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Original article

Biophysical and molecular docking studies of naphthoquinone derivatives on the ATPase domain of human Topoisomerase II

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ABSTRACT

Numerous naphthoquinone derivatives, such as rhinacanthins function as anticancer drugs, which target hTopoII. The structure of hTopoII contains both an ATPase domain and a DNA binding domain. Several drugs bind to either one or both of these domains, thus modifying the activity of hTopoII. The naphthoquinone esters and amides used in this study showed that their hTopoIIα inhibitory activity was inversely proportional to ATP concentration. In order to better characterize the inhibitory action of these compounds, sufficient quantities of soluble functional hTopoII-ATPase domain were required. Therefore, both the alpha and beta isoforms of the hTopoII-ATPase domain were over-expressed in *Escherichia coli*. The hTopoIIα-ATPase activity was reduced in the presence of naphthoquinone derivatives. Additionally, a molecular docking study revealed that the selected naphthoquinone ester and amide bind to the ATP-binding domain of hTopoIIα. Collectively, the results here provide for the first time a novel insight into the interaction between naphthoquinone esters and amides, and the ATP-binding domain of hTopoIIα. The further elucidation of the mechanism of action of the naphthoquinone esters and amides inhibitory activity is essential.

1. Introduction

DNA topoisomerases are necessary and ubiquitous enzymes involved in chromosome condensation and segregation, and in regulating intracellular DNA supercoiling [1,2]. DNA topoisomerases can be classified into two types, Type I and Type II topoisomerases. Type I topoisomerases (TopoI) act by generating a transient single-stranded break in the DNA double helix, followed by either a single-stranded DNA passage event or the controlled rotation about the break, whilst Type II topoisomerases (TopoII) catalyze DNA topological changes by breaking both strands of the double helix and transporting another double-stranded DNA segment through the break and then reannealing the break. TopoI enzymes are involved in all DNA processes and play an important role in maintaining genomic integrity [3,4]. TopoII enzymes on the other hand play essential roles in DNA transaction in vivo, including chromosome condensation and segregation, and the removal of the supercoils generated during replication and transcription [5]. In addition to such essential functions in the cell, TopoII enzymes have become important targets of many widely used antibiotics and antitumor drugs [6–8]. For instance, eukaryotic Type II topoisomerases are targets of anticancer drugs [9]. These enzymes are homodimers with some variability in molecular weights. For example, the enzyme from *Saccharomyces cerevisiae* has a monomer molecular mass of 164 kDa [5] while the two isoforms of the human enzyme, α and β, are 170 and 180 kDa, respectively [10]. Topoisomerase-targeting anticancer drugs can be divided into two broad classes that vary widely in their mechanisms of action [9]. Within Class I drugs or "TopoII poisons" two main types exist: the first increases levels of enzyme – DNA cleavage complexes by interacting with TopoII at the protein – DNA interface in a non-covalent manner and the second acts by covalently modifying the enzyme. These drugs act by stabilizing covalent topoisomerase-DNA complexes, which are the intermediates during the catalytic cycle of the enzyme. Although this type of inhibitor has been used in cancer therapy for many years, it is very toxic to normally dividing cells and shows a narrow therapeutic window [11]. Unlike Class I inhibitors, Class II drugs or "catalytic topoisomerase inhibitors" interfere with the catalytic function of the enzyme without trapping the covalent complex. Catalytic TopoII inhibitors are a heterogeneous group of

Abbreviations: ATP, adenosine 5′-triphosphate; EDTA, Ethylenediaminetetraacetic acid; ICRF-187, (S)-4,4′-(1-methyl-1,2, ethanediyl)bis-2,6-piperazinedione; IPTG, isopropyl-β-D-thiogalactopyranoside; NADH, β-nicotinamide adenine dinucleotide; PK/LDH couple assay, pyruvate kinase/lactate dehydrogenase couple assay; hTopoII, human topoisomerase II; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; Tris-Cl, Tris (hydroxymethyl) amino methane.

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compounds that might interfere with the binding between DNA and TopoII, stabilize non-covalent DNA TopoII complexes or inhibit ATP-binding.

Rhinacanthins are naphthoquinone ester derivatives isolated from the methanolic extract of the roots of the medicinal plant *Rhinacanthus nasutus* (Acanthaceae) [12–14]. In Thailand, the roots and leaves of the plant have been incorporated in Thai traditional medicine for treatment of several diseases including cancer and inflammation [15]. Some of the naphthoquinone derivatives showed potent antiallergic activity against antigen-induced β-hexosaminidase release [16,17]. Additionally, these compounds exhibited cytotoxicities against the human carcinoma cell lines, KB, HeLa and HepG2 [18]. Based on the cytotoxicity results, all naphthoquinone esters containing an –OH group at C-3 of the naphthoquinone ring (Fig. 1) showed high toxicity against the said cancer cell lines, while those with a methoxy group or without the –OH group displayed no activity or were much less cytotoxic [18].

Naphthoquinone naphthoate esters with an–OH group at 2-position of the naphthalene moiety (e.g., Rhinacanthin-N [Rhi-N]) were significantly more cytotoxic than those with –OMe (e.g., Rhinacanthin-Q, [Rhi-Q]) or without –OH group (e.g., NKPSL4 [18]). Additionally, naphthoquinone naphthoate esters exhibited greater cytotoxicity than naphthoquinone benzoate esters. This structure-activity relationship demonstrates that the scaffold of Rhi-N can be used to develop novel anticancer agents as also confirmed by Sharma et al. (2008) [19]. Some of the naphthoquinones such as 1, 2-naphthoquinone and 1, 4-naphthoquinone are potent inducers of the TopoII-DNA "cleavable complex", which leads to the inhibition of TopoII activity [20–23], whereas Rhi-N and Rhi-Q interfere with DNA relaxation of yeast TopoII [18]. Other naphthoquinones such as β-lapachone have been reported to inhibit against various cancers cell lines in vitro and also inhibit the activity of TopoI and induce TopoIIα-mediated DNA strand breaks [23]. Recently, novel naphthoquinone esters containing cyclopentyl and cyclohexyl substituents at C-2́of the propyl chain exhibited relatively good cytotoxicities towards cancer cells but much less cytotoxicities towards Vero cells compared to the naphthoquinone esters with 2′,2′-dimethyl group (such as Rhi M, RhiN, RhiQ and NKPSL4) [24]. In addition to naphthoquinone esters, the biological activity of novel naphthoquinone amides has been evaluated [25]. Even though, most of naphthoquinone amides showed moderate cytotoxicity towards tested cancer cell lines, they exhibited weak toxicity against normal Vero cell lines and much less toxic than the previous naphthoquinone esters.

In this study, we report a biophysical approach to investigate the effect of naphthoquinone derivatives on the ATPase domain of hTopoII. The presence of the compounds moderately inhibits the ATPase activity of the protein. Using the molecular docking approach, the structures of the ligands-protein-complex were examined. The results reported here can lead to the development of further novel naphthoquinone derivatives which act as hTopoIIα ATPase inhibitors.

2. Materials and methods

2.1. Materials

Buffer A contained 50 mM Tris-Cl pH 8, 0.5 M NaCl, 5 mM imidazole. Buffer B contained 20 mM Tris-Cl, pH 8, 50 mM NaCl. The plasmid miniprep kit was purchased from Fermentas (EU). The primers were synthesized by Bio Basic INC. The HiTrap Chelating HP column was purchased from GE Healthcare (Thailand). ATP, NADH, phosphoenolpyruvate, pyruvate kinase and lactate dehydrogenase were purchased from Sigma (USA). The other chemicals are analytical reagent grade. Naphthoquinone compounds were synthesized according to Kongkathip, et al. [18] and Pradidphol, et al. [25].

2.2. Plasmid construction

The gene encoding ATPase region of hTopoIIα (residues 29-428) and hTopoIIβ (residues 45-444) was amplified from plasmids pCM1 and YEPTOP2, respectively (gifts from Prof. Osheroff, N, Vanderbilt University School of Medicine, USA) in a polymerase chain reaction using synthetic primers: F_hTopoIIA_29, 5′ GAGCAGCTAGCTCTGTTGAAAGAATCTATCAAAAG 3′; R_hTopoIIA_428, 5′ GTCGGCCTCGAGTTATGAACACTTCTTGTTTAACTG 3′, F_hTopoIIB_45, 5′ GAGCAGCTAGCTCTGTTGAGAGAGTGTATCAG 3′ and R_hTopoIIB_444, 5′ GTCGGCCTCGAGTTATGAACACTTCTTATTC 3′. The resulting *Nhe*I/*Xho*I fragment was subcloned into *Nhe*I/*Xho*I sites of the pET28b-expression vector (Novagen) yielding a fusion protein of 400 amino acids with *N*-terminal His-tags (6x His). The sequence of a positive clone containing the hTopoII-ATPase insert was determined in its entirety to ensure that no mutations had been introduced during the polymerase chain reaction.

2.3. Expression and purification of ATPase domain

Plasmids pET28b-hTIIa-ATPase and pET28b- hTIIb-ATPase-constructed as described above were used to express the recombinant ATPase domain of hTopoII. Expression and purification of the enzymes was adapted from [26]. In brief, the expression of hTopoII-ATPase was carried out in *Escherichia coli* BL21 (DE3) cells. Cells were grown at 37 °C to an optical density of ~0.6 at 600 nm in LB broth containing 50 μg/ml kanamycin and then induced with 1 mM IPTG overnight at 30 °C. After expression, the cells were harvested by centrifugation, resuspended in buffer A and lysed by sonication. The fusion protein was purified by HiTrap Chelating HP column attached to HPLC. The protein was eluted by increasing concentration of imidazole and analyzed by 12% SDS-PAGE. Fractions containing the ATPase protein were dialyzed in buffer B. Protein concentration was determined by the BCA protein assay (PIERCE) using BSA as a standard protein.

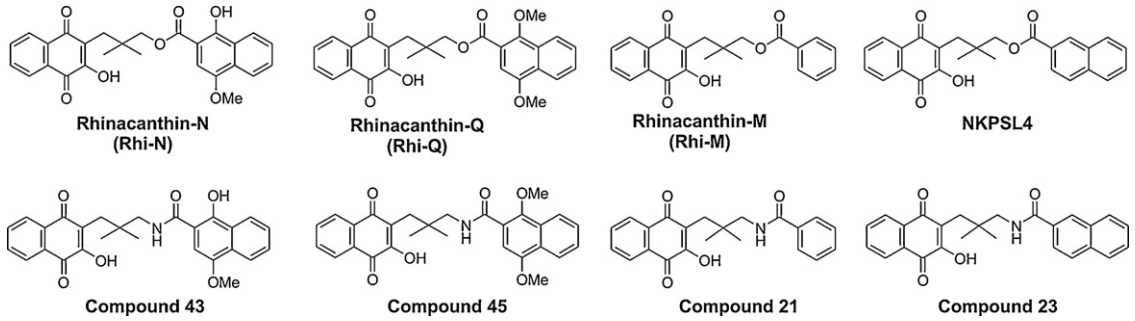

Fig. 1. Chemical structures of naphthoquinone compounds used in this study.