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Spherical polymeric nanoconstructs for combined chemotherapeutic and anti-inflammatory therapies

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Abstract

Nanoparticles can simultaneously deliver multiple agents to cancerous lesions enabling *de facto* combination therapies. Here, spherical polymeric nanoconstructs (SPNs) are loaded with anti-cancer – docetaxel (DTXL) – and anti-inflammatory – diclofenac (DICL) – molecules. In vitro, combination SPNs kill U87-MG cells twice as efficiently as DTXL SPNs, achieving a IC_{50} of 90.5 nM at 72 h. Isobologram analysis confirms a significant synergy (CI = 0.56) between DTXL and DICL. In mice bearing non-orthotopic glioblastoma multiforme tumors, combination SPNs demonstrate higher inhibition in disease progression. At 70 days post treatment, the survival rate of mice treated with combination SPNs is of about 70%, against a 40% for DTXL SPNs and 0% for free DTXL. Combination SPNs dramatically inhibit COX-2 expression, modulating the local inflammatory status, and increase Caspase-3 expression, which is directly related to cell death. These results suggest that the combination of anti-cancer and anti-inflammatory molecules constitutes a potent strategy for inhibiting tumor growth.

Key words: Nanoparticles; Tumor microenvironment; Cancer inflammation; Combinatorial therapy

During the past decade, pre-clinical and clinical studies have demonstrated that the immune system plays a crucial role in the progression, regression, spreading and recurrence of neoplastic diseases.[1-4] The so-called tumor microenvironment (TME) is not solely populated by tumor and stromal cells but also by a multitude of different types of immune cells, including macrophages, dendritic cells, mast cells, natural killer cells, T and B cells. The relative proportion, intra-tumor density and spatial distribution of immune cells vary with the tumor type, disease progression and patient, and together define the 'immune contexture' of cancer.[5] TME progression is orchestrated via a complex milieu of cytokines and chemokines, generated by the multitude of different cells, which often supports progression and spreading of the disease. Indeed, activated immune cells can release bioactive molecules within TME such as growth factors, accelerating proliferation; survival factors, limiting cell apoptosis; enzymes, facilitating the localized digestion of the extracellular matrix and metastatization.[6-9] For all this, 'avoiding immune destruction' is now accepted as an emerging hallmark of cancer and modulating the 'immune contexture' is becoming a novel strategy for controlling disease progression and recurrence.[10]

Non-steroidal anti-inflammatory drugs (NSAIDs), such as diclofenac, celecoxib, ibuprofen, aspirin, and natural

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anti-inflammatory molecules, such as curcumin and many others, have been used in cancer prevention and treatment with the objective of modulating the inflammatory components of the disease.[11-15] In preclinical studies of gliomas and other cancers, diclofenac and ibuprofen have been shown to moderately inhibit cell growth and migration.[14,16] Specifically in gliomas, diclofenac was also implicated with a consistent reduction in lactate formation therefore exerting a direct anti-tumor activity.[16] Similarly, celecoxib has been demonstrated to enhance the anti-proliferative activity of chemotherapeutic molecules in liver, colon-rectal and head and neck cancers.[17,18] Herbs and natural molecules such as curcumin have been used extensively in cancer prevention and adjuvant therapies. Curcumin alone has been shown to provide anti-inflammatory, antioxidant, antiviral, antibacterial and moderate anti-tumor activity by multiple authors.[11,19,20] Despite some successes, the use of NSAIDs and natural anti-inflammatory molecules in anti-tumor therapies remains controversial. This is due to their poor solubility in water and, for NSAIDs, the well-known side effects including gastrointestinal bleeding and thrombosis.[20]

Recently, nanoparticle-based formulations of anti-inflammatory molecules are being explored for a variety of applications. For instance, celecoxib-loaded nanoparticles have been synthesized to modulate angiogenesis in vivo[21] and to exert anti-tumor activity in colon cancer[22]; other nanocarriers have been used to deliver derivatives of ibuprofen for the treatment of lung cancer[23]; and a myriad of manuscripts have been published on 'nano-curcumin'.[24] Works on the nano-formulation and co-administration of anti-inflammatory molecules and potent chemotherapeutics are just starting to demonstrate their potential in cancer treatment.[25] In this paper, spherical polymeric nanoconstructs (SPNs), consisting of a hydrophobic poly(lactic-co-glycolic acid) (PLGA) core stabilized externally by a single phospholipid monolayer, are developed for the co-delivery of docetaxel (DTXL), a potent anti-cancer drug, and diclofenac (DICL). The anti-cancer and anti-inflammatory activity of the combination is proved in vitro and in vivo using non-orthotopic glioblastoma multiforme as a reference cancer model.

Methods

Materials and chemicals

Poly(D,L-lactide-co-glycolic) acid (PLGA, 50:50, Carboxy Terminated, MW ~ 60 kDa) was purchased by Sigma Aldrich (St. Louis, MO). 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[succinyl(polyethylene glycol)-2000] (DSPE-PEG) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (Rhod-Lipid) were purchased by Avanti Polar Lipids (Alabaster, Alabama). Analytical grade dimethyl sulfoxide (DMSO), acetonitrile (ACN), chloroform and other solvents were obtained from Sigma Aldrich.

Preparation of DTXL- and DICL-loaded nanoparticles

Spherical polymeric nanoparticles loaded with DTXL (DTXL SPNs) were prepared by a slightly modified sonication–emulsion technique, already described elsewhere.[26] Briefly, carboxy-terminated PLGA and DTXL, in a 10:1 ratio, were dissolved in chloroform to obtain a homogeneous solution (oil phase). For the superficial lipid monolayer, two lipids were used (DPPC and DSPE-PEG) representing the 20% w/w of the polymer, with a DPPC/DSPE-PEG molar ratio of 7.5:2.5. DPPC was added to the polymer and DTXL solution (oil phase). DSPE-PEG instead was dissolved in the aqueous phase, made of 4% ethanol. The ratio between the oil phase and the aqueous phase was 1:5. To prepare SPNs, the oil phase was added drop-wise to the aqueous phase under ultrasonication. The obtained emulsion was then placed in a reduced pressure environment, under magnetic stirring, to facilitate solvent evaporation. SPNs were centrifuged at first for 2 min at 300×g to settle down any possible debris and the supernatant was centrifuged 3 more times for purification, thus removing also the free drug not encapsulated inside the SPNs. DTXL:DICL SPNs were prepared in a similar way, adding DICL to the oil phase in a ratio 1:1, 3:1 and 5:1 with DTXL. SPNs for internalization studies were prepared by substituting 20% w/w of DPPC with a rhodamine-lipid (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl), while keeping the same lipids amount (20% w/w of the polymer).

SPNs size, stability and morphology characterization

Dynamic light scattering (DLS, Malvern Zetasizer Nano S) and SEM (FEI, Elios Nanolab 650) were employed to characterize SPN size and morphology. DLS was used to assess the radius of SPNs under hydrated conditions, whereas SEM was used for the dried state. For DLS measurements, samples were re-suspended in both water and PBS and kept at 37 ° C. Stability was also checked under the same conditions up to 7 days. For SEM images, a drop of the samples was spotted on a silicon template, previously sputtered with gold to increase the contrast and the signal-to-noise ratio.

Evaluation of drug loading and encapsulation efficiency in SPNs

For estimating the drug amount inside SPNs, DTXL and DICL absorbance peaks were used: 230 nm and 280 nm, respectively. To measure the drug loading and its encapsulation efficiency inside SPNs, samples were first lyophilized and then dissolved in acetonitrile and analyzed by HPLC (Agilent 1260 Infinity, Germany). The drug loading is defined as the weight ratio between the considered drug and the SPN weight, in percentage. The encapsulation efficiency is defined as the percentage weight ratio between the drug amount inside SPNs at the end of their preparation and the initial amount of drug used (the input amount).

Drug release from SPNs

To assess the drug release kinetics from SPNs, samples were poured in a Slide-A-Lyzer MINI dialysis microtubes with a molecular cut off of 10 kDa (Thermo Scientific) and then dialyzed over 4 L of PBS buffer at pH 7.4, while kept at 37 °C. For each time point, triplicate samples were collected and analyzed by HPLC.