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Photochemical internalization enhanced macrophage delivered chemotherapy

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ABSTRACT

Background: Macrophage (Ma) vectorization of chemotherapeutic drugs has the advantage for cancer therapy in that it can actively target and maintain an elevated concentration of drugs at the tumor site, preventing their spread into healthy tissue. A potential drawback is the inability to deliver a sufficient number of drug-loaded Ma into the tumor, thus limiting the amount of active drug delivered. This study examined the ability of photochemical internalization (PCI) to enhance the efficacy of released drug by Ma transport.
Methods: Tumor spheroids consisting of either F98 rat glioma cells or F98 cells combined with a subpopulation of empty or doxorubicin (DOX)-loaded mouse Ma (RAW264.7) were used as in vitro tumor models. PCI was performed with the photosensitizer $AlPcS_{2a}$ and laser irradiation at 670 nm.
Results: RAW264.7 Ma pulsed with DOX released the majority of the incorporated DOX within two hours of incubation. PCI significantly increased the toxicity of DOX either as pure drug or derived from monolayers of DOX-loaded Ma. Significant growth inhibition of hybrid spheroids was also observed with PCI even at subpopulations of DOX-loaded Ma as low as 11% of the total initial hybrid spheroid cell number.
Conclusion: Results show that RAW264.7 Ma, pulsed with DOX, could effectively incorporate and release DOX. PCI significantly increased the ability of both free and Ma-released DOX to inhibit the growth of tumor spheroids in vitro. The growth of F98 + DOX loaded Ma hybrid spheroids were synergistically reduced by PCI, compared to either photodynamic therapy or released DOX acting alone.

1. Introduction

Current treatment for many cancers often start with surgical resection followed by various post-operative treatments such as chemotherapy and/or radiotherapy. The primary goal of post-operative treatment is to reduce or eliminate tumor recurrence due to remaining malignant cells residing in the margin of the resection cavity. Targeted delivery approaches of chemotherapeutic drugs, such as cell-based vectorization, may result in improved outcomes since they can target and maintain elevated drug concentrations at the tumor site and prevent their spread into healthy tissue [1,2]. In contrast to nanoparticles, cells such as monocytes, Ma, and stem cells migrate to and infiltrate into tumors via an active process, despite stromal barriers and the elevated interstitial pressure present in most tumors. Ma are attracted by chemotactic factors secreted by tumors, especially from hypoxic regions, where conventional chemo and radiation therapy are least effective [3,4]. Furthermore, Ma are highly resistant to many anticancer drugs in comparison to tumor cells [5]. Ma also have the important advantage in that they can be obtained from the patient by leukapheresis, loaded *ex vivo* with drugs and reinjected into the blood.

Previous studies have demonstrated the feasibility of delivering loaded nanoparticles or water-soluble drugs to tumors using Ma. These include transport of gold nanorods to target breast cancer cells [6], adenovirus to prostate tumors [7], gold nanoshells to gliomas or squamous cell carcinomas [8–10], DOX to lung metastasis from breast tumors [11] and drug-loaded nanoparticles to human breast tumors [12]. Although the experimental results obtained so far are promising, one important limitation is infiltration of a sufficient number of exogenous drug-loaded Ma into the tumor. This limits the concentration of active drug that can be delivered to the tumor microenvironment. Therefore, methods to enhance the efficacy of the Ma transported and released drug would offer a distinct therapeutic advantage.

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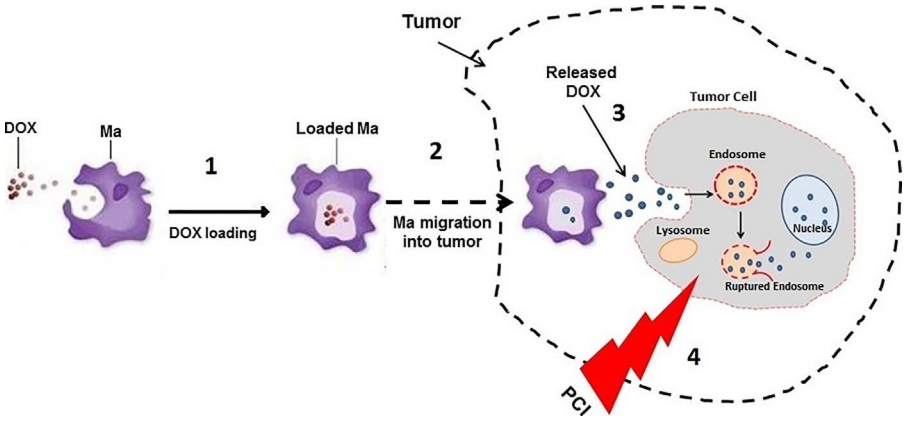

Fig 1. Overview of PCI via Ma-mediated delivery of drug into tumors. (1) Ma incubated with DOX, forming loaded Ma. (2) Loaded Ma migrate to tumor cells incubated with photosensitizer, $AlPcS_{2a}$. (3) DOX, which is released with time, is incorporated into tumor cells by endocytosis. (4) Laser irradiation (PCI) leads to endosomal escape, significantly enhancing drug efficacy.

PCI has previously been demonstrated to enhance the effects of a large number of macromolecules (including chemotherapeutic agents) that are subject to endosome-lysosome entrapment [13–16]. PCI is a technique, which utilizes the photochemical properties of photodynamic therapy (PDT), for the enhanced and site-specific delivery of drugs into the cell cytoplasm. Drugs that are internalized into cells via endocytosis end up trapped in intracellular endosomes and lysosomes. The concept of PCI is based on using photosensitizers, which localize in the cell membrane and are carried into the cell forming the endosome membrane. The photosensitizer remains in the endosome membrane while the macromolecule is localized within the lumen. Specific amphiphilic photosensitizers like disulfonated aluminum phthalocyanine ($AlPcS_{2a}$) or *meso*-tetraphenyl chlorin disulphonate ($TPCS_{2a}$), preferentially accumulate in the membranes of endosomes and lysosomes. Upon light exposure, the photosensitizer interacts with ambient oxygen to produce singlet oxygen, which ruptures the vesicular membrane. The released agent can therefore exert its full biological activity, in contrast to being degraded by lysosomal hydrolases following endosome-lysosome fusion.

The basic concept of drug loaded Ma-PCI is illustrated in Fig. 1.

The aim of the present work was to evaluate the ability of $AlPcS_{2a}$ PCI to enhance the efficacy of DOX released from loaded Ma. Supernatants from DOX-loaded Ma were evaluated for their ability to inhibit the growth of multi-cell three dimensional glioma spheroids with and without PCI. In addition, PCI experiments were performed utilizing a subpopulation of DOX-loaded Ma incorporated into glioma hybrid spheroids, as a simulation of limited Ma penetration into tumors.

2. Materials and methods

2.1. Cell lines and drug

The murine macrophages RAW264.7 (designated Ma) and F98 rat glioma cells were obtained from the American Type Culture Collection (Manassas, VA). Both cell lines were maintained in Advanced DMEM medium (Thermo Fisher Scientific, Carlsbad, CA) supplemented with 2% heat-inactivated fetal bovine serum (FBS), 25 mM HEPES buffer, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C, 5% CO_2 and 95% humidity. The F98 and Ma cells were grown as monolayers in T-25 tissue culture flasks, Greiner BioOne (Frickenhausen Germany) and in 9 cm flat-bottomed square dishes (Simport Scientific Beloeil, QC, Canada) respectively. Doxorubicin Hydrochloride (DOX) was obtained from Sigma Aldrich (St. Louis, MO).

2.2. Ma DOX supernatant production

Ma cells were incubated in 9 cm square dishes until sub-confluent. The medium was replaced with medium containing DOX solution (100 μg/ml) for 2 min. The Ma were washed four times directly in the dishes to remove all non-incorporated drug and the cultures were incubated in fresh medium. Portions of the supernatants were harvested after 5 and 120 min. Supernatants were also harvested from monolayers of "empty" Ma (not pulsed with DOX), which acted as controls.

2.3. Toxicity of DOX on F98 and RAW264.7 Ma

To test the toxic effects of DOX directly on Ma or F98 cell monolayers, 5×10^3 of both cell types were aliquoted into the wells of flat bottomed tissue culture microplates. Monolayer cultures were used in these experiments since the Ma do not form spheroids. Twenty-four hours later, DOX was added to the wells at increasing concentrations and the incubation was continued for 96 h, at which point the culture medium was replaced with fresh clear buffer containing MTS reagent (MTS, Promega, Madison, WI) which was used to determine cell viability. Cells were incubated in MTS reagents for 2 h. The optical density was measured using an ELx800uv Universal Microplate Reader (Bio-Tek Instruments, Inc, Winooski, VT).

2.4. PCI enhanced toxicity of DOX or active supernatants on F98 spheroids

F98 spheroids were formed by a modification of the centrifugation method previously described [15]. F98 glioma spheroids were generated with 2.5×10^3 cells in 200 μl of culture medium per well of an ultra-low attachment surface 96-well round-bottomed plate (Corning Inc., NY). Immediately following centrifugation, the tumor cells formed into a disk shape. The plates were maintained at 37 °C in a 5% CO_2 incubator for 24 h to allow them to take on the usual three-dimensional spheroid form.

Twenty-four hours after spheroid generation, 0.5 μg/ml of the photosensitizer ($AlPcS_{2a,}$ Frontier Scientific, Inc., Logan, UT) was added. Eighteen hours later the spheroids were washed four times. DOX as pure drug or active supernatant at increasing concentrations was added in fresh medium. Four hours after DOX or supernatant was added, light treatment, $\lambda = 670$ nm, from a diode laser (Intense, North Brunswick, NJ) at an irradiance of 2.0 mW/cm^2 was administered for 8.0 or 10 min, corresponding to radiant exposures of 0.96 or 1.2 J/cm^2 respectively.

Control cultures received light treatment but no DOX (PDT control) or DOX but no illumination (drug only control). Following PCI, the plates were returned to the incubator. Typically, 8–16 spheroids were followed for each group for up to 14 days of incubation. Culture medium in the wells was exchanged every third day. Determination of spheroid growth was carried out by averaging two measured perpendicular diameters of each spheroid using a microscope with a calibrated eyepiece micrometer and their volume calculated assuming a perfect sphere.