Bioorganic & Medicinal Chemistry Letters 27 (2017) 2286–2291

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Brefeldin A enhances docetaxel-induced growth inhibition and apoptosis in prostate cancer cells in monolayer and 3D cultures

Huarong Huang [a,*], Ting Liu [a], Junxi Guo [a], Lin Yu [a], Xiaofeng Wu [a], Yan He [a], Dongli Li [b], Junlei Liu [a], Kun Zhang [a], Xi Zheng [a,c,*], Susan Goodin [d]

[a] Allan H. Conney Laboratory for Anticancer Research, Guangdong University of Technology, Guangzhou 510006, China
[b] Department of Chemical Engineering and Environment, Wuyi University, Jiangmen 510060, China
[c] Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, United States
[d] Rutgers Cancer Institute of New Jersey, New Brunswick, NJ 08903, United States

ARTICLE INFO

Article history:
Received 30 January 2017
Revised 12 April 2017
Accepted 13 April 2017
Available online 17 April 2017

Keywords:
Brefeldin A
Docetaxel
Prostate cancer
Bcl-2
3D culture

ABSTRACT

Docetaxel is a commonly used chemotherapeutic drug for patients with late stage prostate cancer. However, serious side effect and drug resistance limit its clinical success. Brefeldin A is a 16-membered macrolide antibiotic from mangrove-derived Fungus *Aspergillus* sp. (9Hu), which exhibited potent cytotoxicity against human cancer cells. In the present study, we determined the effect of brefeldin A on docetaxel-induced growth inhibition and apoptosis in human prostate cancer PC-3 cells. Brefeldin A in combination with docetaxel inhibited the growth of PC-3 cells in monolayer and in three dimensional cultures. The combination also potently stimulated apoptosis in PC-3 cells as determined by propidium iodide staining and morphological assessment. Mechanistic studies showed that growth inhibition and apoptosis in PC-3 cells treated with brefeldin A and docetaxel were associated with decrease in the level of Bcl-2. The present study indicates that combined brefeldin A with docetaxel may represent a novel approach for improving the efficacy of docetaxel, and Bcl-2 may serve as a target for brefeldin A to enhance the effects of docetaxel chemotherapy.

Prostate cancer is one of the most commonly diagnosed visceral malignancies and the second leading cause of cancer-related deaths among men in the United States.[1] Androgen deprivation therapy (ADT) remains the main treatment for advanced and metastatic prostate cancer.[2] Despite initial response, nearly all patients on ADT progress to castration-resistant prostate cancer (CRPC) in 18–24 months.[3] Although the newer and more potent anti-androgen treatment have shown some promise, resistance is already being encountered in the clinic.[4] Chemotherapy with docetaxel is currently a standard treatment for metastatic and CRPC and remains a backbone in current development of treatment regimens.[5]

Docetaxel (Fig. 1), an anti-microtubule agent, was approved by the US FDA as the mainstay treatment against CRPC.[5,6] Docetaxel is produced semi-synthetically from the needles of the Pacific yew tree (*Taxus brevifolia*). Studies had shown that docetaxel was more effective against progressive human prostate cancers than other conventional anti-cancer agents.[6–8] Although initially effective, docetaxel-based regimen has only shown a median survival of 18–20 months and response rate of only 50%.[7,8] In addition, there are severe side effects associated with docetaxel treatment including the suppression of bone marrow function leading to immune dysfunction and anemia.[9] The development of chemoresistance to docetaxel is observed in some patients which limits its clinical success.[10,11] Clearly, it holds high clinical significance to enhance the efficacy of docetaxel at lower doses in a less-toxic manner and to reduce the resistance to this chemotherapeutic drug.

Marine-derived fungi have become a rich source of biologically active natural products.[12] We have focused on screening out natural antitumor metabolites from mangrove-derived fungi.[13,14] Among the isolated metabolites, brefeldin A (Fig. 1), identified as an antitumor macrolide-compound, was newly found to the most abundant metabolite in the extract of the fungus *Aspergillus* sp. (No. 9Hu), derived from the root of the mangrove *Kandelia candel*.[15] Brefeldin A, isolated previously from the *Penicillium decumben* by Singleton et al.,[16,17] has been initially known to play a regulatory role in the intracellular transport system.[18,19] It induces the reversible disassembly of the Golgi complex, resulting in the

* Corresponding authors at: Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, United States (X. Zheng).
E-mail addresses: hrhuang@gdut.edu.cn (H. Huang), xizheng@pharmacy.rutgers.edu (X. Zheng).

http://dx.doi.org/10.1016/j.bmcl.2017.04.047
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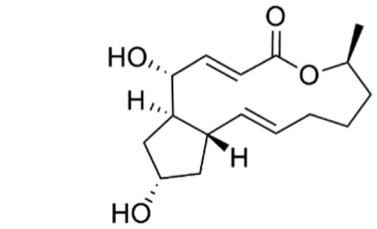
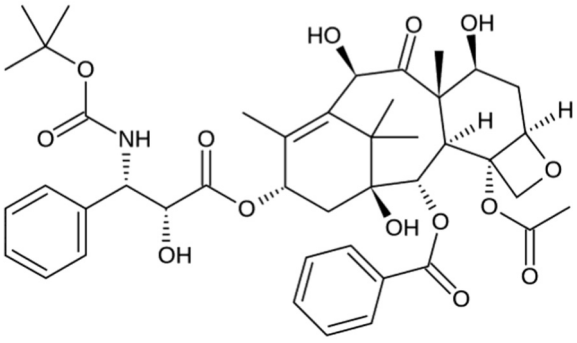


Brefeldin A



Docetaxel

Fig. 1. Structures of docetaxel and brefeldin A.

interruption of protein transport from the endoplasmic reticulum (ER) to the Golgi.[18] Brefeldin A has also been shown to collapse the Golgi complex into the ER, redistributing Golgi-associated proteins/enzymes to the ER.[19] In addition, brefeldin A had been found to induce apoptosis and growth inhibition in several human cancer cells, including leukemia, colon, and prostate cancer cells.[20–23] An earlier study in showed that brefeldin A had anti-proliferative activity in vivo in animal models.[24]

Although earlier studies have demonstrated the anticancer activity of brefeldin A in prostate cancer cells,[22,25,26] the effects of brefeldin A in combination with commonly used chemotherapeutic drugs on growth and apoptosis of prostate cancer cells have not been reported. If brefeldin A can enhance the efficacy of docetaxel at lower doses in a less-toxic manner, it will significantly improve the docetaxel-based chemotherapy and be of great benefit for patients. We hypothesized that brefeldin A will enhance the effectiveness of docetaxel in prostate cancer cells. To test our hypothesis, we determined the effects of brefeldin A isolated from mangrove-derived Fungus *Aspergillus* sp. and docetaxel alone or in combination on growth and apoptosis of human prostate cancer cells.

The extraction and isolation of brefeldin A from mangrove-derived fungus *Aspergillus* sp. (No. 9Hu) were carried out as previous described.[15] The chemical structure of brefeldin A was elucidated by comparing the MS, ^{1}H and ^{13}C NMR, and X-ray diffraction spectroscopic data (crystallographic data for BFA has been deposited with the Cambridge Crystallographic Data Centre, CCDC number 1525069, see supplementary material) with reported values.[16,17,27] The effects of brefeldin A and docetaxel alone or in combination on the growth of human prostate cancer cells were determined using the trypan blue exclusion assay. As shown in Fig. 2A, treatment of human prostate cancer LNCaP (androgen-dependent) and PC-3 (androgen-independent) cells

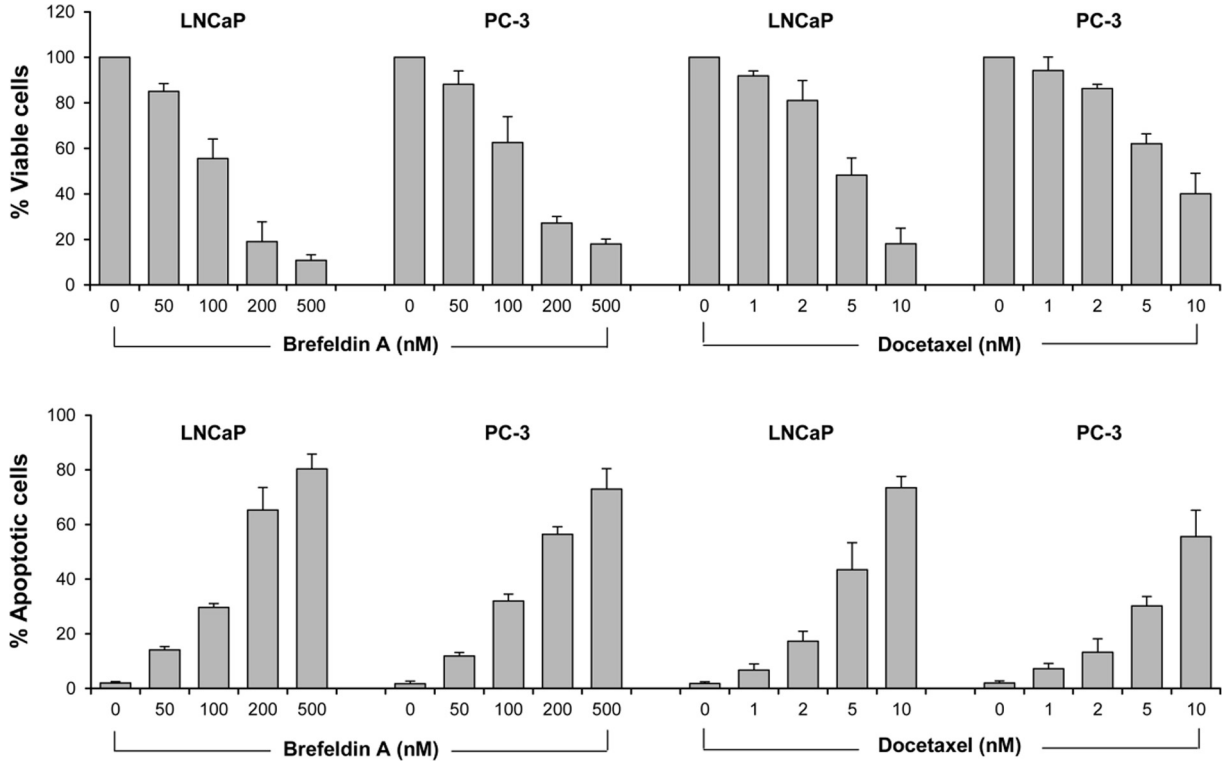


Fig. 2. Effects of docetaxel and brefeldin A on human prostate cancer cells. LNCaP and PC-3 cells were seeded at a density of 0.2×10^5 cells/ml in cell culture dishes and incubated for 24 h. The cells were then treated with different concentrations of docetaxel or brefeldin A for 72 h. The number of viable cells was determined by the trypan blue exclusion assay. Apoptosis was determined by propidium iodide staining and morphological assessment.