कैंसर प्रतिरोध पर विजय

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कैंसर कोशिकाओं को कुशलतापूर्वक नष्ट करने के लिए टोपोआइसोमरेज़ 1 और PARP1 के लिए दोहरे अवरोधक का विकास

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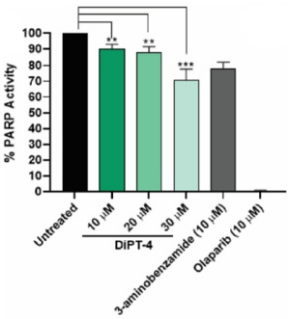

DiPT-4 की उपस्थिति या अनुपस्थिति में शुद्ध मानव PARP1 के साथ इन विट्रो PARP गतिविधि परख।

सारांश

टोपोआइसोमेरस 1 (TOP1) मनुष्यों में क्रमिक रूप से संरक्षित प्रोटीन है जो रेप्लिकेशन और ट्रांसक्रिप्शन के दौरान डीएनए सुपरकॉयल को हटाता है। TOP1, कोशिका उत्तरजीविता के लिए आवश्यक होने के कारण, एक पसंदीदा चिकित्सीय लक्ष्य है। TOP१-लक्षित उपचार TOP1-DNA सहसंयोजक संकुलों (TOP1ccs, जो TOP1 उत्प्रेरक के अनिवार्य मध्यवर्ती हैं) को स्थिर करते हैं, परिणामस्वरूप भारी TOP1-DNA एडक्ट्स और घातक DNA डबल स्ट्रैंड ब्रेक का संचय होता है। पॉली (ADP-राइबोज) पॉलीमरेज़ 1 (PARP1) एक प्रमुख DNA क्षय प्रतिक्रिया नियामक है जो TOP1ccs को दूर करने में महत्वपूर्ण भूमिका निभाता है, और इसलिए TOP1 अवरोधकों के प्रतिरोध के एक प्रमुख तंत्र के रूप में कार्य करता है। परिणामस्वरूप, कई नैदानिक परीक्षणों के दौरान कॉम्बिनेटरियल TOP1 और PARP1 अवरोध का उपयोग किया गया। इस अध्ययन में, हमने एकल अणु दोहरे अवरोधक (DiPT-4) की पहचान की है जो एकल कर्मक TOP1 अवरोधकों के लिए चिकित्सीय प्रतिरोध को दूर करने के लिए TOP1 और PARP दोनों के प्रति महत्वपूर्ण अवरोधक गतिविधि रखता है।

Overcoming Cancer Resistance

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Development of a Dual Inhibitor for Topoisomerase 1 and PARP1 for Efficient Killing of Cancer Cells

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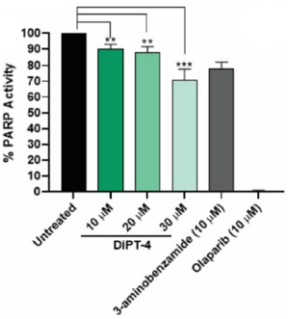

In vitro PARP activity assay with purified human PARP1 in the presence or absence of DiPT-4

ABSTRACT

Topoisomerase 1 (TOP1) is an evolutionarily conserved protein in humans which removes DNA supercoils during replication and transcription. TOP1, being essential for cell survival, is an attractive therapeutic target. TOP1-targeted therapies stabilize TOP1-DNA covalent complexes (TOP1ccs, which are obligate intermediates of TOP1 catalysis), resulting in accumulation of bulky TOP1-DNA adducts and lethal DNA double strand breaks. Poly (ADP-ribose) polymerase 1 (PARP1) is a key DNA damage response regulator which plays instrumental roles in removal of TOP1ccs, and hence acts as a key mechanism of resistance to TOP1 inhibitors. Consequently, combinatorial TOP1 and PARP1 inhibition has been subjected to multiple clinical trials. In this study, we have identified a single molecule dual inhibitor (DiPT-4) having significant inhibitory activity towards both TOP1 and PARP to overcome therapeutic resistance to single agent TOP1 inhibitors.

KEYWORDS: *Topoisomerase 1, PARP1, Dual Inhibitor, Resistance to Topoisomerase 1, TOP1 poison*

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Introduction

Cancer cells have high dependence on TOP1 due to its indispensable roles in replication and transcription. Hence, TOP1-targeted therapies are widely used in colorectal, lung, ovarian and pancreatic cancers. However, TOP1 inhibitors often suffer from development of therapy resistance. PARP1, a key DNA damage response protein, is instrumental in recruiting multiple proteins to TOP1 inhibitor-induced DNA damage sites and also in proteasomal degradation of TOP1ccs [1]. Hence, combinatorial inhibition of TOP1 and PARP1 is expected to improve therapy outcomes [2]. However, clinical trials with combinations of TOP1 and PARP1 have met with limited success due to dose limiting toxicities, often arising from pharmacokinetic incompatibilities between these two classes of drugs [3]. Single molecule dual inhibitors have generated significant interest among medicinal chemists due to their capability of eliminating pharmacokinetic incompatibility between drug classes by combining two different inhibitory activities in one molecule. Here, we report the development of a novel dual inhibitor of TOP1 and PARP which shows promising activity against both TOP1 and PARP, thus providing a proof-of-concept for further development of such inhibitors for clinical use.

Materials and Methods

Colorectal cancer (HCT116), pancreatic cancer (MIA PaCa-2 and PANC-1) and immortalized normal breast epithelium (MCF-10A) cell lines were used in this study. Cytotoxicity was evaluated using clonogenic survival and MTT assays. Rapid Approach to DNA Adduct Recovery (RADAR) assay [4] was used to quantify TOP1cc stabilization. TOP1 catalytic activity was measured using *in vitro* plasmid relaxation assay employing purified human TOP1 and supercoiled pUC19 plasmid. *In vitro* activity of purified PARP1 was measured using a PARP activity kit based on colorimetric estimation of biotinylated poly (ADP-ribose) (PAR) chains on histone substrates. Protein levels were quantified using Western blotting. PARP-trapping was evaluated using Western blotting with chromatin extracts. DNA damage was visualized using immunofluorescence and confocal microscopy.

Results and Discussion

In order to develop promising dual TOP1/PARP inhibitors, we utilized 1,8-naphthalimide and benzylphthalazin-1(2H)-one pharmacophores (for TOP1 and PARP inhibition respectively) connected through a 1, 3, 4-oxadiazole linker (Fig. 1A). Our cytotoxicity screen against HCT116, MIA PaCa-2 and PANC-1 cell lines revealed DiPT-4 (Fig. 1B), a candidate with (a) a fluorine side group in the benzylphthalazin-1(2H)-one phenyl ring, (b) a 1-carbon spacer between benzylphthalazin-1(2H)-one phenyl ring and 1, 3, 4-oxadiazole and (c) Sulphur linker between 1, 3, 4-oxadiazole and naphthalimide as the most promising molecule with superior cytotoxic activity (IC_{50} of 4, 4.5 and 4 μM respectively against HCT116, MIA PaCa-2 and PANC-1) compared to its parent molecules i.e., 1,8-naphthalimide (corresponding IC_{50} values of 15, 18 and 20 μM respectively) and Olaparib (corresponding IC_{50} values of 9, 8 and 7 μM respectively). Further, DiPT-4 showed selectively toxicity towards cancer cells and not cells of non-cancerous origin (MCF-10A).

We found that DiPT-4 induces DNA damage, a robust G2/M arrest, and finally, apoptosis in HCT116 cells.

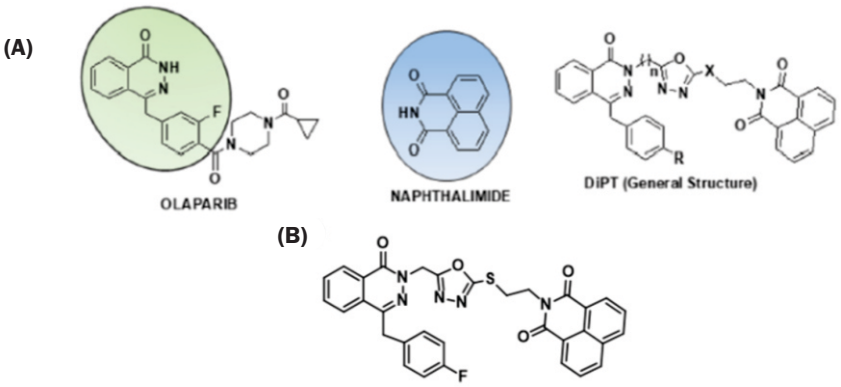

Fig.1: (A) Pharmacophores utilized for development of the DiPT series of molecules. (B) Structure of DiPT-4, the most promising candidate with superior activity compared to both parents. Adapted from [5].

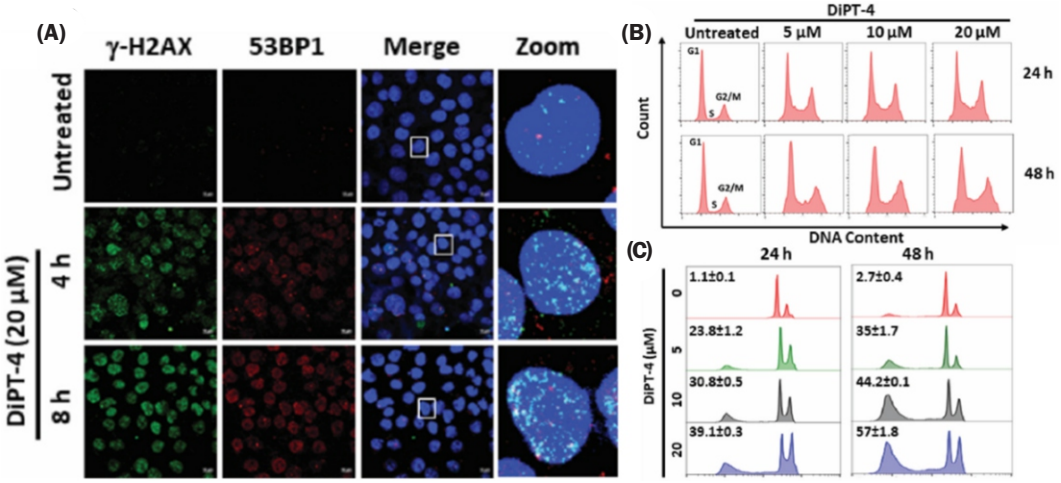

Fig.2: (A) Immunofluorescence-mediated microscopic detection of γ-H2AX and 53BP1 (DNA damage markers) foci in DiPT-4-treated HCT116 cells. (B) Cell cycle and (C) SubG1 (apoptotic population) analysis of HCT116 cells treated with different concentrations of DiPT-4 for 24 or 48 h. Adapted from [5].

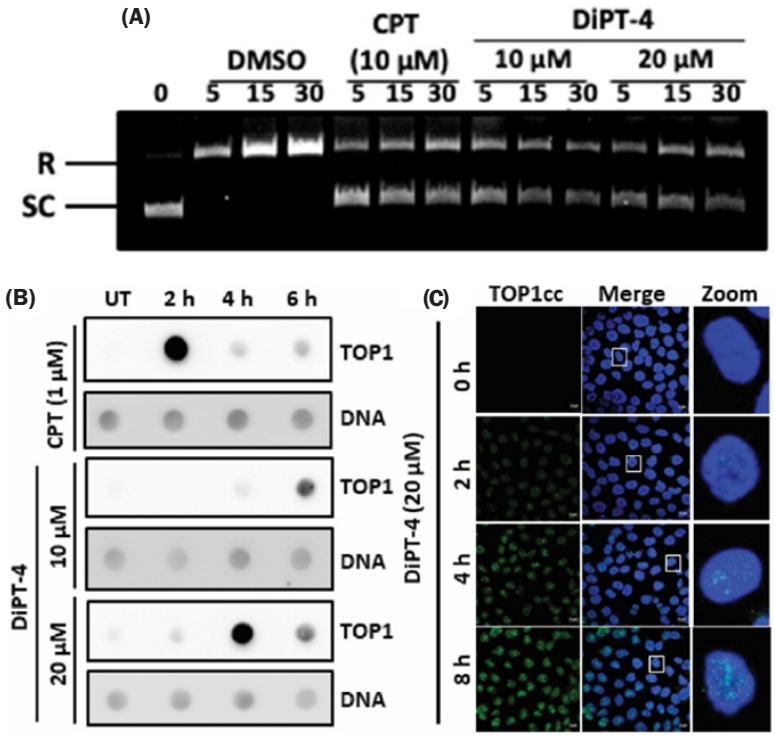

Fig.3: (A) In vitro plasmid relaxation assay with purified human TOP1 in the presence or absence of DiPT-4. R: relaxed DNA; SC: supercoiled DNA. (B) RADAR assay and (C) microscopic detection of time and concentration-dependent TOP1cc stabilization by DiPT-4. Camptothecin (CPT) was used as a positive control. Adapted from [5].

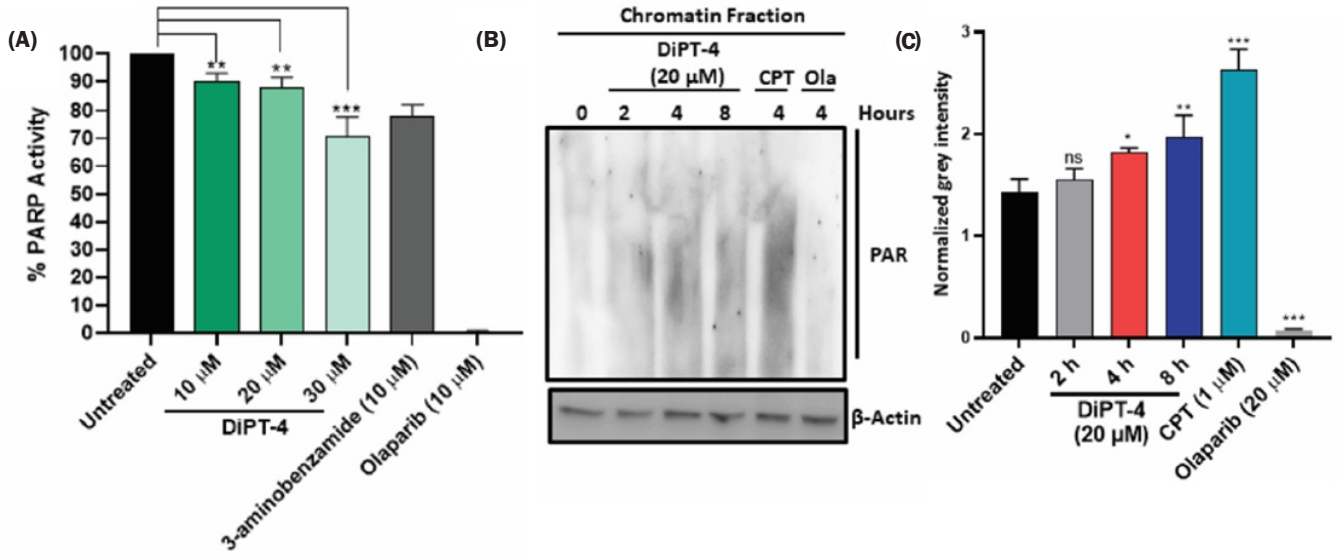

Fig.4: (A) In vitro PARP activity assay with purified human PARP1 in the presence or absence of DiPT-4. (B, C) Total PARylation levels in chromatin fraction of cells treated with DiPT-4, CPT (classical TOP1 inhibitor) and Olaparib (PARP inhibitor). Adapted from [5].

DiPT-4 was found to inhibit TOP1 catalytic activity *in vitro* (Fig. 3A) coupled with TOP1cc stabilization in a time (Fig. 3B, C) and concentration (Fig. 3B) dependent manner.

We also investigated the ability of DiPT-4 to inhibit PARP activity. DiPT-4 was found to inhibit PARP1 catalytic activity *in vitro* (Fig. 4A). Interestingly, DiPT-4 induced significantly lower PARP-mediated poly (ADP-ribosylation) i.e., PARylation in chromatin-bound proteins compared CPT, a classical TOP1 inhibitor (Fig. 4B, C).

Finally, we performed computational calculations to predict pharmacokinetic parameters of DiPT-4. Our calculations revealed that DiPT-4 satisfied both Lipinski and Veber criteria with a single violation (pertaining to molecular weight), suggesting that DiPT-4 possesses favourable drug-like characteristics (Fig. 5).

In addition to the presented findings, other interesting observations were made. As expected of an effective dual inhibitor, DiPT-4 induced copious DNA damage in cells without

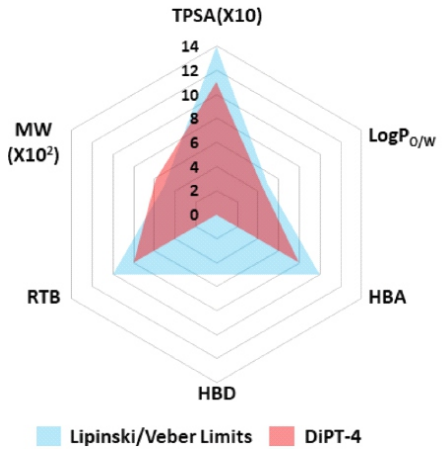

Fig.5: Radar plot of drug-like characteristics of DiPT-4 vs Lipinski and Veber criteria. MW: molecular weight; TPSA: topological polar surface area; LogP$_{O/W}$: lipophilicity; HBA: Hydrogen bond donors; HBD: Hydrogen bond donors; RTB: rotatable bonds. Adapted from [5].

robust induction of PARylation which plays a critical role in removal of TOP1ccs and cellular survival in response to DNA damage. We also observed that TOP1ccs stabilized by DiPT-4 have a longer half-life compared to those stabilized by conventional TOP1-targeted therapies. Also, in contrast to conventional TOP1 inhibitors, treatment with DiPT-4 does not induce global TOP1 downregulation, which is a major protective mechanism which imparts resistance to TOP1 inhibitors. Take together, our results provide a promising proof-of-concept for development of a single molecule dual inhibitor of TOP1 and PARP, which may form the basis for development of more such potent inhibitors with clinical relevance.

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