International Immunopharmacology 31 (2016) 45–49

Contents lists available at ScienceDirect

International Immunopharmacology

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

Niclosamide induces apoptosis in human rheumatoid arthritis fibroblast-like synoviocytes

Mingcheng Huang [1], Shan Zeng [1], Qian Qiu, Youjun Xiao, Maohua Shi, Yaoyao Zou, Xiuyan Yang, Hanshi Xu, Liuqin Liang *

Department of Rheumatology, The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China

ARTICLE INFO

Article history:
Received 8 September 2015
Received in revised form 20 October 2015
Accepted 2 November 2015
Available online 17 December 2015

Keywords:
Fibroblast-like synoviocytes
Niclosamide
Apoptosis

ABSTRACT

To explore the effects of niclosamide on the viability and apoptosis of rheumatoid arthritis of fibroblast-like synoviocytes (rheumatoid arthritis (RA)fibroblast-like synoviocytes (FLS)), FLS obtained from RA patients were treated with niclosamide. Niclosamide significantly inhibited the viability of RA FLS in a concentration-dependent manner. Niclosamide treated FLS showed a significant increase in the percentage of apoptosis and higher intracellular ROS levels. N-acetyl-l-cysteine (NAC) pretreatment significantly attenuated niclosamide-induced apoptosis. The apoptotic response was due to the up-regulation of pro-apoptotic protein, Bax, and down-regulation of antiapoptotic protein, B cell lymphoma 2 (Bcl-2). The activation of mitochondrial pathway in niclosamide-treated RA FLS induced the cytochrome C, cleavage of caspase-9 and caspase-3.
Additionally, niclosamide inhibited the phosphorylation of Akt. Collectively, our results reveal that niclosamide inhibits cell proliferation and induces mitochondrial apoptosis of RAFLSs, which is associated with the modulation of Akt signaling pathways.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by abnormal synovial hyperplasia and progressive joint destruction [1]. Synovial hyperplasia with an increased number and size of rheumatoid fibroblast-like synoviocytes (FLS) is considered a characteristic of RA [2]. In RA, stromal fibroblast-like synoviocytes (FLS) display increased resistance to apoptotic stimuli, which promotes their accumulation in the inflamed joint, as well as the release of products responsible for bone and cartilage damage [3]. For these reasons, the induction of apoptosis has been proposed as a potential therapeutic approach for RA.

Niclosamide is a Food and Drug Administration-approved oral antihelminthic drug and has various biological activities including anticancer, antioxidative, antiviral activities and anti-inflammatory [4,5]. It is reported that niclosamide treatment can reverse the aggressive phenotypes of ovarian carcinoma cells, leading to decreased proliferation, migration, and invasion and increased apoptosis [6,7]. Niclosamide has been found to induce apoptosis in AML partially through alteration of the Bcl-2 family members and causes apoptosis through generation of reactive oxygen species (ROS) [8,9]. Overproduction of ROS can induce oxidative stress, consequently leading to DNA damage and apoptosis [10].

Our recent study has demonstrated that niclosamide possesses anti-inflammatory effect in collagen-induced arthritis [5]. However, the effects of niclosamide on the viability and apoptosis of RA FLS are still unclear. In this study, we aimed to check whether niclosamide exerts suppressive effects against RA FLS and the molecular mechanisms involved.

2. Materials and methods

2.1. Cell culture

Synovial tissues were obtained from active RA patients, according to the revised criteria of the American College of Rheumatology, who were undergoing synovectomy or joint replacement, and who had given informed consent. Normal control synovial tissues were obtained by arthroscopic biopsy from five patients that had menisci injuries or cruciate ligament rupture without history of acute or chronic arthritis, and who had given informed consent. Synovial tissues were washed with phosphate-buffered saline(PBS) and cut into small pieces and treatment with 1 mg/ml type I collagenase (Sigma-Aldrich, St. Louis, MO, USA) in DMEM/F12 medium for 2 h at 37 °C. After washing with PBS, isolated RA FLS were suspended in DMEM/F12 containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin. RA FLS obtained from the 4th to 6th passages were used for experiments.

* Corresponding author at: Department of Rheumatology, The First Affiliated Hospital, Sun Yat-sen University, No. 58 Zhongshan Road 2, Guangzhou, Guangdong 510080, China.
E-mail address: lliuq@mail.sysu.edu.cn (L. Liang).
[1] Mingcheng Huang and Shan Zeng contributed equally to this work

http://dx.doi.org/10.1016/j.intimp.2015.11.002
1567-5769/

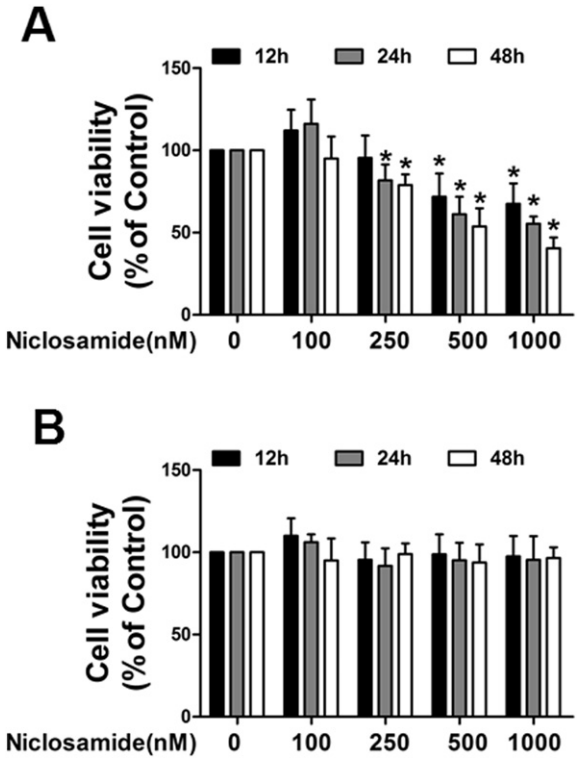

Fig. 1. Niclosamide inhibits the viability of RA FLS. A–B, RA FLS and normal FLS were treated with indicated concentrations of niclosamide for 12 h, 24 h and 48 h, and cell viability was assessed using the MTT assay. DMSO-treated cells were used as control. *P < 0.05 vs. control. Bar graphs represent data from three independent experiments.

2.2. MTT assay

Cells were seeded in 96-well plates and then treated with either 0.1% dimethylsulfoxide (DMSO) as a diluent control or the indicated concentration of niclosamide in serum free medium. After 48 h of incubation, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (Sigma-Aldrich, USA) solution was added for 4 h. After the used media containing MTT solution was removed, the formed formazan crystals were dissolved in DMSO. The plates were read at 540 nm in a microplate reader (BioRad, Hercules, CA).

2.3. Induction of RA-FLS apoptosis by flow cytometry

RA FLS were collected, centrifuged and washed with PBS after treated the indicated concentration of niclosamide for 72 h. This was followed by staining Annexin-V-FLOUS and propidium iodide(Sigma) for 15 min at room temperature. For each example, 20,000 cells were analyzed on a flow cytometer (FACStar, BDBiosciences). Data are expressed as mean ± S.D. of three independent experiments.

2.4. TUNEL assay

Apoptotic events were measured by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. The cells were seeded in glass coverslips and incubated overnight. After being treated by niclosamide for 48 h, TUNEL assay was performed using In Situ Cell Death Detection Kit (Promega, Madison, WI) according to the manufacturer's instructions. Briefly, RA FLS were incubated with niclosamide for 48 h, and were fixed with 4% paraformaldehyde for 25 min at 4 °C. After washing with PBS, permeabilization solution (0.2% Triton X-100 in PBS) was added for 5 min and cells were

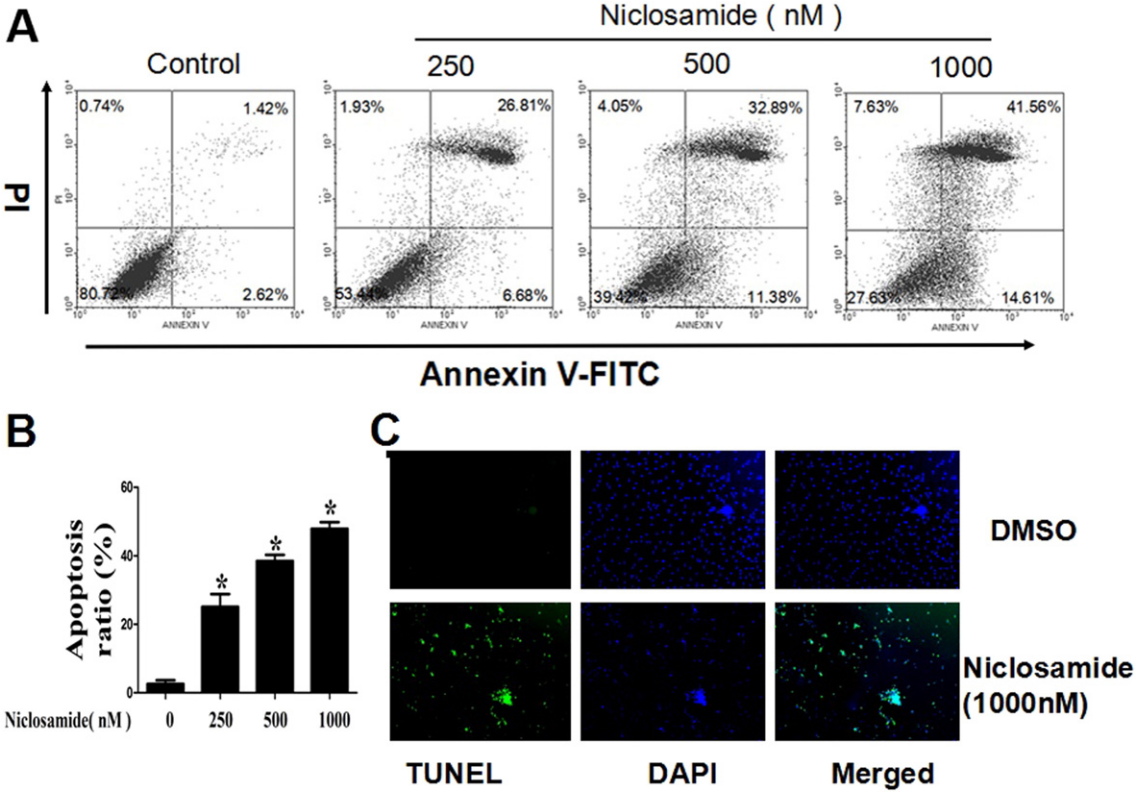

Fig. 2. Niclosamide induces apoptotic death in RA FLS. A–B, Measurement of apoptosis in RA FLS treated with or without niclosamide for 72 h using annexin V/PI staining assay. Representative dot plots of flow cytometry are shown in upper panels. Bar graphs show quantitative analysis of apoptosis from three independent experiments. C, The niclosamide-induced apoptosis of RA FLS as measured by TUNEL staining. FLS were exposed to niclosamide for 48 h. The images were captured under a fluorescence microscope. The nuclei are shown in blue, and the TUNEL-positive cells are shown in green. Results are expressed as fold change relative to the control. *P < 0.05 vs. control.