Development of nanoparticles for delivery of S4BR, a novel competitive inhibitor of polynucleotide kinase/phosphatase (PNKP), in colorectal cancer therapy

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ABSTRACT

Background: Polynucleotide kinase/phosphatase (PNKP) is a bifunctional DNA repair enzyme which phosphorylates DNA 5′-termini and dephosphorylates DNA 3′-termini making the damaged DNA termini amenable for ligation. PNKP inhibitors (PNKPi) can make cancer cells more sensitive to DNA damage by ionizing radiation or Topoisomerase I inhibitors. Purpose: Our team has identified S4BR as a novel competitive PNKPi (IC50= 0.9 µM). We aimed to develop a nanocarriers of S4BR and investigate its potential anticancer activity in colorectal cancer (CRC). **Methods:** Three nano-formulations of S4BR were prepared by dissolving it and either of the following three polymers in DMSO with dropwise addition to distilled water followed by dialysis against water using S4BR: polymer 1:10 w/w ratio. The polymers used were poly(ethylene oxide)-poly(benzyl-caprolactone (PEO-PBCL), poly(ethylene oxide)-poly(caprolactone) (PEO-PCL) or poly(ethylene oxide)-polymer(D, L-lactic acid) (PEO-PDLA). The prepared formulations were characterized for the level of encapsulated S4Br using UV/vis spectroscopy at 445 nm, and average diameter using dynamic light scattering (DLS). Cytotoxicity of S4BR was measured in wild type HCT116 (WT HCT116) and its Phosphatase and tensin homolog (PTEN) knock-out (HCT116 PTEN-/-) phenotype using MTT and colony forming assay. **Results:** The different polymeric micelles were all in the nano-size range, not exceeding an average diameter of 137 nm. Highest encapsulation efficiency and loading content of S4BR was achieved in PEO-PBCL micelles (91.18% and 8.78%, respectively). A lower IC_{50} for S4BR was observed against HCT16 PTEN-/- (IC50 =17.7 µM) compared to WT HCT 116 (IC50= 50.35 µM) indicating synthetic lethality of S4BR in HCT116 PTEN-/-. Clonogenic survival assay showed HCT116 /PTEN-/- to be more sensitive to S4Br at 12.5 µM while no toxicity was observed in WT HCT116 confirming synthetic lethality of S4Br in PTEN deficient CRC. **Conclusions:** The data confirms the anti-cancer activity of S4BR in PTEN negative CRC in line with what is expected from a PNKPi. The data also shows a good potential for PEO-PBCL nanocarriers for solubilization and delivery of S4BR in CRC.

1.INTRODUCTION

Human polynucleotide kinase/phosphatase (PNKP) is a bifunctional DNA repair enzyme which phosphorylates DNA 5′-termini and dephosphorylates DNA 3′-termini of damaged DNA termini making it ready for DNA ligation, **Fig.1A** . Inhibition of PNKP can make cancer cells sensitive to DNA damage by ionizing radiation or Topoisomerase I inhibitors. In previous studies by our group, S4BR has been identified as a novel competitive inhibitor of PNKPI with an IC50 of 0.9 µM, **Fig.1B.**

The **objective** of this study was develop a nano-formulation for preferential delivery of S4Br to solid tumors and assess its activity as a synthetically lethal monotherapy in PTEN deficient colorectal cancer (CRC) cells.

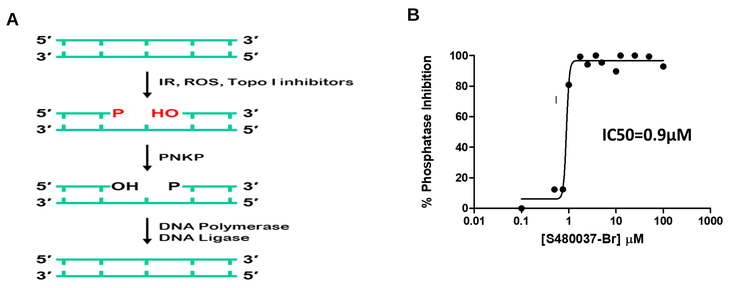

Figure1.A. PNKP enzyme activity, B. Phosphatase inhibition activity of S4BR.

2.METHODS

1.Anticancer activity of S4Br in CRC.

1.1. *Invitro* cytotoxicity assay.

The cytotoxicity of S4Br free drug was assessed in both HCT116/PTEN$^{+/+}$ and HCT116/PTEN$^{-/-}$cell lines. Initially, 2000 cells were plated in each well of 96-well flat-bottomed plates 24 h prior to the treatments. Then, cells were treated with S4Br as free drug with a concentration range of 0.1:50 µM. Control cells received only 0.1% DMSO. After experimental incubation time points, 20 µL of MTS reagent was added in each well and further incubated for 2 h at 37°C before measuring the absorbance at a wavelength of 490 nm[1].

$$\textit{Cell viability (\%)} = \frac{\textit{Absorbance of treated cells} - \textit{Absorbance of blank well}}{\textit{Absorbance of untreated cells} - \textit{Absorbance of blank well}} \times 100 \quad (1)$$

1.2.Clonogenic survival assay.

Based on MTs results, HCT116/PTEN$^{+/+}$ and HCT116/PTEN$^{-/-}$ were treated for 48 h with S4Br nontoxic concentrations for clonogenic survival assay. Cells were seeded in 6 well plates for 24 h in advance and cultured for 10−13 consecutive days. Colonies then were stained with a crystal violet (Sigma, Oakville, ON, Canada) for 30 min, after which the plates were washed in warm water and left to dry overnight, and colonies were counted [2].

$$\textit{\%Cell survival} = \frac{\textit{Colonies counted after treatment}}{\textit{Cells seeded x (plating efficiency)}} \times 100 \quad (2)$$

2.Preparation of S4Br polymeric micelles.

Block copolymers of poly(ethylene oxide)-poly(α-benzyl-ε-caprolactone (PEO-PBCL) with degree of polymerization (DP) of 32 for the PBCL block, poly(ethylene oxide)-poly(e-caprolactone) (PEO-PCL) with DP of 44 for the PCL block or poly(ethylene oxide)-polymer(D, L-lactic acid) (PEO-PDLA), with DP of 36 for the PDLLA block were synthesized and self-assembled to polymeric micelles. The DP of PEO in all three polymers was 114.

S4br was loaded to polymeric micelles using S4Br:polymer (1:10 w/w)ratio via dialysis method, **Fig.2**. Polymeric micelles were then characterized in terms of average diameter by dynamic light scattering, polydispersity index (PdI), encapsulation efficiency % and loading content %.

$$\textit{Encapsulation efficiency \%} = \frac{\textit{Amount of encapsulated S4BR}}{\textit{Initial amount of S4BR}} \times 100 \quad (3)$$

$$\textit{Loading content \%} = \frac{\textit{Amount of encapsulated S4Br}}{\textit{Total amount of drug and polymer}} \times 100 \quad (4)$$

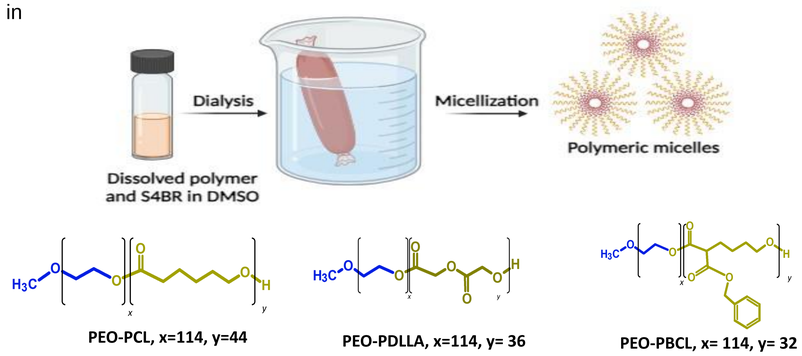

Figure 2. Schematic preparation of micellar nano-formulations composed of S4BR using PEO_{114}-PCL_{44},PEO_{114}-$PBCL_{32}$ or PEO_{114}-$PDLLA_{36}$ copolymers via dialysis method.

3.RESULTS

HCT116/PTEN$^{+/+}$ IC50	50.35 µM
HCT116/PTEN$^{-/-}$ IC50	17.76 µM

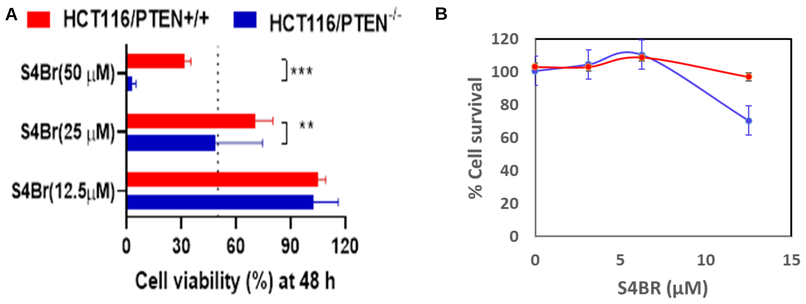

Figure 3. A. *Invitro* cytotoxicity study of S4BR on HCT116 wild type and HCT116/PTEN-/- with IC50 in each cell line. Higher cytotoxicity was observed in HCT116/PTEN-/- indicating PNKPI activity and synthetic lethality in HCT116/PTEN-/- . B. Clonogenic survival assay comparing S4BR (3.12,6.25,12.5 µM) on HCT116 wild type and HCT116/PTEN-/-. HCT116 /PTEN-/- is more sensitive to S4BR at 12.5 µM while no toxicity is observed in HCT116/PTEN+/+ type confirming synthetic lethality of S4BR.

Table 1. Characterization of S4BR loaded polymeric micelles (PM). PEO-PBCL polymeric micelles showed highest encapsulation efficiency and loading content%.

	PEO-PDLLA PM	PEO-PCL PM	PEO-PBCL PM
Encapsulation efficiency %	61.2± 0.23	36.7±2.36	91.2±1.091
Loading content %	5.6±0.976	3.3±0.58	8.8±1.013
Particle size (nm)	94.3± 0.73	107± 0.98	136±2.12
PDI	0.128	0.225± 0.014	0.166±0.002

4.CONCLUSIONS

S4Br has shown anticancer activity in HCT116 CRC cells deficient in PTEN perhaps due to synthetic lethal partnership between PTEN depletion and PNKP inhibition. S4Br was loaded successfully to polymeric micelles of different block copolymers but showed the best loading efficiency in those made from PEO-PBCL.

5.ACKNOWLEDGMENT

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