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Original Research

Edaravone Inhibits Inflammation in Toll-Like Receptor 4-Stimulated PBMNCs from Multiple Sclerosis Patients

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Abstract

Background: Multiple sclerosis (MS) is a neurological disorder that is directly linked to inflammation in the central nervous system (CNS). The activation of toll-like receptors (TLRs) exacerbates neuroinflammation by increasing the production of reactive oxygen species (ROS) and proinflammatory cytokines. Edaravone (EDV) has been proposed as a potential therapy for CNS diseases because of its free radical scavenging and anti-inflammatory properties. This study investigated the effects of EDV on the inflammatory response in TLR4-stimulated peripheral blood mononuclear cells (PBMNCs) from MS patients and a healthy control group. **Methods**: The impact of EDV on ROS production in lipopolysaccharide (LPS)-stimulated PBMNCs was assessed using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) reduction and luminol-dependent chemiluminescence assays. The interleukin (IL)-6 concentration in the PBMNC supernatants was measured using enzyme-linked immunosorbent assay (ELISA). **Results**: The results showed that ROS production in PBMNCs stimulated using LPS (a TLR4 activator) was significantly inhibited ($p < 0.05$) by EDV in the MS patients and control group. Additionally, EDV significantly reduced IL-6 secretion in TLR4-stimulated PBMNCs in these groups ($p < 0.05$). No significant differences were observed between the groups. **Conclusion**: Our findings suggest that EDV may serve as an adjunctive therapy for MS by reducing ROS and IL-6 production in TLR4-stimulated PBMNCs in MS patients, highlighting its potential in modulating neuroinflammation and oxidative stress.

Keywords: edaravone; multiple sclerosis; toll-like receptors; reactive oxygen species; IL-6; inflammation

1. Introduction

Emerging evidence has highlighted the crucial role of the immune system in a wide range of neurological disorders. Indeed, neuroinflammation, the inflammatory response within the central nervous system (CNS), involves immune cells, such as microglia and astrocytes, becoming activated and infiltrating peripheral immune cells. Although this response is a normal mechanism aimed at maintaining homeostasis by repairing damage and eliminating pathogens, chronic or excessive inflammation can contribute to the pathogenesis of numerous neurological conditions [1–4]. Multiple sclerosis (MS) is a neurological disorder directly linked to CNS inflammation. In MS patients, the immune system targets and damages the myelin sheath, resulting in nerve damage and impaired neurological function; this process results in the gradual loss of motor, sensory, and cognitive functions. MS primarily affects young adults aged 20 to 50 and exhibits geographic variability, with a higher prevalence in Europe and North America. MS also presents a higher incidence in females, with a sex ratio of 3:1. However, males with MS often experience a more rapid disease progression and accumulate disabilities quicker than females [5–10]. The inflammatory response in MS involves both innate and adaptive immune systems. Subsequently, various pathways initiate inflammation, which promotes the activation of Toll-like receptors (TLRs), a class of pattern-recognition receptors (PRRs) expressed by innate immune cells [11,12]. TLR-mediated inflammatory responses occur by detecting pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). In MS patients, TLRs recognize myelin debris and CNS antigens, exacerbating inflammation and increasing blood–brain barrier (BBB) permeability, allowing immune cell infiltration, which worsens tissue damage [13,14]. TLR4 plays a key role in various inflammatory signaling pathways, and its overexpression can result in the development of neurological diseases [15]. Increased TLR expression in MS drives neuroinflammation, which promotes the production of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, and IL-6. This process also generates reactive oxygen species (ROS) and nitric oxide (NO), which contribute to oxidative stress [13,16–19].

ROS, generated primarily by the NADPH–oxidase (NOX) complex and mitochondria, play a crucial role in

the pathogenesis of MS. ROS activate diverse signaling pathways that drive inflammation, including the activation of nuclear factor-kappa B (NF-κB), which increases the production of proinflammatory cytokines and chemokines. Additionally, ROS contribute significantly to demyelination, axonal/neuronal injury, and the disruption of the BBB integrity in MS [20–22]. The development of novel therapeutic approaches has been driven by advancements in understanding immune responses and inflammatory mechanisms. Indeed, immunomodulatory and immunosuppressive drugs for MS, such as interferon-beta, glatiramer acetate, and monoclonal antibodies (e.g., natalizumab and ocrelizumab), target various aspects of the immune response to reduce inflammation and slow disease progression. However, some patients do not respond to these treatments or develop tolerance over time [23,24]. Moreover, oxidative stress has been implicated in various neurological conditions; meanwhile, current treatments do not directly address the signaling pathways involved in ROS generation. In this context, the free radical scavenger edaravone (EDV) has been studied for its neuroprotective properties and ability to reduce oxidative stress [24,25]. Originally approved in Japan for treating acute ischemic stroke, EDV has also shown promise in treating amyotrophic lateral sclerosis (ALS) [26–31]. Therefore, understanding the mechanisms through which EDV modulates ROS production and cytokine secretion in TLR4-stimulated peripheral blood mononuclear cells (PBMNCs) could significantly affect inflammation management in MS and other related pathologies. Thus, this study aimed to evaluate the effects of EDV on TLR4-stimulated PBMNCs obtained from MS patients and a healthy control group.

2. Material and Methods

2.1 Study Population

This parallel group cross-sectional study was approved by the Ethics Committee of Santa Casa Hospital in Belo Horizonte, Brazil (approval number 69385917.7.0000.5138). All participants provided informed consent. This study included 30 MS patients and 30 healthy controls. None of the participants with MS smoked, and all were receiving immunotherapy. The exclusion criteria included pregnancy, dementia, malignancy, infections, and substance dependence.

2.2 Preparation of PBMNCs

PBMNCs were isolated from peripheral venous blood using a modified Ficoll–Hypaque density gradient method described by Bicalho *et al.* [32]. Cell suspensions were adjusted to a concentration of 1×10^6 cells/mL, and viability was confirmed (>90%) using the trypan blue (Trypan Blue solution, 1%, T6146, Merck KGaA, Darmstadt, Germany) exclusion assay.

2.3 Assessment of ROS Production

2.3.1 MTT Reduction Method

A direct 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) reduction assay was used to measure intracellular ROS levels, reflecting the actions of both mitochondria and NADPH oxidase. MTT is a yellow tetrazolium salt dehydrogenase that is reduced in isopropanol to form soluble purple formazan crystals. The absorbance values after the reaction were analyzed spectrophotometrically at 570 nm [33]. PBMNC suspensions (5×10^5 cells/mL) were incubated at room temperature (23 °C) for 30 min or under four different conditions at 37 °C for 24 h: (i) phosphate-buffered saline (PBS, control without stimulation), (ii) EDV (3-methyl-1-phenyl-2-pyrazolin-5-one; cat. #M70800 Merck KGaA; 1 µM) [29], (iii) lipopolysaccharides (LPS from *Escherichia coli*; cat. #5010 Merck KGaA, Darmstadt, Germany; 50 µg), and (iv) LPS + EDV (to evaluate the influence of EDV on ROS production in TLR4-stimulated cells). Next, 30 µL MTT solution (cat. #M2128 Merck KGaA, Darmstadt, Germany; 5 mg/mL) was added, and cells were incubated at 37 °C for 2 h. Subsequently, 1.5 mL isopropanol-HCl was added to each tube, and samples were vortexed vigorously. Following centrifugation at 3000 rpm for 5 min, the supernatant absorbance was measured at 570 nm using an Ultrospec 2100 Pro spectrophotometer (GE Healthcare, Chicago, IL, USA). Experiments were performed in triplicate for each condition. PBMNCs were identified based on their morphology. All primary cells used in the study tested negative for mycoplasma contamination.

2.3.2 Luminol-Based Chemiluminescence Method

Luminol-based chemiluminescence has been extensively used to detect ROS production. Luminol (5-amino-2,3-dihydro-1,4-phthalazine-dione, 10^{-4} M, A8511, Merck KGaA, Darmstadt, Germany) is a redox-sensitive molecule that exhibits blue luminescence upon oxidation. Cells naturally produce baseline luminescence, known as native or endogenous chemiluminescence. However, this luminescence can be amplified using chemical reagents that emit intensified luminescence upon reacting with the produced ROS. This method is highly sensitive and can detect intracellular and extracellular ROS because of its ability to permeate cell membranes [34–37]. In each test, 200 µL luminol (10^{-4} M, dissolved in 0.4 M DMSO, 472301, Merck KGaA, Darmstadt, Germany) was mixed with 100 µL PBMNCs (1×10^6 cells/mL) in PBS (P2272, Merck KGaA, Darmstadt, Germany). Baseline ROS levels were measured over 20 min, and reactions were monitored using a luminometer (model 20/20n, Promega, Madison, WI, USA). The effects of Edaravone (EDV, M70800, Merck KGaA, Darmstadt, Germany, 1 µM, 100 µL) and lipopolysaccharide (LPS, L4391, Merck KGaA, Darmstadt, Germany, 50 µg/50 µL) on ROS production in PBMNCs were evaluated in sequential reactions for an additional 20 min. To

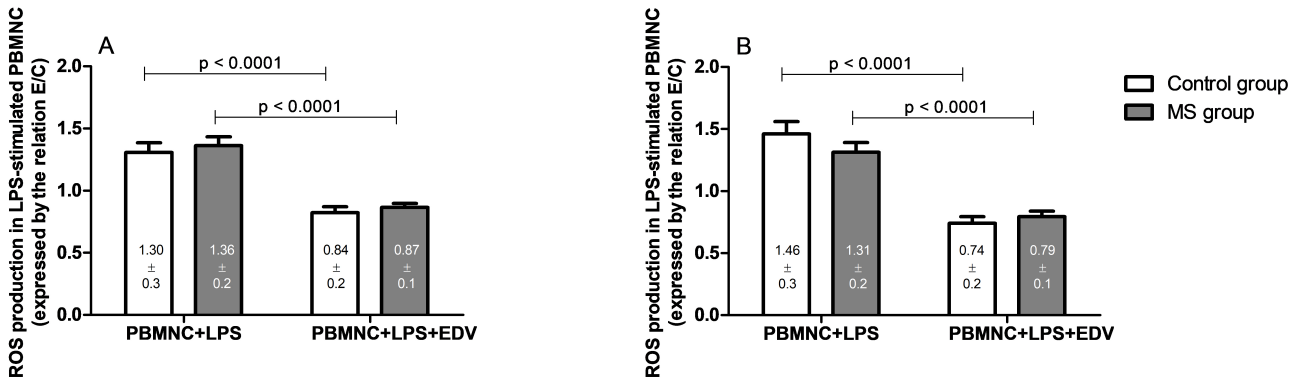

Fig. 1. Effect of edaravone (EDV) on intracellular reactive oxygen species (ROS) production in LPS-stimulated peripheral blood mononuclear cells (PBMNCs) from multiple sclerosis (MS) patients and healthy individuals. PBMNCs were incubated with EDV for (A) 30 min and (B) 24 h. Values are expressed as E/C (E = PBMNCs in the presence of LPS as a TLR4 activator; C = PBMNCs in the absence of the activator) ± SD; n = 20 for each group. Basal levels for ROS production ($OD_{570} \times 10^2 \pm SD$): control group 30 min = 31 ± 10, control group 24 h = 32 ± 6; MS group 30 min = 37 ± 10, MS group 24 h = 36.5 ± 8. ROS, reactive oxygen species; LPS, lipopolysaccharide; TLR, Toll-like receptor.

Table 1. Study population characteristics.

N	Control group	Multiple sclerosis group	*p*-value
	30	30	ns
No. of females (as determined at birth) (%)[a]	23 (76.7%)	19 (63.4%)	ns
Age in years[a]	40 ± 8.6	44 ± 12	ns
Disease duration in years[b]	na	11 ± 8	-
MS disease course			
Relapsing-remitting, N (%)	na	21 (70%)	-
Primary progressive, N (%)	na	6 (20%)	-
Secondary progressive, N (%)	na	3 (10%)	-

[a] Values are expressed as a percentage of the total, χ^2 test; [b] values are expressed as the mean ± standard deviation, Student *t*-test; MS, multiple sclerosis; na, not applicable; ns, not significant ($p > 0.05$).

assess the influence of EDV on ROS generation in TLR4-stimulated cells, a combined treatment using LPS and EDV was investigated. The results are presented as relative light units (RLUs) per minute. Cells treated with H_2O_2 (386790-M, Merck KGaA, Darmstadt, Germany), a known ROS inducer, were included as a positive control to validate the assay's sensitivity.

2.4 IL-6 Detection in PBMNC Supernatants

IL-6 production was analyzed in culture supernatants. PBMNCs (1×10^5/100 µL) were incubated in 96-well plates with or without EDV (1 µM, 100 µL) and LPS (50 µg/50 µL) at 37 °C for 24 h. To assess the effects of EDV on IL-6 production in TLR4-stimulated cells, a combination of LPS + EDV was investigated. After incubation, supernatants from PBMNC cultures were collected, and IL-6 concentrations were measured using a human IL-6 DuoSet ELISA kit (R&D Systems, Inc., Minneapolis, MN, USA).

2.5 Statistical Analysis

Data were analyzed using GraphPad Prism version 5 (GraphPad Software, Inc., San Diego, CA, USA) and presented as E/C ratios (in which E = PBMNCs in the presence of the TLR4 activator LPS, whereas C = PBMNCs in the absence of activators) or mean ± standard deviation (SD). An unpaired Student's *t*-test was used for continuous data, while the χ^2 test was applied as needed. Statistical significance was set at $p < 0.05$.

3. Results

Table 1 presents a comprehensive overview of the study population. The study included 30 healthy individuals (control group) with a mean age of 40 ± 8.6 years and 30 individuals with MS with a mean age of 44 ± 12 years and an average disease duration of 11 ± 8 years. No significant differences were observed between the groups regarding age or sex at birth. According to the International Advisory Committee on Clinical Trials of MS, 21 patients were diagnosed with relapsing-remitting MS, six with primary progressive MS, and three with secondary

Table 2. Effect of edaravone on ROS production in TLR4-stimulated peripheral blood mononuclear cells from multiple sclerosis patients and healthy controls, determined via luminol-based chemiluminescence.

Experimental conditions	Control group				Multiple sclerosis group			
	Mean (pg/mL)	Standard deviation	Standard error	Activation ↑ Inhibition ↓ (%)	Mean (pg/mL)	Standard deviation	Standard error	Activation ↑ Inhibition ↓ (%)
I. PBMNCs + PBS	168.1	26.8	7.1	-	185.6	19	6.5	-
II. PBMNCs + EDV	165.7	24	6.4	-	186.9	7.4	2.5	-
III. PBMNCs + LPS	199.4*	25	7.3	19 ↑ [a]	205.9*	23	6.9	11 ↑ [a]
IV. PBMNCs + LPS + EDV	166.8#	21	5.5	16 ↓ [b]	182.1#	13	4.3	12 ↓ [b]

N = 10 for each group. *$p < 0.05$ vs. PBMNCs + PBS, Student *t*-test; #$p < 0.05$ vs. PBMNCs + LPS, Student *t*-test. [a] The activation percentage (↑) was calculated as follows: ((mean of PBMNC + LPS/mean of PBMNC + PBS) – 1) × 100. [b] The inhibition percentage (↓) was calculated as follows: (1 – (mean of PBMNC + LPS + EDV/mean of PBMNC + LPS)) × 100. EDV, edaravone; LPS, lipopolysaccharide (TLR4 activator); PBMNCs, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; RLUs, relative light units; ROS, reactive oxygen species; TLR, Toll-like receptor.

Table 3. Modulation of IL-6 production by edaravone in TLR4-stimulated peripheral blood mononuclear cells from multiple sclerosis patients and healthy controls.

Experimental conditions	Control group				Multiple sclerosis group			
	Mean (pg/mL)	Standard deviation	Standard error	Activation ↑ Inhibition ↓ (%)	Mean (pg/mL)	Standard deviation	Standard error	Activation ↑ Inhibition ↓ (%)
I. PBMNCs + PBS	32.3	2.3	0.34	-	34.4	2.5	0.38	-
II. PBMNCs + EDV	32.4	2.0	0.25	-	33.6	2.8	0.46	-
III. PBMNCs + LPS	41.2*	1.8	0.42	27.5 ↑ [a]	42.8*	2.8	0.65	24.4 ↑ [a]
IV. PBMNCs + LPS + EDV	32.7#	1.5	0.39	20.6 ↓ [b]	33.9#	1.6	0.41	20.8 ↓ [b]

N = 15 for each group. *$p < 0.05$ vs. PBMNCs + PBS, Student *t*-test; #$p < 0.05$ vs. PBMNCs + LPS, Student *t*-test. [a] The activation percentage (↑) was calculated as follows: ((mean of PBMNC + LPS/mean of PBMNC + PBS) – 1) × 100. [b] The inhibition percentage (↓) was calculated as follows: (1 – (mean of PBMNC + LPS + EDV/mean of PBMNC + LPS)) × 100. EDV, edaravone; LPS, lipopolysaccharide (TLR4 activator); PBS, phosphate-buffered saline; PBMNCs, peripheral blood mononuclear cells; TLR4, Toll-like receptor 4.

progressive MS [38–41]. The sample number in the table represents the collective number of subjects used across all tests illustrated in the figures.

3.1 EDV Downregulated ROS Production in TLR4-stimulated PBMNCs

The oxidative response of PBMNCs was measured using the MTT reduction method, with values expressed as E/C ± SD (Fig. 1). ROS production was significantly enhanced in LPS-stimulated PBMNCs from MS patients and controls ($p < 0.05$), showing similarly increased levels after both the 30-minute and 24-hour incubation periods. Moreover, the addition of EDV significantly reduced ROS generation in TLR4-stimulated PBMNCs in both groups ($p < 0.05$), with both showing equivalent inhibition during both each incubation period.

The oxidative response in PBMNCs was also measured using the luminol-based chemiluminescence method, and the results are expressed as the mean ± SD (Table 2). These data reveal comparable basal ROS levels in resting PBMNCs from MS patients and healthy controls. Stimulation with LPS significantly increased ROS production in the PBMNCs from both groups ($p < 0.05$). However, this effect was significantly attenuated when EDV was added to the assay ($p < 0.05$). These results highlight the ability of EDV to effectively suppress ROS production in TLR4-stimulated PBMNCs, suggesting a modulation of the innate immune response.

3.2 EDV Inhibited Cytokine Secretion in TLR4-Stimulated PBMNCs

IL-6 levels were determined in TLR4-stimulated PBMNC culture supernatants from MS patients and the control group treated with or without EDV to explore whether the EDV-mediated downregulation of ROS production in TLR4-stimulated PBMNCs was linked to modulated proinflammatory cytokine production. As shown in Table 3, LPS activated IL-6 production to similar levels in PBMNCs from MS patients and the control group. Additionally, LPS-stimulated IL-6 production was similarly inhibited in PBMNCs from both the MS and control groups following EDV treatment.

4. Discussion

The current study investigated the possible immunomodulatory effects of EDV on TLR4-stimulated PBMNCs from MS patients. The results demonstrate that EDV effectively reduced ROS generation and IL-6 secretion in TLR4-stimulated cells from both MS patients and the control group (Tables 1,2,3, and Fig. 1). These findings suggest that the antioxidant and anti-inflammatory properties of EDV could serve as an adjunctive therapy for MS by targeting the chronic neuroinflammation that drives disease progression.

MS is an immune-mediated inflammatory demyelinating disease primarily driven by the inflammatory activity of recruited peripheral immune cells and resident glial cells in the CNS [1,9,40]. Although leukocytes play a crucial role in resolving inflammation or infection, their infiltration into the CNS can exacerbate inflammation by releasing ROS, cytokines, and chemokines. This excessive inflammation contributes to demyelination, axonal damage, and MS symptom progression [14,20,21].

Emerging evidence highlights the critical role of innate immune cells in the progression of MS. TLRs trigger specific intracellular signaling that promotes innate immune responses, contributing to the development of MS and experimental autoimmune encephalomyelitis (EAE) [41–43]. Moreover, TLR4 has been linked to MS symptoms, and studies have shown that silencing TLR4 can alleviate these symptoms [44–49]. TLR4 activation leads to mitochondrial ROS production, alongside NADPH oxidase and inflammatory cytokine secretion through NF-κB activation [50–52].

ROS production is essential, given that these molecules have a physiological role in cellular activity, proliferation, cell death, inflammation, and infection. However, excessive ROS levels can cause demyelination, axonal/neural injury, disrupt BBB integrity, and secretion of proinflammatory cytokines. Meanwhile, low antioxidant enzyme expressions limit the ability to neutralize ROS in the CNS [53–56]. Additionally, ROS reacts with lipids, proteins, and nucleic acids, resulting in functional deficiencies [13,14,16–18]. Our study demonstrated that EDV significantly modulated ROS production in TLR4-stimulated PBMNCs from MS patients and healthy controls ($p < 0.05$) (Fig. 1 and Table 2). These results suggest that EDV acts either on the TLR4 signaling pathway or indirectly on the mitochondrial respiratory chain and NOX complex.

Dysregulated cytokines such as IL-6 have been shown to significantly contribute to tissue injury and neurological deficits in MS. Indeed, elevated IL-6 levels are often detected in the cerebrospinal fluid and blood of MS patients, exacerbating chronic inflammation and demyelination by promoting Th17 cell differentiation [1,57–61]. In this study, stimulation using the TLR4 activator LPS caused a significant increase in IL-6 secretion in PBMNCs from MS patients and the control group compared to resting cells ($p < 0.05$). However, EDV effectively inhibited IL-6 secretion, demonstrating its potential to reduce inflammation ($p < 0.05$) (Table 3).

Given that ROS and proinflammatory cytokines are central to the pathogenesis of MS, leading to severe CNS damage [62], therapeutic approaches targeting inflammation and oxidative stress are promising for altering the progression of MS. Anti-inflammatory and antioxidant therapies are also being explored to protect myelin and neural cells [28,63,64]. Moreover, EDV, a low-molecular-

weight synthetic antioxidant with free radical-scavenging and anti-inflammatory properties, has high permeability across the BBB. Intravenous EDV was approved in 2017 by the United States Food and Drug Administration (FDA) to delay ALS progression, with clinical trials focusing primarily on motor function [25,65,66]. Subsequently, the FDA approved an oral suspension formulation of EDV in 2022 for use in patients with ALS [67]. Importantly, the antioxidant properties of EDV may confer neuroprotection against ALS, acute ischemic stroke, and other comorbidities [26,31]. The exact mechanism of action of EDV remains unclear; however, it is thought to donate electrons to free radicals and inhibit lipid peroxidation, which is implicated in autoimmune and inflammatory diseases. Furthermore, the antioxidant and anti-inflammatory effects of EDV are likely mediated through the nuclear factor erythroid 2-related factor 2/heme oxygenase-1 (NRF2/HO-1) signaling pathway, which is critical for cellular antioxidant responses and maintaining BBB integrity. By reducing ROS levels and inhibiting proinflammatory cytokines such as IL-6, EDV could provide a general approach to MS treatment, complementing current therapies that predominantly target immune cell activity. These properties suggest that EDV is a promising candidate for reducing the long-term neurodegeneration observed in patients with MS, potentially improving clinical outcomes and quality of life.

One of the main challenges in developing novel drugs to treat MS is the complexity and variability of the disease. Hence, overcoming these challenges requires a deeper understanding of the MS pathogenesis and therapeutic strategies that can precisely and effectively target specific disease mechanisms. Immunomodulatory treatments have demonstrated promising outcomes in reducing the occurrence of new lesions and relapse rates, thereby slowing disease progression. However, treatment adherence varies considerably owing to the frequent occurrence of adverse effects, with most MS patients experiencing increased disability and persistent symptoms. Therefore, the exploration of complementary therapies, including the use of vitamins and dietary supplements, to reduce disease severity and symptoms is increasing [25,26,68–70].

Given that neurodegenerative disorders are devastating and incurable, it is pivotal that new treatments are continually developed. Therapies that reduce ROS production by modulating oxidative stress and regulating inflammation may be beneficial for treating MS. The neuroprotective effects of EDV are believed to be related to its antioxidant properties, particularly the ability to eliminate free radicals. Immune activation, which is characterized by inflammation and oxidative damage, contributes to demyelination, axonal injury, and disease progression in MS patients. Thus, elucidating the mechanisms underlying these processes is essential to develop effective therapeutic strategies to interrupt the damage cycle and improve the outcomes of patients with MS.

This study has several limitations. We did not evaluate the effects of EDV on other cellular pathways or oxidative stress markers, such as malondialdehyde (MDA) and glutathione (GSH), or the activities of antioxidant enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT). Additionally, cytokines, such as TNF-α and IL-1β, were not measured. However, the evaluation of these markers in future studies could provide a more comprehensive understanding of the impact of EDV on oxidative stress and inflammation in MS. Additionally, all the patients with MS in this study were receiving immunotherapy, which likely influenced the activation state and responsiveness of their PBMNCs. This could potentially explain the absence of observed differences between the groups and highlights the need for further research to improve our definition of the conditions under which edaravone exerts its effects.

5. Conclusion

Our findings suggest that EDV may serve as an adjunctive therapy for MS by reducing ROS and IL-6 production in TLR4-stimulated PBMNCs. These results highlight the potential of EDV in modulating neuroinflammation and oxidative stress, which are critical drivers of MS progression. Future studies should explore the long-term benefits of EDV in clinical settings and investigate its effect on additional oxidative stress pathways to improve our understanding of its therapeutic potential.

Abbreviations

ALS, amyotrophic lateral sclerosis; BBB, blood-brain barrier; CAT, catalase; CNS, central nervous system; DAMPs, damage-associated molecular patterns; EAE, experimental autoimmune encephalomyelitis; EDSS, expanded disability status scale; EDV, Edaravone; FDA, Food and Drug Administration; GPx, glutathione peroxidase; GSH, glutathione; HMGB1, high mobility group box 1; IL, interleukin; MDA, malondialdehyde; MS, multiple sclerosis; NF-κB, nuclear factor-kappa B; NO, nitric oxide; NOX, NADPH-oxidase; NRF2/HO-1, nuclear factor erythroid 2 related factor 2/heme oxygenase-1; PAMPs, pathogen-associated molecular patterns; PBMNC, peripheral blood mononuclear cells; PBMNCs, peripheral blood mononuclear cells; PRR, pattern-recognition receptors; ROS, reactive oxygen species; SOD, superoxide dismutase; TLR, toll-like receptor.

Availability of Data and Materials

Some datasets generated and analyzed in this study are not publicly available but are available from the corresponding author upon reasonable request.

Author Contributions

CMOV, PHVD, LFA analyzed the data, wrote the original article, designed and drew the figures and tables. LFA, RPS, JVC collected the data and conducted the experiments. PPC selected the patients. CMOV and PHVD designed the study and supervised it. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All procedures involving human participants performed in this study were in accordance with the ethical standards of the institutional and national research committee, were approved by Dr. Francisco das Chagas Lima and Silva Ethics Committee of Santa Casa Hospital of Belo Horizonte, Brazil (approval number 69385917.7.0000.5138), and complied with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent to participate in the study was obtained from all participants.

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Conflict of Interest

The authors declare no conflict of interest.

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