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THE IMPACT OF COMBINED ADMINISTRATION OF PARAQUAT AND MANEB ON MOTOR AND NON-MOTOR FUNCTIONS IN THE RAT

A. TINAKOUA,[a,b,c] S. BOUABID,[a,b,c] E. FAGGIANI,[a,b] P. DE DEURWAERDÈRE,[a,b] N. LAKHDAR-GHAZAL[c] AND A. BENAZZOUZ[a,b]*

[a] *Univ. de Bordeaux, Institut des maladies neurodégénératives, UMR 5293, Bordeaux, France*

[b] *CNRS, Institut des maladies neurodégénératives, UMR 5293, Bordeaux, France*

[c] *Université Mohammed V, Faculté des Sciences, Equipe Rythmes Biologiques, Neurosciences et Environnement, Rabat, Morocco*

Abstract—Paraquat (PQ) and maneb (MB) are potential risk factors for Parkinson's disease. However, their impact on non-motor disorders, monoamine neurotransmission and basal ganglia function is not clearly determined. Here we investigated the effects of combined treatment with PQ/MB on motor behavior, anxiety and "depressive-like" disorders, tissue content of monoamines, and subthalamic nucleus (STN) neuronal activity. Male Sprague–Dawley rats were intoxicated by PQ (10 mg/kg) and MB (30 mg/kg) twice a week. Two weeks later, the majority of animals (group 1, 16/26) showed a severe loss of body weight with tremor and respiratory distress and others (group 2, 6/26) showed only tremor. Animals of group 2 received PQ/MB during four weeks before developing weight loss. A last group (group 3, 4/26) was insensitive to PQ/MB after 6 weeks of injections. Groups 1 and 2 displayed a failure of motor activity and motor coordination. Group 3 showed slight motor deficits only after the last injection of PQ/MB. Moreover, PQ/MB induced anxiety and "depressive-like" behaviors in animals of groups 2 and 3. Biochemical analysis showed that PQ/MB reduced striatal dopamine (DA) tissue content paralleled by changes in the activity of STN neurons without changing the content of norepinephrine and serotonin in the cortex. Our data provide evidence that individuals are not equally sensitive to PQ/MB and show that the motor deficits in vulnerable animals, are not only a result of DA neuron degeneration, but may also be a consequence of peripheral disabilities. Nevertheless, the parkinsonian-like non-motor impairments may be a direct consequence of the bilateral DA depletion. © **2015 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: paraquat, maneb, Parkinson's disease, dopamine, norepinephrine, serotonin.

INTRODUCTION

Parkinson's disease (PD) is a neurological disorder, characterized by motor symptoms, such as rigidity, resting tremor, postural instability and bradykinesia. Non-motor disabilities (depression, anxiety, cognitive impairment, sleep disorder. . .) are also a hallmark of the disease. The motor and non-motor symptoms are supposed to be a result of the degeneration of dopaminergic (DAergic) neurons in the substantia nigra pars compacta (SNc) (Ehringer and Hornykiewicz, 1960) and also of other monoaminergic neurons, including noradrenergic (Bertrand et al., 1997) and serotonergic (Kish, 2003) systems. The cause of sporadic cases of PD is still unknown, but recent epidemiological and experimental studies suggest the implication of environmental factors. In fact, this notion of association between environmental factors and PD is born from early observations by Davis and Colleagues (1979) who reported chronic parkinsonism secondary to intravenous injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrine (MPTP) in young people. MPTP is a meperidine analog, which does not exist in nature, but its molecular structure presents similarities with other industrially and naturally occurring substances (Langston et al., 1983). In addition to heavy metals, elevated exposure to manganese has been suggested as a high risk factor for the development of PD, by altering neurobiological systems and producing neurological deficits in patients (Guilarte, 2013) and animal models (Sabbar et al., 2012; Bouabid et al., 2014). Indeed, cumulative exposure to lead and/or manganese induced motor and non-motor impairments paralleled by changes in the electrical and metabolic activities of subthalamic nucleus (STN) neurons in the rat (Sabbar et al., 2012; Bouabid et al., 2014). These studies provide solid arguments for the involvement of environmental toxicant in the induction of atypical PD-like deficits. Furthermore, several epidemiological studies suggested a link between pesticides and PD (Tanner, 1989; Ascherio et al., 2006; Dick et al., 2007; Hatcher et al., 2008). Among pesticides, Paraquat (PQ) is one of the most widely used herbicide in the developing

*Correspondence to: A. Benazzouz, Institut des maladies neurodégénératives, CNRS UMR 5293, Université de Bordeaux, 146 Rue Léo-Saignat, 33076 Bordeaux Cedex, France. Tel: +33-557-574-625.
E-mail address: abdelhamid.benazzouz@u-bordeaux.fr (A. Benazzouz).

Abbreviations: 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, serotonin; 6-OHDA, 6-hydroxydopamine; ANOVA, analysis of variance; CV, coefficient of variation; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; EPM, elevated plus maze; HPLC, high performance liquid chromatography; MB, maneb; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrine; NE, norepinephrine; PD, Parkinson's disease; PQ, Paraquat; SNc, substantia nigra pars compacta; STN, subthalamic nucleus.

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countries. PQ is a possible candidate risk factor for PD, as it bears structural similarities with MPP+, the active component of MPTP (Snyder and D'Amato, 1985). It has been suggested that PQ penetrates the blood–brain barrier (Shimizu et al., 2001) causing the loss of nigro-striatal DAergic cells, which results in the induction of motor deficits (McCormack et al., 2002; Thiruchelvam et al., 2003). In addition to PQ, the fungicide Maneb (MB) is known to reduce locomotor activity (Morato et al., 1989) and already experienced as an enhancer of MPTP (Takahashi et al., 1989) and PQ (Thiruchelvam et al., 2000b; Kachroo et al., 2010) neurotoxicity. The fact that both PQ and MB share the ability to alter the DAergic system and their use in geographically overlapping areas, encouraged by lack of models using environmental neurotoxins, prompted numerous research teams to use the PQ/MB combination as an animal model of PD (Dauer and Przedborski, 2003; Xu et al., 2011; Blesa et al., 2012; Campos et al., 2013b). However, the relevance of the model has not been studied in detail, taking into account all aspects of PD, including motor and non-motor symptoms, the changes in basal ganglia neuronal activity and the impact of peripheral disabilities.

The present study aimed to investigate the effects of combined treatment with PQ and MB on: (1) locomotor activity and motor coordination, (2) anxiety behavior, (3) "depression-like" behavior, (4) tissue contents of dopamine (DA) in the striatum and norepinephrine (NE) and serotonin (5-HT) in the frontal cortex, (5) and the neuronal activity of subthalamic nucleus, which is a basal ganglia structure playing a key role in the pathophysiology of PD.

EXPERIMENTAL PROCEDURES

Animals and PQ/MB treatment

All animal experiments were carried out in accordance with European Communities Council Directive 2010/63/UE, and all efforts were made to minimize the number of animals used and their suffering. The study received approval from the local Ethics Committee (Comité d'éthique pour l'expérimentation animale Bordeaux, France).

Two-month-old male Sprague–Dawley rats (n = 34) weighing 280 g in the beginning of the experiments were used. Animals were obtained from the "Centre d'Elevage Depré" (Centre d'Elevage Depré, Saint Doulchard, France), and maintained in a room with constant temperature (24 °C) and humidity (45%), exposed to a 12-h light/12-h dark cycle (light on at 7:00 A.M.) with access to food and water *ad libitum*. Animals were initially divided into two groups as follows: (i) 26 treated animals received intra-peritoneal (i.p.) injections of a solution containing PQ (10 mg/kg) and MB (30 mg/kg), dissolved in saline (0.9% NaCl), twice a week for two, four or six weeks depending on their vulnerability to treatment. (ii) 8 control animals received saline (0.9% NaCl) in the same conditions. The doses used were chosen on the basis of previous published protocols (Thiruchelvam et al., 2000a; Cicchetti et al., 2005). The animal's weight was monitored twice a week. The general health of the animals was daily observed throughout the duration of the experiment. The tremor has not been quantified but its presence or absence was determined by careful observations of video recordings.

Evaluation of locomotor behaviors (Open Field)

Spontaneous horizontal and vertical (rearing) activities as well as stereotyped movements (movements with low amplitudes and independent of locomotor activity) were measured using a photoelectric actimeter (Actitrack, Panlab, Barcelona, Spain), as previously described (Belujon et al., 2007; Chetrit et al., 2009). Briefly, the apparatus consisted of a transparent cage that was connected to a photoelectric cell. Light beams detected movements and the total motor activity of each rat was recorded. All recordings in the actimeter were done in an isolated room between 8:0 A.M. and 2:00 P.M. The protocol consisted of two phases: (i) Habituation: spontaneous motor activity was recorded during four consecutive days in two consecutive sessions of 10 min before the beginning of injections. (ii) The test was made each week after two injections of PQ/MB or saline and consisted of two consecutive sessions of 10 min. Only the second session of 10 min was retained for data analysis to evaluate the motor behavior of the animals. Statistical analyses were done using Prism (GraphPad Software, San Diego, CA, USA).

Evaluation of motor coordination (rotarod)

To assess the effect of PQ/MB treatment on motor coordination, rats were trained to remain on a rotarod (BIOSEB, *in vivo* Research Instruments, Spain), as previously described (Papp and Bal, 1987; Rozas et al., 1997). All rats underwent a 3-day training program on a 7-cm diameter rotarod. During the training period, each rat was placed on a horizontal rod rotating at a gradually increasing speed from 4 to 20 rotations per minute (rpm) for a maximum of 15 min by which time a steady baseline level of performance was attained. The day after training, the motor coordination was recorded for each animal during five trials. The latency to fall off the rotarod was recorded and the time limit was fixed to 3 min.

Evaluation of anhedonia (sucrose preference test)

The sucrose preference test was used as previously described (Delaville et al., 2012). Rats were housed in individual cages with food and water *ad libitum*. Three days before the experiment onset, rats were housed in the presence of two bottles of water and the position of the bottles was randomly changed to prevent a place preference. On the test day, when the light turns off at 7:00 P.M., pre-weighted water and 1% sucrose-containing bottles were placed on the home cage and rats were allowed to drink for 2 h. During these 2 h, the position of the bottles was changed four times. Water or sucrose absorption was measured by weighing the bottles before and after the test. Sucrose preference was calculated using the following formula as previously reported (Delaville et al., 2012):