Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

In vitro and *in vivo* evaluation of resveratrol and 3,5-dihydroxy-4'-acetoxy-*trans*-stilbene in the treatment of human prostate carcinoma and melanoma

Gregory W. Osmond, MD, MPH,[a] *Elizabeth M. Masko, PhD,*[b,1] *Douglas S. Tyler, MD,*[b,c] *Stephen J. Freedland, MD,*[a,b,c] *and Salvatore Pizzo, MD, PhD*[a,b,*]

[a] *Department of Pathology, Duke University Medical Center, Durham, North Carolina*
[b] *Department of Surgery, Duke University Medical Center, Durham, North Carolina*
[c] *Department of Surgical Service, Durham Veteran's Affairs Medical Center, Durham, North Carolina*

ARTICLE INFO

Article history:
Received 31 August 2011
Received in revised form
25 January 2012
Accepted 28 February 2012
Available online 28 March 2012

Keywords:
Resveratrol
3,5-dihydroxy-4'-acetoxy-*trans*-stilbene
3,5,4'-trihydroxy-*trans*-stilbene
Prostate carcinoma
Melanoma
4'-acetoxy resveratrol

ABSTRACT

Background: Resveratrol (RESV) is a naturally occurring compound that may possess anticancer capabilities in both prostate carcinoma and melanoma.

Methods: The *in vitro* and *in vivo* cytotoxic activity of RESV and 3,5-dihydroxy-4'-acetoxy-*trans*-stilbene (4-ACE) was tested using cellular assays and a xenograft model. Five prostate carcinoma cell lines were used for *in vitro* evaluation. A melanoma cell line (Duke melanoma 738 [DM738]) and the prostate carcinoma line CWR22 were used for *in vivo* experiments. Mice were randomized to osmotic mini pumps with 200 μL of RESV (250 mg/mL), 4-ACE (335 mg/mL), or vehicle (50% dimethyl sulfoxide, 50% polyethylene glycol). Serum drug and metabolite levels were calculated by high-performance liquid chromatography with diode-array detection. Western blots were performed on treated tumors. Results were analyzed using a student's t-test, analysis of variance, and the Mann–Whitney rank sum test.

Results: RESV and 4-ACE were cytotoxic in a time- and dose-dependent manner in all prostate carcinoma cell lines tested. Enhanced growth compared with controls was seen at the 24 h time point in four lines treated with RESV and two lines treated with 4-ACE (Ps $<$ 0.048). *In vivo*, no difference in either tumor growth or postmortem tumor weight was detected in either DM738 ($P = 0.555$, $P = 0.562$) or CWR22 ($P = 0.166$, $P = 0.811$) xenografts treated with either drug. Serum drug levels did not correlate with tumor growth rates for any treatment group (all Ps $>$ 0.11). Treated tumors demonstrated protein changes by western blot.

Conclusion: Although *in vitro* data were promising, RESV and 4-ACE have limited potential as single agents in the treatment of prostate carcinoma and melanoma.

* *Corresponding author*. Department of Pathology, Duke University Medical Center, Box 3712, Durham, NC 27710, USA. Tel.: 919 684 3528; fax: 919 684 8689.
E-mail address: salvatore.pizzo@duke.edu (S. Pizzo).
[1] Co-first author.

0022-4804/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
doi:10.1016/j.jss.2012.02.057

1. Introduction

Resveratrol (RESV), or 3,5,4'-trihydroxy-*trans*-stilbene, is a natural molecule that has shown experimental promise in various disease models, including cancer [1], cardiovascular disease [2], and ischemic injuries [3,4]. RESV was first identified as a potential anticancer agent in 1997 when it was shown to inhibit carcinogenesis at multiple stages [1]. Subsequent studies have shown RESV to possess both antiproliferative and pro-apoptotic actions in many cancer types [5], including melanoma [6] and prostatic carcinoma [7,8]. Despite powerful *in vitro* effects observed with its administration, *in vivo* studies have yielded mixed results [5].

We have previously reported that RESV is selectively and considerably cytotoxic to malignantly transformed melanocytes, sparing normal human fibroblasts [9,10]. However, the impressive *in vitro* effects of RESV did not translate to a mouse xenograft model, with administration of daily intraperitoneal injections [10]. We then showed that transient exposure of RESV was insufficient to elicit its cytotoxic capacity *in vitro* [10]. In the present study, we build on these findings, evaluating the therapeutic potential of RESV and 3,5-dihydroxy-4'-acetoxy-*trans*-stilbene (4-ACE), an analogue of RESV, in prostatic carcinoma and melanoma both *in vitro* and *in vivo*. We attempted to overcome the barriers of *in vivo* translation by administering elevated, sustained drug doses through the implantation of an osmotic mini pump, coupled with the utilization of 4-ACE, an analogue of RESV shown to have a longer half-life [11].

2. Materials and methods

2.1. Cell lines and chemicals

Prostate cell lines LNCaP, PC3, CWR22, and DU145 were obtained from American Type Culture Collection, and LAPC4 was obtained from William J. Aronson at University of California, Los Angeles. LNCaP [12] and LAPC4 [13] cells were maintained as previously described. PC3, CWR22, and DU154 were maintained in Roswell Park Memorial Institute 1640 media (Gibco by Life Technologies, Grand Island, NY), 10% phosphate-buffered saline, and 1% Pen Strep (Sigma-Aldrich, St. Louis, MO). Duke melanoma 738 (DM738), a previously characterized melanoma cell line derived from human tumor tissue, also was chosen for this study because of its cytotoxic sensitivity to RESV [10]. DM738 cells were maintained in Iscove's Modified Dulbecco's Medium, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin/L-glutamate. RESV was purchased from Sigma-Aldrich (St. Louis, MO), and 4-ACE was provided by the Merritt Andrus lab of Brigham Young University (Provo, UT).

2.2. In vitro cytotoxicity assays

Prostatic carcinoma-derived cell lines LAPC4, CWR22, LNCaP, PC3, and DU145 were plated at 6×10^4 cells/mL and incubated overnight in 96 well plates. RESV, 4-ACE, or vehicle was then added and cells were incubated for 24, 48, 72 or 96 h at 37°C, 5% CO_2. Time points beyond 96 h were not performed as our prior results showed no additional cytotoxic effects [10]. Cell viability was then quantified using an MTS assay [14]. At each drug concentration and time point, the fraction of surviving cells was calculated compared with control wells and plotted as a function of drug concentration for each cell line at each time point.

2.3. In vivo testing of RESV and 4-ACE in a systemic mouse model

To explore the *in vivo* effects of RESV and 4-ACE, we used a xenograft mouse model of melanoma and prostate carcinoma. All animal protocols were approved by the Duke University Medical Center Institutional Animal Care and Use Committees. Cell lines demonstrated to be RESV and 4-ACE sensitive in our *in vitro* analyses, DM738 [9] and CWR22, were chosen for testing. Male nude mice aged 6–8 wk were purchased from Harlan Laboratories (Indianapolis, IN) and given a single subcutaneous injection with a 25 gauge needle into the right hind limb of either 5×10^9 DM738 cells or 1×10^5 CWR22 cells suspended in 200 uL of 50% PBS and 50% Matrigel. Before injection, cell lines were confirmed free of infectious pathogens. Injected mice were monitored every third day, and tumors were measured with vernier calipers. Tumor volume was calculated as $[(\text{length})\ (\text{width})^2]/2$, with width being the smaller of the two measurements. Osmotic mini pumps (ALZET, Cupertino, CA) were implanted subcutaneously 1 wk after injection of DM738 and 2 wk after injection of CWR22. At the time of implantation a radiofrequency identification chip was placed, and mice were randomly assigned to a treatment group. Ten mice per treatment arm were used. On day 1 of treatment, all DM738 tumors were less than 320 mm^3 and CWR22 tumors less than 300 mm^3 with a single exception at 395 mm^3. Pumps were filled with 50 mg RESV (250 mg/mL), 67 mg 4-ACE (335 mg/mL), or vehicle (50% dimethyl sulfoxide, 50% polyethylene glycol). Pumps calibrated to release their contents over a 14-d (Model 2002) and 42-d (Model 2006) duration were implanted in the DM738 and CWR22 xenografts, respectively. Mice were monitored for loss of body weight, tumor ulceration, and depression. Animals were euthanized before day 14 for DM738 and day 42 for CWR22 of treatment if tumor ulceration occurred, body weight decreased by >15%, or tumor volume was greater than 1,500 mm^3.

2.4. Pump implantation

Before the implantation of the pumps, animals were anesthetized using Isoflorane in a chamber and then maintained with a nose cone. The area of implantation was sterilized with chlorhexidine (×3) and rinsed with 70% ethanol (×3). A small incision was made over the scapula, and the sterile pump was then inserted into the subcutaneous space. The wound was then closed with 9 mm wound clips, and the incision site was treated with bupivacaine drops and triple antibiotic ointment. Mice were handheld until recovery and monitored daily thereafter. Instruments were sterilized via a bead sterilizer between animals.

2.5. Quantification of serum drug levels

Animal serum obtained by bleeding at sacrifice was sent to RINP International (Laguna Hills, CA) for quantification of