Nitric Oxide 29 (2013) 17–24

Contents lists available at SciVerse ScienceDirect

Nitric Oxide

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

Contribution of either YY1 or Bcl_{XL}-induced inhibition by the NO-donor DETANONOate in the reversal of drug resistance, both *in vitro* and *in vivo*. YY1 and Bcl_{XL} are overexpressed in prostate cancer

Sara Huerta-Yepez [a,b], Stavroula Baritaki [a], Guillermina Baay-Guzman [b], Marco A. Hernandez-Luna [b], Angeles Hernandez-Cueto [c], Mario I. Vega [a,d], Benjamin Bonavida [a,*]

[a] *Department of Microbiology, Immunology & Molecular Genetics, David Geffen School of Medicine, Jonsson Comprehensive Cancer Center, University of California at Los Angeles, Los Angeles, CA 90095, USA*
[b] *Unidad de Investigación en Enfermedades Oncológicas, Hospital Infantil de México, Federico Gómez, Mexico City, Mexico*
[c] *Unidad de Investigacion en Inmunologia e Infectologia CMN "La Raza", IMSS, Mexico City, Mexico*
[d] *Oncology Research Unit, Oncology Hospital, Siglo XXI National Medical Center IMSS, Mexico City, Mexico*

ARTICLE INFO

Article history:
Received 22 August 2012
Revised 3 December 2012
Available online 11 December 2012

Keywords:
Nitric oxide
Sensitization
YY1
Bcl_{XL}
Chemoresistance
Prostate cancer

ABSTRACT

Nitric oxide (NO) donors have been shown to activate or inhibit constitutively-activated survival/anti-apoptotic pathways, such as NF-κB, in cancer cells. We report here that treatment of drug-resistant human prostate carcinoma cell lines with high levels (500–1000 μM) of the NO-donor DETANONOate sensitized the resistant tumor cells to apoptosis by CDDP and the combination was synergistic. We hypothesized that DETANONOate inhibits previously identified NF-κB-regulated resistant factors such as Yin Yang 1 (YY1) and Bcl-2/Bcl_{XL}. Lysates from tumor cells treated with DETANONOate showed inhibition of YY1 and Bcl_{XL} expressions. Transfection with either YY1 or Bcl_{XL} siRNA resulted in the inhibition of both YY1 and Bcl_{XL} expressions and sensitized the cells to CDDP apoptosis. Mice bearing PC-3 tumor xenografts and treated with the combination of DETANONOate and CDDP resulted in significant inhibition of tumor growth; treatment with single agent alone did not have any effect on tumor growth. Analysis of patients TMA tissues with prostatic cancer revealed higher expression of both YY1 and Bcl_{XL} as a function of tumor grades and their levels were directly correlated. Thus, both YY1 and Bcl_{XL} are potential prognostic biomarkers. Overall, the above findings suggest that one mechanism of DETANONOate-induced sensitization of resistant tumor cells to CDDP correlated with the inhibition of NF-κB and its targets YY1 and Bcl_{XL}. The examination of the combination of NO donors and cytotoxic therapy in the treatment of resistant prostate cancer may be warranted.

Published by Elsevier Inc.

Introduction

Currently, treatment of various cancers relies on conventional chemotherapy, radiation, hormonal therapy, and immunotherapy [1]. While such treatments result in objective clinical responses, however, several patients experience relapse and metastasis [2]. The failure to respond to such therapies is due, in large part, to acquired tumor resistance to apoptotic signals via genetic and epigenetic alterations [3,4].

We and others have reported novel approaches to overcome resistance via the application of various chemo and immuno-sensitizing agents [5,6]. For instance, we have reported that treatment of resistant tumor cells *in vitro* with an NO-donor, such as DETANONOate, resulted in the sensitization to TRAIL-mediated apoptosis via inhibition of the constitutively activated NF-κB survival/anti-apoptotic pathway [7,8]. In addition, we have reported that treatment with DETANONOate also resulted in the inhibition, downstream of NF-κB, of the transcription repressor Yin Yang 1 (YY1) that regulates resistance to TRAIL apoptosis [8].

Based on these findings, we investigated whether treatment with DETANONOate also results in the chemosensitization of drug-resistant tumor cells and examined underlying mechanisms of sensitization. A previous report by the Wink's group demonstrated that, under appropriate conditions, NO can sensitize tumor cells to various chemotherapeutic agents although the mechanism was not investigated [9]. The present study analyzed the

Abbreviations: CDDP, cisplatin-diamine-dichloro platinum; DETANONOate, diethylenetriamine NONOate; DR5, death receptor 5; EMSA, electrophoretic mobility shift assay; NO, nitric oxide; siRNA, small interfering RNA; TMA, tissue microarray; TRAIL, tumor necrosis factor (TNF)-related apoptosis-inducing ligand; TESS, transcriptionally element search system; YY1, Yin Yang 1.

* Corresponding author. Fax: +1 310 206 2791.
E-mail address: bbonavida@mednet.ucla.edu (B. Bonavida).

1089-8603/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.niox.2012.12.001

chemosensitizing activity of the NO-donor DETANONOate, used at high concentrations, on drug-resistant human prostate carcinoma cell lines, and its clinical significance. The followings were investigated: (1) the chemosensitizing activity of DETANONOate to CDDP-induced apoptosis in resistant DU-145 and PC-3 human prostate carcinoma cell lines (2) the DETANONOate-mediated effect on the NF-κB targeted resistant factors YY1 and Bcl_{XL} (3) the direct role each of YY1 and Bcl_{XL} in sensitization (4) the antitumor activity of DETANONOate *in vivo* in mice bearing PC-3 tumor xenografts following treatment with DETANONOate, CDDP and combination and (5) the expression levels of both YY1 and Bcl_{XL} in human prostate cancer tissue microarrays (TMA) as a function of tumor grade.

Materials and methods

Cells and culture conditions

The human androgen-independent PC-3 and DU145 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained as previously described [8].

Reagents

The anti–actin and the anti-tubulin monoclonal antibodies were purchased from Biosource International (Camarrillo, CA, USA) and from Calbiochem (San Francisco, CA, USA), respectively. Anti-YY1 and anti-Bcl_{XL} antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Phycoerytrin (PE)-conjugated anti-active caspase 3 and PE-conjugated IgG were purchased from PharMingen (San Diego, CA, USA). DETANONOate was purchased from Alexis (San Diego, CA, USA). The SureSilencingTM siRNA kits were purchased from SuperArray Bioscience Corporation (Frederick, MD, USA). CDDP was purchased from Sigma-Aldrich, St. Louis, MO.

Cell treatments

Log-phase prostate carcinoma cell lines cells were seeded into six-well plates at approximately 6×10^5 cells/ml and grown in 2 ml of medium containing 5% FBS for 24 h to approximately 70% confluence. The PC-3 cells were synchronized by treatment with 1% FBS for 18 h prior to each experiment.

Western blot analysis

PC-3 cells were cultured at a low FBS concentration (1%) for 18 h prior to each treatment. After incubation, the cells were maintained in FBS-free medium (control) or treated with DETANONOate (500 or 1000 μM). Lysates were prepared and analyzed by western as previously described [10].

Semiquantitative reverse transcription–polymerase chain reaction (RT–PCR)

Total RNA of PC-3 cells was extracted and purified from $\sim 1 \times 10^6$ cells by a single-step monophasic solution of phenol and guanidine isothiocyanate-chloroform using Trizol® reagent (Life Technologies, Inc.) as previously described [8].

siRNA transfections

PC-3 cells were cultured in 1 ml of RPMI medium supplemented with 5% FBS. Transfection of PC-3 cells with siRNAs was performed as previously described [11].

Determination of apoptosis

Apoptosis was determined by flow cytometry for the activation of caspase 3 as previously described [7,8]. The cells were treated with DETANONOate (500–1500 μM) for 18 h and then followed by treatment with CDDP (1,5, 10, or 15 μg/ml) for an additional 24 h.

Assessment of in vivo tumor growth

All animal experiments were done in accordance with institutional guidelines for animal welfare. The animals were maintained under clean room conditions in sterile rodent microisolator cages. Animals received sterile rodent chow and water *ad libitum*. For tumor growth experiments, 8-week-old athymic nude mice were used. Human PC-3 prostate carcinoma cells were injected s.c. with an 27-gauge needle into the right hind limb (1×10^6 cells in 100 μl of PBS) under sevofluorane anesthesia. When the animals developed palpable tumors (50–100 μM^3), the mice were randomly assigned to different groups of 6 mice each: the first experimental animal group was treated with 2.0 mg/kg of CDDP via i.p. twice a week for two weeks (weeks four and five); the second experimental group was treated with 0.4 mg/kg of DETANONOate injected directly into the tumor under sevofluorane anesthesia (in 50 μl) every 2 d for 2 weeks (weeks four and five); and the third experimental group was treated with DETANONOate similar to the second experimental group and followed by treatment with CDDP like the first experimental group. The dosages used were as previously described [12,13].

Tumor volume was measured with a caliper once a week and calculated according to the following formula: A (length) $\times$ B (width) $\times$ C (height) $\times$ 0.5236. The efficacy of each treatment was evaluated by the change of tumor volume during the treatment period. After treatments, the mice were euthanized and tumors were harvested for histological studies.

Immunohistochemistry

Four-micrometer slices of the PC-3 tumor cell line were placed on slides and either stained with Mayer's hematoxylin and eosin (H&E) for the histopathological examination or used for immunohistochemistry. The expression levels of YY-1 and Bcl_{XL} were determined using the antibodies directed against YY-1 and Bcl_{XL}. Analysis and quantification were done as previously described [8].

Patients with prostate cancer

The study cohort consisted of 60 randomly selected hormonenaïve patients who underwent radical retropubic prostasectomy between 2002 and 2006 from the Department of Pathology of Hospital de Especialidades del CMN "La Raza" IMSS (Mexico City, Mexico). The cohort consisted of 20 PCa low grade (PCaLG), 20 PCa medium grade (PCaMG) and 20 PCa high grade (PINHG) samples [14].

Prostate tissue microarray

At least three core tissue paraffin embedded biopsies (each 0.6 μM in diameter) were taken from morphologically representative regions of each prostate tumor and precisely arrayed as previously described [15]. Tumor samples were accompanied by matching benign (morphologically normal or hypertrophic) and PIN lesions, when available. Case material was arrayed into five TMA blocks. For staining, sections (4 μ) were transferred to glass slides using an adhesive slide system (PSA-CS 4, Instrumedics Inc.) to support cohesion of the array elements.