Evaluating the Synergistic Effect of Various Ibuprofen Concentrations in Combination with Doxorubicin-Loaded Liposomes to Target Tumor-Associated Macrophages

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Statement of Purpose:

Macrophages (M0) and their phenotypes (M1 and M2) are phagocytic cells and perform different tasks. M1 are pro-inflammatory cells, while M2 produce anti-inflammatory cytokines, contributing to tissue healing [1]. However, in a tumor microenvironment, the M2 phenotype macrophages (anti-inflammatory macrophages) act as protumoral macrophages and are known as tumor-associated macrophages (TAMs). These TAMs can stimulate angiogenesis while enhancing tumor cell invasion, tumor metastasis, intravasation, chemoresistance, and motility [2]. Furthermore, when these M2 cells are converted into TAMs, they no longer produce the anti-inflammatory cytokines; inflammation is not suppressed, resulting in the proliferation of the tumor. Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) that inhibits tumor growth, metastasis, and chemoresistance.

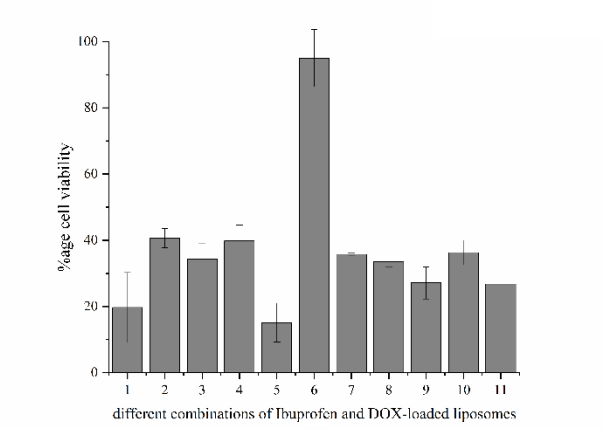
Moreover, liposomes have been used as a successful targeting agent for decades and can be successfully used to target these phagocytic TAMs. Therefore, this study aims to investigate the synergy effect of ibuprofen as an anti-inflammatory drug and doxorubicin (DOX) loaded liposomes together. The ibuprofen will compensate for the loss of anti-inflammatory cytokines not produced by TAMs, fight chemoresistance, and inhibit tumor growth. At the same time, DOX-loaded liposomes will attack the TAMs resulting in the death of TAMs and shrinkage of the tumor.

Methods:

1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) was combined with 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) in 2:1 (DOPE:DOPC) using thin film hydration method for the synthesis of liposomes. The liposomes were then dissolved in citric acid (pH 4.0) and extruded through a 100 nm filter 21 times to homogenize the size of liposomes. The pH of the solution was adjusted to 7.4 using sodium hydroxide; 10 mg/mL DOX solution (in PBS) was added to the liposome solution and kept at 65 ^{o}C for 1 hr to load the liposomes with DOX. Next, the liposomes were centrifuged at 5,000 rpm for 5 min, the supernatant was removed, and the amount of DOX not encapsulated was calculated using a plate reader and a calibration curve. The liposomes were now ready to be tested in combination with ibuprofen. The half-minimal inhibitory concentration (IC_{50}) for these DOX-loaded liposomes and ibuprofen was calculated against M0. Based on the result, four different concentrations of ibuprofen (0.25 μM, 0.5 μM, 1.0 μM, and 2.0 μM) were made from a stock solution of ibuprofen in dimethyl sulfoxide. The dilutions were performed before the cell experiments using Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum, 1% streptomycin, and 1% penicillin. RAW 264.7 cells were used as M0 and transformed to M1 and M2 using lipopolysaccharide (LPS) and interleukin-4 (IL-4), respectively. MTT assay was performed using a constant concentration of DOX-loaded liposomes (50 mg/mL) with the 4 above-stated concentrations of ibuprofen for M0, M1, and M2. Finally, the synergy effects were calculated.

Results:

The approach resulted in a 96% encapsulation efficiency of DOX in the liposomes. IC_{50} values for free DOX, DOX-loaded liposomes and ibuprofen were 8.98 ± 2.2 mg/mL, 4.79 ± 1.2 mg/mL, and 61.15 ± 1.97 mg/mL, respectively. Figure 1 shows the percentage cell viability of various combinations of liposomes and ibuprofen concentrations, where 1= 50 μg /mL DOX-loaded liposomes (DLL) + 0.25 μM ibuprofen, 2= 50 μg/mL DLL + 0.5 μM ibuprofen, 3= 50 μg/mL DLL + 1.0 μM ibuprofen, 4= 50 μg/mL DLL + 2.0 μM ibuprofen, 5 = 50 μg/mL DLL only, 6= 50 μg/mL DOX free liposomes, 7= 10 μg/mL free DOX, 8= 0.25 μM ibuprofem, 9= 0.5 μM ibuprofen, 10= 1.0 μM ibuprofen, and 11= 2.0 μM ibuprofen.



(Figure 1: cell viability of 50 mg./mL DOX-loaded liposomes with various concentrations of ibuprofen)

Further experiments with M1 and M2 macrophages are currently being performed, along with an ongoing synergy study.

References:

[1] Saqib, Uzma, et al. Oncotarget 9.25 (2018): 17937.

[2] Noy, Roy, and Jeffrey W. Pollard. Immunity 41.1 (2014): 49-61.