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3′-Hydroxy-4′-methoxy-β-methyl-β-nitrostyrene inhibits tumor growth through ROS generation and GSH depletion in lung cancer cells

Chun-Hao Tsai [a,b], Pei-Wen Hsieh [c,d,k], Yi-Chen Lee [a,e], Chie-Hong Wang [a,f,h], Wen-Chin Chiu [a,g], Chun-Wun Lu [a], Yen-Yun Wang [h], Stephen Chu-Sung Hu [i,j], Tain-Lu Cheng [b], Shyng-Shiou F. Yuan [a,b,f,h,*]

[a] Translational Research Center, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[b] Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[c] Graduate Institute of Natural Products, School of Traditional Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan
[d] Department of Anesthesiology, Chang Gung Memorial Hospital, Taoyuan, Taiwan
[e] Department of Anatomy, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[f] Department of Obstetrics and Gynecology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[g] Division of Thoracic Surgery, Department of Surgery, Kaohsiung Medical, University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[h] Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[i] Department of Dermatology, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
[j] Department of Dermatology, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[k] Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan

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ABSTRACT

Aims: Members of the β-nitrostyrene family are known to suppress tumor growth, with the underlying mechanisms of β-nitrostyrene remain mostly unclear. Herein, we synthesized a β-nitrostyrene derivative, 3′-hydroxy-4′-methoxy-β-methyl-β-nitrostyrene (CYT-Rx20), and explored its anticancer activities in human lung cancer cells in vitro and in vivo.
Main methods: Cell viability was measured by XTT assay. Apoptosis was detected by Annexin V/PI staining. Caspase activation was determined by western blotting. ROS (reactive oxygen species), MMP (mitochondrial membrane potential) and mitochondrial mass were determined by flow cytometry. GSH level was detected by ELISA assay.
Key findings: In this study, we found that CYT-Rx20 significantly reduced cell viability, accompanied by G2/M arrest in lung cancer cells. Increased protein levels of cleaved-caspase families indicated apoptotic cell death upon CYT-Rx20 treatment. Furthermore, increased level of intracellular reactive oxygen species (ROS), loss of mitochondrial membrane potential (ΔΨm), glutathione (GSH) depletion and inhibition of GSH reductase were observed after CYT-Rx20 treatment. The effects of CYT-Rx20 on cell viability and the loss of ΔΨm were significantly reversed when cells were pretreated with thiol antioxidants NAC, GSH, or 2-ME. Finally, xenograft animal study demonstrated that CYT-Rx20 significantly suppressed lung tumor growth in vivo.
Significance: Our data demonstrated that CYT-Rx20 triggered apoptotic cell death in lung cancer cells and suppressed lung tumor growth through GSH depletion, suggesting that CYT-Rx20 may have the potential to be further developed as an anticancer compound for treating lung cancer.

1. Introduction

Lung cancer is a leading cause of cancer death worldwide with a five-year survival rate < 18% [1]. Surgical resection provides a curative treatment for early stage lung cancer, while chemotherapeutic agents, including platinum-based carboplatin and non-platinum-based paclitaxel, are commonly used for the treatment of stage III to IV patients [2,3]. In addition, Erlotinib, an EGFR-targeting antibody, has been used for lung cancer treatment [4,5]. However, chemoresistance remains a serious problem in the management of advanced lung cancer, and there is a urgent need for development of effective chemotherapeutic agents [4].

The β-nitrostyrene family members exert various biological effects, including anticancer, anti-inflammatory, antimicrobial, and antiplatelet activities [6–8]. For example, 3, 4-methylenedioxy-β-nitrostyrene inhibits ATPase and decreases NLRP3 inflammasome activation, resulting in immunosuppression [6]. For anticancer activity, 3, 4-methylenedioxy-β-nitrostyrene inhibits β1 integrin and surface protein disulfide isomerase,

Abbreviations: CYT-Rx20, 3′-hydroxy-4′-methoxy-β-methyl-β-nitrostyrene; IC_{50}, 50% inhibitory concentration; XTT, 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide; ΔΨm, mitochondrial membrane potential; 2-ME, 2-mercaptoethanol.

* Corresponding author at: Translational Research Center, No. 100, Tzyou 1st Road, Kaohsiung 807, Taiwan.
E-mail address: yuanssf@ms33.hinet.net (S.-S.F. Yuan).

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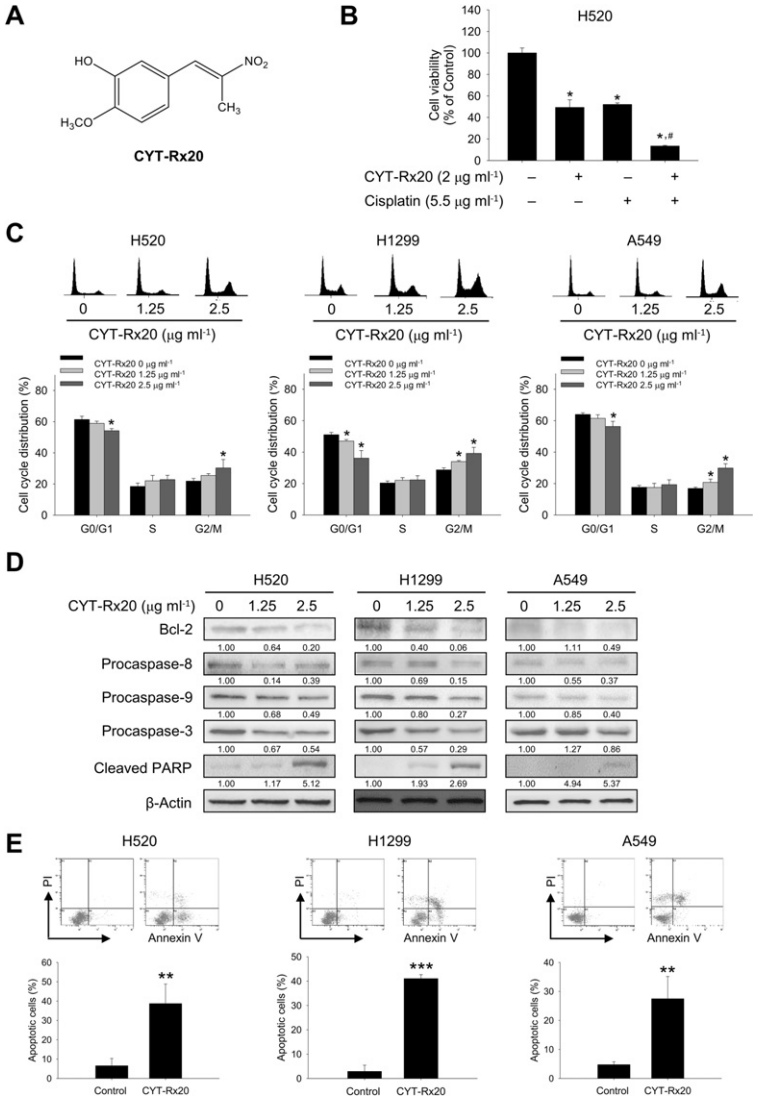

Fig. 1. Effects of CYT-Rx20 on cytotoxicity and cell cycle distribution in lung cancer cells. (A) Chemical structure of CYT-Rx20. (B) CYT-Rx20 synergistically enhanced the cisplatin-induced cytotoxicity. (C) Cells were treated with CYT-Rx20 for 24 h, and cell cycle distribution was examined by flow cytometric analysis. (D) The expression of caspase-associated proteins was determined by immunoblotting after treatment with CYT-Rx20 at the indicated concentrations for 24 h. (E) Cells were treated with CYT-Rx20 (0 or 2.5 μg ml^{-1}) for 24 h, and apoptotic cell death was determined by annexin V/PI staining followed by flow cytometric analysis. *, significant difference ($p < 0.05$) compared with the control group by Student's *t*-test. #, significant difference ($p < 0.05$) compared with the CYT-Rx20 group by Student's *t*-test.

and suppresses breast cancer cell adhesion and migration [9]. In addition, β-nitrostyrene derivatives also induced cancer cell apoptosis by both Ca^{2+}-dependent and –independent pathways [10]. Furthermore, 2-aryl-3-nitro-2H-chromenes, synthetic hybrid analogs of β-nitrostyrene and flavanone, promotes breast cancer cell apoptosis by inducing DNA damage and activating caspase-3 [11].

In a previous report, we showed that CYT-Rx20, a synthetic derivative of β-nitrostyrene, inhibited platelet aggregation and promoted breast cancer cell death [8] accompanied with ROS-mediated autophagic cell death. The activation of autophagy can be significantly reversed by thiol group and partically reversed by ERK inhibitors in breast cancer cells [12]. In current study, the anti-lung cancer activity of CYT-Rx20 and the underlying mechanisms are explored both in vitro and in vivo.

2. Materials and methods

2.1. Reagents

CYT-Rx20 was synthesized according to our previous reports [8, 13]. RPMI 1640, H_2DCFDA, and JC-1 were purchased from Invitrogen (Carlsbad, CA, USA). Fetal bovine serum, penicillin, streptomycin, and amphotericin B were ordered from Biological Industries (Beit Haemek, Israel). XTT, propidium iodide (PI), *N*-acetyl-L-cysteine (NAC), glutathione (GSH), 2-mercaptoethanol (2-ME), SB203580, SC79, DPI, and DMSO were obtained from Sigma-Aldrich (St Louis, MO, USA). All other reagents used in this study were described wherever suitable.

Table 1
Cytotoxicity of CYT-Rx20 and cisplatin on human lung cancer cell lines.

	Cell line	H520	H1299	A549	H441
IC_{50} (μg ml^{-1})[a]	CYT-Rx20	2.30 ± 0.10	2.12 ± 0.11	3.28 ± 0.28	2.57 ± 0.06
	Cisplatin	5.31 ± 0.03	8.99 ± 0.30	3.83 ± 0.25	3.61 ± 0.23

[a] Data were presented as mean ± SD.