Available online at www.sciencedirect.com
SCIENCE DIRECT
Cancer Letters 241 (2006) 301–308
CANCER Letters
www.elsevier.com/locate/canlet

Effects of the histone deacetylases inhibitors sodium butyrate and trichostatin A on the inhibition of gap junctional intercellular communication by H_2O_2- and 12-O-tetradecanoylphorbol-13-acetate in rat liver epithelial cells

Ji-Won Jung [a], Sung-Dae Cho [a], Nam-Shik Ahn [a], Se-Ran Yang [a], Joon-Suk Park [a], Eun-Hye Jo [a], Jae-Woong Hwang [a], Okezie I. Aruoma [a,b], Yong-Soon Lee [a,*], Kyung-Sun Kang [a,*]

[a] *Department of Veterinary Public Health, College of Veterinary Medicine, Seoul National University, San 56-1, Sillim-dong, Gwanakgu, Seoul 151-742, South Korea*
[b] *Department of Applied Science, London South Bank University, 103 Borough Road, London SE1 0AA, UK*

Received 3 August 2005; received in revised form 24 October 2005; accepted 24 October 2005

Abstract

The histone deacetylase (HDAC) inhibitors, trichostatin A (TSA) and sodium butyrate (NaBu) are considered as potent therapeutic agents for cancer treatment presenting therapeutic benefits with less risk of side effects. The microbial metabolite, TSA is a potent reversible and highly specific inhibitor of mammalian histone deacetylases. NaBu causes hyperacetylation of core histones with effects similar to TSA but it is not a specific inhibitor of HDACs. The gap junction is a channel in the plasma membrane of most cell types which allows direct communication (gap junctional intercellular communication; GJIC) of small molecules and ions. Modulation of GJIC is a known cellular event associated with tumor promotion. The effects of NaBu and TSA on the H_2O_2- and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced GJIC inhibition of WB cells and the mechanisms involved in the process were assessed. TSA and NaBu exerted differential preventive effects on the H_2O_2 and TPA-induced inhibition of GJIC as well as hyperphosphorylation of connexin43 (Cx43) in WB-F344 rat liver epithelial cells (WB cells). NaBu prevented the TPA-induced GJIC inhibition via ERK1/2 inactivation whilst TSA restored the H_2O_2-induced GJIC inhibition and Cx43 hyperphosphorylation by preventing p38 MAP kinase. The inhibition of tyrosine phosphorylation and down-regulation of src protein observed may also contribute to Connexin 43 dephosphorylation and GJIC restoration by TSA and NaBu partly through depletion of src protein pool. Thus, TSA and NaBu exert differential effects on chemically induced GJIC inhibition via modulation of MAP kinases and partly, tyrosine kinases.
© 2005 Elsevier Ireland Ltd. All rights reserved.

Keywords: Histone deacetylase inhibitor; Sodium butyrate; Trischostatin A; Gap junctional intercellular communication; Rat liver; Cancer promotion

1. Introduction

There is increasing evidence that HDAC inhibitors could contribute to cell differentiation and/or apoptosis through modulation of various protein kinases which

* Corresponding author. Tel.: +82 2 880 1298/1246; fax: +82 2 876 7610.
E-mail addresses: leeys@snu.ac.kr (Y.-S. Lee), kangpub@snu.ac.kr (K.-S. Kang).

0304-3835/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2005.10.029

include mitogen-activated protein (MAP) kinases and tyrosine kinases as well as HDAC inhibition resulting in conformational changes in chromosomes [1–3].

Modulation of MAP kinases including extracellular signal-regulated protein kinases (ERKs) 1/2, p38 MAP kinase and c-Jun N-terminal kinases (JNKs) is involved in various cellular responses such as regulation of cell cycle, apoptosis, proliferation, intercellular communication via gap junction, and signaling molecules depending on cell type, strength and duration of signal [4–6].

The gap junction is a channel in the plasma membrane of most cell types, which allows direct communication (gap junctional intercellular communication; GJIC) of small molecules and ions through two juxtaposed hemi-channel comprised with hexamerized connexin proteins called connexons [7,8]. It functions in homeostasis, cell growth, differentiation, and other physiological processes [5,9]. The inhibition of GJIC by either chemical tumor promoters or oncogenes is implicated in the mechanism of tumor promotion and progression [10–14]. The conformational changes of Cx43, a major connexin in astrocytes, cardiac myocytes and WB-F344 rat liver epithelial cells (WB-cells), leading to GJIC inhibition are mediated with phosphorylation of its serine or tyrosine residue of C-terminus induced by MAP kinases including ERK1/2 and p38 MAP kinase [15,16]. Most of tumor-promoting factors including phorbol esters, hydrogen peroxide (H_2O_2), epidermal growth factor and oncogenic ras protein inhibit GJIC via phosphorylation of Cx43 subsequently with modification of phosphorylation pattern of MAP kinases [15,17,18].

The HDAC inhibitor 4-phenylbutyrate has been shown to enhance GJIC, which is accompanied by an elevation of Cx43 expression in human glioblastoma, and rat glioma cells [19,20]. However, the GJIC modulation by HDAC inhibitors during chemically induced tumor promotion and the associated molecular mechanisms are yet to be clarified. The microbial metabolite, TSA, is not only a potent reversible inhibitor of mammalian histone deacetylases [21] but also a highly specific inhibitor of HDACs. NaBu cause hyperacetylation of core histones and although inducing similar effects as TSA, it is not a specific inhibitor of HDACs, however, both induce apoptotic cell death in tumor cells [22,23]. In the present study, the chemopreventive effect of two HDAC inhibitors, sodium butyrate (NaBu) and trichostatin A (TSA), in the 12-*O*-tetradecanoylphorbol-13-acetate (TPA)- and H_2O_2-induced GJIC inhibition of WB cells and the mechanisms involved were assessed. NaBu could prevent TPA-induced GJIC inhibition via ERK1/2 inactivation, on the other hand, TSA could restore H_2O_2-induced GJIC blockage by preventing p38 MAP kinase. The inhibition of tyrosine phosphorylation and down-regulation of src protein were observed and suggested to contribute to Cx43 dephosphorylation and GJIC restoration.

2. Materials and methods

2.1. Chemicals

Lucifer yellow CH and anti-β-actin monoclonal antibody were obtained from Sigma Chemical (St Louis, MO, USA). Anti-Cx43 monoclonal antibody was supplied from Chemicon Laboratories (Temecula, CA, USA). Anti-phospho MAPK and anti-phospho p38 antibodies were purchased from Promega (Madison, WI, USA). Anti-phospho JNK, anti-p38 polyclonal and anti-JNK1 monoclonal antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-MAP polyclonal antibody was supplied from Zymed Laboratories Inc. (San Francisco, CA, USA). Anti-phospho MKK3/6 polyclonal antibodies were from Cell Signaling Technology Inc. (Beverly, MA, USA). Anti-p-Tyr, anti-v-src monoclonal antibodies were obtained from Oncogene Research Products (San Diego, CA, USA).

2.2. Cell culture

WB-F344 (WB) cell, a kind gift of Dr J. Trosko of Michigan State University (East Lansing, MI, USA) was cultured in D-media (Formula No. 78-5470EF, Gibco BRL, Grand Island, NY, USA) containing 3 ml/l PSN mixture (Gibco BRL, Grand Island, NY, USA) in the presence of 5% fetal bovine serum (Gibco BRL, Grand Island, NY, USA). Cells were incubated in a 37 °C humidified incubator containing 5% CO_2 and 95% air. Cells were grown in 75 mm tissue culture plates and the culture medium was changed every other day.

2.3. Cell viability assay

The cytotoxic effects of each chemical on WB cells were measured by MTT assay, based on the ability of live cells to convert tetrazolium salt into purple formazan. In brief, the cells were seeded in 24-well microplates and incubated overnight. Then the cells were treated with different concentrations of the test chemicals or its vehicle, DMSO (0.1%) or deionized water, for 24 h. At the end of these periods, 50 μl of MTT stock solution (5 mg/ml, Sigma) was added to each well and the plates were further incubated for 4 h at 37 °C. The supernatant was removed and 500 μl of DMSO was added to each well to solubilize the water insoluble purple formazan crystals, and then transferred into 96-well microplate for reading. The absorbency at a wavelength of 570 nm was measured with EL800 microplate reader (BIO-TEK Instrument, Winooski, VT). All the measurements were performed in triplicate.

Download English Version:

https://daneshyari.com/en/article/2115679

Download Persian Version:

https://daneshyari.com/article/2115679

Daneshyari.com