Published in final edited form as:

J Surg Res. 2013 January ; 179(1): e141–e148. doi:10.1016/j.jss.2012.02.057.

***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,*}

^aDepartment of Pathology, Duke University Medical Center, Durham, North Carolina

^bDepartment of Surgery, Duke University Medical Center, Durham, North Carolina

^cDepartment of Surgical Service, Durham Veteran's Affairs Medical Center, Durham, North Carolina

Abstract

Background—Resveratrol (RESV) is a naturally occurring compound that may possess anti-cancer 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 ($P < 0.048$). *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 $P > 0.11$). 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.

Keywords

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

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% CO₂. 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 timepoint, 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 μ L 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 mm³ and CWR22 tumors less than 300 mm³ with a single exception at 395 mm³. 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 mm³.

2.4. Pump implantation

Before the implantation of the pumps, animals were anesthetized using Isoflurane in a chamber and then maintained with a nose cone. The area of implantation was sterilized with chlorhexidine ($\times 3$) and rinsed with 70% ethanol ($\times 3$). 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 tripleantibiotic 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 native RESV and 4-ACE, as well as their metabolites, using high-performance liquid chromatography with diode-array detection (HPLC-DAD) [15].

2.6. Preparation of tissue lysate

Using the QProteome Mammalian Protein Preparation kit (Qiagen, Valencia, CA), a total of 1 mL of cell lysis buffer was added to 50–60 g of prostate or melanoma xenograft tumor tissue and processed using a mechanical tissue homogenizer. Homogenates were centrifuged at 10,000 rpm for 20 min to clarify the lysates, and total protein concentration was determined using the bicinchoninic acid Protein Assay Reagent (Thermo Fisher Scientific, Rockford, IL). All lysates were stored at -80°C until further analysis.

2.7. Western blot analysis

Denatured samples of tissue homogenates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and subsequent immunoblotting to determine phosphorylation and expression of target proteins using the Fast Western Blot kit (Pierce-Thermo Scientific, Rockford, IL). Primary antibodies used for immunoblotting were anti-Cyclin B1 (#4138), Cyclin D (#2926), and β -actin (#4970) from Cell Signaling Technology

(Beverly, MA). Densitometric analysis was performed using the ImageJ software (National Institutes of Health, Bethesda, MD).

2.8. Statistical analysis

In vitro toxicity assays were analyzed using a student's t-test. Analysis of tumor response to therapy was performed using the analysis of variance and Mann–Whitney rank sum tests.

3. Results

3.1. RESV and 4-ACE are effective cytotoxic agents against prostate carcinoma cell lines *in vitro*

Studies were designed to determine the efficacy of RESV and 4-ACE as single agents in treating prostatic carcinoma cell lines before their use in xenografts. Administration of 4-ACE and RESV resulted in significant cytotoxicity in both a dose- and time-dependent manner in all prostate carcinoma cell lines tested, with the earliest cytotoxic effects seen in CWR22, LNCaP, and DU145 (Figs. 1 and 2). Cytotoxic effects were not seen until after 48 h of treatment with actual enhancement of cellular growth compared with control wells seen at the 24-h time point in four cell lines treated with RESV (LAPC4, CWR22, LNCaP, and DU145) and two cell lines treated with 4-ACE (LAPC4 and LNCaP) (all P s < 0.048).

3.2. Mouse xenografts of DM738 and CWR22 treated with RESV or 4-ACE showed no effect compared with controls

Nude mice carrying DM738 or CWR22 subcutaneous xenografts were used to examine the cytotoxic effects of RESV and 4-ACE *in vivo*. DM738 xenografts were sacrificed on day 14 of treatment. The decision was made to kill all CWR22 xenografts prematurely (day 28 of treatment) as 10 of the 30 mice had calculated tumor volumes, which exceeded end point. No difference in either tumor growth or postmortem tumor weight was detected in either DM738 ($P = 0.555$, $P = 0.562$) (Fig. 3A and B) or CWR22 ($P = 0.166$, $P = 0.811$) xenografts (Fig. 4A and B). A large amount of variability was present, however, prompting reanalysis using the Mann–Whitney rank sum test. Although no statistical significance was found in comparing RESV or 4-ACE to control mice for either CWR22 ($P = 0.456$ and 0.58) or DM738 ($P = 0.218$ and 0.476 , respectively), CWR22 tumors treated with 4-ACE tended to have higher growth rates compared with control. Also of note, seven of 20 mice treated with 4-ACE were sacrificed prematurely because of signs of severe distress (4) or poor wound healing at the site of pump insertion (3).

3.3. Serum drug concentrations did not correlate with tumor growth rate

Serum drug levels of native RESV and 4-ACE, as well as their metabolites, were quantified using HPLC-DAD. Glucuronide and sulfate metabolites were detected in the serum of all xenografts treated with either RESV or 4-ACE. Only two of the 10 analyzed xenografts treated with RESV had detectable levels of native RESV in their serum. None of the analyzed xenografts treated with 4-ACE had detectable levels of native 4-ACE in their serum, though nine of the 11 analyzed had detectable levels of native RESV (Fig. 5). Total serum drug concentrations, including native drug and metabolites, did not correlate with tumor growth rates for any treatment group, including melanoma xenografts treated with RESV ($P = 0.383$) or 4-ACE ($P = 0.115$) (Fig. 3C and D) or prostate carcinoma xenografts treated with RESV ($P = 0.441$) or 4-ACE ($P = 0.643$) (Fig. 4C and D). No detectable levels of RESV, 4-ACE, or their drug metabolites were found in control serum.

3.4. Xenograft tumors treated with RESV or 4-ACE resulted in altered expression of cyclins

To demonstrate cellular changes within tumors after drug administration, protein lysates from xenograft tumors were collected. Western blots show increased expression of cyclin D1 and cyclin B in melanoma (Fig 6A), whereas cyclin D1 is decreased in prostate carcinoma (Fig 6B).

4. Discussion

RESV is effective *in vitro* against many different types of malignant cell lines, including melanoma and prostate carcinoma [7,8,10]. The current experiments confirm RESV's previously observed cytotoxic efficacy against prostate carcinoma. We have previously shown that both RESV and 4-ACE are selectively cytotoxic *in vitro* in treating DM738 relative to normal human dermal fibroblasts [9,10]. Comparing the data in Figs. 1 and 2 with our previously published data, we find that RESV and 4-ACE also are selectively cytotoxic to all malignant prostate cell lines (P s < 0.007) except for LNCaP and PC3 when treated with RESV (P = 0.3245 and 0.2661, respectively) (Fig 7). These characteristics, together with RESV's ability to enhance the cytotoxic effects of chemotherapeutic agents [10], make RESV a good candidate for further study in the context of cancer therapeutics.

Although *in vitro* experiments are very promising in nearly all cancer cell lines tested, subsequent *in vivo* studies have yielded mixed results, with most researchers finding either no effect or a mild response, both positive and negative [5]. There are interesting exceptions to this statement, such as tumor growth restriction in neuroendocrine tumor mouse xenografts [16,17], but overall RESV has not proven to be the miracle drug *in vivo* that it was *in vitro*.

We also observed previously that the powerful cytotoxic effects seen with RESV administration *in vitro* were not elicited with transient daily exposures of RESV via intraperitoneal injections [10]. This caused us to conclude that the *in vitro* effects of RESV may indeed translate to *in vivo* models if adequate serum levels of RESV were achieved and maintained.

Some researchers have unsuccessfully attempted to overcome such barriers in animals by placing high levels of RESV in rodent food and water [5]. These experiments likely failed because of the extremely low bioavailability of RESV [18,19]. In fact, Walle *et al.* [15] reported a study where native RESV was undetectable in human plasma at any time point when administered orally. Metabolites of RESV are readily absorbed and cleared, but native RESV remains undetectable [15]. Because our compelling *in vitro* data were regarding native RESV and its analogues, we elected not to administer drug via an oral route.

Niles *et al.* [20] published an exception to the *in vivo* studies that have been performed with RESV, where in addition to oral administration of RESV, a pellet charged to release 100 mg of RESV over 21 d was implanted subcutaneously next to growing melanoma tumors. They found no statistical effect in tumor growth compared with controls. Given the implications of this study, we felt that it needed to be challenged and/or confirmed. As such the present study uses a distinct delivery system wherein RESV is delivered in a continuous fashion with a different formulation. Further, we also evaluated 4-ACE, an analogue of RESV, which has shown similar *in vitro* effects compared with RESV [9] but with a longer *in vivo* half-life [11]. Whereas we were hopeful that these measures would lead to biologically effective concentrations in the tumor that were not reached by Niles *et al.*, we have confirmed their findings that this approach does not alter *in vivo* tumor growth and strengthened the case against RESV as an anti-cancer therapeutic agent.

Our western blots confirm that drug in fact reached the tumor. Prior studies have demonstrated marked decreased cyclin expression with RESV therapy [10]. In the present study we saw decreased cyclin D expression in prostate carcinoma xenografts, with increased expression in melanoma xenografts. This could be because of a number of things. First, our western data represent the protein expression within tumors after multiweek continuous therapy. It may be that the mechanistic effects in such a situation differ from short-term drug administration. Alternatively, and more likely in our estimation, it may be that native RESV levels reaching the tumor are decreased to the point that they are yet inadequate to induce the cytotoxic and growth restriction effects seen with *in vitro* RESV therapy.

We, with others [21], hypothesize that the mechanism by which RESV acts to selectively induce death in malignantly transformed cells while sparing normal counterparts is via autophagy. If indeed this is true, we would expect to see survival-enhancing effects after early and/or very low levels of drug administration. Indeed this is the case, as manifested by enhanced cellular growth at the 24-h period among our prostate cell lines (see Figs. 1 and 2). Interesting, Niles *et al.* similarly found that their melanoma xenografts treated with RESV tended to have larger tumors than controls, though not statistically significant [20]. On balance, it is highly improbable that RESV will be able to induce its impressive anticancer *in vitro* effects in a prostate carcinoma or melanoma xenograft model. We conclude therefore that although *in vitro* results were encouraging, RESV and 4-ACE have limited potential as single agents in the treatment of melanoma and prostate carcinoma.

Our findings interestingly correlate with conclusions made by researchers in other fields regarding RESV's disease-fighting ability. Perhaps the most compelling case is made by Crozier *et al.* [22], who in using census data and regional differences in RESV wine concentrations make a compelling case that the so-called "French paradox," where a relatively low incidence rate of cardiovascular disease is seen in a population with a diet rich in saturated fats, was incorrectly attributed to RESV and instead is more likely to be the result of high procyanidin concentrations. In addition, Pacholec *et al.* [23] recently found that RESV does not directly activate SIRT1, calling into question its previously purported ability to delay the onset and reduce the incidence of age-related diseases, such as diabetes. Taken as a whole, doubt has been cast on RESV's ability to combat disease *in vivo*.

We recommend that further research concerning RESV, especially in the setting of cancer therapeutics, be focused on understanding and exploiting the underlying mechanisms by which RESV acts as a selectively cytotoxic and chemosensitization agent—something that is currently unclear but seems to take place at *in vitro* concentrations between 50 and 100 μM [10]. Such mechanistic investigation ought to be focused on cell lines, which have been shown susceptible to RESV therapy *in vivo* as the critical targets and relevant players may be easier to detect.

Acknowledgments

This work was supported in part by CA131235 and a VA Merit Review Grant.

References

1. Jang M, Cai L, Udeani GO, et al. Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science*. 1997; 275:218. [PubMed: 8985016]
2. Bradamante S, Barengi L, Villa A. Cardiovascular protective effects of resveratrol. *Cardiovasc Drug Rev*. 2004; 22:169. [PubMed: 15492766]
3. Sinha K, Chaudhary G, Gupta YK. Protective effect of resveratrol against oxidative stress in middle cerebral artery occlusion model of stroke in rats. *Life Sci*. 2002; 71:655. [PubMed: 12072154]

4. Wang Q, Xu J, Rottinghaus GE, et al. Resveratrol protects against global cerebral ischemic injury in gerbils. *Brain Res.* 2002; 958:439. [PubMed: 12470882]
5. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: The in vivo evidence. *Nat Rev Drug Discov.* 2006; 5:493. [PubMed: 16732220]
6. Yang S, Irani K, Heffron SE, et al. Alterations in the expression of the apurinic/aprimidinic endonuclease-1/redox factor-1 (APE/Ref-1) in human melanoma and identification of the therapeutic potential of resveratrol as an APE/Ref-1 inhibitor. *Mol Cancer Ther.* 2005; 4:1923. [PubMed: 16373707]
7. Wang TT, Schoene NW, Kim YS, et al. Differential effects of resveratrol and its naturally occurring methylether analogs on cell cycle and apoptosis in human androgen-responsive LNCaP cancer cells. *Mol Nutr Food Res.* 2010; 54:335. [PubMed: 20077416]
8. Aziz MH, Nihal M, Fu VX, et al. Resveratrol-caused apoptosis of human prostate carcinoma LNCaP cells is mediated via modulation of phosphatidylinositol 3'-kinase/Akt pathway and Bcl-2 family proteins. *Mol Cancer Ther.* 2006; 5:1335. [PubMed: 16731767]
9. Wong Y, Osmond G, Brewer KI, et al. Synthesis of 4'-ester analogs of resveratrol and their evaluation in malignant melanoma and pancreatic cell lines. *Bioorg Med Chem Lett.* 2010; 20:1198. [PubMed: 20022501]
10. Osmond GW, Augustine CK, Zipfel PA, et al. Enhancing melanoma treatment with resveratrol. *J Surg Res.* 2012; 172:109. Epub 2010 August 6. [PubMed: 20855085]
11. Yang H, Baur JA, Chen A, et al. Design and synthesis of compounds that extend yeast replicative lifespan. *Aging Cell.* 2007; 6:35. [PubMed: 17156081]
12. Mavropoulos JC, Buschemeyer 3rd WC, Tewari AK, et al. The effects of varying dietary carbohydrate and fat content on survival in a murine LNCaP prostate cancer xenograft model. *Cancer Prev Res (Phila).* 2009; 2:557. Epub 2009 May 26. [PubMed: 19470786]
13. Thomas JA, Antonelli JA, Lloyd JC, et al. Effect of intermittent fasting on prostate cancer tumor growth in a mouse model. *Prostate Cancer and Prostatic Dis.* 2010; 13:350. published online 24 August 2010. 10.1038/pcan.2010.24
14. Mossman T. Rapid colorimetric assay for cellular growth and survivals: Application to proliferation and cytotoxicity assays. *J Immunol Methods.* 1983; 65:55. [PubMed: 6606682]
15. Walle T, Hsieh F, DeLegge M, et al. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metab Dispos.* 2004; 32:1377. [PubMed: 15333514]
16. Pinchot SN, Jaskula-Sztul R, Ning L, et al. Identification and validation of Notch pathway activating compounds through a novel high-throughput screening method. *Cancer.* 2011; 117:1386. [PubMed: 21425138]
17. Truong M, Cook MR, Pinchot SN, et al. Resveratrol induces Notch2-mediated apoptosis and suppression of neuroendocrine markers in medullary thyroid cancer. *Ann Surg Oncol.* 2011; 18:1506. [PubMed: 21184191]
18. Wenzel E, Soldo T, Erbersdobler H, et al. Bioactivity and metabolism of trans-resveratrol orally administered to Wistar rats. *Mol Nutr Food Res.* 2005; 49:482. [PubMed: 15779067]
19. Ferrer P, Asensi M, Segarra R, et al. Association between pterostilbene and quercetin inhibits metastatic activity of B16 melanoma. *Neoplasia.* 2005; 7:37. [PubMed: 15736313]
20. Niles RM, Cook CP, Meadows GG, et al. Resveratrol is rapidly metabolized in athymic mice and does not inhibit human melanoma xenograft tumor growth. *J Nutr.* 2006; 136:2542. [PubMed: 16988123]
21. Opipari AW Jr, Tan L, Boitano AE, et al. Resveratrol-induced autophagocytosis in ovarian cancer cells. *Cancer Res.* 2004; 64:696. [PubMed: 14744787]
22. Crozier A, Borges G, Ryan D. The glass that cheers: Phenolic and polyphenolic constituents and the beneficial effects of moderate red wine consumption. *The Biochemist.* 2010; 32:4.
23. Pacholec M, Bleasdale JE, Chrunk B, et al. SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1. *J Biol Chem.* 2010; 285:8340. Epub 2010 January 8. [PubMed: 20061378]

Fig. 1.

Human prostate carcinoma cell lines treated with RESV. (A) through (E) demonstrate the dose response curves of DU145, CWR22, PC3, LNCaP, and LAPC4 cells, respectively, when treated at doses between 0 and 100 uM RESV *in vitro* for 24–96 h. RESV cytotoxicity is both dose and time dependent. Error bars are the relative standard error of measurement (SEM) of six measurements.

Fig. 2.

Human prostate carcinoma cell lines treated with 4-ACE. (A) through (E) demonstrate the dose response curves of DU145, CWR22, PC3, LNCaP, and LAPC4 cells, respectively, when treated at doses between 0 and 100 uM 4-ACE *in vitro* for 24–96 h. 4-ACE cytotoxicity is both dose and time dependent. Error bars are the relative SEM of six measurements.

Fig. 3.

In vivo testing of RESV and 4-ACE in melanoma xenografts. (A) Calculated xenograft tumor volumes are shown over the 14-d course of therapy with vehicle, RESV, or 4-ACE. No statistical difference is seen between the treatment groups ($P = 0.555$). (C) Postmortem tumor weights at sacrifice are graphically shown. No statistical difference between treatment groups is detected ($P = 0.562$). (B) and (D) Total serum drug concentrations quantified by HPLC-DAD, including administered drug and metabolites, are shown on the X-axis, and tumor growth rates are shown on the Y-axis for the RESV and 4-ACE treatment groups, respectively. Serum drug levels did not correlate with tumor growth rates for either treatment group ($P = 0.383$ and 0.115 , respectively).

Fig. 4.

In vivo testing of RESV and 4-ACE in prostate carcinoma xenografts. (A) Calculated xenograft tumor volumes are shown over the 28-d course of therapy with vehicle, RESV, or 4-ACE. No statistical difference is seen between the treatment groups ($P = 0.166$). (C) Postmortem tumor weights at sacrifice are graphically shown. No statistical difference between treatment groups is detected ($P = 0.811$). (B) and (D) Total serum drug concentrations quantified by HPLC-DAD, including administered drug and metabolites, are shown on the X-axis, and tumor growth rates are shown on the Y-axis for the RESV and 4-ACE treatment groups, respectively. Serum drug levels did not correlate with tumor growth rates for either treatment group ($P = 0.441$ and 0.632 , respectively).

Treated Melanoma Mice

Treatment Group	Resveratrol Glucuronides (uM)	Resveratrol Sulfates (uM)	Native Resveratrol (uM)	Acetyl Resveratrol (uM)	Total (uM)	Change in Tumor Volume (mm ³)
Control	0	0	0	0	0	3.166
Control	0	0	0	0	0	7.031
Control	0	0	0	0	0	5.078
Control	0	0	0	0	0	3.366
Control	0	0	0	0	0	3.298
Resveratrol	0.296	0.69	0	0	0.986	1.762
Resveratrol	0.348	0.306	0	0	0.654	1.044
Resveratrol	0.376	0	0	0	0.376	8
Resveratrol	0.151	0.344	0	0	0.495	1.664
Resveratrol	0.475	0	0	0	0.475	17.016
4-ACE	0.152	0.549	0.05	0	0.751	4.509
4-ACE	0.432	0	0.359	0	0.791	4.369
4-ACE	0.239	0	0.242	0	0.465	1.57
4-ACE	0.758	0	0.369	0	1.127	4.63
4-ACE	0.209	0	0.551	0	1.065	2.79

Treated Prostate Mice

Treatment Group	Resveratrol Glucuronides (uM)	Resveratrol Sulfates (uM)	Native Resveratrol (uM)	Acetyl Resveratrol (uM)	Total (uM)	Change in Tumor Volume (mm ³)
Control	0	0	0	0	0	3.271
Control	0	0	0	0	0	1.224
Control	0	0	0	0	0	2.945
Control	0	0	0	0	0	3.631
Resveratrol	0.111	0.348	0.465	0	0.924	4.82
Resveratrol	0.081	0	0.592	0	0.673	2.933
Resveratrol	0	0.822	0	0	0.822	1.271
Resveratrol	0.256	0.199	0	0	0.455	2.018
Resveratrol	0.325	0.53	0	0	0.855	11.738
4-ACE	0.173	0.23	0.091	0	0.494	9.094
4-ACE	0.146	0.226	0.088	0	0.46	6.119
4-ACE	0.143	0	0	0	0.143	8.94
4-ACE	0.385	0	0	0	0.385	4.031
4-ACE	0.136	0.268	0.582	0	0.986	6.142

Fig. 5.

Serum drug concentrations of RESV, 4-ACE, and their metabolites. Serum drug levels taken before sacrifice and quantified using HPLC-DAD are shown in the table. Glucuronide and sulfate metabolites were detected in the serum of all xenografts treated with either RESV or 4-ACE. Only two of the 10 analyzed xenografts treated with RESV had detectable levels of native RESV in their serum. None of the analyzed xenografts treated with 4-ACE had detectable levels of native 4-ACE in their serum, though nine of the 11 analyzed had detectable levels of native RESV. No detectable levels of RESV, 4-ACE, or their drug metabolites were found in control serum.

Fig. 6. Western blots performed on (A) melanoma and (B) prostate carcinoma xenograft tumors after therapy with RESV or 4-ACE. Cyclin D1 is decreased relative to controls in prostate carcinoma tumors (B), whereas it is increased along with cyclin B in melanoma tumors (A).

Fig. 7.

Selective cytotoxicity of RESV and 4-ACE. Prostate carcinoma cell lines and normal human dermal fibroblasts treated with 50 uM of RESV or 4-ACE for 96 h are compared, demonstrating that RESV and 4-ACE are selectively cytotoxic to all malignant prostate cell lines ($P < 0.007$) except for LNCaP and PC3 when treated with RESV ($P = 0.3245$ and 0.2661 , respectively).