Neuroprotection and reduction of glial reaction by cannabidiol treatment after sciatic nerve transection in neonatal rats

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Abstract

In neonatal rats, the transection of a peripheral nerve leads to an intense retrograde degeneration of both motor and sensory neurons. Most of the axotomy-induced neuronal loss is a result of apoptotic processes. The clinical use of neurotrophic factors is difficult due to side effects and elevated costs, but other molecules might be effective and more easily obtained. Among them, some are derived from Cannabis sativa. Cannabidiol (CBD) is the major non-psychotropic component found on the surface of such plant leaves. The present study aimed to investigate the neuroprotective potential of CBD. Thus, 2-day-old Wistar rats were divided into the following experimental groups: sciatic nerve axotomy + CBD treatment (CBD group), axotomy + vehicle treatment (phosphate buffer group) and a control group (no-treatment group). The results were analysed by Nissl staining, immunohistochemistry and terminal deoxynucleotidyl transferase dUTP nick end labeling at 5 days post lesion. Neuronal counting revealed both motor and sensory neuron rescue following treatment with CBD (15 and 30 mg/kg). Immunohistochemical analysis (obtained by synaptophysin staining) revealed 30% greater synaptic preservation within the spinal cord in the CBD-treated group. CBD administration decreased the astroglial and microglial reaction by 30 and 27%, respectively, as seen by glial fibrillary acidic protein and ionised calcium binding adaptor molecule 1 immunolabeling quantification. In line with such results, the terminal deoxynucleotidyl transferase dUTP nick end labeling reaction revealed a reduction of apoptotic cells, mostly located in the spinal cord intermediate zone, where interneurons promote sensory–motor integration. The present results show that CBD possesses neuroprotective characteristics that may, in turn, be promising for future clinical use.

Introduction

The immature central nervous system is very sensitive to injury, so that tissue repair does not occur or is incomplete, leading to irreversible sequelae (Moran & Graeber, 2004). Peripheral nerve axotomy is a well-established model that allows understanding of nervous system regeneration mechanisms and limitations, particularly during the neonatal phase (Svensson & Aldskogius, 1993; Tiraishi & Rezaie, 2004). Such lesions retrogradely affect the cell body as well as the surrounding microenvironment of motor and sensory neurons, triggering several changes (Moran & Graeber, 2004). Among these, chromatolysis is a hallmark feature (Aldskogius & Svensson, 1993), together with synaptic loss (Cullheim et al., 2002; Oliveira et al., 2002; Tiraishi & Rezaie, 2004). Simultaneously, close to the lesioned neurons, the glial cells become reactive (Derouiche & Frotsher, 2001), which has been related to the acceleration of neuronal and synaptic loss (Hermansson et al., 1995; Koyama & Baba, 1999; Rogers et al., 2003; Moran & Graeber, 2004; DeLeo et al., 2006).

The immature neurons are still morphologically and functionally undeveloped (Lowrie et al., 1994), being dependent on appropriate neurotrophic factor supply from the target (Levi-Montalcini, 1987; Oppenheim, 1991; Tiraishi & Rezaie, 2004; Huh et al., 2008). In this regard, many therapeutic approaches have been used to avoid neuronal death. Thus, the use of several molecules with neuroprotective properties, such as neurotrophic factors, has been tested after injury, during the regenerative process. Although effective, clinical use of such substances has been delayed due to side effects as well as high production costs (Gold, 1997; Mohiuddin et al., 1999; Matsuura et al., 2013).
Among alternative substances that may have neuroprotective capabilities, cannabinoids have emerged as candidates for the treatment of several conditions (Pertwee, 2004; Mechoulam et al., 2007; Hayakawa et al., 2010). Cannabidiol (CBD) is the major non-psychoactive component derived from Cannabis sativa and its use has been reported as positive in various neurodegenerative pathologies (Braid et al., 2003; Pertwee, 2004; Mishima et al., 2005; Mechoulam et al., 2007; Sagredo et al., 2007; Hayakawa et al., 2008; Castillo et al., 2010; Pazos et al., 2012). CBD can enhance endocannabinoid action by inhibiting anandamide uptake and metabolism (Fernández-Ruiz et al., 2012). It is also a potent anti-inflammatory and antioxidant (Hampson et al., 1998; Pertwee, 2004; Mechoulam et al., 2007).

The neuroprotective action of CBD has already been examined in cortical neuron cultures exposed to toxic levels of glutamate. The results showed that CBD decreased glutamate excitotoxicity (Hampson et al., 1998). Additional data also showed that CBD treatment prevents cell death by necrosis and apoptosis and modulates inflammatory processes, including the decrease of nitric oxide synthesis (Alvarez et al., 2008; Lafuente et al., 2011).

Considering the above-mentioned positive effects of CBD, the present study investigated the survival of dorsal root ganglia (DRG) neurons and motoneurons, preservation of spinal cord circuits and reduction of glial reactivity following sciatic nerve transection in neonatal rats. The results show a significant rescue of DRG neurons, spinal motoneurons and pre-synaptic terminals, coupled with reduction of astroglisis and apoptosis, reinforcing the possible future clinical usefulness of CBD.

Materials and methods

Animals

Neonatal Wistar rats (2 days old) were obtained from the Multidisciplinary Center for Biological Investigation (Multidisciplinary Center for Biological Investigation/UNICAMP) and housed using a 12 h light/dark cycle and controlled temperature (23 °C), with free access to food and water. The study was approved by the Institutional Committee for Ethics in Animal Experimentation (Institutional Committee for Ethics in Animal Experimentation/IB/UNICAMP, proc. no. 2371-1) and the experiments were performed in accordance with the guidelines of the NIH and the Brazilian College for Animal Experimentation. The animals were subjected to unilateral sciatic nerve transection at mid-thigh and divided into three groups: axotomy alone, axotomy + vehicle treatment (saline phosphate buffer with 2% tween) (Ren et al., 2009; Kwiatkosi et al., 2012; Gomes et al., 2013) and axotomy + CBD treatment. The animals were killed with anesthetic overdose at 5 days post-lesion (post-natal day 7) and their lumbar spinal cords (n = 6 for each group) and L5 DRG (n = 5 for each group) were processed for neuronal survival counting, immunohistochemistry (n = 5 for each group) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) reaction (n = 4 for each group).

Surgical procedures and cannabidiol administration

The animals were anesthetised by hypothermia, being immersed up to the neck in crushed ice for 5 minutes (1 °C–2 °C). They were then subjected to a left sciatic nerve transection. A 2-mm-long segment of the distal stump was removed to avoid regeneration. CBD (C21H30O2, cat. no. THC-1073, THC Pharm, Germany) was diluted in saline phosphate buffer with 2% tween. In sequence, the rats were intraperitoneally injected with 5, 15 or 30 mg/kg of CBD. CBD administration was repeated every 24 h for 5 days, at which point the animals were killed. All doses were evaluated in terms of neuro-protection by Nissl-stained motoneuron counting. Only the 15 mg/kg dose was used for immunohistochemistry and TUNEL.

Tissue preparation

At post-natal day 7, all animals received an overdose of a mixture of Kensor (xylasim, 10 mg/kg, Köning, Argentina) and Vetaset (cetamin, 50 mg/kg, Fort Dodge, USA), and were subjected to transcardial perfusion with 0.1 M phosphate-buffered saline (PBS), followed by fixative (10% formaldehyde in 0.1 M PBS, pH 7.4). The lumbar intumescences and L5 DRG were dissected out, post-fixed for 12 h in the same fixative solution and then washed with phosphate buffer and stored in 10, 20 and 30% sucrose, for 12 h each, before freezing in liquid nitrogen at −25 °C. Cryostat transverse sections (12 µm thick) of the spinal cords and DRG were obtained and transferred to gelatin-coated slides, dried at room temperature for 30 min and stored at −20 °C until use.

Immunohistochemistry

Spinal cord sections (12 µm thick) were obtained in a cryostat (HM525, Microm, USA) and incubated with the following primary antibodies: mouse anti-synaptophysin (Dako, 1 : 100), rabbit anti-glia fibrillary acidic protein (Abcam, 1 : 1500) and rabbit anti-ionised calcium binding adaptor molecule 1 (Dako, 1 : 700).

With regard to specificity, the anti-human synaptophysin labels a single band of 38 kDa, corresponding to the protein P38 (Navone et al., 1986; Buffa et al., 1988). The immunolabeling pattern observed in the present study was similar to that observed in other studies, providing a characteristic punctuate labeling (Essrich et al., 1998; King et al., 2006). The anti-glia fibrillary acidic protein identifies a single band of 52 kDa on immunoblotting (Farjo et al., 2007). The antibody used herein provided the expected immunostaining pattern of astroglial cells, compatible with previous publications (Bulloch et al., 2008). Ionised calcium binding adaptor molecule 1 (a calcium-binding protein) antiserum has previously been characterised in the rat brain displaying a typical microglia staining (Ito et al., 1998). The same immunolabeling pattern was recognised in the present work.

Sections were incubated overnight in a moist chamber at 4 °C. The primary antisera were diluted in a solution containing bovine serum albumin and Triton X (0.3%) in 0.01 M PBS. After rinsing, the secondary antibodies were applied and incubated for 45 min at room temperature (23 °C), according to the host of the primary antibodies (CY-2 and CY-3, 1 : 250, Jackson Immunoresearch). The sections were then rinsed in phosphate buffer, mounted in a mixture of glycerol/phosphate buffer (3 : 1) and examined using a fluorescence microscope (TS-100, Nikon, Tokyo, Japan) equipped with a CCD camera (DMX1200F, Nikon). For quantitative measurements, three representative images of the ipsilateral and contralateral ventral horn were captured from each animal for all experimental groups. For analysis of synaptophysin immunolabeling, the integrated density of pixels was measured in eight areas surrounding each lateral motor nucleus motoneuron, in the anterior horn of the spinal cord. For the analysis of anti-glia fibrillary acidic protein and anti-ionised calcium binding adaptor molecule 1 antibodies, the integrated density of pixels was measured in a fixed area, in the lateral region of the spinal cord ventral horn, as described by Oliveira et al. (2004) and Freire et al. (2010, Supporting Information). Quantification was performed.
with IMAGEJ software (version 1.33u, National Institutes of Health, USA). The integrated density of pixels was measured for each animal and then a mean value ± SE was calculated for each group.

**Survival counting of spinal motoneurons and dorsal root ganglia neurons**

Cell counting was performed in 12 μm alternate sections obtained from the lumbar intumescences (n = 6 animals/group) and DRG (n = 5 animals/group) at 5 days after surgery. Sections were stained with cresyl violet (Nissl staining) for 50 s, dehydrated, diaphanised and the specimens mounted with Entellan (Merck). The motoneurons were localised based on the morphology and ventral horn localisation (dorsolateral region, lamina IX). In spinal cord sections, cell counting was performed in approximately 20 sections on both the ipsilateral and contralateral sides. In DRG sections, cell counting was performed in five adjacent sections on both the ipsilateral and contralateral sides. Only cells with a visible nucleus and nucleolus were counted. To correct the double counting of neurons, the Abercrombie formula (Abercrombie & Johnson, 1946) was used

\[
N = \frac{nt}{(t + d)}
\]

where \(N\) is the corrected number of counted neurons, \(n\) is the counted number of cells, \(t\) is the thickness of between sampled sections (48 μm for spinal cord and 36 μm for DRG) and \(d\) is the average diameter of the cells. As neuronal size significantly affects cell counts, the value of \(d\) was calculated specifically for each experimental group. For this purpose, the diameter of neurons was measured in 15 randomly chosen neurons (per group), using IMAGE TOOL software (version 3.0, The University of Texas Health Center, TX, USA) and the mean value was calculated.

**Staining of apoptotic cells (terminal deoxynucleotidyl transferase dUTP nick end labeling)**

The TUNEL reaction was carried out according to the literature (Lawson et al., 1997; Oliveira et al., 1997; Lawson & Lowrie, 1998). Frozen spinal cord sections were post-fixed in ethanol/acetic acid (2 : 1) for 5 min at −20 °C and rinsed twice for 5 min in

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**Fig. 1.** Neuronal survival at 5 days after post-natal day 2 sciatic nerve transection. (A and B) Contralateral (CL) and ipsilateral (IL) sides, respectively, of the axotomy-alone group. (C and D) CL and IL sides, respectively, of the vehicle group. (E and F) 5 mg/kg CBD group. (G and H) 15 mg/kg CBD group. (I and J) 30 mg/kg CBD group. (K) Percentage of neuronal survival after sciatic nerve transection. The group treated with 15/30 mg/kg CBD displayed significantly increased neuronal survival compared with untreated groups (**p = 0.0001). Scale bar = 50 μm. **p < 0.01.
PBS. The slides were transferred to a humid chamber and the equilibration buffer solution was applied (s7110-1, Chemicon) and incubated for 5 min at room temperature. The equilibration buffer was shaken off and the reaction buffer, containing the TdT enzyme (s7110-4, Chemicon), was applied for 30 min at 37 °C. After washing with PBS for 10 min, the sections were incubated with the fluorescein solution (s7110-5, Chemicon) for 30 min. The slides were then rinsed in PBS and mounted with glass coverslips in a mix of glycerol/PBS (3 : 1). Images were obtained using a fluorescence microscope (Eclipse TS100, Nikon) equipped with a digital camera (DXM1200F, Nikon). In order to ensure the presence of TUNEL-positive neurons, double labeling with neuronal nuclei (Abcam, USA; 1 : 500) was carried out and visualised in a DM5500B epifluorescence microscope (Leica) equipped with a DFC345FX camera and LAS AF software (version 4.2).

**Statistical analysis**

Statistical analyses were performed by using ANOVA (one-way ANOVA) with Bonferroni post-test, and two-way repeated-measures ANOVA. Differences between groups were considered significant when the P-value was < 0.05 (*); P < 0.01 (**) and P < 0.001 (***)

**Results**

**Motoneuron survival**

The neuronal survival was analysed after sciatic nerve transection and compared between the experimental groups through the counting of motor neurons in spinal cord, both ipsilateral and contralateral to the lesion. No statistical differences were observed in the number of motoneurons found on the contralateral side from all experimental groups. However, there was a reduction of the motoneuron population, in all experimental groups, at 5 days after sciatic nerve transection on the ipsilateral side. Figure 1 represents the neuronal survival percentage in all experimental groups. As shown, a statistically significant CBD neuroprotective effect was observed (at 15 and 30 mg/kg), leading to a 21% increase in neuronal survival (59.9% surviving neurons; $F_{4,20} = 18.62, P < 0.0001$, Bonferroni post-test) when compared with the non-treated, vehicle and 5 mg/kg CBD groups (neuronal survival, respectively: 34.9, 36.5, 37.3%; $P < 0.001$).

![Fig. 2. Dorsal root ganglia (DRG) sensory neuron survival at 5 days after post-natal day 2 sciatic nerve transection. (A and B) Contralateral (CL) and ipsilateral (IL) sides, respectively, of the vehicle group. (C and D) CL and IL sides, respectively, of the 15 mg/kg CBD group. (E) Counting of sensitive neuronal survival after sciatic nerve transection. The group treated with CBD displayed a greater number of sensitive neurons when compared with the CL side ($P = 0.026$), indicating a decrease of programmed cell death. The group treated with CBD displayed significantly increased neuronal survival after axotomy compared with the vehicle group ($**P = 0.001$). Scale bar = 200 μm.](image)
Dorsal root ganglia neuron survival

Sensitive neuronal survival was investigated by the counting of L5 DRG neurons on both the ipsilateral and contralateral sides (Fig. 2A–D). CBD treatment led to a 10% increase in neuronal preservation ($F_{1,8} = 7.43, \ P < 0.05$) on the contralateral side of the experimental groups (Fig. 2E). As shown in the same graph, a statistically significant CBD neuroprotective effect was observed in the ipsilateral : contralateral ratio, leading to a 23.2% increase in neuronal survival when compared with the vehicle group (neuronal survival: CBD treated: 78.6%; vehicle: 60.3%; $F_{1,8} = 28.10, \ P < 0.05$, repeated-measures ANOVA; Fig. 2E).

Fig. 3. Immunohistochemistry of anti-synaptophysin in the ventral horn of the spinal cord at 5 days after post-natal day 2 sciatic nerve transection. (A and B) Contralateral (CL) and ipsilateral (IL) sides, respectively, of the axotomy-alone group. (C and D) CL and IL sides of the vehicle group. (E and F) 15 mg/kg CBD group. (G) Triple labeling with synaptophysin (Syph), neuronal nuclei (NeuN) and 4′,6-diamidino-2-phenylindole, dilactate (DAPI), indicating the motoneuron and interneuron cell bodies at lamina IX. (H) Quantification of the synaptic covering at lamina IX at 5 days after lesion. A significant preservation of synapses, in the group treated with 15 mg/kg CBD ($**P = 0.0003$), can be observed. Scale bar = 50 μm. **$P < 0.01$. 

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Synaptophysin, glial fibrillary acidic protein and ionised calcium binding adaptor molecule 1 immunoreactivity

Synaptophysin is a protein localised in pre-synaptic vesicles that can be used as a marker for synaptic covering evaluation. Figure 3 shows a reduction of immunoreactivity after lesion on the ipsilateral side of all experimental groups. The comparison between groups, however, revealed that synaptophysin expression was 30% more preserved in the CBD-treated group (15 mg/kg CBD, 77.50%; axotomy alone, 53.88%; axotomy + vehicle, 62.74%; $F_{2,13} = 31.62$, $P < 0.001$, Bonferroni post-test).

Glial fibrillary acidic protein is a component of astrocyte intermediate filaments and was used to analyse the astroglial reactivity. There was no statistical difference between the experimental groups when contralateral sides were compared. However, CBD treatment reduced the astrogliosis by 30% on the ipsilateral side, when com-

Fig. 4. Immunohistochemistry of anti-glial fibrillary acidic protein (GFAP) in the ventral horn of the spinal cord at 5 days after post-natal day 2 unilateral sciatic nerve transection. (A and B) Contralateral (CL) and ipsilateral (IL) sides, respectively, of the axotomy-alone group. (C and D) CL and IL sides of the vehicle group. (E and F) 15 mg/kg CBD group. (G) Quantification of the reactive astrogliosis at 5 days after lesion, in the lamina IX microenvironment. A significant reduction of the reactive astrogliosis in the group treated with 15 mg/kg CBD (*$P = 0.0149$) can be observed. Scale bar = 50 μm.
pared with untreated and vehicle-treated groups (15 mg/kg CBD, 31.14%; axotomy alone, 44.99%; axotomy + vehicle, 44.06%; $F_{2,16} = 5.535, P < 0.05$; Bonferroni post-test, Fig. 4).

Anti-ionised calcium binding adaptor molecule 1 immunolabeling was used to evaluate microglial reactivity, which is increased after injury. CBD treatment reduced by 27% the microglial reactivity when compared with non-treated and vehicle-treated groups (15 mg/kg CBD, 25.36%; axotomy alone, 35.77%; axotomy + vehicle, 34.17%; $F_{2,16} = 6.489, P < 0.01$; Bonferroni post-test, Fig. 5).

### Apoptotic cell detection

In neonatal rats, neuronal death through apoptosis is increased after axotomy, not only in the region where the injured neurons are

**Fig. 5.** Immunohistochemistry of anti-ionised calcium binding adaptor molecule 1 (Iba-1) in the ventral horn of the spinal cord at 5 days after post-natal day 2 unilateral sciatic nerve transection. (A and B) Contralateral (CL) and ipsilateral (IL) sides, respectively, of the axotomy-alone group. (C and D) CL and IL sides of the vehicle group. (E and F) 15 mg/kg CBD group. (G) Quantification of the microglial reaction at 5 days after lesion. Observe the significant reduction of immunoreactivity in the group treated with 15 mg/kg CBD. Scale bar = 50 μm. **P < 0.05.**
located but also in other spinal cord areas, mostly because of the intense interaction established between these cells, by forming intraspinal circuits. In this sense, we evaluated the number of cells in the apoptotic process in the ventral, intermediate and dorsal spinal cord regions. No differences were observed in the ventral and dorsal regions, but the 15 mg/kg CBD group showed a reduction of 25% in the number of apoptotic cells on the ipsilateral side at the intermediate region, when compared with the non-treated and vehicle-treated groups ($F_{3,12} = 6.800$, $P < 0.0063$, Bonferroni post-test). Also, the TUNEL/neuronal nuclei double labeling indicated that most of the apoptotic cells were neurons (Fig. 6).

**Discussion**

In the neonatal period, nerve damage results in acute retrograde changes that take place within the spinal cord, resulting in motoneuron and interneuron death soon after injury. Sensory neurons, present in the DRG, are equally affected. This is a result of the immature central nervous system stage, which is dependent on trophic support from the target (Lowrie et al., 1994).

Taking into consideration that neonatal experimental models are useful for the study of putative neurotrophic substances (Lieberman, 1971; Chen, 1978; Reisert et al., 1984; AldSkogius & Svensson, 1993), the present study used post-natal day 2 neonatal Wistar rats subjected to unilateral sciatic nerve transection as the experimental model to test the potential neuroprotective effects of CBD at different concentrations. This molecule has been proposed as a promising cannabinoid derivate, as it is non-psychotropic and yet possesses important pharmacological properties (Scuderi et al., 2009). For example, it has been shown to have anxiolytic properties at different doses. Also, relatively high doses of CBD (e.g. 10 × or more greater than used herein) have not shown important side effects (e.g. 212 mg/kg) in different species (for review, see Scuderi et al., 2009).

Wolff & Missler (1992) reported that transection of the sciatic nerve causes the degeneration of large percentage of spinal motoneurons in newborn rats, predominantly by apoptosis. Nevertheless, the programmed cell death of certain neurons also takes place during the first post-natal week and has to be taken into account (Wright et al., 1983; Oppenheim, 1991). This is in line with the present findings by the TUNEL technique, which revealed a number of putative neurons undergoing apoptosis in different locations of the spinal cord. Another important fact is that sciatic nerve axotomy leads not only to the death of spinal motoneurons, but also to the loss of interneurons, as shown in other studies (Oliveira et al., 1997). In our study, we also observed, by the TUNEL technique, the presence of cells undergoing apoptosis in other regions of the spinal cord, such as the dorsal and intermediate regions, where the presence of interneurons is characteristic. Remarkably, the group receiving CBD (15 mg/kg) showed a decreased number of cells undergoing apoptosis. In line with this, the DRG neuron death ratio improved.
from 39.7 to 21.4% (vehicle and CBD groups, respectively), indicating that CBD treatment possibly rescued neurons from both programmed death as well as axotomy-induced degeneration. The effects of CBD, however, seemed to be relatively milder on DRG neurons as compared with spinal neurons. Although we cannot provide a conclusive explanation for this, a possibility is that satellite cells present in the DRG are less responsive to CBD than astrocytes and microglial cells. In line with this, Schmidt et al. (2012) described the upregulation of CB1 and CB2 receptor in reactive astrocytes and microglial cells following cerebral ischemia. However, the work by Veress et al. (2013) indicates that satellite cells do not express detectable levels of CB1 receptor, although it can be found in sensory neurons. Also, CB2 receptor has recently been shown in DRG neurons by Hsieh et al. (2011). Nevertheless, no information is provided regarding satellite cells.

Cannabinoids may also have effects on synaptic plasticity, as already reported by Kim & Thayer (2001). In that study, using hippocampal neurons in culture, they have shown that cannabinomimetic drugs, such as WIN 55,212-2, prevented the recruitment of new synapses by inhibiting the formation of cyclic adenosine monophosphate. However, Tagliafierro et al. (2006) have demonstrated an increase in synaptophysin expression, as well as the number of synaptic vesicles, in the cerebellum and hippocampus. In this sense, our data also indicate that CBD strengthens synaptic contacts, decreasing the loss of inputs to lesioned motoneurons. This may have different implications, such as the preservation of neurotrophic factor levels, provided by pre-synaptic boutons and the maintenance of more complex intrasynaptic circuits.

It has been reported that apoptosis of axotomized motor neurons may be related to the synaptic elimination process, which is a characteristic reaction following axotomy (Wolff & Missler, 1992). Interestingly, in the present study, the group treated with CBD maintained more elevated immunoreactivity against synaptophysin, indicating a better synaptic preservation.

The mechanisms by which CBD exerts its neuroprotective effects are not well understood. One hypothesis is that it may be anti-inflammatory and antioxidant (Hampson et al., 1998; Pertwee, 2004; Mechoulam et al., 2007). Nevertheless, the effects of CBD may also be connected to cannabinoid CB1 and CB2 receptors (Howlett, 2002), which will be investigated in future studies in our laboratory.

Another important finding of the present work is related to the astroglial and microglial reaction. As seen by the immunohistochemistry, CBD administration reduced both the astroglial reactivity and microglial reaction. Interestingly, such glial cells have been correlated with synaptic elimination processes (Aldskogius & Svensson, 1993; Oliveira et al., 2002, 2004; Wekerle, 2005). Thus, the reduction of such glial reactivity may have an additional influence on neuronal rescue by the synaptic stabilization in the spinal cord microenvironment after peripheral injury.

As already mentioned, the occurrence of apoptosis is a hallmark of neonatal injury. It is possible that CBD treatment also influenced the occurrence of this process. It has been shown that CBD decreases the levels of caspase-9 (pro-apoptotic pathway) and reduces the expression of glutamate (glutamatergic excitotoxicity). Also, it reduces the concentrations of tumor necrosis factor-alpha, cyclo-oxygenase-2 and inducible nitric oxide synthase (Castillo et al., 2010). Such data reinforce the findings herein, which showed a decrease of apoptosis, particularly of neurons in the intermediate region of the spinal cord. Such neurons are specially related to the sensory–motor integration and have been shown to be influenced by the survival of motoneurons and sensory neurons (Oliveira et al., 2004). The results described above are also probably related to the reduction of microglial and astroglial reactivity, characteristic of the inflammatory process in the nervous system after injury.

Taken together, the present results demonstrate the neuroprotective and antiapoptotic roles of CBD following neonatal lesion to the peripheral nervous system. They are reinforced by the preservation of synapses within the spinal cord coupled with reduced astrogliosis. In this way, the data herein reinforce the possible clinical usefulness of this molecule for future strategies related to neuronal survival and regeneration, as well as to the decrease of astroglial reaction and inflammatory responses within the nervous system.

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Abbreviations

CBD, cannabidiol; DRG, dorsal root ganglia; PBS, phosphate-buffered saline; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

Conflict of interest

The authors declare that there is no conflict of interest.

References


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