Anti-leukemia Effect of Resveratrol

MIN-FU TSAN*, JULIE E. WHITE*, JEWRAJ G. MAHESHWAR† and G. CHIKKAPPA†

*Regional Office (10R), Office of Research Compliance and Assurance, Department of Veterans Affairs, 50 Irving Street, N.W., Washington, DC 20422, USA; †Charles River Laboratories, Troy, NY 12180, USA; ‡Stratton VA Medical Center, and Albany Medical College, Albany, NY 12208, USA

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Resveratrol is a phytoalexin naturally present in fruits, medicinal plants and wines. It has a diversity of biological activities. While its role in the protection against coronary heart disease (CHD) in people with moderate wine consumption, remains unclear, resveratrol preferentially inhibits the growth of leukemia cells in culture. Potential mechanisms for its anti-leukemia effect include induction of leukemia cell differentiation, apoptosis, and cell cycle arrest at S-phase; and inhibition of DNA synthesis by inhibiting ribonucleotide reductase or DNA polymerase. Preliminary results suggest that resveratrol also inhibits the viability of freshly isolated leukemia cells, especially promyelocytic leukemia cells. Because of its low in vivo toxicity, resveratrol deserves further investigation as an anti-leukemia agent.

Keywords: Resveratrol; Leukemia; Apoptosis

INTRODUCTION

Trans (t)-resveratrol (3,4',5-trihydroxystilbene) is present naturally in a variety of fruits and medicinal plants [1–3]. It functions as a phytoalexin that protects plants against fungal infection [2]. Resveratrol is the active component of “kojokon” prepared from the root of Polygonum cuspidatum which has been used in the traditional Chinese herb medicine for centuries for the treatment of several diseases, particularly lipid disorders [3,4]. Because of its high concentration in grape skin, significant amount of resveratrol is present in wine, especially red wine [5].

Resveratrol and the “French Paradox”

Certain populations, e.g. the French, suffer little coronary heart disease (CHD) despite a diet relatively high in fat content. It has been postulated that regular consumption of red wine in moderate amounts by these people may be responsible for the phenomenon (the French Paradox) [6,7]. Resveratrol, being a potent antioxidant and a metal (especially copper) chelator [8], possesses a diversity of biological effects that may prevent the development of CHD. These include, but are not limited to, the inhibition of platelet aggregation [9], oxidation of low-density lipoprotein [10] and the expression of tissue factor [11]. It has been suggested that resveratrol may be responsible for the beneficial effect of red wine in protecting against CHD [6,7,12]. However, Wilson et al. [13] reported that oral administration of resveratrol (1 mg/kg/day) promoted, rather than reduced, the formation of atherosclerotic lesions in hypercholesterolemic rabbits. Thus, the role of resveratrol in the French Paradox remains controversial. Recent evidence suggests that it is the alcohol, not other constituents, present in the wine that is responsible for the protection against CHD by its ability to increase the blood level of high-density lipoprotein [14].

Resveratrol and Cancer Chemoprevention

In search for new cancer chemopreventive agents, hundreds of plant extracts have been evaluated for their ability to inhibit cyclooxygenase activity due to its potential role in carcinogenesis. An extract derived from Cassia quinquangulata Rich. (Leguminosae), collected from Peru in 1974, was found to be a potent inhibitor of cyclooxygenase, and resveratrol was identified as the active principle [15]. In 1997, Jang et al. [15] reported that resveratrol demonstrated cancer chemopreventive activity in assays representing three major stages of carcinogenesis. Resveratrol was shown to act as an antioxidant and antimutagen and to induce phase II drug-metabolizing enzymes (anti-initiation activity). It mediated anti-inflammatory effects and inhibited cyclooxygenase and hydroperoxidase functions (anti-promotion activity); and it induced HL60 human promyelocytic leukemia cell
differentiation (anti-progression activity). In addition, it inhibited the development of pre-neoplastic lesions in carcinogen-treated mouse mammary glands in culture and inhibited tumorigenesis in a mouse skin model [15]. Since then, resveratrol has been shown to inhibit the growth of human leukemia and solid cancer cell lines in vitro [16–20]. This concise review summarizes the anti-leukemia effect of resveratrol.

ANTI-LEUKEMIA EFFECT OF REVERATROL

Resveratrol has been shown to inhibit the in vitro growth of human acute lymphocytic and non-lymphocytic leukemia cell lines, including HL60 (promyelocytic leukemia) [15,17,18,21,22], THP-1 (acute monocytic leukemia) [23], U937 (myelomonocytic leukemia) [22,24], K562 (myeloid leukemia/chronic myelogenous leukemia in blast crisis) [25], CEM-C7H2 (T-acute lymphocytic leukemia) [26], and Jurkatt (T-lymphoblastic leukemia) [26] cell lines. The estimated 50% effective dose (ED₉₀) is approximately 10 µmol/l in several studies [17,18,23]. The effect of resveratrol appears reversible, since leukemic cells regain their growth capability almost completely after removal of resveratrol from the culture medium [18,23,24].

Mechanisms of Resveratrol’s Growth Inhibitory Effect

A number of mechanisms have been proposed for the growth inhibitory effect of resveratrol on leukemia cells including induction of differentiation, apoptosis, cell cycle arrest and inhibition of DNA synthesis.

Differentiation Induction

Using HL60 promyelocytic leukemia cells, Jang et al. [15] reported that resveratrol induced differentiation of the leukemia cells toward myelo-monocytic phenotype. Ragione et al. [18] also reported that resveratrol induced differentiation, and caused HL60 cell cycle arrest at S/G₂ phase transition. However, other investigators were unable to demonstrate differentiation induction of HL60 [17] or THP-1 [23] cells by resveratrol.

Induction of Apoptosis

Recently, chemotherapeutic agents, irrespective of their intracellular targets and mechanisms of action, have been shown to exert their biological effect by triggering a common final death pathway (apoptosis) in their target cells including leukemias, lymphomas and solid cancers [27–29]. Apoptosis is a programmed cell death accomplished by specialized cellular machinery that results in DNA fragmentation, chromatin condensation, membrane blebbing, cell shrinkage, and disassembly into membrane enclosed vesicles (apoptotic bodies) [27,29]. Exactly how cytotoxic agents induce apoptosis in chemo-sensitive cancer cells, remains incompletely understood [27,29].

The morphological and biochemical changes that occur in apoptotic cells are the results of caspase-mediated cleavage of cellular polypeptides. Caspases are cysteine proteases with an absolute requirement for cleavage after aspartic acid. In apoptosis, they function in both cell disassembly (effector caspases, e.g. caspases-3, 6 and 7) and in initiating this disassembly in response to apoptotic signals (initiator caspases, e.g. caspases-8 and 9) [30].

There are two archetypal pathways of caspase activation: death receptor-mediated and mitochondria-mediated pathways (Fig. 1). Ligation of death receptors such as Fas (CD95, APO-1) to their ligands such as Fas ligand (FasL, CD95L), results in recruitment of adaptor molecules such as FADD (Fas-associated death domain) to the cytoplasmic domain of the receptors. As a consequence, procaspase-8 is activated. Activated caspase-8 in turn activates downstream caspases-3 and 7 [29,31]. In the mitochondrial pathway, the initial stimuli/insults remain poorly understood. However, these stimuli cause loss of mitochondrial transmembrane potential, and release of cytochrome c from mitochondria, either as a result of rupture of the outer mitochondrial membrane, or as a consequence of an alteration in the pore-forming outer membrane protein, voltage-dependent anion channel [29,32]. Once released, cytochrome c binds to the cytoplasmic scaffolding protein, Apaf-1 (apoptosis-protease activating factor-1), which together with ATP or dATP, activates procaspase-9. Activated caspase-9 in turn activates caspases-3 and 7 [29,33].

Earlier studies suggest that chemotherapeutic agent-induced apoptosis involves Fas-receptor-mediated apoptotic pathway, either by inducing upregulation of Fas receptor and/or Fas ligand expression [28,34], or by causing clustering of Fas receptors [35], in the target cells. Exactly how chemotherapeutic agents induce/enhance Fas and/or FasL expression in cancer cells is not clear. On the other hand, more recent studies suggest that most anticancer drugs induce apoptosis independent of Fas/FasL [36,37]. Instead, they induce mitochondrial release of cytochrome c, followed by the activation of caspase-9 and downstream caspases [38,39].

\[ kDa \]

\[ -17.6 \]

\[ -7.5 \]

1 day 2 days 3 days

FIGURE 1 Effect of resveratrol on mitochondrial cytochrome c release. THP-1 cells were treated with or without resveratrol (30 µM) for 1.2 or 3 days, or with etoposide (50 µM) for 4h (positive control for apoptosis). Cell extracts were then immuno-blotted for cytochrome c using a polyclonal antibody. Lanes 1, 3 and 5, control; lanes 2, 4 and 6, resveratrol; lane 7, etoposide, 4h.
Clement et al. [17] showed that resveratrol induced an enhanced expression of FasL in HL60 cells leading to Fas/FasL-mediated apoptosis. Suri et al. [21] also reported the induction of apoptosis by resveratrol in HL60 cells, however, no detailed study on the mechanism of apoptosis was carried out. In contrast, Ragione et al. [18] was unable to detect any increased apoptosis in resveratrol-treated HL60 cells. On the other hand, Tsan et al. [23] demonstrated that induction of apoptosis in THP-1 cell by resveratrol was independent of Fas/FasL signaling pathway. Likewise, Bernhard et al. [26] reported that resveratrol-induced apoptosis of both CEM-C7H2 and Jurkat cells, was independent of Fas/FasL pathway. It is possible that whether resveratrol-induced apoptosis of leukemia cells is dependent on Fas/FasL signaling pathway, is cell type specific. However, Dirks et al. [40] have previously demonstrated that HL60 and THP-1 cells, despite the presence of Fas receptors, are resistant to Fas/FasL-mediated apoptosis. As shown in Fig. 1, resveratrol causes a progressive increase of cytochrome c in the cytoplasm of THP-1 cells, presumably released from the mitochondria. These preliminary data suggest that resveratrol induces THP-1 cell apoptosis through the mitochondrial pathway.

Cell Cycle Arrest

Flow cytometric analysis of DNA contents has revealed that resveratrol-induced growth inhibition is associated with cell cycle arrest at the S-phase in HL60 [18], U937 [24] and CEM-C7H2 [26] cells. This cell cycle arrest is reversible; removal of resveratrol from the culture medium stimulates U937 cells to reenter the cell cycle synchronously [24]. The biochemical mechanism for the resveratrol-induced cell cycle arrest at the S-phase is not clear.

Inhibition of DNA Synthesis

Resveratrol has been shown to be a potent inhibitor of ribonucleotide reductase and inhibits DNA synthesis in K562 cells [25]. Sun et al. [41] also reported that resveratrol was an inhibitor of DNA polymerase. Whether inhibition of ribonucleotide reductase and/or DNA polymerase by resveratrol is responsible for the above described cell cycle arrest and/or apoptosis, needs further investigation.

Effect of Resveratrol on Normal Hematopoietic Progenitors and Blood Cells

Resveratrol at concentrations that inhibit the growth of leukemia cells, has no effect on the viability of human lymphocytes [17] or THP-1 cells that have been induced to differentiate into monocytes/macrophages by phorbol ester [23]. Resveratrol also has no inhibitory effect on the phytohemagglutinin-induced lymphocyte proliferation [17].

Gautam et al. [22] showed that murine hematopoietic progenitors are more resistant to resveratrol than murine leukemia cells, 32Dp210 and L1210, and that hematopoietic reconstitution in lethally irradiated mice from resveratrol-treated bone marrow was comparable to that of non-treated cells. These results suggest the potential of resveratrol for ex vivo pharmacological purging of leukemia cells for bone marrow autographs.

Effect of Resveratrol on Freshly Isolated Human Leukemia Cells

So far, the anti-leukemia effect of resveratrol has only been demonstrated in established leukemia cell lines under in vitro culture conditions. Whether resveratrol has a similar effect on leukemia cells freshly isolated from patients, has not been examined. We had the opportunity to study acute leukemia cells from two patients. Patient #1 was a 71-year-old man who developed acute myeloblastic leukemia four years after the diagnosis of myelodysplastic syndrome with sideroblasts. Patient #2 was an 87-year-old man who developed promyelocytic leukemia with chromosomal translocation, t(15;17) (q22;q21), 10 years after radiation therapy for a prostate cancer and one year after chemotherapy (melphalan and prednisone) for multiple myeloma.

As shown in Fig. 2, isolated acute leukemia cells did not proliferate under the culture condition, but maintained their viability as ascertained by the exclusion of trypan blue dye. Resveratrol at high concentrations reduced the viability of the leukemia cells, more so in the promyelocytic leukemia cells isolated from Patient #2 than the acute myeloblastic leukemia cells isolated from Patient #1. The estimated EC50s were approximately 45 and 150 μM for the promyelocytic and myeloblastic

![Figure 2: Effect of resveratrol on the growth/viability of freshly isolated acute leukemia cells. Leukemia cells were isolated from a patient (Patient #1) with acute promyelocytic leukemia and a patient (Patient #2) with promyelocytic leukemia. Leukemia cells (1 x 10⁶ cells in 1 ml RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum and antibiotics) were treated with or without resveratrol (0–200 μM) for three days. The number of viable cells was then determined using trypan blue dye exclusion. Similar results were obtained in the presence of growth factor, granulocyte/macrophage colony stimulating factor (GM-CSF, 100 units/ml).]
leukemia cells, respectively. These ED₉₀S are considerably higher than the ED₁₀ for THP-1 or HL60 cells (approximately 10 μM). Similar results were obtained using a MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide]-based cell proliferation assay kit (Molecular Probe, Eugene OR). Retinoic acid at concentrations ranging from 0.1 to 10 μM, had no effect on the viability of the promyelocytic leukemia cells obtained from Patient #2. However, it induced differentiation of these cells as determined by a marked decrease in nitroblue tetrazolium reduction activity. In contrast, resveratrol did not induce differentiation of the promyelocytic leukemia cells (data not shown).

**CONCLUSION AND FUTURE DIRECTIONS**

Resveratrol, a phytoalexin present in a number of fruits, medicinal plants and wines, has a diversity of biological effects. While its contribution to the protection against CHD in individuals with moderate wine consumption, remains uncertain, it inhibits efficiently the growth of cancer, including leukemia, cell lines in vitro. Because of its low in vivo toxicity [4,13,15], and its preferential anti-leukemia effect over normal blood cells and marrow progenitor cells [17,22,23], it has great potential of becoming an anti-leukemic agent. However, much more needs to be done to achieve this goal. Further studies are required to elucidate the mechanism of its anti-leukemic effect; its effect on freshly isolated human leukemia cells, particularly promyelocytic leukemia cells, in vitro and its pharmacokinetics and in vivo effect against acute leukemias in animal models.

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**References**


