Conjugation of Bicyclol-Polymer Conjugates for Protecting Liver from Injury

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Statement of Purpose: Bicyclol, a novel anti-hepatitis drug originated from Chinese Herb *Fructus Schizandrae,* was used to treat chronic hepatitis B. It would boost immune system by increasing hepatic activity. However, it is insoluble in water, resulting in poor absorption. The purpose of this work is to increase bicyclol water solubility and bioavailability. Our strategies include conjugation of bicyclol to water soluble N-(2-hydroxy propyl) methacrylamide (HPMA) polymer through pre-polymerization and post-polymerization modifications.

Methods: Two series of bicyclol-conjugated copolymers are prepared in this work. First, the bicyclol copolymer of was synthesized by conventional radical polymerization from N-(2-hydroxy propyl) methacrylamide and functionalized hydrophobic monomer. The coupling reaction between primary amine and carboxylic group was employed to construct the monomer.[1] Alternatively, using a post-polymerization modification strategy, the bicyclol moieties were appended to poly(N-(3-amino propyl) methacrylamide) copolymer with controllable attachment at room temperature. The monomer and bicyclol conjugated copolymers were purified by column and dialysis and characterized by ^{1}H NMR spectroscopy and FT-IR. The solubility of the copolymers and bicyclol were measured in water at room temperature. The toxicity of bicyclol conjugation polymer at 0.5, 2.5, 25 μM to Huh7-SJR12 replicon cells was measured by Firefly luciferase activity experiments.

Results: The appearance of the resonances characteristic ester protons (–OCOCH_2–) was observed at ~2.60 ppm along with the resonances of methylene protons (–CH_2NHCO–) at 3.20 ppm in the ^{1}H NMR spectra, which indicated the successful coupling between amine and carboxylic group in the monomer. Furthermore, the appearance of the resonances characteristic methyl protons (–PhOCH_3–) in the bicyclol copolymers was observed at ~4.90 ppm in ^{1}H NMR spectroscopy, indicative successful incorporation of bicyclol moieties in the final water soluble copolymers. The solubility of the bicyclol conjugated on HPMA polymer in water was 60 mmol·L^{-1}, which was much greater than that of the bicyclol alone. The bicyclol-HPMA conjugates induced the similar Firefly luciferase activity in Huh7-SJR12 replicon cells as the control medium.

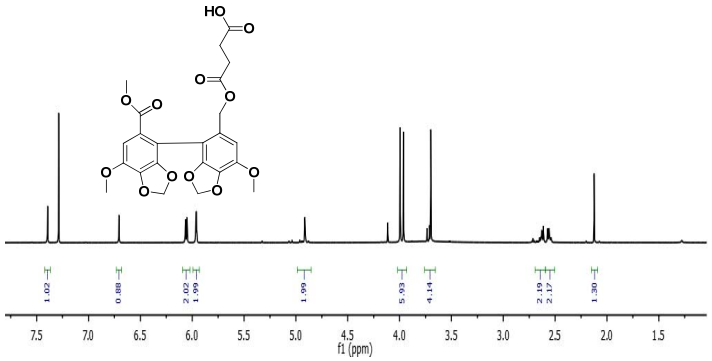

Figure 1. ^{1}H NMR (400 MHz) spectrum of Bicyclol and succinic anhydride adducts.

Conclusions: The bicyclol containing water soluble copolymers were prepared by radical polymerization and polymerization modification approach. The bicyclol conjugated copolymers were not toxic to Huh7-SJR12 replicon cells. The solubility of bicyclol in water was significantly improved by conjugating bicyclol on to the HPMA polymer. The future study will focus on bicyclol conjugated polymer protection from liver injury.

Reference:

1. Lee, H.K. et al. *J. Med. Chem.* **2012**, *55*, 5413.