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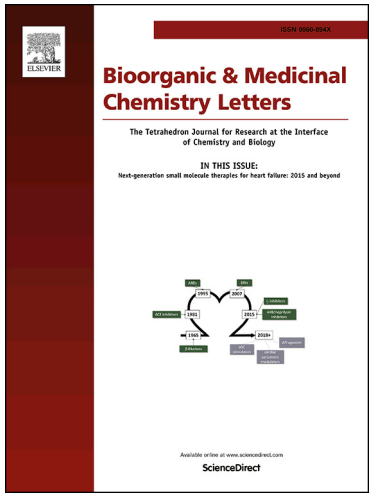

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Design, characterization, and in vitro antiproliferative efficacy of gemcitabine conjugates based on carboxymethyl glucan

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ABSTRACT

Gemcitabine (GEM) is widely used in clinical practice in the treatment of cancer and several other solid tumors. Nevertheless, the antitumor effect of GEM is partially prevented by some limitations including short half life, and lack of tumor localizing. Carboxymethyl glucan (CMG), a carboxymethylated derivative of β-(1-3)-glucan, shows biocompatibility and biodegradability as well as a potential anticarcinogenic effect. To enhance the antiproliferative activity of GEM, four water soluble conjugates of GEM bound to CMG via diverse amino acid linkers were designed and synthesized. ^{1}H NMR, FT IR, elementary analysis and RP-HPLC chromatography were employed to verify the correct achievement of the conjugates. In vitro release study indicated that conjugates presented slower release in physiological buffer (pH 7.4) than acidic buffer (pH 5.5) mimicking the acidic tumor microenvironment. Moreover, A549, HeLa and Caco-2 cancer cell lines were used to evaluate the in vitro cytotoxicity of conjugates and the results showed that binding GEM to CMG significantly enhanced antiproliferative activity of GEM on A549 cells. Therefore, these conjugates may be potentially useful as a delivery vehicle in cancer therapy and worthy of further study on structure-activity relationship and antiproliferative activity in vitro and in vivo, especially for lung tumor.

Gemcitabine (2', 2'-difluoro-2'-deoxycytidine, GEM), a nucleoside analogue, is widely used as antimetabolites in clinical practice to treat sevreral types of solid tumors including lung,[1] ovarian,[2] pancreatic cancer[3] and several others.[4] GEM has no efficacy outside the cell until undergoing phosphorylation in a stepwise manner to be final active triphosphate derivatives of GEM.[5] However, its chemotherapeutic efficacy is hampered by poor intracellular uptake depending on different nucleoside transporters,[6] rapid inactivation by cytidine deaminase (CDA), and indiscriminately targeting both cancer cells and normal cells, resulting in severe side effects.[7] Furthermore, the small molecular weight of GEM also contributes to a rapid renal clearance. To compensate for these drawbacks, a larger dose of GEM is administered to satisfy effectiveness of clinical treatment, further provoking adverse effects. To address these issues, conjugation of GEM with synthetic or natural polymers has been developed, for example, covalent attachment of GEM to synthetic polymers including polyethylene glycol (PEG),[8, 9] polyisoprene,[10] methoxy poly (ethylene glycol) - poly (lactic acid) (mPEG-PLA)[11] and poly (lactic-co-glycolic acid)[12] by modifying at its 4-amino group has been reported in literature, these conjugates can improve the cytotoxicity of GEM in vitro while reducing drug associated side effects, enhancing its stability and prolonging its circulation time in plasma. Poly-L-glutamic acid (PLGA)[13] and albumin,[14] nature carriers, introduced into 5'-hydroxyl and 4-amino group of GEM, respectively, have a similar efficacy with the above mentioned conjugates. Unfortunately, a mumber of drugs with PEGy-lated agents can activate the complement system, which may cause hypersensitivity reactions.[15]

Very few published reports are available regarding the use of GEM conjugated with polysaccharides. Compared with synthetic polymers, a polysaccharide has a variety of advantages, such as lower cost, higher safety, and adequate loading

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