Biomedicine & Pharmacotherapy 106 (2018) 403–410

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

In vitro combinatorial anti-proliferative and immunosuppressive effects of *Brucea javanica* extract with CX-4945 and imatinib in human T-cell acute lymphoblastic leukemia cells

Jung-Il Jung[a], Se Young Kim[a], Kyeong-Yong Park[b], Kongmany Sydara[c], Sang Woo Lee[d], Soon Ae Kim[e,**], Jiyeon Kim[a,*]

[a] Department of Biomedical Laboratory Science, School of Medicine, Eulji University, Daejeon 34824, Republic of Korea
[b] Department of Integrated Material's Development, CHA Meditech Co., Ltd, Daejeon 34025, Republic of Korea
[c] Institute of Traditional Medicine, Ministry of Health, Vientiane Capital, Lao People's Democratic Republic
[d] International Biological Material Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 34141, Republic of Korea
[e] Department of Pharmacology, School of Medicine, Eulji University, Daejeon 34824, Republic of Korea

ARTICLE INFO

Keywords:
Brucea javanica extract
T-ALL
Jurkat
NF-κB
IL-2
CK2

ABSTRACT

Since 1970, the isolated and identified components of *Brucea javanica* (L.) Merr. have been known to contain anticancer effects, particularly antileukemic effect. In this study, the inhibitory effect of *Brucea javanica* (BJ) on cell growth and inflammation was confirmed in human T-cell acute lymphocytic leukemia (T-ALL) cells, and its efficacy as an antileukemic agent was verified. Our results showed that BJ extract induced caspase-dependent apoptosis of T-ALL Jurkat cells through inhibition of the CK2-mediated signaling pathway, while exerting no significant cytotoxicity in normal peripheral blood mononuclear cells. Moreover, BJ extract suppressed the NF-κB signaling pathway, thus, inhibiting the interleukin (IL)-2 expression induced by phorbol 12-myristate 13-acetate (PMA) and phytohemagglutinin (PHA). Notably, combined treatment with BJ extract plus CX-4945 or imatinib exerted enhanced inhibitory effects on T-ALL cell growth and IL-2 production. Overall, these results suggest that BJ extract can be a potent therapeutic herbal agent for T-ALL treatment and prevention of IL-2 mediated inflammatory immune responses.

1. Introduction

Among the hematopoietic disorders, leukemia is a progressive disease, characterized by clonal hematopoietic cells in the peripheral blood and bone marrow, poor prognosis, and high mortality. T-cell acute lymphoblastic leukemia (T-ALL) is the most commonly diagnosed blood cancer with chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), and chronic myeloid leukemia (CML) [1]. T-ALL occurs mainly in children, with a peak in adults over 40 years of age and frequently have a poor prognosis [2]. The signs of T-ALL are characterized by anemia, and abnormal white blood cells in the blood and bone marrow, which interfere with the immune response. Notably, helper CD4 + T lymphocyte mediated inflammatory responses and autoimmune responses are regulated by interleukin (IL)-2 expression [3–5]. IL-2, a major T-cell growth factor, has critical role in helper T cell activation. Mitogen activated protein kinase (MAPK) and NF-κB signaling pathways stimulate IL-2 production, which in turn promotes T cell growth and immune responses [6–9]. Since IL-2 production is associated with T cell survival, inhibition of IL-2 expression in ALL may contribute to T-ALL treatment and prevention. Despite the increasing amounts of researches and results for new drug development, the prognosis for T-ALL patients is poor and inflammatory response related side effects are remained.

Brucea javanica (L.) Merr. has various synonyms of *Alianthus gracilis Salisb.*, *Brucea amarissima Desv. ex Gomes*, *Brucea glabrata Decne.*, *Brucea gracilis (Salisb.) DC.*, *Brucea sumatrana Roxb.*, *Brucea sumatrensis Spreng.*, *Gonus amarissimus Lour.*, *Rhus javanica* L.. Since 1900, *Brucea javanica* (BJ), an evergreen shrub, its extract has been used to treat infectious

Abbreviations: BJ extract, *Brucea javanica* twig and leaves extract; T-ALL, T-cell acute lymphoblastic leukemia; CK2, Casein kinase 2; PMA, phorbol 12-myristate 13-acetate; PHA, phytohemagglutinin; 7-AAD, 7-aminoactiomycin; IL-2, Interleukin-2

* Corresponding author at: Department of Biomedical Laboratory Science, School of Medicine, Eulji University; 77, Gyeryong-ro 771beon-gil, Jung-gu, Daejeon 34824, Republic of Korea.
** Corresponding author at: Department of Pharmacology, School of Medicine, Eulji University; 77, Gyeryong-ro 771beon-gil, Jung-gu, Daejeon 34824, Republic of Korea.
E-mail addresses: 96k620@gmail.com (J.-I. Jung), fate1903@naver.com (S.Y. Kim), pky@chamc.co.kr (K.-Y. Park), kmnkong@gmail.com (K. Sydara), ethnolee@hanmail.net (S.W. Lee), sakim@eulji.ac.kr (S.A. Kim), yeon@eulji.ac.kr (J. Kim).

https://doi.org/10.1016/j.biopha.2018.06.164
Received 5 February 2018; Received in revised form 25 June 2018; Accepted 27 June 2018

and inflammatory diseases [10]. Especially, BJ has been known to exert remarkable anti-leukemic activity. Bioactive constituents of BJ, including nigakilactones, alkaloids, lignan, terpenoids, steroids, and flavonoids, showed anti-tumor and anti-leukemic activity [10,14]. BJ extract or its constituents induced cytotoxic effect and apoptosis in several types of human cancer and leukemia cells [10–16]. In addition, BJ whole extract or seed oil or isolated single compounds such as Bruceine D, bruceoside D, E, and F and yadanzioside P exhibited anti-cancer and anti-leukemic activity and their potential as therapeutic agent [10–16]. The extract of BJ seeds also has been used for the treatment of inflammation and allergy related diseases [17]. Moreover, combined treatment with conventional drugs, the emulsion of BJ oil exerted nontoxic anti-proliferative effect in lung adenocarcinoma and gastric cancer [18,19]. These previous reports represent that BJ extracts and isolated single compounds have anti-proliferative and anti-inflammatory activity in many types of diseases, including blood cancers. However, the mechanism of cell growth inhibition and anti-inflammatory effects of BJ extract has not been studied in blood cancers, particularly T-ALL.

In this study, methanol extract from BJ twig and leaves and combined treatment with inhibitors having suppression effect on CK2/Akt expression or phosphorylation, such as CX-4945 and imatinib, showed anti-proliferative and immunosuppressive effect through the inhibition of NF-κB signaling pathway in T-ALL Jurkat cells. We assessed the inhibitory effects of BJ extract on phorbol 12-myristate 13-acetate (PMA) and phytohaemagglutinin (PHA) induced activation of NF-κB signaling pathway and secretion of IL-2 using several assays. Our results suggest that concomitant administration of BJ extract and existing therapies can be a potent therapeutic strategy for T-ALL treatment.

2. Materials and methods

2.1. Materials

CX-4945 and imatinib were obtained from Selleck Chemicals (USA), and NE-PER nuclear and cytoplasmic extraction reagent from Thermo Fisher Scientific (USA). Ficoll Histopaque solution, phytohemagglutinin (PHA), and phorbol 12-myristate 13-acetate (PMA) were purchased from Sigma-Aldrich (USA). Fetal bovine serum (FBS), phosphate-buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 medium, and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) were purchased from Corning Life Science (USA). MUSE® Annexin V and Dead Cell Assay Kit was purchased from Merck Millipore (Germany), the Human IL-2 ELISA kit from KOMABIOTECH (Korea), the Caspase-Glo® 3/7 assay system from Promega (USA), and the Cell Counting Kit-8 from Dojindo Molecular Technologies (USA). Primary antibodies specific for phosphorylated (p)-p65, p65, CK2α, CK2α′, CK2β, and actin were purchased from Santa Cruz Biotechnology, Inc. (USA). Antibodies raised against p-Akt (S473) and p-Akt (T308) were purchased from Cell Signaling Technology, Inc. (USA).

2.2. Preparation of plant materials

A methanol extract of *B. Javanica* (twig and leaves) was obtained from the Foreign Plant Extract Bank (no. FBM180-045; Daejeon, Korea) (Fig. S1). *B. Javanica* was collected from Nong Tao village, Mahaxai district, Kham Muan Province of Laos in 2013, and was authenticated by the Chief of the Institute of Traditional Medicine, Kongmany Sydara. A voucher specimen (LK831) is stored at the herbarium of the Korea Research Institute of Bioscience and Biotechnology (KRIBB). Briefly, 71 g of dried and refined *B. Javanica* twig and leaves was extracted using 1000 mL 95% methanol with a sonicator (SDN-900H, SD Ultrasonic Cleaner, Seoul, Korea) at 45 °C for 3 days (15 min sonication followed by 2 h standing; repeated 10 times per day). The extract was percolated with filter paper (3 mm; Whatman PLC, Kent, UK), condensed using a rotary evaporator (Buchi, Swiss), and lyophilized using a freeze dryer (Christ, Germany). To make a stock solution, the powder (71 g; yield ca. 5.68%) was dissolved in distilled water and dimethylsulfoxide (DMSO 20 mg/mL). Before use in the in vitro assay, the stock solution was diluted with culture medium.

2.3. PBMC isolation and cell culture

The International Review Board of Eulji University (EU 17-03) approved our use of human primary peripheral blood mononuclear cells (PBMCs). PBMCs were isolated from heparinized peripheral blood as previously described [20]. Isolated PBMCs were washed with PBS, and then resuspended in DMEM supplemented with 10% heat-inactivated FBS and 1% antibiotics. Human acute T lymphoblastic leukemia Jurkat clone E6-1 cells (No. 40152) were purchased from the Korean Cell Line Bank (KCLB) and maintained in RPMI 1640 medium containing 5% FBS and 1% antibiotics. Both PBMCs and Jurkat cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2.

2.4. Cell viability assay

PBMCs or Jurkat cells (1.0×10^4 cells/well) were seeded into a 96-well plate. After a 24-h incubation, the cells were treated for 24–72 h with BJ extract alone or combined with CX-4945 or imatinib in complete media containing 5% FBS. Cell viability was measured using the Cell Counting Kit-8 following the manufacturer's instructions. Absorbance was measured using a Multiscan FC microplate photometer (Thermo Fisher Scientific, USA). All experiments were performed in triplicate.

2.5. Flow cytometry

Jurkat cells (1×10^5 cells/mL) were treated with BJ extract (0–10 μg/mL) and incubated for 48 h. The cells were then washed with PBS and treated with MUSE (Annexin V and 7-AAD) solution, followed by analysis with the Dead Cell Assay Kit. The proportion of apoptotic cells was measured using the MUSE® Cell Analyzer (Merck Millipore, Germany). Data were analyzed using the MUSE® Annexin V and Dead Cell software module (Merck Millipore).

2.6. Measurement of caspase-3/7 activity

Jurkat cells (1×10^5 cells/mL) were treated for 48 h with BJ extract in culture media containing 5% FBS. Then caspase-3/7 activities were measured using the Caspase-Glo® 3/7 assay system, following the manufacturer's instructions.

2.7. Western blot analyses

Jurkat cell lysates were prepared using NE-PER nuclear and cytoplasmic extraction reagent. After protein quantification, the cytoplasmic fractions (40 μg) were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were incubated with primary antibodies for specific protein detection, followed by incubation with HRP-conjugated secondary antibodies. Protein expression was visualized using the Luminata™ Forte Western HRP Substrate (Merck Millipore). To determine relative protein expression, band intensities were measured using X-ray films and development solution (Fujifilm, Tokyo, Japan). The detected bands were quantified by ImageJ software, and the relative ratio between each sample and control was presented in western blot results (Fig. 3C, Fig. 4B and C).

2.8. Quantitative real-time PCR (qRT-PCR)

Jurkat cells were pretreated for 3 h with BJ extract alone or combined with CX-4945 or imatinib, followed by a 1-h incubation with PMA (50 ng/mL) plus PHA (1 μg/mL) in culture media containing 5%