International Immunopharmacology 11 (2011) 128–135

Contents lists available at ScienceDirect

International Immunopharmacology

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

Possible role of macrophages induced by an irridoid glycoside (RLJ-NE-299A) in host defense mechanism

Tabasum Sidiq [a], Anamika Khajuria [a,*], Pankaj Suden [a], Rohit Sharma [a], Surjeet Singh [a], K.A. Suri [b], N.K. Satti [b], R.K. Johri [a]

[a] Division of Pharmacology, Indian Institute of Integrative Medicine (CSIR), Jammu-180001, India
[b] Division of Natural Products Chemistry, Indian Institute of Integrative Medicine (CSIR) Jammu-180001, India

ARTICLE INFO

Article history:
Received 19 April 2010
Received in revised form 6 October 2010
Accepted 26 October 2010
Available online 11 November 2010

Keywords:
Picrorhiza kurroa
RLJ-NE-299A
Irridoid glycoside
Phagocytosis
Nitric oxide
Candida albicans
Salmonella typhimurium

ABSTRACT

In order to explore the possible role of macrophages and other necessary immune competent (T and B) cells in the modulation of immune responses, an attempt was made to study the immunomodulatory effect of an irridoid glycoside (RLJ-NE-299A) isolated from the roots of *Picrorhiza kurroa*. Both *in vitro* and *in vivo* studies were used to evaluate the effect of RLJ-NE-299A on humoral, cellular, and phagocytic activity of macrophages. The data obtained in the present study showed that RLJ-NE-299A significantly increased sheep red blood cell (SRBC) and induced antibody (IgM and IgG) titer and delayed type hypersensitivity (DTH) reaction in mice. Besides augmenting the humoral and cell-mediated immune response, it induced macrophage phagocytosis and stimulated cytokine-induced macrophage activation and nitric oxide (NO) production, which resulted in a high degree of protection against *Candida albicans* and *Salmonella typhimurium* infections. Flow cytometric analysis indicated the enhanced expression of co-stimulatory surface molecules CD80 and CD86. The ability of RLJ-NE-299A to upregulate these cell surface antigens involved in antigen presentation may provide an explanation for the increased T-cell mediated immunity involving macrophages. Taken together this *in vitro* and *in vivo* preclinical data suggests that RLJ-NE-299A acts as an effective immunomodulator specifically to improve macrophage function during infections. The effects of this agent on these cells at concentrations relevant to *in vivo* therapy support its immunopharmacologic application to modify cellular immunity.

1. Introduction

Plant-based immunomodulators are currently receiving inadequate attention and are investigated for immune modifying activity. Many natural plant products and synthetic compounds have been evaluated for their potential to modulate immune responses [1,2]. Medicinal plants have become an integral part of standard health care, based on combination of time-honored traditional usage and scientific research. However, interest in medical herbs has increased and prompted for scientific scrutiny of their therapeutic potential and safety. Many refined natural products are in clinical use and exert their anti-infective effects by directly affecting the pathogen and increasing the resistance of body to infections [3].

An attempt has been made to explore the promising immunostimulator out of the selected plant extracts which were evaluated for immunopharmacological properties. One among them, *Picrorhiza kurroa*, was identified to possess potent immunostimulatory properties [4]. *P. kurroa* represents a remarkable reputation among the indigenous system of medicine and modulates the biologic response of immune cells that enhance the host's ability to resist infections. The immunopharmacological properties have been attributed in general to roots [5]. On the basis of the importance of roots for phytochemical aspects, an irridoid glycoside, RLJ-NE-299A, isolated from the roots of *Picrorhiza kurroa*, was evaluated in detail to observe its effect on mouse polymorphonuclear lymphocytes leukocytes and peritoneal macrophages for the process of phagocytosis. Besides, the effects of RLJ-NE-299A on humoral immunity keeping neutralizing antibodies in mind, cellular immune responses via delayed type hypersensitivity reaction, release of NO by the activated macrophages, changes in cytokines and several other pathways were investigated. Macrophages play an important role in innate and acquired immunity and can be activated by a variety of stimuli [6]. These are the antigen processing cells, and they participate in antibody synthesis, induce phagocytosis of foreign particles, and release soluble mediators like cytokines, TNF-α, and IFN-γ involved in the destruction and in host defense. In the present study, detailed study with RLJ-NE-299A has been undertaken to find out whether RLJ-NE-299A accounts for its substantial

* Corresponding author. Tel.: +91 191 2574425, +91 9906120729.
E-mail address: anamikakhajuria@yahoomail.com (A. Khajuria).

1567-5769/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.intimp.2010.10.017

activity in the activation of macrophages and which may help in elucidating the mechanism of host immune responses to infectious diseases. It has been conceived that agents capable of modifying the immune response according to the clinical situation would be required to treat or prevent immune based disorders [7]. Such agents may act by increasing the antibody responses, by enhancing the phagocytic activity of macrophages, or by modifying the cell-mediated immune response. Novel cell surface and soluble signaling molecules, CD80 and CD86 produced by cells of the immune system have been discovered that regulate host responses to microorganisms [8]. It is now widely appreciated that these molecules interact in a concerted fashion to maintain a balance that governs an appropriate response to infectious organisms. We have observed that discovering compounds like irridoid glycosides, which positively or negatively modulate the biologic response of immune cells and mediators like cytokines that enhance the host's ability to resist microbial infection directed specifically against bacterial and fungal infections, is critical for understanding the interplay between the bacteria, fungus, and the host immune system. By characterizing the immunological correlates of protection against infections, such measurements will aid in the development of efficacious prophylactic drugs.

2. Materials and methods

2.1. Reagents

The organic solvent exhausted material (0.5 kg) of the plant *P. kurroa* obtained from commercial source was used in this study. Trichloroacetic acid (TCA) and trifluoroacetic acid (TFA) were purchased from Merck (India). Medium RPMI 1640 (Himedia, Bombay, India), 96-V-wells microtitration plates and microtissue culture plates (96-U-wells) from Tarson, trypan blue (Microlabs, Bombay), fetal calf serum (FCS), lipopolysaccharide (LPS, *Escherichia coli* 055 B5), dimethylsulphoxide (DMSO), Hank's balanced salt solution (HBSS), HEPES, 2-mercaptoethanol, penicillin, streptomycin, and levamisole (Sigma), fluoroisothiocyanate (FITC)-labeled anti-CD80 (B7-1) and anti-CD86 (B7-2) mAbs (BD Biosciences) were used. Horseradish peroxidase-conjugated anti-mouse IgG and OPD substrate (*ortho*-phenylenediamine dihydrochloride) were purchased from Sigma Chemicals, USA. L-Glutamine, sulfanilamide, and naphthylenediamine hydrochloride were likewise purchased from Sigma. Assay kits for all cytokines IFN-γ and TNF-α were purchased from BD, USA. *Candida albicans*, *Salmonella typhimurium*, and LPS (ultra pure *E. coli*, O111:B4 strain) from the list were procured from of Biological Laboratories, USA.

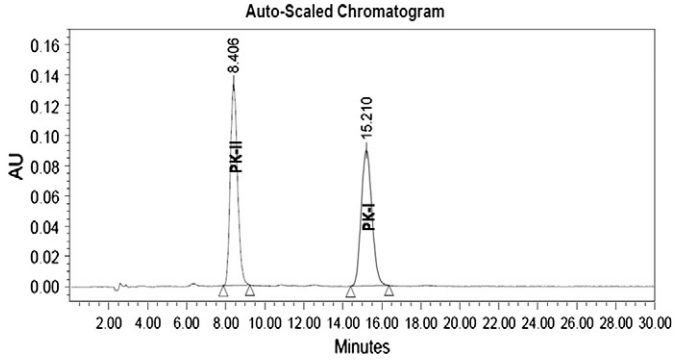
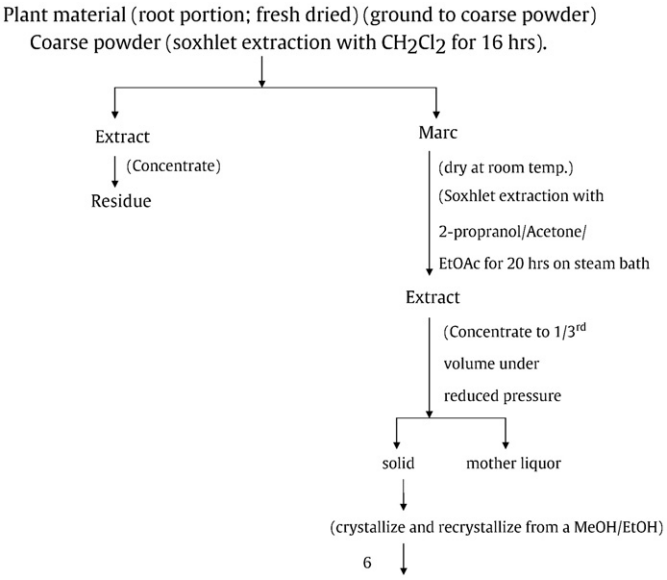


Fig. 1. RLJ-NE-299A using HPLC shimadzu system consisting of HPLC column RP-18 (250 nm × 4.0, i.d. 5 μm) and mobile phase methanol:water (2:3) with flow rate of 0.7 ml/min. The chemical constituents were analyzed by PDA detector at 270 nm.

2.2. Plant material and extraction

The root parts of *P. kurroa* were collected from the Institutional Herbarium and authenticated by the HOD Botany Department of IIIM, Jammu (CSIR). A voucher specimen was submitted at the Institute's Herbarium Department for future references with the voucher number 21545. RLJ-NE-299A is a standardized mixture of two iridoid glycosides: Picroside I and Picroside II [9,10]. The root powder (500 g) of *P. kurroa* was extracted with dichloroethane while refluxing and the extract was rejected. The marc was extracted with EtOAc while refluxing in a Soxhlet for 20 h. The EtOAc extract was centrifuged to remove suspended matter and concentrated under vacuum to 1/4th of its volume and allowed to stand at 20 ± 5 °C for 36 h. The separated solid was filtered off and recrystallized from MeOH, yielding RLJ-NE-299A (2.23gm). HPLC analysis of RLJ-NE-299A is shown in Fig. 1.



2.3. Animals

Study was conducted on male Balb/c mice (18–22 g) from Institutional Animal House. The Ethical Committee of the Indian Institute of Integrative Medicine (CSIR) instituted for animal handling approved all protocols. The animals were bred and maintained under standard laboratory conditions: temperature (25 ± 2 °C) and photoperiod of 12 h. Commercial pellet diet and water were given *ad libitum*.

2.4. Immunization schedule

Sheep red blood cells (SRBC) were used as a source of T-dependent antigen. For this purpose, the blood was withdrawn from a healthy sheep in Alsever's solution [11]. SRBC used for immunization were prepared in pyrogen-free normal saline. Mice were divided into eight groups, each consisted of six animals. RLJ-NE-299A at 3, 10, and 30 mg/kg (in 200 μl of normal saline) was administered orally by gavage for 15 days, daily. The dose volume was 0.2 ml. Control group received normal saline. Levamisole, a known immunostimulator reported to augment the antibody response [12], was given orally as positive control, at a dose of 2.5 mg/kg body weight. All groups were immunized with 0.2 ml of SRBC (5×10^9) per mouse intraperitoneally (i.p.) on day 0 of drug treatment. Additional three immunized groups, challenged on day 7 with SRBC, were used for DTH and different immunoglobulin and phagocytic assays.