Biochemical and Biophysical Research Communications xxx (2016) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Anthelmintic drug niclosamide enhances the sensitivity of chronic myeloid leukemia cells to dasatinib through inhibiting Erk/Mnk1/eIF4E pathway

Zhong Liu [a, 1], Yong Li [a, 1], Cao Lv [a], Li Wang [b, **], Hongping Song [a, *]

[a] Department of Pharmacy, Puai Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, 430033, China
[b] Department of Obstetrics and Gynecology, Puai Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, 430033, China

ARTICLE INFO

Article history:
Received 4 August 2016
Accepted 8 August 2016
Available online xxx

Keywords:
Chronic myeloid leukemia
Niclosamide
Erk/Mnk/eIF4E
TKI resistance

ABSTRACT

Chronic myeloid leukemia (CML) responds well to BCR-ABL tyrosine kinase inhibitors (TKI), but becomes resistant to TKIs after it progresses to blast phase (BP). Here we show that niclosamide, a FDA-approved anthelmintic drug, enhances the sensitivity of BP-CML cells to dasatinib (2nd generation of BCR-ABL TKI) through inhibiting Erk/Mnk1/eIF4E signaling pathway. Niclosamide dose-dependently inhibits proliferation and induces apoptosis in a panel of CML cell lines. It also selectively targets BP-CML CD34 stem/progenitor cells through inducing apoptosis, inhibiting colony formation and self-renewal capacity while sparing normal bone marrow (NBM) counterparts. In addition, combination of niclosamide and dasatinib is synergistic in CML cell lines and BP-CML CD34 cells. Importantly, niclosamide inhibits phosphorylation of Erk, Mnk1 and eIF4E in CML cells. Overexpression of phosphomimetic but not nonphosphorylatable form of eIF4E reverses the inhibitory effects of niclosamide, suggesting that eIF4E inhibition is required for the action of niclosamide in CML. Compared to NBM, the increased levels of eIF4E and its activity in CML CD34 cells might explain the selective toxicity of niclosamide in CML versus NBM. We further show that dasatinib time-dependently induces eIF4E phosphorylation. The combination of eIF4E depletion and dasatinib results in similar effects as the combination of niclosamide and dasatinib, suggesting that niclosamide enhances dasatinib through targeting eIF4E. Our work is the first to demonstrate that niclosamide is a potential drug to overcome resistance to BCR-ABL TKI treatment in BP-CML. Our findings also suggest the therapeutic value of Erk/Mnk/eIF4E in CML treatment.

1. Introduction

Chronic myeloid leukemia (CML) is a hematological stem cell malignancy caused by the reciprocal translocation t(9:22) [1]. The chromosome translocation produces oncogene BCR-ABL that drives the pathogenesis of CML by activating proliferation and survival pathways. Therefore, treatment with the BCR-ABL tyrosine kinase inhibitors (TKIs), such as imatinib, has dramatically improved the treatment of CML with the majority of chronic phase (CP) patients [2]. Although imatinib is effective in managing CML, it is not effective in eliminating residual leukemia progenitor cells that may serve as a source for relapse [3,4], suggesting that BCR-ABL-independent pathways support leukemia stem/progenitor cells survival. Alternative therapeutic strategies targeting these essential pathways are required for better clinical management of CML.

Niclosamide is a FDA-approved anthelmintic drug, particularly for cestodes infection [5]. It inhibits oxidative phosphorylation in the mitochondria leading to cestodes death [6]. Recently, niclosamide was reported to exhibit anti-tumorigenic activity in a number of human cancers, including colorectal, ovarian cancers and myeloma [7–10]. Niclosamide also enhances the inhibitory effects of conventional chemotherapeutic drugs (eg, cisplatin) in cancer [11,12]. Several work demonstrate that niclosamide acts on cancer cells through inhibiting Wnt/β-catenin, Stat3, NK-κB, Notch and ROS [7–11,13–15]. Importantly, niclosamide has been shown to have cytotoxicity on acute myeloid leukemia stem cells [15]. However, the effects of niclosamide in CML is unknown.

* Corresponding author.
** Corresponding author.
E-mail addresses: 932259164@qq.com (L. Wang), yjkpuai@126.com (H. Song).
[1] Zhong Liu and Yong Li contributed to this work equally.

http://dx.doi.org/10.1016/j.bbrc.2016.08.047
0006-291X/

In this study, we have investigated the effects of niclosamide and its underlying mechanisms in CML using cell lines and primary CD34 stem/progenitor cells derived from patients with BP-CML. Our results show that niclosamide selectively inhibits proliferation, colony formation and self-renewal capacity, and induces apoptosis of multiple CML cell lines and patient CD34 cells while sparing normal bone marrow (NBM) counterparts. Importantly, niclosamide is synergistic with dasatinib (2nd generation of BCR-ABL TKI) in CML. We further demonstrate that the inhibitory effects of niclosamide in CML cells are attributed to its inhibition of Erk/Mnk1/eIF4E signalling pathways. Finally, we find that niclosamide enhances the effects of dasatinib through targeting eIF4E. These findings suggest that niclosamide might serve as an alternative therapeutic strategy, either alone or in combination with dasatinib, for CML treatment, especially those resistant to dasatinib.

2. Materials and methods

2.1. Patient samples

CML samples were obtained from patients seen at Puai Hospital after signed informed consent under protocols approved by the institutional review board. Patients' mononuclear cells were separated by using Ficoll. CD34 cells were selected using CD34 MicroBead kit (Miltenyi Biotec, Germany). NBM CD34 cells were purchased from StemCell Technologies, Inc. BP-CML and NBM CD34 cells were cultured using serum-free StemPro complete medium (Life Technologies, US) as previously described [16]. Stem cell factor (0.2 ng/mL), macrophage inflammatory protein-1α (0.2 ng/mL), granulocyte colony-stimulating factor (1 ng/mL), granulocyte-macrophage colony-stimulating factor (0.2 ng/mL), leukemia inhibitory factor (50 pg/mL) and interleukin 6 (1 ng/mL) were added in the culturing medium.

2.2. Cell culture and drugs

Human CML cell lines K562, KU812 and KCL22 were obtained from American Type Culture Collection and KBM7 was obtained from Haplogen GmbH. CML cells were cultured in RPMI1640 medium (Life Technologies, US) supplemented with 10% fetal bovine serum (FBS) (Hyclone, UK) and 2 mM glutamine (Invitrogen, US). Dasatinib (LC, laboratories, US) and niclosamide (Sigma, US) were dissolved in DMSO.

2.3. Western blotting

Whole protein from cells were lysed by RIPA lysis buffer (Life Technologies Inc, US) supplemented with protease inhibitor cocktail (Roche, US). Total proteins were processed for western blot analysis using antibodies recognizing Erk, phosphor-Erk, Mnk1, phosphor-Mnk1, eIF4E, phosphor-eIF4E and β-actin (Cell Signaling Technologies, US).

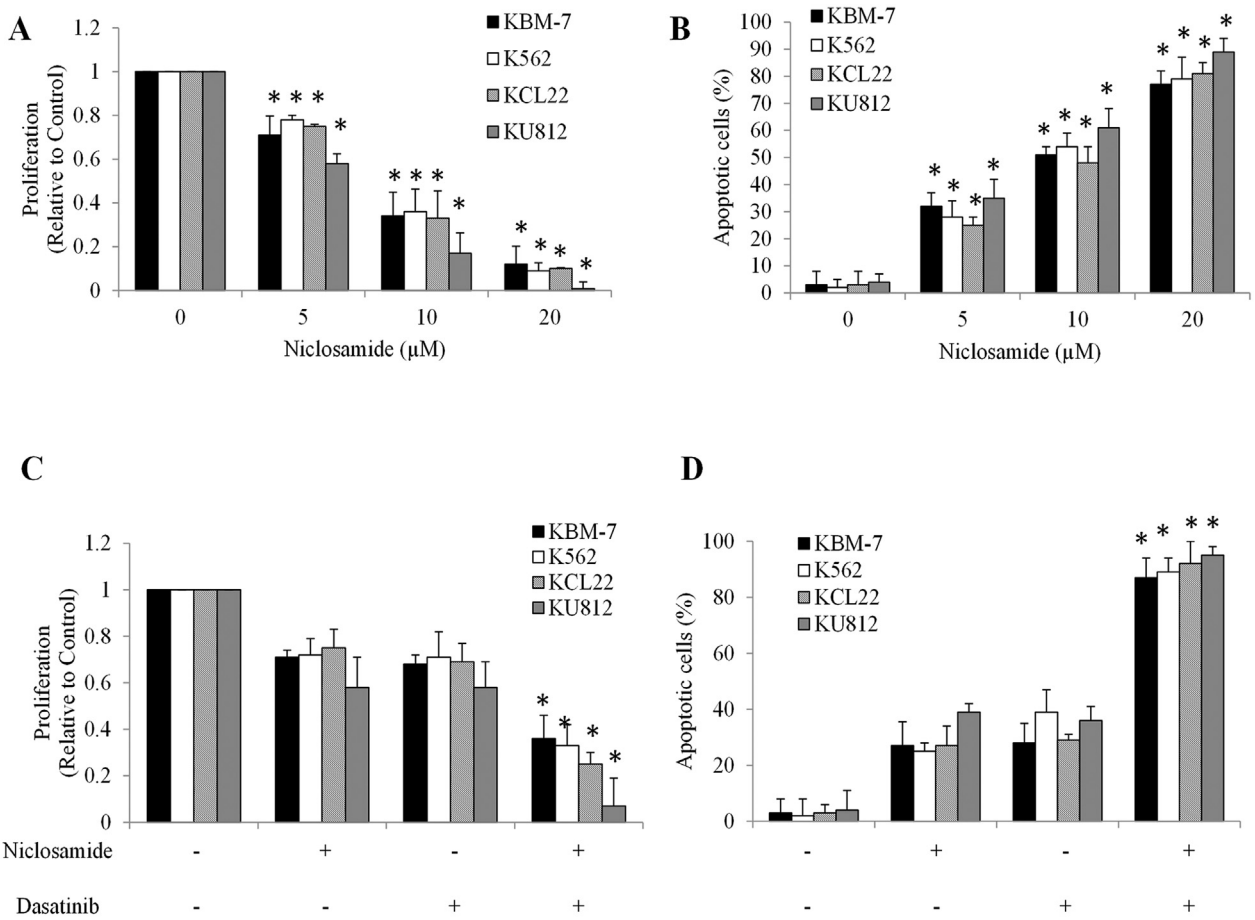

Fig. 1. Niclosamide is active against multiple CML cell lines. Niclosamide significantly inhibits proliferation (A) and induces apoptosis (B) of KBM7, K562, KU812 and KCL22 cells. Combination of niclosamide and dasatinib inhibits more proliferation (C) and induces more apoptosis (D) of KBM7, K562, KU812 and KCL22 cells compared to single drug alone. Cells were treated with niclosamide, dasatinib alone or both for 72 h. Niclosamide at 5 μM and dasatinib at 100 nM were used in combination studies. *, $p < 0.5$, compared to control or single arm.

Please cite this article in press as: Z. Liu, et al., Anthelmintic drug niclosamide enhances the sensitivity of chronic myeloid leukemia cells to dasatinib through inhibiting Erk/Mnk1/eIF4E pathway, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.08.047