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Prophylactic administration of *Withania somnifera* extract increases host resistance in *Listeria monocytogenes* infected mice

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Abstract

In this study, we demonstrated that *Withania somnifera* L. extract (WSE) protects mice from a lethal dose of *Listeria monocytogenes* when administered prophylactically at 100, 250 and 500 mg/kg for 10 days, with survival rates up to 30%. These doses also prevented the myelosuppression and the splenomegaly caused by a sublethal infection with *L. monocytogenes*, due to increased numbers of granulocyte–macrophage progenitors (CFU-GM) in the bone marrow. Investigation of the production of colony-stimulating factors (CSFs) revealed increased colony-stimulating activity (CSA) in the serum of normal and infected mice pre-treated with WSE. Further studies to investigate the levels of interferon-gamma (INF-γ) and lymphocyte cell proliferation were undertaken. We observed dose-dependent increases in cell proliferation and in the levels of INF-γ in mice infected with *L. monocytogenes* and treated with WSE. All together, our results suggest that WSE indirectly modulates immune activity and probably disengages *Listeria*-induced suppression of these responses by inducing a higher reserve of myeloid progenitors in the bone marrow, proliferation of lymphocytes and increased INF-γ levels.

Keywords: *Withania somnifera* L.; *Listeria monocytogenes*; Haematopoiesis; Interferon-gamma

1. Introduction

Withania somnifera L. Dunal (*W. somnifera*), a solanaceae, known as Hindi–Ashwagandha, English–Indian Ginseng, winter cherry, is classified in the ancient Indian system of medicine (Ayurveda) as a rasayana, a group of plant-derived drugs that improve overall physical and mental health and prevent diseases in the elderly [1–4]. This plant has been extensively studied and over 35 chemical constituents have been extracted, isolated and identified. Its biologically active constituents are alkaloids (isopelletierine, anaferine), steroidal lactones (withanolides, withaferins), saponins

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containing an additional acyl group (sitoindoside VII and VIII), and withanolides with a glucose at carbon 27 [5]. Studies have demonstrated its anti-inflammatory [6], anti-tumor [7–9], anti-stress [10], anti-oxidant [11–13], anti-aging [14,15], analgesic [16] and hemato/immunomodulator activities [17–21]. The use of *W. somnifera* has been mainly associated to its modulatory effect on the immune system [22]. Preparations obtained from this plant have been shown to enhance circulating antibody titre, increase the activity of lysosomal enzymes, increase phagocytosis by macrophages, stimulate the generation of cytotoxic T lymphocytes, and prevent cyclophosphamide-induced myelosuppression in mice [22]. It has also been suggested that the increased production of nitric oxide by macrophages, induced by the plant, might contribute to the immunostimulant properties of *W. somnifera* [23].

Listeria monocytogenes has been extensively used as an experimental model to study the mechanisms involved in innate and cell-mediated antimicrobial defense. Following infection with *L. monocytogenes*, innate immune responses are rapidly triggered and are essential for host survival [24–28]. Early resistance to infection is attributed to the efficient mobilization of bone marrow-derived cells, particularly granulocyte/macrophage cells, production of IFN-γ by natural killer cells and the resultant activation of macrophages [29]. IFN-γ and TNF are essential for primary defense against infection with *L. monocytogenes* [30,31], and mice that lack these cytokines or their cognate receptors rapidly succumb to infection [32,33].

Based on the above reports, this work was designed to investigate the prophylactic effects of the oral administration of the WSE in *L. monocytogenes* infected mice. Since this plant increases the functional activity of macrophages, we investigated the effects of the WSE on the production of granulocyte–macrophage progenitor cells [colony-forming unit-granulocyte–macrophage (CFU-GM)] of infected mice in parallel to the survival rate. In addition, in these animals, we also investigated the lymphocyte proliferation and IFN-γ production.

2. Materials and methods

2.1. Animals

The mice used in this study were bred at Unicamp Central Animal Facilities and raised under specific pathogen-free condition. Male BALB/c mice, 8–10 weeks old, were matched for body weight before use. The animals were housed 10/cage and allowed free access to laboratory chow and water. Animal experiments were done in accordance with institutional protocols and the guidelines of the Canadian Council on Animal Care Institutional Animal Care [34].

2.2. L. monocytogenes infection

L. monocytogenes obtained from the Microbiology Laboratory, Department of Clinical Pathology (Universidade Estadual de Campinas) was used to infect the animals. Bacterial virulence was maintained by serial passages in BALB/C mice. Fresh isolates were obtained from infected spleens, grown in BHI medium and stored at − 80 °C in 1 ml aliquots. Before use, each sample was thawed and diluted to appropriate concentrations in 0.9% NaCl. Mice were inoculated intraperitoneally (i.p.) with a sublethal dose of 1×10^3 viable *L. monocytogenes* per mouse for the study of CFU-GM and colony-stimulating activity (CSA). For the evaluation of survival, mice were inoculated i.p. with a lethal dose of 4×10^4 *L. monocytogenes* per mouse. In both cases, mice were infected at the end of a 10-day treatment with each different concentration of WSE used in this study.

2.3. Treatment regimen

WSE was provided as an orange medium brown powder with slight characteristic odor, containing 1% of total alkaloids and 1.5% of withanolides, by Galena Química e Farmacêutica Ltda (Campinas, SP, Brazil).

WSE was dissolved in sterile water and diluted to the appropriate concentrations immediately before use. Doses of 100, 250 or 500 mg/kg were administrated orally to mice for 10 days, prior to infection, in a 0.2 ml volume/mouse. Mice were infected with a sublethal dose of the bacteria 3 h after the end of the treatment and assays were performed 24, 48 and 72 h after bacterial inoculation. Each experiment included parallel control groups of normal and infected mice treated with an equivalent volume of the vehicle.

2.4. Progenitor cell assay

Bone marrow cells were prepared aseptically from one complete femur shaft in RPMI 1640 medium (Sigma Chemical Co, St Louis, MO). Spleens were then removed aseptically and converted to disperse cell suspensions in RPMI 1640 medium by gently pressing through a stainless-steel mesh net. Bone marrow and spleen cells were placed in duplicate 1 ml semi-solid agar cultures in 35 mm Petri dishes using 1×10^5 bone marrow cells or 2.5×10^5 spleen cells per culture. The medium used was Dulbecco's modified Eagle's medium (DMEM, Sigma) containing 20% foetal calf serum and 0.3% agar. Colony formation was stimulated by the addition of 0.5 ng/ml of recombinant murine granulocyte–macrophage colony-stimulating factor (rmGM-CSF; Sigma). The cultures were incubated for 7 days in a fully humidified atmosphere of 5% CO_2 in air and colony formation (clones > 50 cells) was scored at × 35 magnification using a dissection microscope.