Neuropharmacology 116 (2017) 151–159

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Cannabidiol reduces brain damage and improves functional recovery in a neonatal rat model of arterial ischemic stroke

Maria Ceprián [a, b], Laura Jiménez-Sánchez [b], Carlos Vargas [b], Lorena Barata [b], Will Hind [c], Jose Martínez-Orgado [b, d, *]

[a] Department of Biochemistry and Molecular Biology III, CIBERNED, IRICYS, School of Medicine, Universidad Complutense de Madrid, Spain
[b] Instituto de Investigación Puerta de Hierro Majadahonda, Madrid, Spain
[c] GW Research Ltd, Cambridge, UK
[d] Department of Neonatology, Hospital Clínico San Carlos – Instituto de Investigación Sanitaria San Carlos, Madrid, Spain

ARTICLE INFO

Article history:
Received 18 November 2016
Received in revised form
14 December 2016
Accepted 19 December 2016
Available online 21 December 2016

Keywords:
Cannabidiol
Neuroprotection
Newborn
Stroke
Rats

ABSTRACT

Background: and purpose: Currently there is no effective treatment for neonatal arterial ischemic stroke (AIS). Cannabidiol (CBD) is neuroprotective in models of newborn hypoxic-ischemic brain damage and adult stroke. The purpose of this work was to study the protective effect of CBD in a neonatal rat model of AIS.

Methods: Middle Cerebral Artery Occlusion (MCAO) was achieved in neonatal Wistar rats by introducing a nylon filament to the left MCA for 3 h; 15 min after removing the occluder vehicle (MCAO-V) or CBD single dose 5 mg/kg (MCAO-C) were administered i. p. Similarly manipulated but non-occluded rats served as controls (SHM). A set of behavioral tests was then conducted one week (P15) or one month (P38) after MCAO. Brain damage was then assessed by magnetic resonance imaging (MRI), proton magnetic resonance spectroscopy (H^+-MRS) and histologic (TUNEL for cell death, immunohistochemistry for neuron, astrocyte and microglia identification) studies.

Results: CBD administration improved neurobehavioral function regarding strength, hemiparesis, coordination and sensorimotor performance as assessed at P15 and P38. MRI indicated that CBD did not reduce the volume of infarct but reduced the volume of perilesional gliosis. H^+-MRS indicated that CBD reduced metabolic derangement and excitotoxicty, and protected astrocyte function. Histologic studies indicated that CBD reduced neuronal loss and apoptosis, and modulated astrogliosis and microglial proliferation and activation.

Conclusions: CBD administration after MCAO led to long-term functional recovery, reducing neuronal loss and astrogliosis, and modulating apoptosis, metabolic derangement, excitotoxicity and neuroinflammation.

1. Introduction

Brain damage due to arterial ischemic stroke (AIS) is usually considered an adult condition, however, the neonatal period is a time of particular susceptibility and risk (Kratzer et al., 2014); one out of 4000 live newborns suffer from perinatal AIS (PAIS) although just 25% are diagnosed at that time (Armstrong-Wells and Ferriero, 2014; Rutherford et al., 2012). The complex pathophysiology of ischemic brain damage may be the reason for the scant effectiveness of any treatment acting just on some of the determining factors. Thus, only therapies encompassing multiple mechanisms can be really effective. However hypothermia, considered the gold standard for neuroprotection after global hypoxic-ischemic immature brain damage, has resulted in mild and yet contradictory results in animal models of PAIS (Harbert et al., 2011). More promising are the results from studies on the neuroprotective effects of erythropoietin (Epo) or melatonin, which in rat models of PAIS demonstrate reduced brain damage and enhanced

Abbreviations: CBD, cannabidiol; *CRT*, cylinder rearing test; *H^+-MRS*, proton magnetic resonance spectroscopy; *MCAO*, middle cerebral artery occlusion; *MRI*, Magnetic resonance imaging; *NOR*, novel object recognition; *PAIS*, perinatal arterial ischemic stroke.

* Corresponding author. Head of the Department of Neonatology – INA, Hospital Clínico "San Carlos" – IdISSC, Profesor Martin Lagos s/n, 28040 Madrid, Spain.
E-mail address: jose.martinezo@salud.madrid.org (J. Martínez-Orgado).

http://dx.doi.org/10.1016/j.neuropharm.2016.12.017
0028-3908/

neurogenesis and remyelinization (Chang et al., 2005; Gonzalez et al., 2013, 2009; Villapol et al., 2011).

Together with all these, cannabidiol (CBD) emerges as a solid candidate for multifactorial treatment of ischemic immature brain damage (Alvarez et al., 2008; Castillo et al., 2010; Lafuente et al., 2011; Pazos et al., 2013, 2012). CBD demonstrates robust antioxidant and anti-inflammatory activity, and also reduces glutamate release, stabilizes the mitochondrial membrane, augments extracellular concentration of adenosine and prevents NF-κB activation (Castillo et al., 2010; Mechoulam et al., 2007; Pertwee, 2004). All these properties contribute to the neuroprotective potential of CBD for AIS, which was initially demonstrated in adult mice models of stroke where CBD reduces the area of infarct even when administered more than 24 h post-insult, at least in part by increasing intra-lesional blood flow (Hayakawa et al., 2010). However, there are no studies on the neuroprotective potential of CBD in the PAIS models to date.

The aim of this work was to study the neuroprotective potential of CBD in a neonatal rat model of PAIS.

2. Methods

The experimental protocol met European and Spanish regulations for protection of experimental animals (86/609/EEC and RD 53/2013) and was approved by the Ethics Committee for Animal Welfare from the University Hospital Puerta de Hierro Majadahonda (Madrid, Spain). The number of animals used was determined to be the minimum number necessary to attain statistical significance in the reduction of brain volume as assessed by magnetic resonance imaging (MRI).

2.1. Experimental model

The model is an adaptation to 7-to-9 day-old Wistar rats of the temporary middle cerebral artery occlusion (MCAO) model in adult rodents (Derugin et al., 1998).

Pups from each litter were randomly assigned to MCAO or control. All experimental groups were gender balanced. Each MCAO animal was anesthetized by sevoflurane (5% induction, 1% maintenance). The left carotid artery was dissected up to the internal and external branches division to introduce a nylon filament 0.21 mm in diameter, that was progressed 8.5–9 mm through the internal carotid artery until occlusion of the left MCA. All procedures lasted less than 30 min during which rectal temperature was kept at 38 ± 0.5 °C using a servo-controlled heat mattress. The occlusion was maintained for 3 h. Pups were anesthetized similarly to carefully remove the filament. Then, after sealing carotid and skin wounds the pup returned to the dam. Control group pups (SHM) were similarly managed but without MCAO.

2.2. Treatment

Thirty minutes after the end of MCAO pups were randomly assigned to receive vehicle (MCAO-V) or CBD (MCAO-C) both provided by GW Research Ltd (Cambridge, UK). CBD was obtained from a formulation containing CBD 3 mg/mL and further diluted in saline to administer 0.2 mL i. p. to each pup. After testing different doses (1, 5, 10, 50 or 100 mg/kg) 5 mg/kg was the CBD dose selected for further experiments as it revealed the best combination of major efficacy with few side effects.

2.3. Functional studies

All tests were video-recorded to be assessed by three different examiners blinded to the experimental group.

Seven days (P15) after MCAO (or equivalent period) three motor tests were performed (Bona et al., 1997; Bouet et al., 2010; Fan et al., 2005; Ten et al., 2003) (SHM, n = 15; MCAO-V, n = 16; and MCAO-C, n = 13):

- Negative geotaxis: time required to turn 180° after being placed heading down on a ramp tilted at 45°.
- Wire hang test: time to fall down after being suspended with both forelimbs on a wire.
- Grip: grasp reflex score after leaning a thin rod against each paw palm: 2 = digit flexion; 1 = delayed response (more than 2 s) or partial flexion; 0 = digit extension.

At thirty days (P38) after MCAO (or equivalent period), three sensorimotor and one cognitive test were performed (Bouet et al., 2010; Goldstein, 2003; Pazos et al., 2012; Piot-Grosjean et al., 2001) (SHM, n = 13; MCAO-V, n = 18; and MCAO-C, n = 13):

- Beam test: time to cross and foot faults by each hind limb during crossing a beam 1 m-long with a flat surface 1 cm wide held at a 50 cm height.
- Cylinder rearing test (CRT): initial forepaw (left, right, or both) preference after placing the rat in a methacrylate transparent cylinder 20 cm in diameter and 30 cm in height (2 min trial with a minimum of 4 wall contacts).
- Adhesive removal test: time to detect and then remove two adhesive tapes of equal size applied on the palm of each forepaw.
- Novel object recognition (NOR): time spent on exploration of the familiar and the novel object after being allowed to explore a methacrylate box (40 × 40 × 35 cm) containing two identical objects and then returned to the box replacing one of the original objects with a new one.

2.4. Sampling

After the functional test, rats were sacrificed by lethal injection of diazepam and ketamine and transcardially perfused with cold formaline (4%). Brains were then harvested and stored in 4% formalin to perform MRI and histologic studies or snap frozen in isopentane and stored at −80 °C to perform Proton Magnetic Resonance Spectroscopy (H^+-MRS) studies.

2.5. Measurement of the extent of brain injury by MRI

Brain MRI scans were performed in the MRI Unit of the Instituto de Investigaciones Biomédicas “Alberto Sols”, (CSIC-UAM, Madrid, Spain) on a BIOSPEC 70/16 (Bruker-Medical, Ettlingen, Germany) as reported elsewhere (Pazos et al., 2012).

Using the ImageJ 1.43u software (U.S. National Institute of Health), the entire brain volume and volume of increased intensity area were calculated as reported elsewhere (Pazos et al., 2012).

2.6. H^+-MRS

Ex vivo 1H spectra were performed on a Bruker Avance 11.7 T spectrometer (Bruker BioSpin, Karlsruhe, Germany) equipped with a 4-mm triple channel 1H/13C/31P HR-MAS (High Resolution Magic Angle Spinning) resonance probe at the MRI Unit of the Instituto Investigaciones biomédicas “Alberto Sols” to calculate several ratios, including: lactate/N-acetylaspartate (Lac/NAA), glutamate/N-acetylaspartate (Glu/NAA) and myoinositol/creatine (mI/Cr) ratios, as reported elsewhere (Pazos et al., 2013, 2012).