเป็นสารเคมีที่ใช้เหนี่ยวนำให้หนูขาวเกิดอาการคล้ายอาการแสดงของโรคพาร์กินสัน เช่น อาการสั่น เคลื่อนไหวช้า กล้ามเนื้อแข็งเกร็ง และสูญเสียการทรงตัว ในการศึกษานี้ผู้วิจัยใช้โรติโนนในการเหนี่ยวนำให้เกิดอาการคล้ายอาการแสดงของโรคพาร์กินสันในหนูขาว โดยแบ่งหนูขาวสายพันธุ์ Wistar จำนวน 18 ตัวเป็น 3 กลุ่มคือ กลุ่มควบคุม กลุ่มที่ได้รับโรติโนนในขนาด 2.5 มิลลิกรัม/กิโลกรัม และกลุ่มที่ได้รับโรติโนนในขนาด 2.5 มิลลิกรัม/กิโลกรัม ร่วมกับยา Sinemet® ในขนาด 10 มิลลิกรัม/กิโลกรัม สัตว์ทดลองได้รับโรติโนนหรือน้ำมันถั่วเหลืองโดยการฉีดเข้าใต้ผิวหนังทุกวัน ยกเว้นวันหยุดสุดสัปดาห์ จำนวน 9 ครั้ง หลังจากนั้น หนูขาวได้รับ 3% (w/v) acacia หรือ 10 มิลลิกรัม/กิโลกรัม Sinemet® ทางปากทุกวันเป็นเวลา 5 วัน ตามด้วย acacia+น้ำมันถั่วเหลือง, acacia+โรติโนน หรือ Sinemet®+โรติโนน 1 ครั้ง แล้วทำการประเมินพฤติกรรมสัตว์ทดลองแต่ละตัวโดยใช้เครื่อง force plate actimeter ผลการศึกษาพบว่าหนูขาวที่ได้รับโรติโนนมีการเคลื่อนไหวลดลงอย่างมีนัยสำคัญเมื่อเปรียบเทียบกับกลุ่มควบคุม และหนูที่ได้รับยา Sinemet® ในขนาด 10 มิลลิกรัม/กิโลกรัม มีการเคลื่อนไหวดีขึ้น ผลจาก power spectra แสดงให้เห็นว่าอาการคล้ายอาการแสดงของโรคพาร์กินสันที่เกิดในหนูขาวที่ได้รับโรติโนนมีความถี่ระหว่าง 0.5-2.5 เฮิรตซ์และ 4-12 เฮิรตซ์ รูปแบบการศึกษานี้เป็นครั้งแรกที่มีการประยุกต์ใช้เครื่อง force plate actimeter ในการประเมินพฤติกรรมในหนูขาวที่มีอาการคล้ายโรคพาร์กินสันจากการได้รับโรติโนน ซึ่งอาจนำไปใช้ประเมินประสิทธิภาพของยาต้านพาร์กินสันชนิดใหม่ได้

คำสำคัญ: โรติโนน, ความถี่, force plate actimeter

Introduction

Parkinson's disease (PD) is a complex neurodegenerative disorder that has been identified as a health problem in elderly population. It is characterized by movement related disorders such as resting tremor predominating only one side of body, bradykinesia, muscle stiffness, postural instability.[1,2] The increased occurrence of this disease encourages the exploration of better anti-parkinsonian agents which could abolish or minimize these movement disorders. The neurodegeneration in PD is highly specific to neuromelanin containing dopaminergic neurons of substantia nigra pars compacta (Snpc), a structure of brain deeply located within the midbrain (mesencephalon).[3,4] Regardless of being common, the etiology of PD is not completely understood. Rare cases of PD have been shown to be associated with genetic mutation but the role of genetics is not yet clearly elucidated.[5-7] Nevertheless, many researchers believe that PD may arise from the combination of various genetics and environmental factors.[8] Epidemiological studies revealed a higher risk of PD in people chronically exposed to pesticides.[9,10]

Rotenone is a naturally occurring cytotoxic compound found in the roots and stem of plants belonging to the species *Lonchocarpus* or *Derris*.[11] Due to its lipophilicity, it can easily pass or be incorporated into cell membrane and mitochondrial membrane.[12] Rotenone is commercially available as herbicides and pesticides. It is thought to induce parkinsonism-like behaviors by inhibiting complex I of mitochondria, resulting in ATP exhaustion. This energy insufficiency then leads to cell death.[13-15] Moreover, it can also enhance the aggregation of α-synuclein to form Lewy bodies, which is a biomarker of PD with dementia.[13,15] With all the above mentioned properties, rotenone has now become a valuable tool in PD research.[16]

Force plate actimeter (FPA) is a modern device with a sensitive force plate fixed horizontally inside. It is used to quantitatively monitor the neurological behaviors of rodents such as locomotor activity, tremor, distance of travelling, gait disturbances, and rhythmicity. Hence, FPA can be used for studying effects of drugs on neurological behavior in animals.[17,18]

Up till now, there are no reliable studies for quantifying parkinsonism induced by rotenone. In this study, we demonstrated the method for quantitative determination of parkinsonism-like behaviors in rotenone-treated rats using FPA.

Materials and Methods

Chemicals

Rotenone and soybean oil were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acacia obtained from Srichand professional (Bangkok, Thailand) and Sinemet® (levodopa 100 mg + carbidopa 25 mg) manufactured by M&H manufacturing (Bangkok, Thailand) were used in this study.

Animals

A total of 18 male Wistar rats (25-35 g) were obtained from the National Laboratory Animal Center, Salaya, Nakorn Pathom. They were housed in cages under standard and controlled conditions (22±2ºC, 50-70% humidity, and 12/12 h light/dark cycle) and were fed water and diet *ad libitum*. The protocol of animal handling and procedure was approved by the Animal Care and Use Committee, Thailand Institute of Scientific Technological Research (TISTR) and Animal Ethic Committee of Faculty of Pharmacy, Mahidol University.

Experimental design

The protocol of this study was similar to those described by Xiong et al.[20] and Alam et al.[14] as summarized in Table 1.

In brief, animals were randomly divided into three groups of six each. The control (C1) group were subcutaneously injected with soybean oil while rats in R1 and T1 groups were administered with 2.5 mg/kg rotenone daily during weekdays. After receiving 9 doses, rats in R1 and T1 groups developed severe parkinsonian features such as postural instability, rigidity and secretion from eyes and nose. One day gap was scheduled for monitoring their behavior. Then rats in C1 and R1 groups were orally administered with 3% (w/v) acacia for 5 days while rats in T1 group were treated with 10 mg/kg Sinemet® for the same length of time. On the last day of experiment, the animals were injected subcutaneously with either rotenone or soybean 30 min after receiving the last dose of either 3% (w/v) acacia or Sinemet®. The animals were individually placed on FPA for 2 h for behavioral assessment, i.e., locomotor activity and power spectra analysis.

Table 1. Experimental design

Treatment group	Day 1-9		Day 10-14	Day 15
Control (C1)	Soybean oil [a]		3% (w/v) acacia [b]	3% (w/v) acacia [b] + soybean oil [a]
Rotenone (R1)	2.5 mg/kg rotenone suspended in soybean oil [a]	**One day gap**	3% (w/v) acacia [b]	3% (w/v) acacia [b] + 2.5 mg/kg rotenone [a]
Treatment (T1)	2.5 mg/kg rotenone suspended in soybean oil [a]		10 mg/kg Sinemet® suspended in 3% (w/v) acacia [b]	10 mg/kg Sinemet® + 2.5 mg/kg rotenone [a]

[a] Subcutaneous injection; [b] oral gavage

Measurement of locomotor activity

For the assessment of locomotor activity, FPA (CX-9000, BASi®, West Lafayette, IN, USA), a rigid horizontal graphite plate supported by four force transducers placed on all corners of the plate was used in this experiment. When an animal was placed on the graphite plate, the movement was sensed by transducers and a signal was further processed by a computer to generate behavior data. The forces on each corner of the graphite plate, f1, f2, f3 and f4, were arranged in a

counterclockwise direction, starting with f1 in Quadrant 1 (where both X and Y are positive), f2 in Quadrant 2 (where X axis is negative and Y is positive), and so on.[17,18]

We hypothesized that the rotenone-treated rats would experience some motor activity abnormality and might not travel at all. Thus the distance of travelling and bouts of low mobility (BLM) were used as dependent variables to quantify locomotor activity of animals. BLM was measured when animal stayed in one place and performed other activities such as head bobbing, grooming, and rearing. BLM scale ranges from 0 to 8 with higher scores indicative of lower movement, i.e., a score of 0 represents high motor activity whereas 8 indicates no movement.[17,18] Rats with high mobility were expected to show longer distance traveled and hence would have lower BLM.

Power spectra analysis

Power spectra analysis reveals how much power is required in order to induce parkinsonian features such as tremor and lateral movement of jaws. Time intervals measured by FPA were divided into different number of frames, with one frame equaled to 80 sec. After each 80 sec, a new frame was started. For 2-h recording, the time interval was divided into 88 frames. The frames in which the rat did not moved were selected for data analysis to reduce the noises in power spectra; for example, paws of rats. Trajectory motion pictures showed the locomotor position of a rat at that frame. Y-axis depicts the intensity of power and X-axis represents frequency in hertz (Hz). When the rat was motionless, a clear, noticeable peak was expected, which indicated parkinsonian symptoms in rotenone-treated rats.

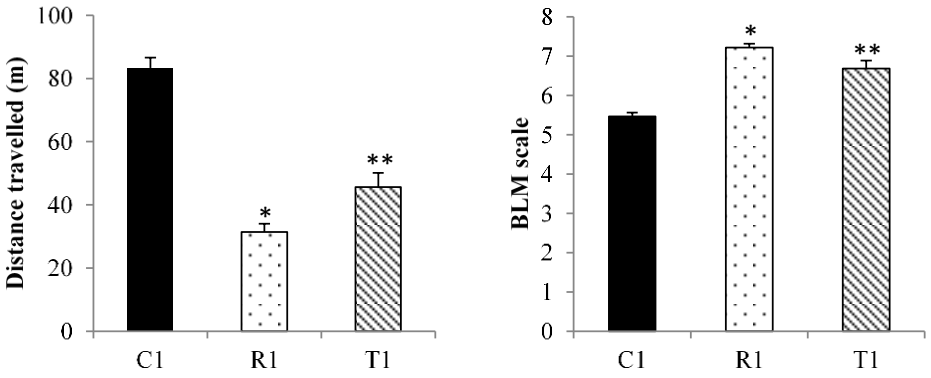
Statistical analysis

All data were expressed as mean±standard error (SE). Comparison between the groups was analyzed using ANOVA with post hoc analysis (LSD). Probability of 5% ($p<0.05$) was considered to be significant. All data were analyzed using IBM SPSS Statistics version 22.

Results

Effect of rotenone and Sinemet® on locomotor activity of rats

Subcutaneous injection of rotenone started to induce parkinsonian features in rats after 6-7 dose administration. Distance travelled and BLM of each group were shown in Figure 1. Rats injected with 2.5 mg/kg rotenone showed a significant reduction in distance travelled compared with control (31.36±2.68 *vs.* 83.29±3.29 m) ($p=0.00$). BLM was significantly increased in rats injected with 2.5 mg/kg rotenone compared with control (7.21±0.09 *vs.* 5.44±0.1) ($p=0.00$). Changes in both parameters were significantly ameliorated by oral administration of 10 mg/kg Sinemet® (45.59±4.49 m and 6.68±0.19, respectively) ($p=0.012$ and $p=0.0017$) compared with 2.5 mg/kg rotenone group.



*p<0.05 compared with C1; **p<0.05 as compared with R1

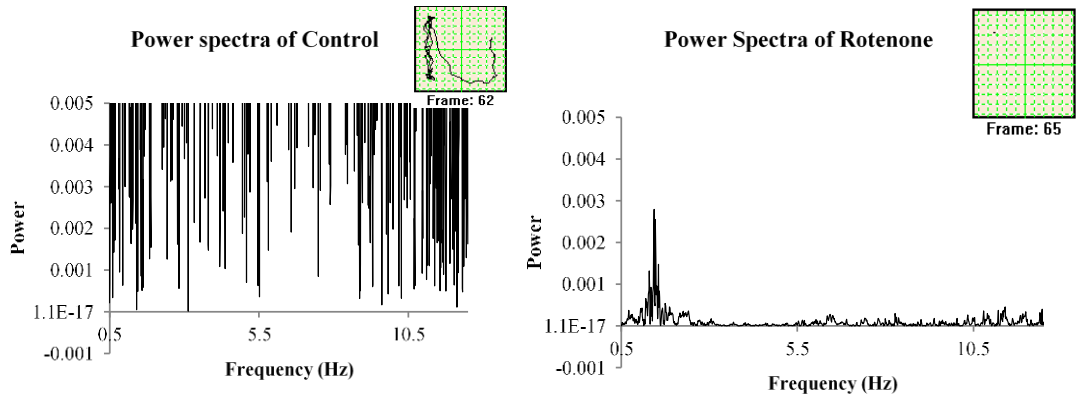
(A) (B)

Figure 1. Locomotor activity of rats. (A) Distance travelled (m) and (B) BLM. C1 = control group, R1 = 3% (w/v) acacia + 2.5 mg/kg rotenone group, and T1 = 10 mg/kg Sinemet®+ 2.5 mg/kg rotenone group. Data are presented as the group means±SE.

Power spectra analysis and trajectory motion pictures

Figure 2 illustrates the trajectory motion and power spectra of rats from C1 and R1 groups. The results showed that the control rat moved around the frame while the rat injected with 2.5 mg/kg rotenone stood still. Upon comparing the spectra at the same power, peaks of C1 were not clear and out of the range, indicating the noise produced by paws of rat while walking around the plate.

Comparison of power spectra results of R1 with T1 along with their trajectory motion pictures are displayed in Figure 3. Grey line represents R1, and black line denotes T1. R1 showed significant peaks at around 0.5-2.5 Hz and 4-12 Hz, and the peaks were suppressed by the oral treatment of 10 mg/kg Sinemet®.



(A) (B)

Figure 2. Trajectory motion pictures and power spectra of (A) control group (C1) and (B) 3% (w/v) acacia + 2.5 mg/kg rotenone group (R1).

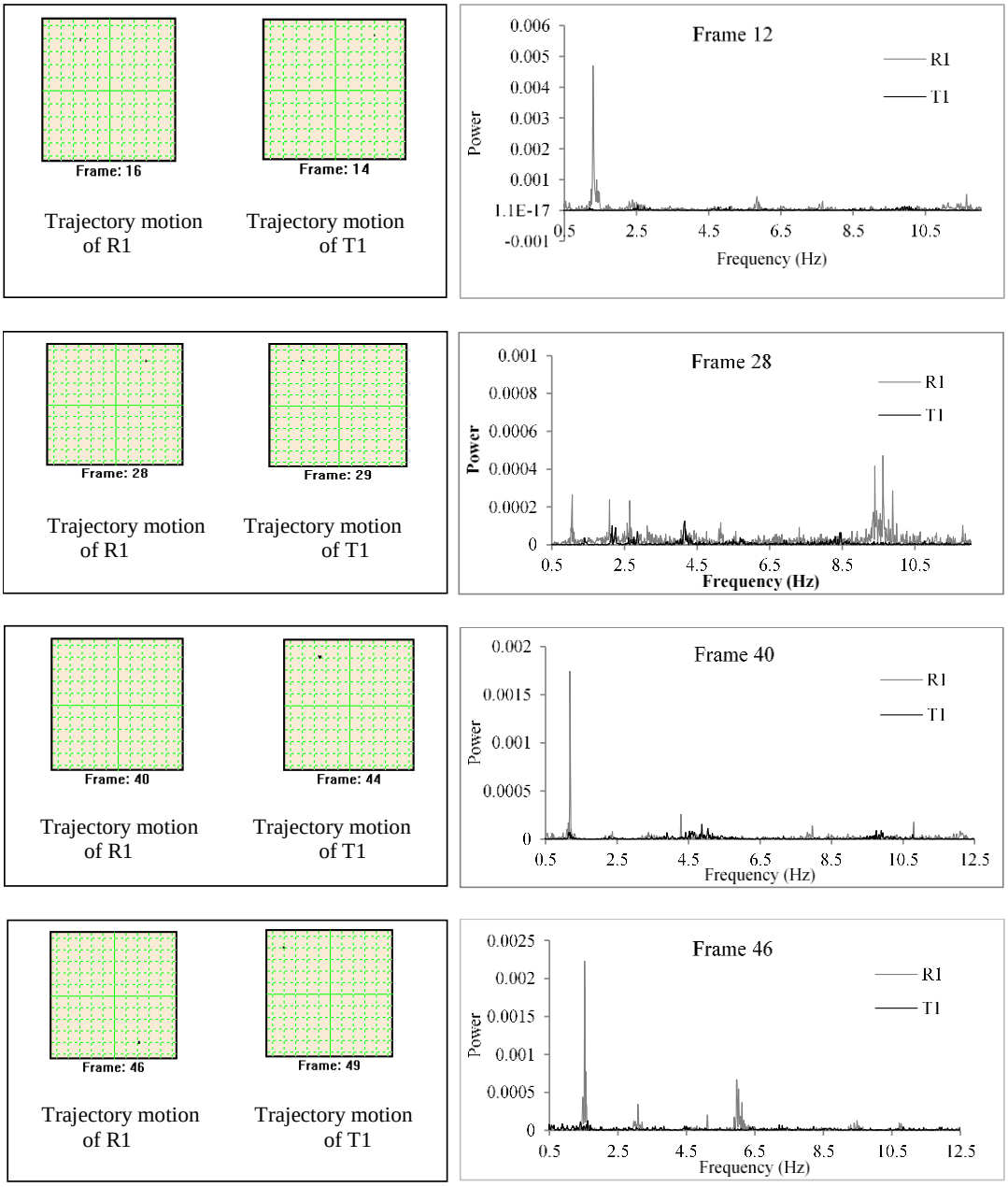


Figure 3. Comparison of trajectory motion pictures and power spectra between 3% (w/v) acacia + 2.5 mg/kg rotenone group (R1) and 10 mg/kg Sinemet® + 2.5 mg/kg rotenone group (T1).

Discussion

Zbigniew[21] and Klein[22] have demonstrated the association between motor disability and chronic exposure to rotenone. Rotenone is selective towards dopaminergic neurons and repeated exposure of rotenone may affect the motor ability of rats. ATP depletion in dopaminergic neurons may result in oxidative

stress and cell death, leading to reduction of dopamine level.[14,21,22] Although dopamine in nigrostriatal pathway is critical for smooth and coordinated movement, depletion of dopamine may adversely affect motor features of rats as well as induce tremor and rigidity.[14-16] The effect of Sinemet® on reduction of the intensity of tremor, rigidity and motor function induced by rotenone was caused by the replacement of dopamine at the degenerated area of Snpc.

During our preliminary study, apart from tremor and rigidity, we also noticed the lateral movement of jaw in rotenone-treated rats, which may correlate with involuntary "chewing effect" observed in human patients. This behavior was similar to that shown by tacrine, a jaw movement-inducing drug. The power spectra of jaw movement in tacrine-treated rats gave the frequency around 0.5-3 Hz[23] whereas the remarkable peaks ranging from 0.5-2.5 Hz and 4-12 Hz were observed in this study. Hence, the peak at 0.5-2.5 Hz found in rotenone-treated rats may also be the power spectra of lateral movement of jaw. Presence of multiple peaks from 4-12 Hz may represent body tremor. Sinemet® was found to reduce the intensity of tremor, rigidity and motor function induced by rotenone.

Conclusion

Our findings showed that 2.5 mg/kg rotenone can induce a parkinsonism-like behavior in rats. Since tremor and motor dysfunction are classic symptoms of parkinsonism, it is possible to use these symptoms as quantitative parameters for designing animal models for Parkinson's disease. By using FPA, the frequency of parkinsonian characteristics induced by rotenone such as whole body tremor and locomotor activity along with other abnormal behaviors of each animal can be quantitatively measured simultaneously.[17-19] Therefore our model may be used to evaluate the capability of new anti-parkinsonian drugs.

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