Polymeric Micellar Co-delivery of Resveratrol and Curcumin to Mitigate *In Vitro* Doxorubicin-Induced Cardiotoxicity

LISA JANSSEN CARLSON,[1] BRIANNA COTE,[1] ADAM WG ALANI,[2] DEEPA A. RAO[1]

[1]School of Pharmacy, Pacific University, Hillsboro, Oregon 97123
[2]Department of Pharmaceutical Sciences, College of Pharmacy, Oregon State University, Corvallis, Oregon 97331-3507

Received 4 April 2014; revised 19 May 2014; accepted 20 May 2014

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.24042

ABSTRACT: Resveratrol (RES) and curcumin (CUR) have free radical scavenging ability and potential chemosensitizing effects. Doxorubicin hydrochloride (DH) is a potent chemotherapeutic with severe cardiotoxicity. We hypothesize that RES and CUR co-loaded in Pluronic® micelles and co-administered with DH will result in cardioprotective effects while maintaining/improving DH anti-proliferative effect *in vitro*. RES–CUR at a molar ratio of 5:1 in F127 micelles (mRC) were prepared and characterized for size, drug loading, and release. *In vitro* cell viability and apoptosis assays in ovarian cancer cells (SKOV-3) and cardiomyocytes (H9C2) with either individual drugs or RES–CUR or mRC in combination with DH were conducted. Combination index (CI) analysis was performed to determine combination effects. Reactive oxygen species (ROS) were quantified in H9C2 for DH, and combinations. The mRC solubilized 2.96 and 0.97 mg/mL of RES and CUR, respectively. Cell viability and CI studies indicated that the combinations were synergistic in SKOV-3 and antagonistic in H9C2 cells. Caspase 3/7 activity in combination treatments was lower than with DH alone in both cell lines. ROS activity was restored to baseline in H9C2 cells in the micelle combination groups. Co-administration of mRC with DH *in vitro* mitigates DH-induced cardiotoxicity through reduction in apoptosis and ROS while improving DH potency in ovarian cancer cells. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: natural products; cancer chemotherapy; toxicity; nanoparticles; polymeric drug delivery systems; physicochemical

INTRODUCTION

Doxorubicin hydrochloride (DH) is a cytotoxic, antineoplastic, anthracycline antibiotic that blocks replication by intercalating cell nucleotide bases in RNA and DNA.[1] It is a commonly used chemotherapeutic agent in various cancers involving solid tumors, including ovarian cancer.[1] Incorporating DH into a therapeutic treatment strategy comes with the risk of developing cumulative, dose-related myocardial toxicity with increasing total lifetime doses at and above 400 mg/m^2.[1] There is presently no clinically proven treatment established for doxorubicin cardiomyopathy.[2] Several mechanisms have been suggested as potentially contributing to DH-induced cardiomyopathy. These mechanisms include the inhibition of nucleic acid and protein synthesis, release of vasoactive amines, changes in adrenergic function, abnormalities in the mitochondria, lysosomal alterations, altered sarcolemmal calcium transport, changes in enzymes [specifically adenylyl cyclase, sodium–potassium adenosine triphosphatase (ATPase), and calcium ATPase], imbalance in myocardial electrolytes, free radical formation, reduction in myocardial antioxidant enzyme activities, lipid peroxidation, and depletion of non-protein tissue sulfhydryl compounds.[2] Thus, many mechanisms have been suggested for DH-induced cardiomyopathy suggesting a multifactorial and complex process, but free oxygen radicals and lipid peroxidation appear to play an important role.[2] One of the postulated mechanisms of production of free radicals like reactive oxygen species (ROS) by DH is because of the redox cycling of the quinolone–semiquinolone ring of the DH.[3] Thus, combining DH with an agent or agents that can counteract this redox cycling might be a potential strategy to mitigate DH-induced cardiotoxicity.

Resveratrol (RES) is a phytoalexin commonly found in grapes, berries, and peanuts that can prevent or slow the progression of a wide variety of illnesses, including cancer, cardiovascular disease, and ischemic injuries, as well as enhance stress resistance and extend the life spans of various organisms from yeast to vertebrates.[4] RES has an intrinsic antioxidant capacity that likely contributes to its chemoprotective effects, and *in vivo* RES has been shown to increase the plasma antioxidant capacity and decrease lipid peroxidation.[4] Emerging studies have indicated that RES may also induce endogenous antioxidants within the cell.[5] RES, in addition to its antioxidant properties, has also been shown to have chemosensitization effects.[6] The nuclear factor-kappa B (NF-κB) pathway seems to play an important role in RES-mediated chemosensitization.[6] Thus, RES with its multiple mechanisms of action as an antioxidant and a chemosensitizer is a promising candidate for adjunct or combination therapy with DH. RES has two major limitations for its use: first, its low-intrinsic aqueous solubility (30 μg/mL[7]); and second, its poor oral bioavailability. RES undergoes extensive metabolism in the intestine and liver leading to an oral bioavailability of less than 1%.[8]

Abbreviations used RP-HPLC, reverse-phase high-performance liquid chromatography; DAD, diode array detector; DH, doxorubicin hydrochloride; RES, resveratrol; CUR, curcumin; DMSO, dimethylsulfoxide; RCD, RES–CUR:DH at a molar ratio of 10:2:1; mRC, RES–CUR at a molar ratio of 5:1 in F127 micelles; mRC—DH, mRC dosed in combination with DH dissolved in DMSO at 10:2:1 molar ratio; NF-κB, nuclear factor-kappa B; ROS, reactive oxygen species; CI, combination index; PBS, phosphate-buffered saline; RT, room temperature; DLS, dynamic light scattering; ATPase, adenosine triphosphatase; MWCO, molecular weight cutoff.

Correspondence to: Deepa A. Rao (Telephone: +503-352-2647; Fax: +503-352-7270; E-mail: deeparao@pacificu.edu)

Journal of Pharmaceutical Sciences

Curcumin (CUR) is the primary curcuminoid found in turmeric.[6] CUR has strong antioxidant properties and has been shown to be chemosensitizing.[6] CUR also plays a role in both inhibition of inflammatory cytokines and prevention of proliferation of cancer cells *in vitro*.[6] Thus, the mechanisms by which CUR can act as an antioxidant, and a chemosensitizer, may be complementary to the actions of RES. Therefore, there is a strong rationale for using CUR along with RES to mitigate DH-induced cardiotoxicity while maintaining and/or improving its potency in cancer cells. As with RES, the limited bioavailability of CUR makes the therapeutic use of this compound challenging. CUR has poor absorption, rapid metabolism, and rapid systemic elimination.[6] Another pharmacokinetic limitation for CUR is that the aqueous solubility has been reported to be 0.6 μg/mL.[9]

RES and CUR, both have low aqueous solubility and low oral bioavailability requiring a formulation that can improve their respective solubilities and enhance their bioavailability. Polymeric micelles are nanocarriers that spontaneously self-assemble with hydrophobic cores and hydrophilic shells.[10] Polymeric micelles have demonstrated that they can solubilize hydrophilic drugs, alter their pharmacokinetics and improve their bioavailability.[10] Micelle formulations alleviate the necessity for adjuvants for drug solubilization such as ethanol or Cemaphor EL®, commonly associated with toxic side effects.[11] Pluronics® are ABA triblock copolymers with polyethylene oxide (PEO) hydrophilic groups flanking a polypropylene oxide (PPO) hydrophobic region that can spontaneously self-assemble to form micelles above their critical micelle concentration.[10] Pluronic® polymers have many advantages including versatility in varying ratios of PEO and PPO, biocompatibility, and approval from the US Federal Drug Administration (FDA) as generally regarded as safe agents.[12]

Many studies have demonstrated that when one natural product is used as pretreatment or in combination with DH, DH-induced cardiotoxicity can be mitigated.[1,2,13] However, thus far, no one has explored the use of multiple natural products dosed in combination with DH as a viable adjuvant therapy for cancer while mitigating DH-induced cardiotoxicity. We hypothesize that RES and CUR in Pluronic® micelles co-administered with DH will result in cardioprotective effects by scavenging free radicals while maintaining or improving DH antiproliferative effects through chemosensitization *in vit*ro. Thus, the purpose of our work is to determine the feasibility of using a combination of phytochemicals as a viable adjunct to chemotherapy while mitigating the cardiotoxicity associated with drugs such as DH.

MATERIALS AND METHODS

Materials

RES, CUR, and DH were purchased from TCI (Portland, Oregon), Alfa Aesar (Ward Hill, Massachusetts), and LC Labs (Woburn, Massachusetts), respectively. Lutrol F127 Pluronic® was kindly donated by BASF (Florham Park, New Jersey). Slide-A-Lyzer Dialysis Cassettes, 20 K molecular weight cutoff (MWCO), were obtained from Thermo Scientific (Rockford, Illinois). Human Caucasian ovarian adenocarcinoma (SKOV-3) and rat embryonic cardiomyocyte (H9C2) cell lines were purchased from ATCC (Manassas, Virginia). Cell culture supplies including RPMI 1640 media, Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum, trypsin Ethylenediaminetetraacetic acid (EDTA), and penicillin/streptomycin were purchased from VWR (Radnor, Pennsylvania). Cell Titer Blue® and Caspase-Glo 3/7 Apoptosis® assays were purchased from Promega (Madison, Wisconsin). OxiSelect™ Intracellular ROS® assay was purchased from Cell Biolabs (San Diego, California). All other chemicals and solvents were of HPLC grade and purchased through VWR.

Methods

Preparation and Characterization of RES–CUR at a Molar Ratio of 5:1 in Micelles

RES–CUR at a molar ratio of 5:1 in F127 micelles (mRC) were prepared by the solvent casting method.[14] Briefly, RES, CUR, and Pluronic® F127 were dissolved in acetone to final stock concentrations of 24, 7.75, and 100 mg/mL, respectively. RES (0.5 mL), CUR (0.5 mL), and Pluronic® F127 (2 mL) stock solutions were added to a 15-mL round-bottom flask and placed on a rotary evaporator under vacuum in a 60°C water bath to remove the acetone. A homogenous drug–polymer film was formed over 8 min. The film was allowed to cool to room temperature (RT) for 5 min and rehydrated in 4 mL of deionized water with gentle agitation followed by sonication for 5 min to obtain uniform size distribution. The micellar solution was filtered through a 0.45-μm, 13-mm nylon filter and characterized for loading, drug retention, and size.

Drug loading of mRC was quantified by reverse-phase high-performance liquid chromatography (RP-HPLC). An Agilent 1200 Series HPL (Agilent Technologies, Inc., Santa Clara, CA)C equipped with a degasser, binary pump, autosampler, thermostated column oven, and a diode array detector (DAD) detector was used for quantifying the drugs. A Zorbax SB-C18 Rapid Resolution HT column (2.1 × 15 mm^2, 1.8 μm; Agilent) was maintained at 40°C throughout the run. Samples of freshly prepared mRC were diluted 1:100 in acetonitrile and quantified by RP-HPLC for drug loading and drug retention over 48 h. The mobile phase was a mixture of 40% aqueous phase containing 1% methanol, 0.1% H_3PO_4 in deionized water, 60% acetonitrile, and an injection volume of 3 μL was used. The flow rate was 0.3 mL/min with a run time of 10 min. RES and CUR were quantified at 306 and 435 nm, respectively, using the DAD detector. The retention times of RES and CUR by RP-HPLC were 0.75 and 4.5 min, respectively. The limit of detection of RES and CUR by RP-HPLC were 0.002 and 0.004 mg/mL, respectively. The data have been presented as mean ± SD for quadruplet measurements.

Dynamic light scattering (DLS) was used to determine the size of the mRC using a ZETASIZER Nano-ZS (Malvern Instruments Ltd., Worcestershire, UK) equipped with He–Ne laser (4 mW, 633 nm) light source and 173° angle scattered light collection configuration. Deionized water was used to dilute freshly prepared drug-loaded micellar solutions to obtain a final F127 concentration of 0.2 mg/mL, and the samples were equilibrated for 2 min at 25°C prior to measurements. The hydrodynamic diameter of F127 micelles was calculated based on the Stokes–Einstein equation. Mean size and polydispersion index (PDI) was calculated using the correlation function curve fitted by cumulant method. The data have been presented as the average diameter with the deviation of volume-weighted particles measured in triplicate along with PDI.

 DOI 10.1002/jps.24042