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Quinolone analogue inhibits tubulin polymerization and induces apoptosis via Cdk1-involved signaling pathways

Ying-Cheng Chen [a], Pin-Hsuan Lu [a], Shiow-Lin Pan [b], Che-Ming Teng [b], Sheng-Chu Kuo [c], Tsung-Ping Lin [c], Yunn-Fang Ho [a], Yu-Chun Huang [d], Jih-Hwa Guh [a,*]

[a] School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan
[b] Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan
[c] Graduate Institute of Pharmaceutical Chemistry, China Medical University, Taichung, Taiwan
[d] Ching Kuo Institute of Management and Health, Keelung, Taiwan

ARTICLE INFO

Article history:
Received 5 February 2007
Accepted 9 March 2007

Keywords:
Quinolone
Mitotic arrest
Cdk1
Bcl-2 family of proteins
Caspase
AIF

ABSTRACT

Cancer chemotherapeutic agents that interfere with tubulin/microtubule function are in extensive use. Quinolone is a common structure in alkaloids and its related components exhibit several pharmacological activities. In this study, we have identified the anticancer mechanisms of 2-phenyl-4-quinolone. 2-Phenyl-4-quinolone displayed anti-proliferative effect in several cancer types, including hormone-resistant prostate cancer PC-3, hepatocellular carcinoma Hep3B and HepG2, non-small cell lung cancer A549 and P-glycoprotein-rich breast cancer NCI/ADR-RES cells. The IC_{50} values were 0.85, 1.81, 3.32, 0.90 and 1.53 μM, respectively. 2-Phenyl-4-quinolone caused G2/M arrest of the cell-cycle and a subsequent apoptosis. The turbidity assay showed an inhibitory effect on tubulin polymerization. After immunochemical examination, the data demonstrated that the microtubules were arranged irregularly into dipolarity showing prometaphase-like states. Furthermore, 2-Phenyl-4-quinolone induced the Mcl-1 cleavage, the phosphorylation of Bcl-2 and Bcl-xL (12-h treatment), and the caspase activation including caspase-8, -2 and -3 (24-h treatment). The exposure of cells to 2-phenyl-4-quinolone caused Cdk1 activation by several observations, namely (i) elevation of cyclin B1 expression, (ii) dephosphorylation on inhibitory Tyr-15 of Cdk1, and (iii) dephosphorylation on Ser-216 of Cdc25c. Moreover, a long-term treatment (36 h) caused the release reaction and subsequent nuclear translocation of AIF. In summary, it is suggested that 2-phenyl-4-quinolone displays anticancer effect through the dysregulation of mitotic spindles and induction of mitotic arrest. Furthermore, participation of cell-cycle regulators, Bcl-2 family of proteins, activation of caspases and release of AIF may mutually cross-regulate the apoptotic signaling cascades induced by 2-phenyl-4-quinolone.

1. Introduction

Microtubules are composed of a backbone of α- and β-tubulin heterodimers and microtubule-associated proteins [1]. They are major cellular components that play crucial roles in a lot of cellular functions, including the maintenance of cell shape, cell adhesion and movement, cell signaling, cell replication and cell division. Microtubules are in a highly dynamic process

* *Corresponding author at*: School of Pharmacy, National Taiwan University, No. 1, Section 1, Jen-Ai Road, Taipei, Taiwan. Tel.: +886 2 2391 9098; fax: +886 2 2391 9098.
E-mail address: jhguh@ha.mc.ntu.edu.tw (J.-H. Guh).
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doi:10.1016/j.bcp.2007.03.015

of polymerization and depolymerization in cells undergoing replication and division, while they are easily affected by numerous endogenous regulators and exogenous factors, and are considered as a susceptible target by numerous therapeutic drugs [2]. Several cancer chemotherapeutic drugs show their anticancer effects through the disturbance of microtubule dynamics, leading to dysregulation of mitotic spindles and the causative mitotic arrest in cancer cells. For example, paclitaxel and docetaxel bind to the microtubule lattice, stabilize microtubule bundles and impair cell mitosis in numerous types of cancer cells [3,4]. In contrast, *Vinca* alkaloids bind to and inhibit microtubule polymerization, resulting in a blockade in mitosis and causing apoptotic cell death [5].

Tubulins of α- and β-subunits are encoded in vertebrates by six and seven different genes, respectively. Each tubulin isotype can be distinguished by its different C-terminal sequence [6,7]. β-Tubulin isotypes, which are the predominant targets of anti-mitotic agents, can be identified by sequence alignment. To date, there are seven β-tubulin isotypes being identified in mammals. Among these isotypes, β_{III}-tubulin is a central target for taxanes. Several studies suggest that the overexpression of β_{III}-tubulin involves in microtubule destabilization and resistance to taxanes [8]. Furthermore, there are several lines of evidence that β_{III}-tubulin is one of the cytoskeleton components in neoplastic other than normal differentiated glial cells [9]. Therefore, the investigation of anticancer mechanisms responsible for tubulin-involved pathways may provide significant insights into the regulation of the growth and progression of cancer cells.

In recent decades, the agents that modulate the G2/M checkpoint are of particular interest in the development of cancer chemotherapeutic drugs. Several clinical tubulin-binding drugs, such as taxanes and *Vinca* alkaloids, act as inhibitors in G2/M phase transition, providing evidence that the dysregulation of tubulin dynamics may disrupt G2/M transition and, subsequently, induce the cell-cycle arrest and apoptosis in tumor cells [10]. The activation of the Cdk1/cyclin B1 complex in the nucleus triggers the progression of the cell-cycle from G2- to M-phase. The activity of Cdk1 is regulated by several factors, including the level of transcription of cyclin B1, Cdk1 phosphorylations on different residues, Cdk1 inhibitor $p21^{Cip1/Waf1}$ and Cdc25 phosphatase activity [11]. It has been suggested that Cdk1 could be a target for the induction of apoptosis. It was demonstrated that inactivation of Cdk1 increases apoptotic cell death induced by DNA damage [12]. Nevertheless, there are many lines of evidence that instead of anti-apoptotic role, Cdk1 acts as a pro-apoptotic mediator in numerous tumor cell types. As regards the tubulin-binding agents, taxol and *Vinca* alkaloids can induce the Cdk1 activity and apoptosis in tumor cells [13]. Recently, several lines of evidence suggest that mitochondria may play a central role in signaling pathways to drive the cell-cycle arrest toward apoptotic cell death caused by tubulin-binding agents. Several observations suggest that Cdk1 is able to trigger mitochondrial membrane permeabilization by targeting on Bcl-2 family proteins, such as Bcl-2 and Bad, and subsequently induce the apoptotic cell death [14]. However, the tubulin-binding agents have diverse expression patterns of Bcl-2 family proteins in varied tumor types.

Targeting tubulin leading to a subsequent apoptosis in cancer cells is an effective mechanism for cancer chemotherapy. However, most of the clinical tubulin-binding agents are derived or semi-synthesized from natural products, which have complicated chemical structures and are hardly synthesized. Accordingly, the tubulin-binding agents with simple chemical structures could be of value as lead pharmacophore for future therapeutic agents. Quinolone is a common structure in natural alkaloids. It has been suggested that quinolone alkaloids, in particular 4-quinolone alkaloids, exhibit several pharmacological activities, such as antimycobacterial activity, and inhibition of leukotriene biosynthesis and monoamine oxidase activity [15–17]. 2-Phenyl-4-quinolone fits the criteria as a pharmacological probe. In this study, the mechanism of 2-phenyl-4-quinolone has been identified from the characterization of tubulin isotypes and cell-cycle regulators to mitochondrial proteins and related apoptotic cascades. This study also provides a prototype structure that enables the investigation of tubulin-targeting strategy in cancer chemotherapy.

2. Materials and methods

2.1. Materials

RPMI 1640 medium, fetal bovine serum (FBS), penicillin, streptomycin, and all other tissue culture reagents were obtained from GIBCO/BRL Life Technologies (Grand Island, NY). EGTA, EDTA, leupeptin, dithiothreitol, reagent, phenylmethylsulfonnylfluoride (PMSF), sulforhodamine B (SRB), propidium iodide (PI), antibodies to β_I, β_{II}, β_{III}, and β_{IV} tubulin isotypes, fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG, and tetramethylrhodamine isothiocyanate (TRITC)-conjugated anti-mouse IgG were obtained from Sigma (St Louis, MO). Antibodies to Bcl-2, Bcl-xL, Mcl-1, Bak, Bax, XIAP, CIAP-1, Survivin, Cyclin B1, Cdk1, Cdc25C, apoptosis inducing factor (AIF), and anti-mouse and anti-rabbit IgGs were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Antibodies to caspase-9, caspase-8, phospho-$cdc2^{Tyr15}$, phospho-$cdc2^{Thr161}$, phospho-Bcl-2^{Ser70} and Bid were from Cell Signaling Technologies (Boston, MA). Antibody to caspase-3 was from Imgenex (San Diego, CA). Antibodies to α- and β-tubulins were from Serotec Products (Beverly, MA) and BD Biosciences PharMingen (San Diego, CA), respectively. Antibody to MPM-2 was from Upstate Biotechnology (Lake Placid, NY). 2-Phenyl-4-quinolone was synthesized and provided by one of our colleagues (Dr. Sheng-Chu Kuo). The purity is more than 98% by the examination of HPLC and NMR.

2.2. Cell culture

NCI/ADR-RES cell line was from DTP Human Tumor Cell Line Screen (Developmental Therapeutics Program, NCI). The other cancer cell lines were from American Type Culture Collection (Rockville, MD). Human cancer cells were cultured in RPMI1640 medium with 10% FBS (v/v) and penicillin (100 units/ml)/streptomycin (100 μg/ml). Cultures were maintained in a humidified incubator at 37 °C in 5% CO_2/95% air.