NEUROPROTECTIVE EFFECTS OF CANNABIDIOL IN AN ANIMAL MODEL OF PARKINSON'S DISEASE

Claudio Giuliano [1]; Miriam Francavilla [1]; Cristina Ghezzi [1]; Silvia Cerri [1]; Nora Rossini [2]; Fabio Blandini [1]

[1] Laboratory of Cellular and Molecular Neurobiology, IRCCS Mondino Foundation, Pavia, Italy;
[2] Linnea SA, Riazzino (Locarno) (TI), Switzerland;

Parkinson's disease (PD) is neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNc). The pro-inflammatory response can occur early in the disease, contributing to the nigrostriatal degeneration. Current PD therapies are purely symptomatic and do not modify disease progression. The identification of new molecules able to slow down the degenerative process associated with PD, represents one of the challenges in this field. Recently, cannabinoids molecules, especially cannabidiol (CBD), have raised much attention for their anti-inflammatory and antioxidant activity. **The aim of this study was to evaluate the effects of chronic treatment with CBD (Linnea SA, Riazzino (TI), Switzerland) on neurodegenerative and neuroinflammatory processes, and motor deficits induced by a unilateral intrastriatal injection of 6-hydroxydopamine (6-OHDA) in rat, in order to evaluate the potential neuroprotective properties of this compound.**

METHODS

ANIMAL MODEL AND EXPERIMENTAL DESIGN

Male Sprague-Dawley rats (N=8/group) were treated for 28 days with CBD (10 mg/kg/day, in Tween 80-saline 1:16) or vehicle (Tween 80-saline 1:16, control) starting on the day of surgery (6-OHDA injection in the right striatum).

NIGROSTRIATAL DEGENERATION

The nigrostriatal lesion was assessed by IHC on PFA-fixed coronal brain sections (40 µm) for the neuronal marker for tyrosine hydroxylase (TH) (rabbit anti-TH (1:2000), Chemicon).

Striatum: The striatal degeneration was expressed as the percentage of striatal volume deprived of TH immunoreactivity, with respect to the entire striatal volume.

SNc: Counting of TH+ cell bodies in the SNc was performed bilaterally on every four sections throughout the entire nucleus, and the result was expressed as percentage of lesion compared to the non-lesioned side.

BEHAVIORAL EVALUATION

The effects of CBD on motor behavior of 6-OHDA lesioned rats were evaluated using the following tests:

Cylinder test: The test evaluates the asymmetry in the use of the front limbs to support the weight of the body against the walls of a cylindrical container, during the exploratory behavior. The number of contacts made by left (controlateral to the lesion) or right (ipsilateral to the lesion) front limb with the cylinder wall was counted for 5 min; the limb preference (*P*) was calculated by the following equation:

$$P = \frac{Ipsilateral}{Ipsilateral+controlateral} - \frac{Controlateral}{Ipsilateral+Controlateral}$$

This test was performed before (baseline motor activity) and after 6-OHDA injection.

Apomorphine-induced rotational test: This test is widely used as a functional index of nigrostriatal lesion. The rotational motor behavior in response to apomorphine injection (0.5 mg/kg, i.p.) was evaluated using an automatic rotameter for 30 min. The response was calculated by subtracting the total number of ipsilateral rotations from the total number of contralateral rotations.

Rotarod test: This test is widely used to evaluate the motor coordination of rodents. On test day, animals received three trials on accelerated (4-20 rpm, 180 sec) or constant (12 rpm, 120 sec) road with a resting time of 5 min between each trial. Average falling time of three trials was calculated.

NEUROINFLAMMATION

The neuroinflammatory process was evaluated by IF on PFA-fixed coronal brain sections (40 µm) as follows:

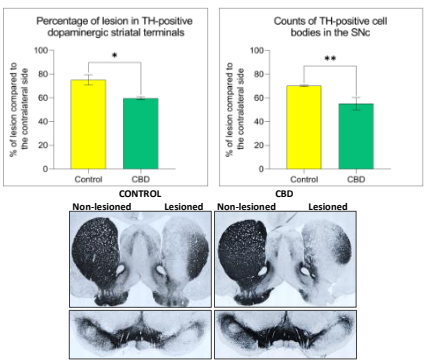
Microglial and astrocytes activation was assessed by counting the number of CD11b+ and GFAP+ cells on three different SNc sections (mouse anti-CD11b (1:300), BIORAD; mouse anti-GFAP (1:1000), SIGMA).

Microglia and astrocytes polarization was assessed by counting the number of CD11b+/CD32+ or CD11b+/CD206+ microglia cells and GFAP+/CD32+ or GFAP+/CD206+ astrocyte cells on three different SNc sections (rabbit anti-CD32/CD206, (1:300) Santa Cruz).

Skeleton Analysis: The microglia process length and number of endpoint were quantified using skeleton plugin in FIJI (NIH, Bethesda, MD, USA). The results obtained were express as branch length (µm)/cell and number of endpoints/cell.

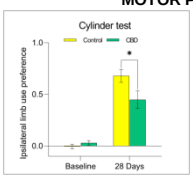
RESULTS

NEURODEGENERATION

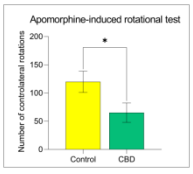


Animals treated with CBD showed a significant reduction of the striatal terminal degeneration and cell body loss in the SNc in comparison with control group (*p<0.05; **p<0.01, Student t-test).

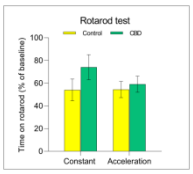
MOTOR PERFORMANCE



Animals treated with CBD showed lower preference in the use of the ipsilateral anterior limb compared to the control group (*p<0.05, Student t-test);



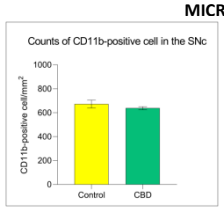
Animals treated with CBD showed a reduction of the number of contralateral rotations induced by apomorphine compared to the control group (*p<0.05, Student t-test).

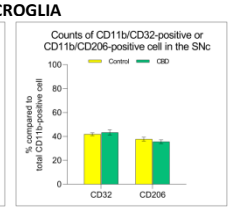


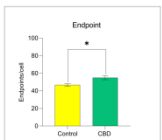
No differences on motor performance evaluated by rotarod test were observed between groups.

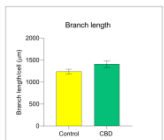
NEUROINFLAMMATION

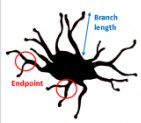
MICROGLIA



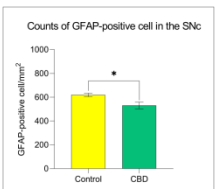


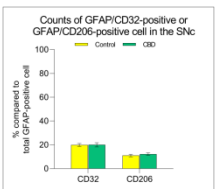


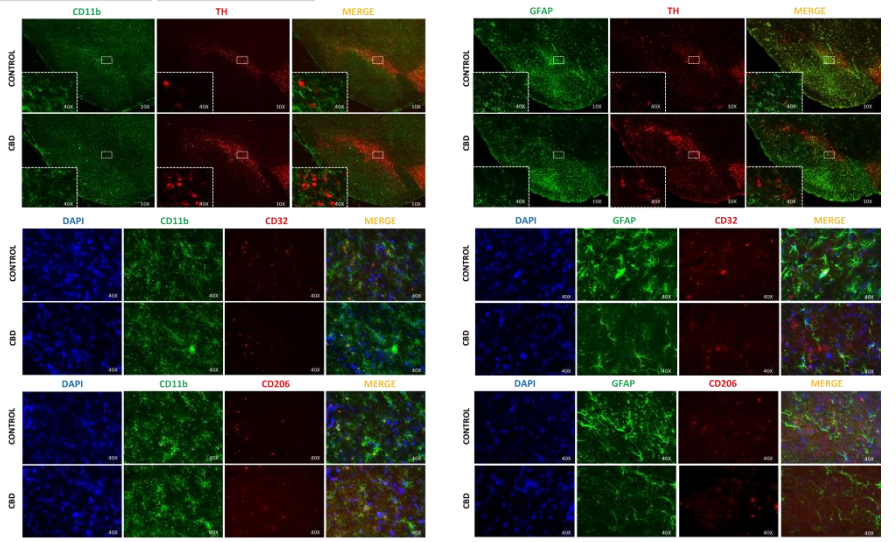




ASTROCYTES







Animals treated with CBD showed a reduction in the number of GFAP⁺ cells/mm² in the SNc (*p<0.05, Student t-test). No differences in the number of CD11b⁺ cells/mm² and in glial cell polarization towards the cytotoxic M1/A1 or cytoprotective M2/A2 phenotype were observed in animals treated with CBD compared to controls. Animals treated with CBD showed a reduction in the number of microglia endpoint, without differences in the microglia branch length.

CONCLUSION

These results further confirm that CBD may have therapeutic potential in PD and suggest intriguing symptomatic properties of this drug. Further analyses are needed to clarify if CBD exerts neuroprotective effects in PD by modulating neuroinflammation or through other mechanisms (i.e. antioxidant action).