Biomedicine & Pharmacotherapy 99 (2018) 96–100

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Crocin improves the proliferation and cytotoxic function of T cells in children with acute lymphoblastic leukemia

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ARTICLE INFO

Keywords:
Crocin
Ara-C
T cells
DNA damage
Acute lymphoblastic leukemia

ABSTRACT

Objective: Immunotherapy is important to improve the survival of children with acute lymphoblastic leukemia (ALL). This study aimed to assess the effects of crocin on the proliferation and function of T cells isolated from children with ALL.

Methods: The mononuclear cells were isolated from peripheral blood of children with ALL and then treated with different final concentrations of crocin. The levels of different cytokines secreted by T cells and the ratio of CD4 and CD8 were measured. Tail DNA% (TDNA), Tail moment (TM), Tail length (TL) and sister chromatid exchange (SCE) were detected to assess DNA damage of T cells.

Results: Crocin significantly promoted T cell proliferation and the secretion of IL-2 and IL-4 in a concentration dependent manner. In addition, crocin increased CD4/CD8 ratio of T subset. Crocin itself caused no significant damage to T cells but reduced DNA damage in T cells treated with Ara-C.

Conclusions: Crocin could improve the proliferation and cytotoxic function of T cells, and reduce DNA damage caused by Ara-C.

1. Introduction

Acute lymphoblastic leukemia (ALL) is the most frequent malignancy of children. The survival rate has increased for children with acute leukemia with the development of intensive chemotherapy, but 30–50% children with leukemia still relapse [1]. The minimal residual disease (MRD) of leukemia induces leukemia relapse, and immunotherapy is one important approach for improving the survival rate of children with leukemia [2].

Crocin is a main water-soluble carotenoid of the saffron extract, which belongs to perential stemless herb of the large Inridaceae family. Saffron extracts inhibited the proliferation of human acute lymphoblastic T-cell human leukemia [3]. Growing evidence has shown that crocin could significantly inhibit the proliferation of cancer cells [4]. Our previous study reported that crocin inhibited the proliferation of HL-60 cells [5].

However, the effect of crocin on immune cells has not been evaluated in detail. In the present study, we performed a series of experiments to assess the effects of crocin on the lymphocytes from children with acute leukemia for complete remission.

2. Materials and methods

2.1. Preparation of T lymphocytes

Peripheral blood mononuclear cells (PBMCs) of ALL children who had complete remission for at least 6 months were isolated by Ficoll's density gradient centrifugation method, and were seeded into 24 well flat-bottom culture plates. All blood samples were obtained with informed consent from their parents, which were approved by the Institute Ethics Committee. After culture for 3 h, non-adherent lymphocytes were taken and further cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) in a humidified incubator of 5% CO_2 at 37 °C.

2.2. Cell proliferation assay

Crocin (Sigma, CAS Number 42553-65-1) was diluted in phosphate-buffered saline for the appropriate concentration. T lymphocytes were divided into different groups and treated with crocin (final concentration 0.625–2.5 mg/ml) for 72 h. Cells were treated with phytohe-

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https://doi.org/10.1016/j.biopha.2018.01.042
Received 5 December 2017; Received in revised form 28 December 2017; Accepted 5 January 2018

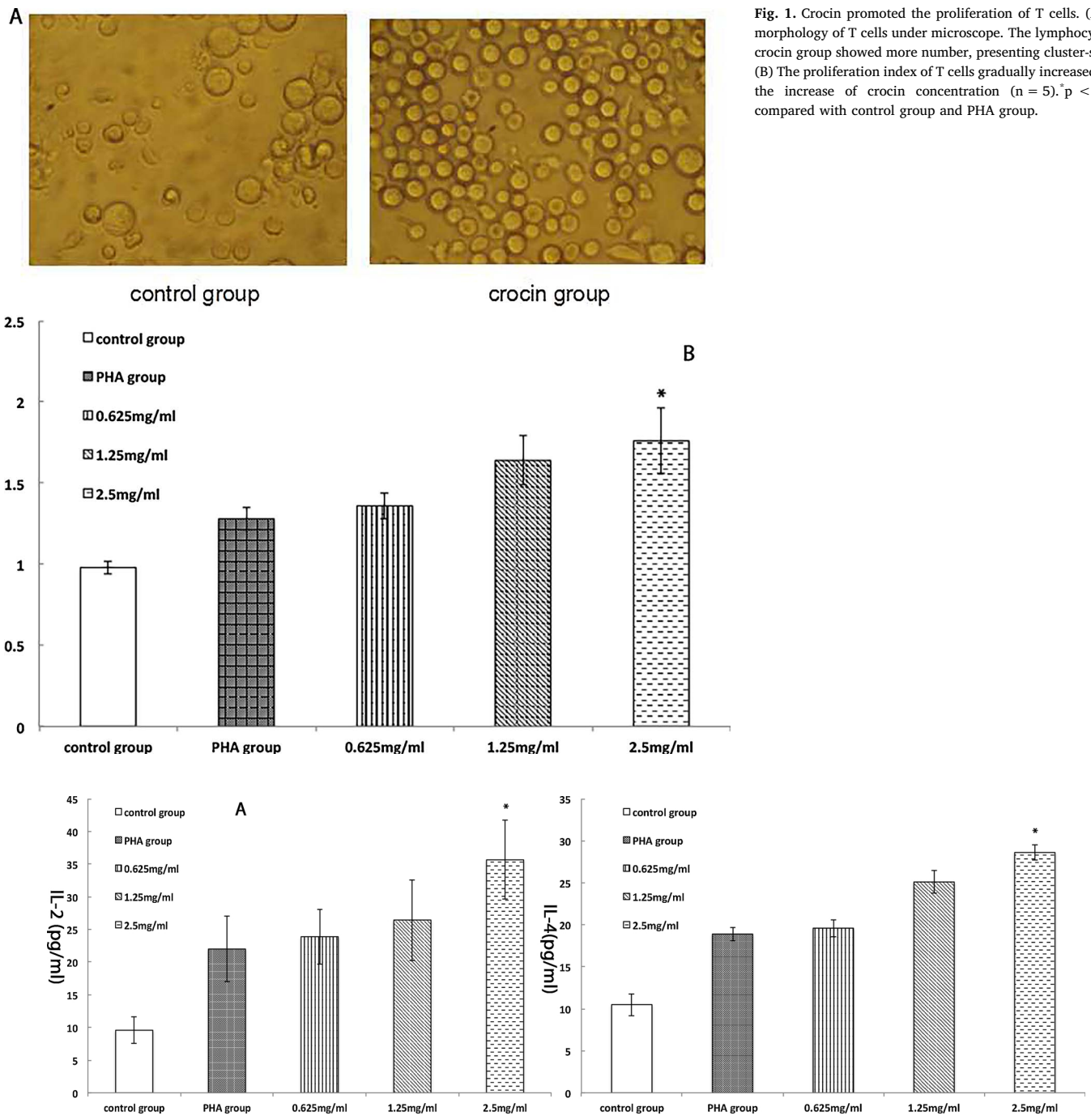

Fig. 1. Crocin promoted the proliferation of T cells. (A)The morphology of T cells under microscope. The lymphocytes in crocin group showed more number, presenting cluster-shape. (B) The proliferation index of T cells gradually increased with the increase of crocin concentration (n = 5).* $p < 0.05$, compared with control group and PHA group.

Fig. 2. Crocin increased the cytokines secreted by T cells. (A) ELISA assay of IL-2 level secreted by T cells treated with PHA or crocin (0.625 mg/ml–2.5 mg/ml) (n = 5). (B) ELISA assay of IL-4 level secreted by T cells treated with PHA or crocin (0.625 mg/ml–2.5 mg/ml) (n = 5). * $p < 0.05$, compared with PHA group and control group.

magglutinin (PHA) as positive control. The cell proliferation was examined by MTT assay kit (Sigma).

2.3. Flow cytometry

T lymphocytes were washed and stained with fluorescence conjugated monoclonal antibodies for CD4 and CD8. Next the cells were fixed in 1% formaldehyde and ≥10,000 cells per sample were immediately analyzed on a flow cytometer (BD Biosciences, San Jose, CA, USA). The data were analyzed by using FlowJo software (FlowJo, Ashland, OR, USA).

2.4. ELISA

The supernatants of T lymphocytes were collected and the concentrations of cytokines IL-2 and IL-4 were examined by using ELISA kits (R&D systems, Minneapolis, MN, USA) following the manufacturer's instructions.

2.5. Comet assay

T lymphocytes were treated with different concentration of crocin for 72 h. Cells treated with Ara-C (Cytarabine) or Ascorbic acid (Zel C) were included as control groups. The length of the comet tail in μm