Paclitaxel-loaded expansile nanoparticles enhance chemotherapeutic drug delivery in mesothelioma 3-dimensional multicellular spheroids

Hongyi Lei, MD, PhD,[a,b] Sophie C. Hofferberth, MBBS,[a] Rong Liu, MD, PhD,[a] Aaron Colby, PhD,[c] Kristie M. Tevis, BS,[c] Paul Catalano, ScD,[a] Mark W. Grinstaff, PhD,[c] and Yolonda L. Colson, MD, PhD[a]

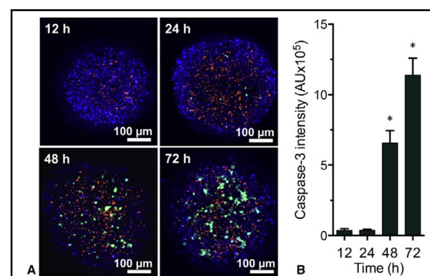
ABSTRACT

Objectives: Intraperitoneal administration of paclitaxel-loaded expansile nanoparticles (Pax-eNPs) significantly improves survival in an in vivo model of malignant mesothelioma compared with conventional drug delivery with the clinically utilized Cremophor EL/ethanol (C/E) excipient. However, in vitro monolayer cell culture experiments do not replicate this superior efficacy, suggesting Pax-eNPs utilize a unique mechanism of drug delivery. Using a mesothelioma spheroid model, we characterized the mechanisms of enhanced tumor cytotoxicity leveraged by Pax-eNPs.

Methods: Human malignant mesothelioma (MSTO-211H) spheroids were coincubated for 24 hours with Oregon Green-conjugated paclitaxel dissolved in C/E or loaded into eNPs. Oregon Green-paclitaxel uptake was measured as Oregon Green intensity via confocal microscopy and kinetics of tumor cytotoxicity were assessed via propidium iodide staining. Pharmacologic endocytotic inhibitors were used to elucidate mechanisms of eNP uptake into spheroids.

Results: Increased drug penetration and a 38-fold higher intraspheroidal drug concentration were observed 24 hours after MSTO-211H spheroids were treated with Oregon Green-conjugated paclitaxel loaded into eNPs compared with Oregon Green-conjugated paclitaxel dissolved in C/E ($P < .01$). Macropinocytosis was the dominant endocytotic pathway of eNP uptake. Spheroids were more susceptible to paclitaxel when delivered via eNP, exhibiting more than twice the propidium iodine intensity compared with an equivalent paclitaxel-C/E dose.

Conclusions: Compared with monolayer cell culture, the in vitro 3-D tumor spheroid model better reflects the superior in vivo efficacy of Pax-eNPs. Persistent tumor penetration and prolonged intratumoral release are unique mechanisms of Pax-eNP cytotoxicity. 3-D spheroid models are valuable tools for investigating cytotoxic mechanisms and nanoparticle-tumor interactions, particularly given the costs and limitations of in vivo animal studies. (J Thorac Cardiovasc Surg 2015;149:1417-25)



Paclitaxel-loaded expansile nanoparticles upregulate caspase-3 activity to enhance apoptosis within tumor spheroids.

Central Message

Monolayer cell culture models do not predict the superior in vivo efficacy of paclitaxel-loaded expansile nanoparticles (Pax-eNPs). Using an in vitro tumor spheroid model, we identified unique mechanisms of Pax-eNP enhanced cytotoxicity. Spheroid resistance to conventional drug exposure suggests this model can identify mechanisms of tumor cytotoxicity relevant to in vivo efficacy.

Perspective

We sought to address the significant differences observed between in vivo animal models and in vitro monolayer cell culture studies investigating the antitumor efficacy of local chemotherapeutic drug-delivery systems. An in vitro 3-dimensional tumor spheroid model was used to elicit unique mechanisms of enhanced tumor cytotoxicity leveraged by paclitaxel-loaded expansile nanoparticles (Pax-eNPs). Because the spheroid model more closely resembles the native tumor environment, these findings provide important clues to understanding the superior in vivo antitumor efficacy of Pax-eNPs. These results suggest that 3-dimensional spheroid models may be a clinically relevant platform to investigate mechanisms of tumor cytotoxicity and drug delivery.

See Editorial Commentary page 1426.

ET/BS

From the Division of Thoracic Surgery,[a] Department of Surgery, Brigham and Women's Hospital, Boston, Mass; Department of Anesthesiology,[b] Zhujiang Hospital, Southern Medical University, Guangzhou, China; and Departments of Biomedical Engineering and Chemistry,[c] Boston University, Boston, Mass.

This work was supported by the Brigham and Women's Hospital International Mesothelioma Program, National Science Foundation (grant No. DMR-1006601) and Boston University's Nanomedicine Program and Cross-Disciplinary Training in Nanotechnology for Cancer (grant No. NIH R25 CA153955), and the Zhuangjiang Hospital Scholarship Program.

HL and SCH are co-first authors.

Read at the 94th Annual Meeting of The American Association for Thoracic Surgery, Toronto, Ontario, Canada, April 26-30, 2014.

Received for publication Dec 1, 2014; revisions received Feb 2, 2015; accepted for publication Feb 7, 2015; available ahead of print April 2, 2015.

Address for reprints: Yolonda L. Colson, MD, PhD, Division of Thoracic Surgery, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St, Boston, MA 02115 (E-mail: ycolson@partners.org).

0022-5223/$36.00

Copyright © 2015 by The American Association for Thoracic Surgery

http://dx.doi.org/10.1016/j.jtcvs.2015.02.020

Abbreviations and Acronyms

2-D	= 2 dimensional
3-D	= 3 dimensional
eNP	= expansile nanoparticle
OG-Pax	= expansile nanoparticles loaded with standard paclitaxel doped with Oregon Green 488 conjugated paclitaxel
Pax-C/e	= paclitaxel dissolved in Cremophor EL/ ethanol
Pax-eNP	= paclitaxel-loaded expansile nanoparticle
PBS	= phosphate buffered saline
PI	= propidium iodide
Rho-eNP	= expansile nanoparticles labeled with rhodamine B

Supplemental material is available online.

Nanoparticle (NP)-based drug-delivery systems have been used to successfully address many of the difficulties encountered during the administration of chemotherapeutic compounds. Encapsulation of drugs within NPs increases drug solubility; alters biodistribution; enhances pharmacokinetics through sustained release; protects sensitive drugs from low-pH environments or enzymatic alteration; and, in some cases, enables targeting of drugs to specific sites.[1-5] Despite these significant advances, challenges remain in the evaluation of local chemotherapeutic drug-delivery systems, hindering their translation into the clinical setting. Importantly, the efficacy of chemotherapeutic delivery systems has been shown to be different for in vitro versus in vivo studies investigating the same agent. Previous studies performed by our group investigating the efficacy of paclitaxel-loaded expansile nanoparticles (Pax-eNPs) have shown that in vitro experiments with monolayer cell cultures do not predict the superior in vivo results demonstrated in animal models. For example, Colson and colleagues[6] demonstrated that Pax-eNP treatment of human intraperitoneal mesothelioma in an orthotopic in vivo xenograft model increases survival 2-fold compared with an equivalent dose of paclitaxel (Pax-C/E), even though Pax-eNPs were less effective than Pax-C/E when investigated using monolayer-based in vitro cytotoxicity assays.[6] These results suggest Pax-eNPs utilize additional mechanistic advantages in vivo that are not represented in traditional 2-dimensional (2-D) monolayer cultures. The inability to screen for efficacy of drug-delivery systems outside of whole organisms highlights the critical need to develop multicellular in vitro models to bridge the gap between conventional 2-D cell experiments and animal studies and further elucidate the mechanisms of tumor penetration and cytotoxicity of NP-based drug-delivery systems.

Multicellular spheroids are three-dimensional (3-D) in vitro microscale tissue analogs shown to serve as important tools for evaluation and optimization of intratumoral drug delivery.[7,8] Compared with 2-D studies, spheroids better model in vivo processes by mimicking many of the physiologic characteristics of the native tumor environment, including complex multicellular architecture, barriers to mass transport, and extracellular matrix deposition.[9-12] Importantly, multicellular spheroid models are more chemoresistant compared with monolayer cells, thus serving as excellent models for the evaluation of drug delivery systems.[13] Our study builds on previous 2-D monolayer in vitro and in vivo animal studies performed by our group investigating the antitumor efficacy of Pax-eNP. We have utilized a 3-D multicellular mesothelioma spheroid model to investigate the mechanisms of early tumor penetration and prolonged intratumoral drug retention as a means to elucidate the enhanced in vivo efficacy of Pax-eNPs.

MATERIALS AND METHODS

NP Preparation

Pax-eNPs were prepared using a previously described technique loading with 5% wt/wt paclitaxel (Sigma Aldrich, St. Louis, Mo).[1,14] For localization studies, eNPs were labeled with rhodamine B (Rho-eNPs) (Polysciences, Inc, Warrington, Pa). For paclitaxel localization studies, eNPs were loaded with standard paclitaxel doped with Oregon Green 488 conjugated paclitaxel (OG-Pax) (Life Technologies Corp, Carlsbad, Calif) at a ratio of 4:1 (ie, Pax:OG-Pax). Free Pax or OG-Pax not contained in eNP was dissolved in Cremophor EL and ethanol (Sigma-Aldrich) and referred to as Pax-C/E or OG-Pax-C/E, respectively.

Cell Culture and Formation of Spheroids

Human malignant mesothelioma cells (MSTO-211H; ATCC, Manassas, Va) were grown at 37°C, 5% carbon dioxide in Roswell Park Memorial Institute 1640 media with 10% fetal bovine serum, 100 μg/mL streptomycin, and 100 units/mL penicillin. To produce MSTO-211H spheroids, 96-well U-bottomed Greiner plates (Sigma-Aldrich, St Louis, Mo) were coated with a 60 μL/well of 5 mg/mL poly-HEMA (poly-2-hydroxyethyl methacrylate; Sigma-Aldrich) in 95% ethanol and air dried. Monolayer MSTO-211H cells were treated with Accutase (BD Biosciences, San Jose, Calif) for 5 minutes, centrifuged, and replated in poly-HEMA-coated U-bottomed plates at 5×10^3 cells/well in 200 μL media. The cells were centrifuged at 216 g for 10 minutes and incubated at 37°C in 5% carbon dioxide for 24 hours to form spheroids.[15,16]

Assessment of Necrosis and Apoptosis via Fluorescence and Confocal Microscopy

Spheroid morphology was examined with a Zeiss AxioImagere M1 Microscope (Carl Zeiss Microscopy, Oberkochen, Germany) at 10× magnification with spheroid size determined as the mean of 2 orthogonal diameters.

At each time point, spheroids were washed 3 times with phosphate buffered saline (PBS). Cell nuclei were stained by incubating with 0.2 μg/mL Hoechst 33342 (blue fluorescent dye; Life Technologies,

ET/BS

Download English Version:

https://daneshyari.com/en/article/2979587

Download Persian Version:

https://daneshyari.com/article/2979587

Daneshyari.com