Rotenone 對老鼠行爲及黑核多巴胺神經元影響之評估

Behavioral Assessment and Immunohistochemical Evaluation of the SNpc in Rats Following Rotenone Treatment

中文摘要

文獻報告指出巴金森氏病罹病率在超過 65 歲的人口爲 1-3%， 佔人類神經退化性疾病的第 2 位。 黑核神經元 (pars compacta of substantia nigra. SNpc) 多巴胺神經細胞數目的減少及細胞質內含 Lewy body 爲此病的病理特徵。巴金森氏病除了小部份屬於家族遺傳病例外，大部份病患的致病機轉尚未完全清楚。最近文獻報告指出長期暴露於殺蟲及殺魚毒物 rotenone 的老鼠會產生類似巴金森氏病的病理特徵：黑核神經元多巴胺神經細胞數目的減少及細胞質內含 Lewy body。 但是對於低劑量的 rotenone 是否會引起移動行爲障礙? 如果低劑量的 rotenone 會引起移動行爲障礙，會在暴露 rotenone 多久後發生? Rotenone 所引起的移動行爲障礙與黑核多巴胺神經元的形態變化是否有任何相關?這些問題仍然有待釐清。本研究的研究目標爲探討: (1) 暴露於 rotenone 多久後 Lewis 老鼠會出現行爲變化， (2) rotenone 引起 Lewis 老鼠黑核多巴胺神經元形態變化所需的時間，及 (3) ?予 rotenone 後 Lewis 老鼠的行爲變化與黑核多巴胺神經元形態變化之間的相關性。

經過兩次前驅試驗，本研究共使用 55 隻實驗鼠，其中 6 隻爲對照組，49 隻爲實驗組。實驗組的老鼠每天接受皮下注射 rotenone 後存活 15 隻，其中有 8 隻爲 rotenone-7 天組 及 7 隻 rotenone-14 天組。 對照組及 rotenone-14 天組兩組分別於第 0、7 及 14 天觀察其移動行爲， rotenone-7 天則於組則於第 0 及 7 天進行移動行爲觀察。 所有動物於實驗結束後犧牲，其腦組織包含黑核多巴胺神經元則進行進一步的形態分析。

本研究結果顯示每天接受皮下注射 rotenone 的實驗鼠會出現高死亡率 (69.4%) 。於給予 rotenone 7 天候，老鼠的體重、步履調整能力及格子攀爬能力等顯著下降。黑核多巴胺神經元具非中斷樹突的比例也顯著地下降。除此之外，老鼠調整步履的能力與黑核多巴胺神經元具非中斷樹突的比例之間有顯著地正相關。

本研究結論顯示儘管所使用 rotenone 劑量較文獻爲低、也能於第 7 天讓 Lewis 鼠產生類似巴金森氏病症狀的行爲變化及黑核多巴胺神經元形態變化，而且這兩者之間存在著顯著的正相關。此研究結果也支持生活環境中的神經毒物如 rotenone 能損害人體黑核多巴胺神經元而產生類似巴金森氏並的主張。

英文摘要

Parkinson’s disease (PD) is the second most common neurodegenerative disease that affects approximately 1 to 3 % of the population with the age over 65. Though genetic

mutations of the α-synuclein, Parkin or ubiquitin carboxy-terminal hydrolase-L1 (UCHL1) causing PD have been reported, the etiology of the majority of PD remains unclear. PD is characterized by a dramatic loss of the dopaminergic neurons and the presence of Lewy bodies in the substantia nigra (SN), especially in the pars compacta (SNpc). Recent evidence suggests that long-term exposure to rotenone, one of the mitochondrial complex I inhibitor and commonly used as a natural botanical insecticide and piscicide, leads to dopaminergic neuronal loss and Lewy body formation in the SN, which mimic the pathology of PD. However, whether exposure to lower doses of rotenone results in behavioral deficits and whether the behavioral deficits correlate with the neuromorphometrical changes in the SN are still undetermined. In this study, we aimed (1) to determine the onset of behavioral changes following rotenone treatment, (2) to examine the dose-response effect of rotenone on dopaminergic neuronal loss in the SNpc and (3) to correlate the behavioral changes with the neuromorphometrical change of the dopaminergic neurons in the SNpc of rats following rotenone treatment.

After evaluation of the delivery routes and doses of rotenone with two pilot studies, fifty-five adult male Lewis rats were allocated into three groups: the control group, the 7-day rotenone group and the 14-day rotenone group. Rotenone (1.5 mg/ml/kg) was given to rats daily for 7 days and 14 days by subcutaneous injection (sc) in the 7-day and 14-day rotenone group, respectively. Control animals received daily sc injection of vehicle for 14 days. The body weight and movement behavior of animals were recorded before treatment, 7, and/or 14 days after rotenone or vehicle treatment and the morphology of the SNpc was also evaluated. Our results indicated that application of rotenone resulted in a high mortality rate (69.4%) and led to a lower body weight, a decreased ability in adjusting steps and a longer latency in the grid test in both rotenone-treated groups. Animals in the 14-day rotenone group also displayed an increased paw retraction time. Both groups of rotenone–treated animals showed a significant reduction in the ratio of dopaminergic neurons with uninterrupted dendrites. Furthermore, there was a positive correlation between the number of adjusting steps and the ratio of dopaminergic neurons with uninterrupted dendrites in the SNpc. Our results demonstrate that exposure to low-dose rotenone leads to behavioral deficits and neuromorphometrical change of dopaminergic neurons in the SNpc of rats and strongly support the idea that exposure to environmental toxin, rotenone, may result in a dramatic loss of dopaminergic neurons in the SNpc of PD patients.