Neuropeptide Galanin Increase ROS and IL-1β Production by Blood Cells from Patients with Multiple Sclerosis

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Introduction

Multiple sclerosis (MS) is a neurodegenerative disease that affects the central (CNS) and peripheral (PNS) nervous system. Neuronal demyelination in the brain and spinal neurons is observed in MS leading to the total or partial interruption of nervous influx [1-3]. It is reported that the neuropeptide Galanin (GAL) is secreted mainly by oligodendrocytes, astrocytes and gastrointestinal apparatus protecting against demyelination and promoting myelination of the neuron [4,5]. Galanin (GAL) is a neuropeptide-containing a 29/30 amino acid and its biological action occur through interaction with three different receptors GALR1, GALR2 and GALR3 [6-8] with main distribution in the CNS, PNS, and intestine [6,9]. Galanin is an immunomodulatory neuropeptide and act regulating several physiological processes, [6,10-14]. ROS and pro-inflammatory cytokines promote the migration of inflammatory cells (neutrophils, macrophages, lymphocytes) to the brain due to the increase of permeability of the blood-brain barrier (BBB) [2,3]. In pathological conditions such as MS, the increase in ROS production exceeds the physiological threshold, generating oxidative stress, an important factor associated with the development of demyelination [15-21]. Among proinflammatory cytokines, IL-1β plays a pivotal role in increasing the permeability of the BBB [22,23]. Its production depends on the inflammasome activation [24], a multiprotein complex of intracellular signalling, sensitive to oxidative stress. Inflammasome induces the maturation and secretion of IL-1β and IL-18 through the activation of caspase-1 besides inducing pyroptosis, a type of inflammatory cell death [25]. This study aimed to evaluate the role of galanin in the modulation of the production of ROS and IL-1β secretion by granulocytes and PBMNC, respectively, from MS patients.

Material and Methods

Ethical approval

The Ethical Committee from Santa Casa Hospital of Belo Horizonte-Brazil approved this study, and the informed consent was obtained from all participants included in the study.

Study population

Patients diagnosed with multiple sclerosis and healthy control, were selected by Dr. Paulo Pereira Christo, at the Neurology service of Santa Casa Hospital (Belo Horizonte, Minas Gerais, Brazil). Volunteers were within the age range of 18 and 65 years. Subjects presenting dementia, inflammation, infection or cancer were excluded from the study, as were pregnant women and individuals with alcohol or tobacco dependency.

Reagents

Human galanin compound was purchased from Merck KGaA, Darmstadt, Germany). For experiments, the concentration (2
μg/100 μL) was used according to previous studies performed by Agasse et al. [26].

**Cell separation**

Granulocytes and PBMNC were obtained from peripheral blood, according to Bicalho et al. [27], with slight modifications. Briefly, heparinized peripheral venous blood samples (10 mL) were subjected to double-gradient density (1.08 and 1.12). The volume proportion among discontinuous gradient and blood were 4:3:3, respectively, from the bottom to the top using siliconized glass or Falcon tubes. After centrifugation during 30-40 min, layers at the top and middle interfaces were collected to yield fractions rich in PBMNC and granulocytes, respectively. The cells were identified and counted based on morphology, granulation and size using a stereoscopic microscope with 400X magnification. Cellular viability was evaluated by the Trypan Blue exclusion test.

**Quantification of ROS production**

A luminol-based chemiluminescence method was employed to assess the oxidative responses of granulocytes. An aliquot (200 μL) of luminol (10⁻⁴ M) was mixed with a 100 μL of granulocytes suspension (1 × 10⁵/mL) in phosphate buffered saline (PBS). The chemiluminescence assay was performed on the Turner BioSystems model 20/20 n luminometer (Promega, Sunnyvale, CA, USA) for 20 min (control without stimulation), following which 100 μL of GAL (2000 nM) was added to the reaction mixture and chemiluminescence was performed for an additional 25 min.

**Quantification of IL-1β in supernatant of cultured PBMNC**

PBMNC (1 × 10⁵/100 μL) from MS patients and healthy controls in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) were incubated in the presence or absence of GAL (2 μM) for 72 h at 37°C under 5% CO₂. The final volume was adjusted to 300 μL in DMEM supplemented with 10% FBS. After incubation, the cells were centrifuged (200 g for 15 min) and the supernatant was collected. The interleukin-1β (human IL-1β; BioLegend, Inc., California, USA, cat. #437006) concentrations were determined through enzyme-linked immunosorbent assay (ELISA) according to the manufacturer instructions.

**Statistical analysis**

The Kolmogorov-Smirnov test was used to assess the normal distribution of the continuous variables; values were expressed as mean ± standard error. The Kolmogorov-Smirnov test was used to evaluate sample normality. Comparisons between groups were performed using unpaired Student t or the χ² test. All analyses were considered significant at values <0.05 using GraphPad Prism 5 (GraphPad Software, Inc).

**Results**

**ROS production by granulocytes from MS patients increases in the presence of galanin**

ROS production by granulocytes of MS patients and healthy controls in the presence or absence of galanin are shown in Figures 1 and 2. The basal ROS production (absence of GAL) by granulocytes from MS patients and control group were similar (p>0.05). In the presence of GAL was observed an inhibition (26%) of ROS generation in cells from healthy control and activation (32%) in granulocytes from MS patients. The comparison was significant at p<0.05.

Galanin increases the secretion of IL-1β by PBMNC from MS patients and healthy controls

Figure 3 shows that Galanin activates IL-1β secretion similarly in both PBMNC from MS patients and healthy controls (p>0.05). The results, expressed as pg/mL (mean ± standard error), were 8.6 ± 2.0 and 9.7 ± 1.2 in the absence of GAL and 41.1 ± 13.0 and 39.9 ± 12.6 for healthy controls and MS patients, respectively. The results on IL-1β secretion in the absence and in the presence of GAL were significantly different at p<0.05.
Galanin (GAL) activates the production of IL-1β in mononuclear cells (PBMCN) of patients with multiple sclerosis and healthy control. Values expressed as mean ± standard error; analysis determined by student’s "t" test. *p<0.05 vs. PBMCN+DMEM. n=20 for each group.

**Conflict of Interest**

The authors declare that they have no conflicts of interest.

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**References**


