Trans-, cis-, and dihydro-resveratrol: a comparative study

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Abstract

Background: Recent studies showed that moderate consumption of red or white wines increased the chances of breast cancer, while similar consumption of red wines, rich in trans-resveratrol (trans-R), decreased the rate of prostate cancer. This prompted us to explore the role of various forms of R in cancer proliferation.

Results: Trans-R was found to be the most potent antiproliferative agent. Cis-R demonstrated somewhat less potency compared to trans-R. Unlike cis-R and trans-R, dihydro-R exhibits moderate proliferative effect on androgen-independent prostate cancer cell lines PC-3 and DU-145 at picomolar concentrations. At higher concentrations, dihydro-R caused proliferation inhibition, similar to cis-R and trans-R. The proliferative effect of dihydro-R at low concentrations can be reversed by trans-R which acts as a partial antagonist in the presence of dihydro-R. Mixtures of dihydro-R and trans-R demonstrated complex non-monotonic cross-modulation activity patterns.

Conclusions: Dihydro-R exhibits proliferative effects in androgen-independent prostate cancer cells at picomolar and nanomolar concentrations. While the exact mechanism of these effects requires further evaluation, our preliminary results point to hormone receptor modulation activity. We also observed strong cross modulation between trans-R and dihydro-R at sub-picomolar concentrations. The role of dihydro-R in cancer proliferation related to moderate consumption of red wine remains an open question because dihydro-R has a very complex activity pattern in the presence of trans-R.

Background

The term “French Paradox” was introduced by S. Renaud in the early 1990s to describe the low incidence of heart disease and obesity among the French in contrast to their relatively high-fat diet [1]. It is widely believed that consumption of red wine, rich in trans-resveratrol (trans-R) and other dietary polyphenols, is the primary cause of this paradox. Given the well-documented anticancer properties of trans-R, it is natural to expect that the moderate consumption of red wine might bring additional benefits of lower cancer rates, especially the most frequent forms of cancer such as breast and prostate cancer. However, recent studies have unequivocally demonstrated that even moderate consumption of red or white wines markedly increased the chances of breast cancer [2-4], while moderate consumption of red wine substantially decreased the rate of prostate cancer [5]. It was also reported that moderate consumption of white wine or beer does not affect prostate cancer rate [5]. Although the alcohol content should be considered as a prime factor responsible for this intriguing disparity between the cancer types [2-5], this unusual situation prompted us to further explore the role of resveratrol (R) in prostate cancer proliferation.

Like any food product, red wine contains a complex mixture of structurally related organic compounds. One of these compounds is R. The predominant trans-R comes along with a lesser-known companion, cis-resveratrol (cis-R), and they are almost always present in red wine together (Figure 1). We recently discovered [6] that the GC-MS peak of cis-R was usually accompanied by a close peak of another polyphenol having molecular weight 2 Da higher than cis-R. Independent synthesis
confirmed that this peak is the peak of the third form of R, dihydro-resveratrol (dihydro-R). Dihydro-R is a well-known metabolite of trans-R [7], but it had not been identified in red wines before the study [6].

In our experiments dihydro-R demonstrated strong proliferative properties in hormone-dependent cell lines (such as MCF-7) at picomolar (10^{-12} M) concentrations [6]. The effect was not observed in cell lines without estrogen receptors, and the proliferative effect could be reversed by the action of estrogen antagonists, such as the known breast cancer drug Tamoxifen. This is consistent with the assumption that dihydro-R is a potent phytoestrogen, even though the conformationally flexible chemical structure of dihydro-R makes it a very unlikely analog of 17β-estradiol and other synthetic nonsteroidal estrogens such as diethylstilbestrol (DES) [6].

Given the preceding, it is reasonable to assume that the presence of dihydro-R in red wines could be partially responsible for the difference observed between breast cancer and prostate cancer in relation to moderate wine consumption. To explore this further, we initiated a comparative study of the biological effects of the three forms of R found in red wines on androgen-independent prostate cancer cell lines (PC-3 and DU-145) and one hormone-dependent breast cancer cell line (MCF-7) in vitro. This paper presents the results of our work.

Results and Discussions
To investigate the ability of trans-R, cis-R, and dihydro-R to control the growth of PC-3 prostate cancer cells, various concentrations of these compounds were added to tumor cell cultures and then incubated for 48 h. Figures 2 and 3 show the tumor cell viability after exposure to these agents.

The cytotoxic effect of trans-R was observed at concentrations of 1 × 10^{-7} to 1 × 10^{-4} M. Similarly, the cytotoxic effect of cis-R was observed at concentrations 1 × 10^{-6} to 1 × 10^{-4} M. The results indicated that trans-R is somewhat more effective as an anticancer agent than cis-R. Dihydro-R, in contrast to trans-R and cis-R, had a biphasic effect. At high concentrations (1 × 10^{-5} to 1 × 10^{-3} M), dihydro-R showed the typical for this class of compounds decrease in the growth rate of tumor cells. However, treatment with 1 × 10^{-10} to 1 × 10^{-8} M of dihydro-R significantly increased the growth of PC-3 prostate cancer cells (Figure 2).

Further comparative evaluation of the biological activity of trans-R and dihydro-R was performed using the DU-145 prostate cancer cell line. Again, trans-R displayed only cytotoxic properties whereas dihydro-R had a biphasic effect, similar to the one observed in PC-3 cells. At high concentrations (1 × 10^{-5} to 1 × 10^{-3} M), dihydro-R decreased the rate of growth of DU-145 tumor cells. Treatment with low concentrations (1 × 10^{-10} to 1 × 10^{-8} M) of dihydro-R significantly increased the growth of DU-145 prostate cancer cells (Figure 3).

The observed cytotoxic effects of both trans-R and cis-R in androgen-independent prostate cancer cell lines are in agreement with the previously reported data. Trans-R is a known inhibitor of human prostate cancer cell growth at concentrations of 1 × 10^{-6} to 1 × 10^{-4} M (e.g., GI50 = 2.41 × 10^{-5} M, DU-145 [8]) depending on the prostate cancer cell line and experimental conditions [9]. The biphasic action of the third form of R, dihydro-R, in

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**Figure 1** Structures of trans-resveratrol (trans-R), cis-resveratrol (cis-R), and dihydro-resveratrol (dihydro-R) isomers

**Figure 2** Biological activity of dihydro-R, trans-R and cis-R in the PC-3 cell line.
comparison with the better studied \textit{trans}-R requires further evaluation, especially in relation to estrogen receptors [6] present in both the PC-3 and the DU-145 prostate cancer cell lines [10-14]. The biological effects of \textit{trans}-R in androgen-independent prostate cancer cell lines can be attributed to a variety of specific molecular targets, including estrogen and androgen receptors [15]. Unfortunately, the exact mechanism of the hormone receptor-mediated activity of \textit{trans}-R is poorly understood because of the apparent complexity of the underlying biological functions of these receptors in prostate cancer cells. For example, it is well documented that 17\textbeta\textendash estradiol (E2) inhibits growth of androgen-independent PC-3 cells [10] but stimulates growth of androgen-dependent LNCaP cells, presumably via its own receptor [16]. The situation is even further complicated by the existence of two estrogen receptors (ERs), ER-\textalpha and ER-\textbeta, in prostate cancer cells [11]. As a result, growth of PC-3 cells, which have both ER subtypes, can be effectively inhibited by estrogens as well as antiestrogens [17]. In contrast, DU-145 cells express only ER-\textbeta, and their growth can be inhibited only by antiestrogens [11].

Given the convolution of hormone receptor functions in prostate cancer cells [18], it could only be presented as a working hypothesis that, similar to MCF-7 breast cancer cells [6], the observed growth stimulation of PC-3 and DU-145 cells by nanomolar concentrations of \textit{dihydro-R} can be attributed to the hormone receptor modulation activity of this compound. A direct comparison between the three cell lines (MCF-7 [6], PC-3, and DU-145, Figure 4) reveals substantial similarities in \textit{dihydro-R} biphatic proliferative action. As expected, the proliferative effects are more pronounced in the “classic” ER-\textalpha-dependent MCF-7 cells compared to PC-3 cells (ER-\textalpha and ER-\textbeta) and then DU-145 cells (only ER-\textbeta).

Additional credence is given to our assumption that \textit{dihydro-R} is a hormone receptor modulator by the recent observation that another dietary polyphenol with a similar biological profile, genistein (Figure 5), also exhibits biphasic behavior in the regulation of prostate cancer cell growth [19]. It was reported that low (5 × 10^-7 M) concentrations of genistein caused increased proliferation of PC-3 cells whereas a pharmacologic dose (5 × 10^-5M) decreased proliferation. Estrogen signaling is mentioned as one of the possible mechanisms of this biphasic action [19] (genistein is a well-known phytoestrogen [14,20,21]).

To explore our working hypothesis further, we evaluated the cancer cell growth effects of \textit{dihydro-R} in the presence of \textit{trans}-R. \textit{Trans}-R is capable of acting as both an estrogen and an antiestrogen [22-26], including non-genomic action [25], but this compound by itself does not significantly affect the growth of PC-3 prostate cancer cells at concentrations below 1 × 10^-7 M. Our experiments clearly demonstrated the interference between \textit{dihydro-R} and \textit{trans}-R (Figure 6). In these experiments \textit{trans}-R acted as a \textit{dihydro-R} antagonist at low concentrations. The maximum antagonistic effect was observed at nanomolar concentrations (1 × 10^-10 to 1 × 10^-8 M). Similar biphasic agonistic/antagonistic properties of \textit{trans}-R in MCF-7 cells in the presence of E2 were reported previously [24].

Somewhat stronger interference between \textit{dihydro-R} and \textit{trans}-R was observed in “classic” ER-\textalpha-dependent MCF-7 cells (Figure 7). As we mentioned previously, \textit{dihydro-R} proliferative action at very low concentrations was more pronounced in the MCF-7 cell line than the PC-3 cell line (Figure 4). As a result, cross-modulation effects of \textit{dihydro-R} and \textit{trans}-R in MCF-7 cells were notable even at sub-picomolar concentrations (1 × 10^-16 to 1 × 10^-14M). This modulation appeared to be non-monotonic and showed a very complex concentration dependence pattern. For example, maximum proliferation effects of a 1/1
mixture of dihydro-\(R\) and trans-\(R\) were observed at \(1 \times 10^{-14}\) and \(1 \times 10^{-7}\) M concentrations, whereas intermediate concentrations (\(1 \times 10^{-11}\) to \(1 \times 10^{-9}\) M) showed little, if any, proliferative activity (Figure 7). Non-monotonic dose response is a well-documented feature of steroid hormone receptors [27,28].

**Experimental Section**

*Trans*-\(R\) was obtained from Sigma (St. Louis, Missouri, USA). *Cis*-\(R\) was prepared from *trans*-\(R\) by photoisomerization [29]. *Dihydro*-\(R\) was synthesized from *trans*-\(R\) by catalytic hydrogenation according to [7,29]. The purity of the compounds was monitored by a combination of high performance liquid chromatography, \(^1\)H nuclear magnetic resonance (NMR) and \(^{13}\)C NMR. MCF-7, a human breast cancer cell line, and PC-3 and DU 145, human prostate cancer cell lines, were obtained from the American Type Culture Collection, Rockville, Maryland. Cell lines were cultured at 37°C in a 5% CO\(_2\) humidified atmosphere and maintained in RPMI-1640 (Sigma, USA) supplemented with 10% heat-inactivated (56°C, 30 min) fetal calf serum (HyClone Laboratories, Logan, UK), 2 mM L-glutamine, and antibiotics (100 \(\mu\)g/ml penicillin sodium salt and 100 \(\mu\)g/ml streptomycin sulfate [Sigma, USA], herein referred to as complete medium [CM]). Resveratrol samples were dissolved in dimethyl sulfoxide (DMSO) (Sigma, USA) at a concentration of 200 mM, and diluted in culture medium just before use. Tumor cells were harvested, counted, suspended in CM, seeded into 96-well tissue culture plates (Costar, France) in 200 \(\mu\)l/well at a concentration of \(1 \times 10^5\) cell/ml, and allowed to adhere overnight (required for particular cell lines to be in log phase). After that, CM was removed from sample wells. Compound sample solutions in CM were added to tumor cells. Final concentrations in sample wells amounted to \(1 \times 10^{-15}\) - \(1 \times 10^{-3}\) M. The plates were incubated at 37°C in a 5% CO\(_2\) humidified atmosphere for 48 h. Three replica wells were used for controls and each compound concentration. The control samples contained DMSO concentrations corresponding to target compound dilutions. Every 24 h cells underwent microscopy and were photographed with the help of an AxioVision 4 system (Zeiss, Germany; see additional file 1). Cell viability assays were performed using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction. After a 48 h incubation of tumor cells (control or drug-treated), MTT solution (5 mg/ml) was added to each well. A 4 h incubation at 37°C was stopped by the addition of 100 \(\mu\)l of DMSO. The absorbances of the samples were measured on a microplate reader Multiscan MS.
Conclusions
We demonstrated that the biphasic proliferative effects of dihydro-\(R\) at micromolar and nanomolar concentrations are not limited to hormone-dependent breast cancer cells but also present, albeit in a reduced scale, in androgen-independent prostate cancer cells. While the exact mechanism of these effects requires further evaluation, our preliminary results point to hormone receptor modulation activity. A significant similarity exists between the phytoestrogens genistein and dihydro-\(R\). We also observed strong cross modulation between trans-\(R\) and dihydro-\(R\), which is noticeable even at subpicomolar concentrations. The role of dihydro-\(R\) in breast cancer and prostate cancer proliferation related to moderate consumption of red wine remains an open question because dihydro-\(R\) exhibits proliferative activity in both prostate and breast cancer cells at low concentrations and has a very complex activity pattern in the presence of trans-\(R\).

Note Added in Proof
The anti-cancer properties of trans-\(R\) go beyond direct growth inhibition of cancer cells. It is known that natural killer (NK) cells, such as mononuclear leukocytes (ML), are capable to kill tumor cells. We subsequently studied immunotropic activity of trans-\(R\) (Table 1, additional file 2). It was found that 10^{-6} M of trans-\(R\) significantly activated lysis of K-562 cells by ML comparing to intact ML: 61 ± 6.8% vs. 46 ± 5.8% for K-562/ML cell ratio 1:10; and 53 ± 8.7% vs. 26 ± 7.3% for K-562/ML cell ratio 1:5.

Our results are consistent with the general trends observed in the previous in vitro studies [30]. The authors [30] reported that in vitro exposure to trans-\(R\) produced a biphasic effect on the anti-CD3/anti-CD28-induced development of both IFN-gamma-IL2 and -IL4-producing CD8+ and CD4+ T-cells. Trans-\(R\) was found to induce a significant enhancement of NK cell cytotoxic activity at low concentrations and to induce suppression at high concentrations.

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Authors’ contributions
NUA carried out biological experiments, participated in design of the study, and drafted the manuscript. MKV and AVS participated in coordination and design of the study. INS carried out analysis of \(R\) samples. AAG participated in design and coordination of the study and edited the manuscript for publication. All authors have read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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References
Phytoestrogens: epidemiology and a possible role in prostate cancer. 


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