School of Pharmacy

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Development and in vitro Biological Evaluation of Polymer-Thalidomide Conjugates for Anti-Cancer Applications

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Introduction

Angiogenesis is essential for tumour growth and metastasis, making it a critical target in cancer therapy[1]. Polymer–drug conjugates have the capacity to inhibit angiogenesis, enhance drug delivery. Thalidomide (THA) is recognized for its anti-angiogenic effects and potential in cancer therapy, However, the stability of THA is considered a limiting factor for its use, due to its relatively short plasma half-life of roughly 5–8 hours[2].

Aim

This project develops conjugation of a THA derivative containing a primary amine group (Thalidomide 4'-ether-alkylC4-amine: THA amine) to a PEG carrier backbone (2 and 6 kDa) via an amide bond, thereby increasing THA's stability while retaining its anti-angiogenic activity. The effects of THA amine and PEG–THA amine conjugates on HUVEC cell migration are evaluated through specific functional assays. Additionally, We aim to investigate whether PEG conjugation enhances drug stability under physiological pHs, thereby maintaining efficacy and reducing degradation.(Fig. 1)

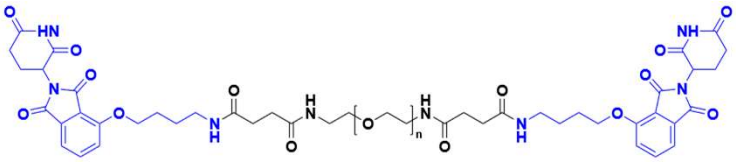

Fig. 1. Structures of PEG-THA amine conjugate.

Methods

RP-HPLC generated a calibration curve for free THA amine, with drug loading and free drug measured by NMR and HPLC. THA and PEG–THA amine stability was tested in PBS (pH 5 and 7.4), The potential conjugates' anti-angiogenic potential was assessed by scratch assay on HUVECs following a published protocol[3].

Results and Discussion

RP-HPLC analysis validated the purity of the PEG-THA amine conjugates (2 and 6 kDa). The 2 kDa conjugate exhibited a higher loading rate (12.6% vs. 5.5%), and both exhibited less than 0.1% free THA. The conjugates exhibited a stability of approximately 45% in PBS (pH 7.4, 37 °C), while the free THA exhibited a stability of 15% after 24 hours This step shows PEGylation improves stability and pharmacokinetics. (Fig. 2).

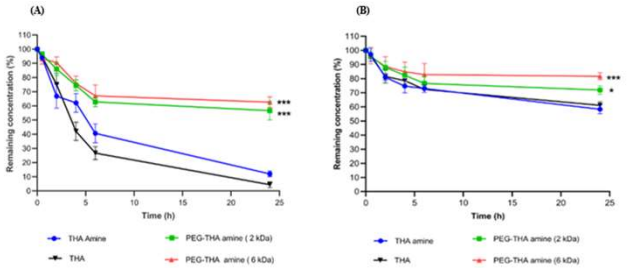

Fig.2. illustrates the stability profiles of free THA compared to its 2 and 6 KDa conjugates in PBS pH A. (5) and B. (7.4) at 37°C over a series of time points. Data expressed as mean ± SEM (n = 3)

Next, for anti-angiogenic effects, PEG-THA mine conjugates were tested for antimigration effects. Scratch tests were performed on VEGF-stimulated HUVECs using free THA amine and its conjugates at a dose of 50 μM (equal to THA). Both conjugates show inhibited HUVECs migration by 45%, comparable to the effect of the free THA compared to the positive control after 12 h of incubation (Fig. 3)

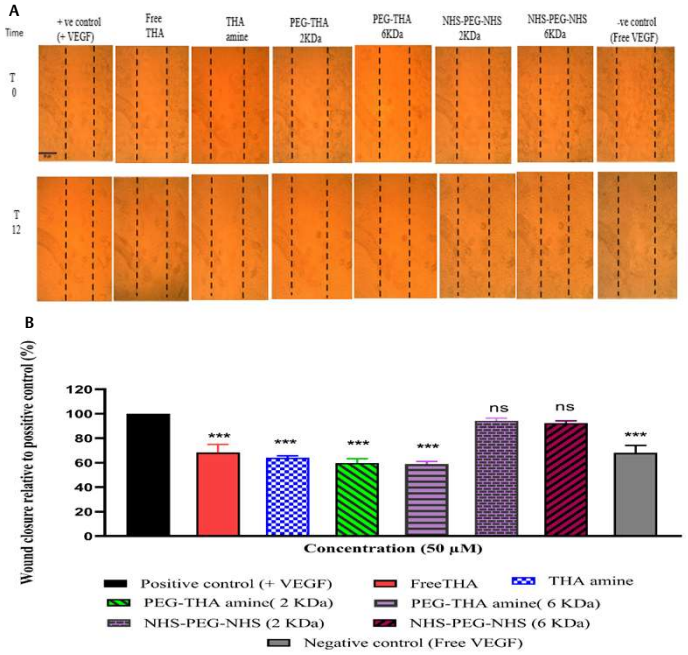

Fig.3 The *in vitro* suppression of wound closure (migration) in HUVECs by free THA, THA amine, and their conjugates is expressed as a ratio to the positive control (10 ng/mL VEGF-enriched medium). (A) Representative images of the scratch assay at 0 h and 12 h at 10× magnification. Images were analysed using ImageJ software; (B) % wound closure after 12 h as a ratio to the control.

Future plans

- ❑ Explore the mechanisms of anti-angiogenic inhibition on HUVEC cells by using Western blotting.
- ❑ Explore the potential of our conjugates' anti-angiogenesis effects *in vivo*.

References

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