Antiproliferative and Pro-apoptotic Activities of a Novel Resveratrol Prodrug Against Jurkat CD4+ T-Cells

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Abstract. *Background/Aim: Resveratrol, a natural polyphenol, possesses many beneficial health properties but its therapeutic application is limited due to its low water solubility and instability against oxidative processes. To improve the stability and lipophilicity of the natural compound, we synthesized a resveratrol prodrug, termed FEHH4-1. In the present study, we compared the antiproliferative and pro-apoptotic effects of resveratrol with FEHH4-1 on Jurkat T-cells. Materials and Methods: Cell proliferation and viability were monitored by 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide assay, annexin-V/7-amino-actinomycin D staining and western blot. To induce interleukin-2 (IL2) expression, cells were stimulated with phorbol 12-myristate 13-acetate/phytohemagglutinin. IL2 production was quantified by enzyme-linked immunosorbent assay. IL2 promoter activity was studied by a Jurkat T-cell line containing an IL2 promoter luciferase reporter construct. Results: Both polyphenols inhibited proliferation, induced apoptotic cell death and blocked IL2 synthesis in Jurkat T-cells. Most importantly, FEHH4-1 was three-to four-times more potent than resveratrol. Conclusion: FEHH4-1 had improved antiproliferative and pro-apoptotic potential against Jurkat T-cells compared to resveratrol.*

Resveratrol (3,4',5-*trans*-trihydroxystilbene) is a natural polyphenol that is present in different plants (red grapes, raspberries, blueberries and other food products). Over the years, resveratrol has become a very popular food supplement due to its many potential positive health effects. Resveratrol has been reported to have potent anti-inflammatory, anti-oxidant and anti-carcinogenic activities (1). The hallmarks of cancer include abnormal cell proliferation and resistance to apoptotic signals. Extensive studies over the past decade have shown both the chemopreventative and chemotherapeutic potential of resveratrol (2). It suppresses the proliferation of wide variety of human tumor cells *in vitro*. Joe *et al*. showed that resveratrol induced growth inhibition, S-phase arrest and apoptosis of several human cancer cell lines (3). Ge *et al*. reported that resveratrol induced apoptosis and autophagy in T-cell acute lymphoblastic leukemia cells by inhibiting the protein kinase B/mammalian target of rapamycin pathway and activating p38-mitogen activated protein kinase (4). It significantly reduced the expression of pro-apoptotic proteins (BCL2-associated X protein, BCL2-like protein 11, and BCL2-antagonist of cell death), and induced caspase-3 cleavage in a time-dependent manner (4). Although a number of cell-culture experiments have shown promising and positive effects of resveratrol, evidence obtained in experimental animals and humans is inconsistent. Resveratrol supplementation in animal models of cancer has shown predominantly positive (5-9), or neutral (10), but also negative effects (11) depending on administration, dose, tumor model, species and other factors. These data suggest that many factors have to be considered before resveratrol can be used for human cancer prevention or therapy. Clinical evidence for resveratrol as an effective supplement for cancer prevention and treatment is scarce. In 2009, the first phase I clinical trial in patients diagnosed with colorectal cancer was published (12). Resveratrol and grape powder administration had no effect on cancerous mucosal Wingless/INT1 (WNT) signaling, but their supplementation did result in reduction of WNT target gene expression in normal mucosa (12).

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Key Words: Resveratrol, FEHH4-1, Jurkat CD4+ T-cells, proliferation, apoptosis.

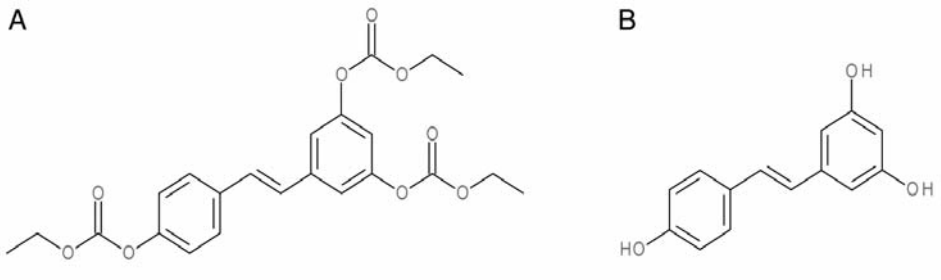

Figure 1. *Chemical structure of FEHH4-1 (A) and resveratrol (B).*

To summarize, the low chemical stability of resveratrol (13), its low bioavailability (13), high metabolic rate (13), and lack of properly controlled clinical studies make the therapeutic use of this polyphenol controversial. Consequently, all these obvious problems have prompted research for improved resveratrol analogs and efficient delivery systems (14,15). Herein we present a novel synthetic resveratrol analog ([4-[(E)-2-[3,5-*bis*(ethoxycarbonyloxy)phenyl]vinyl]phenyl] ethyl carbonate) termed FEHH4-1. Through esterification of all phenolic groups in the resveratrol molecule, a greater stability against oxidative processes and an improvement in lipophilicity can be achieved. Thus, FEHH4-1 can be considered a resveratrol 'prodrug', which is cleaved into its active form through intracellular esterases. In the present study, we report on the anti-proliferative and pro-apoptotic effects of resveratrol and FEHH4-1 on Jurkat T-cells.

Materials and Methods

Chemicals and polyphenolic compounds. Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Carl Roth (Karlsruhe, Germany). Resveratrol was from Sigma-Aldrich, the resveratrol prodrug FEHH4-1 was synthesized at the Department of Pharmaceutical Chemistry (University of Vienna, Austria). Both compounds were dissolved in dimethylsulfoxide (DMSO) (purity ≥99.5%) and diluted into cell culture medium. In contrast to resveratrol, FEHH4-1 was used only up to 50 μM because it precipitated at higher concentrations under cell culture conditions. The final DMSO concentration in all samples including controls was 0.5% (v/v). The selective caspase-3 inhibitor Z-DEVD-FMK was purchased from R&D Systems (Minneapolis, MN, USA).

Synthesis of FEHH4-1. Resveratrol (2.5 mmol) in dry tetrahydrofuran was mixed with sodium hydride (9 mmol) at 0˚C under an argon atmosphere. After 20 min, ethyl chloroformate (9 mmol) was added and the reaction mixture was allowed to reach room temperature (RT). After 60 min at RT, the reaction mixture was filtered and the solvent was removed *in vacuo*. The crude product was purified by column chromatography and characterized by proton-nuclear magnetic resonance (^{1}H-NMR) spectroscopy, carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectroscopy, mass spectrometry (MS), and combustion analysis. Finally, the purity of FEHH4-1 was checked by thin-layer chromatography. Yield: 0.420 g (37.8%) of FEHH4-1. Mp=92-94˚C. ^{1}H-NMR ($CDCl_3$, 200 MHz): ä=7.49 (d, *J*=8.7 Hz, 2H), 7.24-7.14 (m, 4H), 7.13-7.00 (m, 1H), 6.99-6.90 (m, 2H), 4.32 (q, *J*=7.0 Hz, 6H), 1.39 (t, *J*=7.0 Hz, 9H). ^{13}C-NMR ($CDCl_3$, 50 MHz): ä=153.4, 153.1, 151.6, 150.8, 139.6, 134.4, 129.7, 127.7, 127.1, 121.3, 116.4, 113.4, 65.0, 14.1. MS: *m/z*=444 (M^+, 39%), 372 (39%), 256 (48%), 228 (91%), 226 (90%), 181 (100%), 152 (56%). Anal calcd for $C_{23}H_{24}O_9$: C, 62.14%, H, 5.44%. Found C, 61.84%, H, 5.44%.

Cells and cell culture. A Jurkat T-cell line containing a luciferase reporter gene under the control of the interleukin-2 (*IL2*) promoter was obtained from the Department for Laboratory Medicine (Medical University Vienna, Austria). The cells were maintained in Iscove's modified Dulbecco's medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin (Life Technologies, Thermo Fisher Scientific, Carlsbad, CA, USA) at 37˚C and 5% CO_2.

Cell proliferation assay. Jurkat T-cells were seeded in 96-well plates (1×10^4 cells/well) and pre-treated for 24 h with different concentrations of resveratrol or FEHH4-1 before being stimulated with human recombinant IL2 protein (10 ng/ml) for another 24 h. Cell proliferation was measured using the Cell Proliferation Kit II (Roche Diagnostics GmbH, Vienna, Austria) containing 2,3-*bis*-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide reagent. The quantity of the water-soluble formazan product was measured at 490/655 nm using a spectrophotometric microplate reader (iMark™; Bio-Rad Laboratories, Hercules, CA, USA). The results are expressed as the percentage of growth, with 100% representing control cells treated with DMSO (0.5%) alone.

Apoptosis assay. Jurkat T-cells (1×10^5 cells/well) were treated for 72 h with different concentrations of resveratrol or FEHH4-1 and double-stained with annexin-V/7-amino-actinomycin D (Merck Millipore, Darmstadt, Germany) as described by the manufacturer. The cells were then analyzed using the Muse Cell Analyzer (Merck Millipore) as previously described (16).

Western blot analysis. Jurkat T-cells (2×10^6 cells/dish) were treated for 72 h with different concentrations of resveratrol or FEHH4-1, washed twice with phosphate-buffered saline (PBS; pH 7.4), and disrupted in sodium dodecyl sulfate (SDS) sample buffer (Roti-Load1; Roth, Karlsruhe, Germany). The cell lysates were boiled for 5 min at 95˚C and treated with a short impulse of ultrasound (10 s)

to minimize viscosity. Equal amounts of solubilized extracts were separated by SDS polyacrylamide gel electrophoresis on 12% polyacrylamide mini-gels and electrophoretically transferred to polyvinylidene difluoride membranes (Trans-Blot®Turbo™ Transfer Pack; Bio-Rad Laboratories, Inc., Hercules, CA, USA) using the Trans-Blot®Turbo™ Transfer System (Bio-Rad). The membranes were blocked for 1 h with 5% (w/v) nonfat dry milk in PBS containing 0.1% (v/v) Tween 20 (PBST) and probed overnight at 4˚C with specific antibodies to caspase-3/-7 and poly (ADP-ribose) polymerase (PARP; Cell Signaling, New England Biolabs, Frankfurt am Main, Germany), and tubulin (Sigma-Aldrich). The blots were washed 3×5 min with PBST and incubated for 2 h with horseradish peroxidase-coupled anti-rabbit secondary antibody in PBST containing 1% (w/v) nonfat dry milk. Finally, the membranes were washed 3×5 min with PBST and developed with the WesternBright™ ECL-spray (Advansta Inc., Menlo Park, CA, USA). The signals were detected with the GeneGnome device (Syngene, Cambridge, UK).

Stimulation of cells and quantification of IL2 production. Jurkat T-cells were seeded in 12-well plates (2×10^5 cells/well) and stimulated for 24 h with phorbol 12-myristate 13-acetate (PMA; 100 ng/ml) plus phytohemagglutinin (PHA; 1 μg/ml) in the absence or presence of different concentrations of resveratrol or FEHH4-1. The concentration of IL2 in cell culture supernatants was quantified by enzyme-linked immunosorbent assay obtained from eBioscience (San Diego, CA, USA) and used according to manufacturer's instructions.

Luciferase assay. Jurkat T-cells were stimulated for 6 h with PMA/PHA in the absence or presence of different concentrations of resveratrol or FEHH4-1. At the end of each experiment, cells were lysed with CCLR Luciferase Assay System (Promega, Madison, WI, USA) and luciferase activity was measured in a luminometer (GloMax®-Multi Detection System; Promega). The data are reported in relative luciferase units.

Statistical analysis. The results are expressed as the mean±standard error of the mean (SEM). The statistical differences within groups were evaluated by one-factor analysis of variance (ANOVA) or two-way ANOVA followed by *post-hoc* analysis using Bonferroni test as appropriate. A value of $p<0.05$ was considered statistically significant.

Results

Chemical structure of FEHH4-1. The resveratrol prodrug FEHH4-1 was chemically synthesized as described in the Materials and Methods. Through esterification of all phenolic groups, a greater stability against oxidative processes and an improvement in lipophilicity was achieved (Figure 1A).

Resveratrol and FEHH4-1 inhibit proliferation of Jurkat T-cells. Previous studies have demonstrated that resveratrol has potent antiproliferative effects on different leukemia cell lines (3). To assess the impact of resveratrol and FEHH4-1 on cell proliferation, Jurkat T-cells were pre-incubated for 24 h with different concentrations (3.125-100 μM) of each compound before being stimulated with IL2 for another 24 h. Cell proliferation was monitored using 2,3-*bis*-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide reagent as a substrate to assess cell viability. As shown in Figure 2, resveratrol at 50 μM reduced cell proliferation by about 25%, FEHH4-1, however, by about 90%, confirming the enhanced effects of FEHH4-1 on Jurkat T-cells.

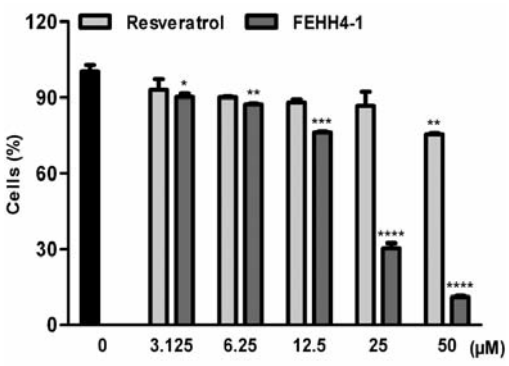

Figure 2. *Resveratrol and FEHH4-1 induce cell-growth arrest of Jurkat T-cells. Jurkat T-cells were pre-incubated for 24 h with vehicle or the indicated concentrations of resveratrol or FEHH4-1 before being stimulated with IL2 for another 24 h. After the addition of 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide reagent, cell proliferation was monitored by measuring the absorption at 490/655 nm. Data are mean values±SEM of two independent experiments. *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 compared to control.*

Resveratrol and FEHH4-1 induce apoptotic death of Jurkat T-cells. To evaluate the potential of resveratrol and FEHH4-1 to induce apoptosis of Jurkat T-cells, cells were incubated for 24 and 72 h with increasing concentrations of each compound (resveratrol: 6.25-100 μM; FEHH4-1: 6.25-50 μM). To study the membrane integrity (externalization of phosphatidylserine), cells were double-stained with annexin-V/7-amino-actinomycin D. The data show that treatment of Jurkat T-cells with resveratrol or FEHH4-1 reduced cell viability in a time and dose-dependent manner. After 24 h, only the highest concentration of resveratrol (100 μM) negatively affected cell viability (Figure 3A); in the cell population treated with 50 μM FEHH4-1, however, only 30% of the cells were still alive (Figure 3B). After 72 h, at 50 μM resveratrol, about 17% of cells were in the early apoptotic stage and the same percentage in the late apoptotic/dead stage (Figure 3C). At 50 μM FEHH4-1, about 30% of cells were in the early apoptotic stage and already 60% were in the late apoptotic/dead stage, highlighting the high degree of sensitivity of Jurkat T-cells to FEHH4-1 (Figure 3D). Interestingly, co-treatment of cells with an antioxidant (L-cysteine; 1 mM) or the selective caspase-3 inhibitor Z-DEVD-FMK (20 μM) did not prevent resveratrol- nor FEHH4-1-induced apoptosis (data not shown).

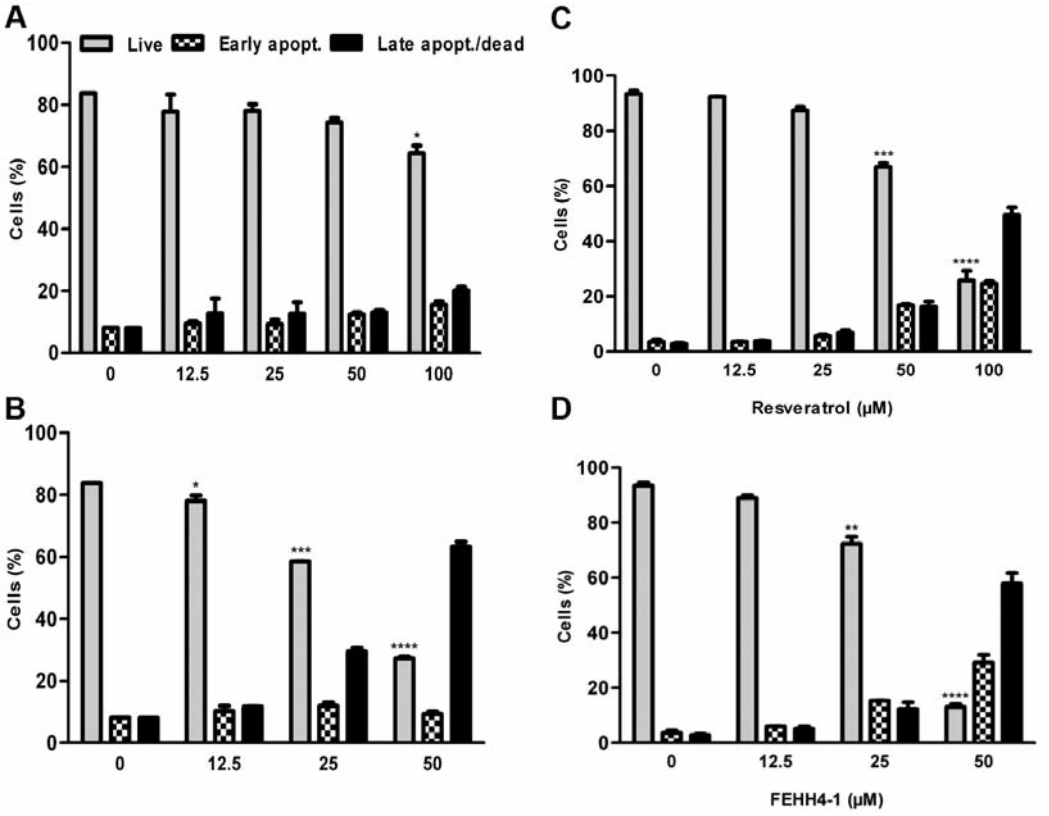

Figure 3. *Resveratrol and FEHH4-1 induce apoptotic death of Jurkat T-cells. Jurkat T-cells were treated for 24 (A, B) and 72 h (C, D) with vehicle or the indicated concentrations of resveratrol or FEHH4-1 and labeled with annexin-V/7-amino-actinomycin D. Data are mean values±SEM of two independent experiments. p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 compared with control.*

Resveratrol induces apoptosis of Jurkat T-cells via cleavage of caspase-3/-7. Next, we investigated whether resveratrol and FEHH4-1 triggered apoptosis *via* the caspase-3 signaling pathway. In this experiment, Jurkat T-cells were exposed for 72 h to different concentrations of resveratrol (12.5-100 μM) or FEHH4-1 (6.25-50 μM) and cleavage products of apoptotic marker proteins such as caspase-3/-7 and poly (ADP-ribose) polymerase (PARP) were analyzed by western blot. As shown in Figure 4, at 50 and 100 μM resveratrol, pro-caspase-3 was partially cleaved into two smaller subunits of 17 and 19 kDa, respectively. At a concentration of 100 μM resveratrol, the total amount of protein dramatically decreased, suggesting that this treatment was already toxic to the cells. In addition, cleavage of pro-caspase-7, another effector caspase, was analyzed. As observed for pro-caspase-3, resveratrol at 50 and 100 μM also induced cleavage of pro-caspase-7. PARP is mainly involved in DNA repair in response to environmental stress. It is one of the main cleavage targets of caspase-3 *in vivo* (17). As shown in Figure 4, at 50 and 100 μM resveratrol, the amount of full length PARP (116 kDa) decreased and a smaller product of 89 kDa appeared, indicating that PARP is also a target of caspase-3 in Jurkat T-cells. Interestingly, in strong contrast to resveratrol, FEHH4-1 did not induce cleavage of pro-caspase-3/-7 nor of PARP (Figure 4), suggesting that FEHH4-1 induced apoptosis *via* a caspase-3/PARP-independent signaling pathway. Treatment with high concentrations of FEHH4-1 (50 μM) also led to a decrease in total protein content as visualized by tubulin staining, an effect that was comparable with that observed with 100 μM resveratrol.

Resveratrol and FEHH4-1 inhibit IL2 gene expression in Jurkat T-cells. IL2 is essential for the sustained proliferation of activated T-cells and their effector functions. Stimulation of Jurkat T-cells with PMA/PHA led to an extensive induction of IL2 synthesis (Figure 5A). To investigate whether resveratrol and FEHH4-1 may have inhibitory effects on IL2 expression, Jurkat T-cells were stimulated for 24 h with PMA/PHA in the absence or presence of different concentrations of resveratrol or FEHH4-1 (3.125-25 μM). Data demonstrate that both polyphenols blocked IL2 production in a dose-dependent manner (Figure 5A). Compared to resveratrol, IL2 levels were significantly lower when the cells were co-incubated

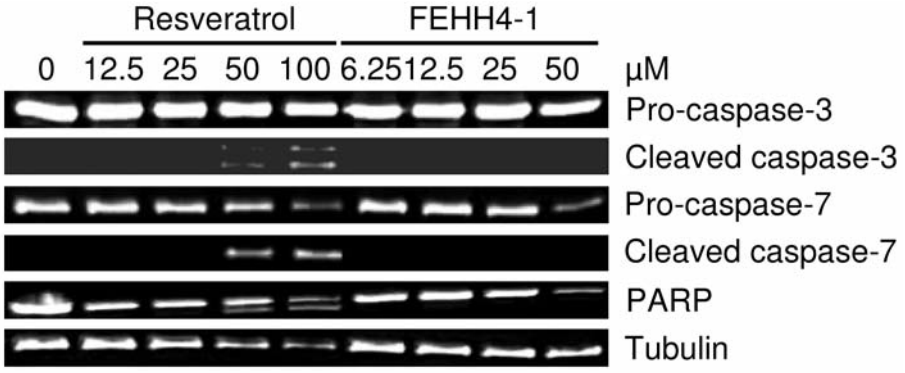

Figure 4. *Resveratrol induces apoptotic death of Jurkat T-cells via the caspase-3 signaling cascade. Jurkat T-cells were treated for 72 h with vehicle or the indicated concentrations of resveratrol or FEHH4-1. The apoptotic marker proteins caspase-3/-7 and poly (ADP-ribose) polymerase (PARP), as well as their cleavage products, were analyzed by western blot. Representative blots of three independent experiments are shown.*

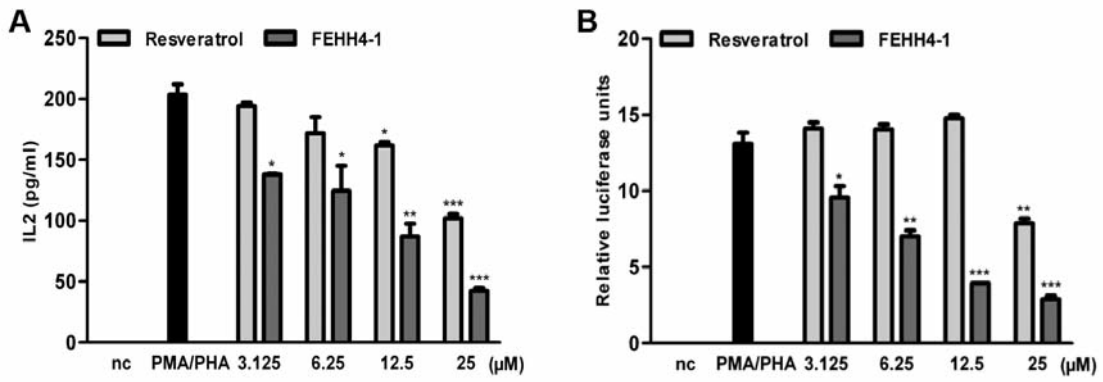

Figure 5. *Resveratrol and FEHH4-1 block IL2 gene expression in Jurkat T-cells. A: Jurkat T-cells were pre-incubated for 30 min with either vehicle or the indicated concentrations of resveratrol or FEHH4-1 before being stimulated for 24 h with phorbol 12-myristate 13-acetate/phytohemagglutinin (PMA/PHA). The IL2 concentration in cell-culture supernatants was quantified by enzyme-linked immunosorbent assay. B: Jurkat T-cells containing an IL2 promoter luciferase reporter construct were stimulated for 6 h with PMA/PHA with/without the indicated concentrations of resveratrol or FEHH4-1. Data are expressed as relative luciferase units. Data are mean values±SEM of two independent experiments. nc, Negative control; *p<0.05, **p<0.01 and ***p<0.001 compared to control.*

with FEHH4-1: 20% inhibition *versus* 60% at 12.5 μM. In addition, we investigated whether resveratrol and FEHH4-1 were directly involved in the transcriptional regulation of the *IL2* gene. In this experiment, Jurkat T-cells containing a luciferase reporter gene under the control of the *IL2* promoter were stimulated for 6 h with PMA/PHA in the absence or presence of resveratrol or FEHH4-1 (3.125-25 μM). In good accordance with the IL2 expression data, we observed that the luciferase activity was significantly reduced when the cells were co-treated with FEHH4-1 instead of resveratrol: at 12.5 μM, 70% reduction was achieved, demonstrating the higher efficacy of FEHH4-1 against Jurkat T-cells (Figure 5B).

Discussion

The polyphenolic compound resveratrol is a naturally-occuring phytochemical that can be found in approximately 72 plant species (18). Red wine and grapes are probably its main sources in Western diets. Many studies have demonstrated its ant-ioxidant and anti-inflammatory activities. Additionally, resveratrol has been shown to have growth-inhibitory activity against several human cancer cell lines and in animal models of carcinogenesis. For example, in HL60 promyelocytic leukeumia cells, treatment of resveratrol led to growth inhibition, induction of apoptosis and S-G_2-phase cell-cycle arrest (19, 20). Resveratrol also

displayed antiproliferative activity against JB6 mouse epidermal, CaCo-2 colorectal, and A431 epidermoid carcinoma cell lines (21-23). Although resveratrol is commercially available as a dietary supplement, controlled clinical trials demonstrating either its efficacy or safety in the treatment or prevention of cancer have not been published so far. Apart from that, pharmacological application of resveratrol is very limited due to its extremely low water solubility and bioavailability. Thus, we chemically synthesized the resveratrol prodrug FEHH4-1 in hope of improving the pharmacological potential of the natural compound. Esterification of the phenolic groups may render FEHH4-1 more resistant to oxidative processes and improve the lipophilicity of the natural compound. FEHH4-1 can be seen as a resveratrol prodrug, which is cleaved into its active form through intracellular esterases.

In the present study, we compared the antiproliferative and pro-apoptotic activities of resveratrol with the novel synthetic resveratrol analog FEHH4-1 against Jurkat leukeumia $CD4^+$ T-cells. The Jurkat T-cell line is an established *in vitro* system used to study the effects of various natural and synthetic compounds on $CD4^+$ T-cell activation, proliferation and apoptosis. However, it must be considered that Jurkat T-cells were derived from a patient with leukemia and therefore may in some aspects differ from T-cells of healthy individuals. Firstly, we studied the effects of resveratrol and FEHH4-1 on the proliferation of Jurkat T-cells. We highlighted the more than threefold greater potential of FEHH4-1 for blocking proliferation of leukemia T-cells *in vitro* after 48-h treatment. Furthermore, our data show that both compounds initiated apoptosis in Jurkat T-cells in a time- and dose-dependent manner. After treatment for 72 h with 50 μM resveratrol, about 70% of the cell population was still alive, whereas incubation with the same concentration of FEHH4-1 resulted in almost complete death of the whole cell population. Interestingly, our data demonstrate that resveratrol but not FEHH4-1 induced cell death *via* the caspase-3/PARP signaling cascade. At 50 μM resveratrol, the effector caspases caspase-3 and -7, as well as PARP were cleaved into smaller subunits, indicating that Jurkat T-cells underwent apoptosis *via* the intrinsic apoptotic pathway. However, in cells treated with FEHH4-1, neither pro-caspase-3/-7 nor PARP cleavage were detected, suggesting that cell death was induced *via* a hitherto non-identified extrinsic pathway. It is important to note that other forms of programmed cell death have been described and other forms of programmed cell death may yet be discovered (24-26). Furthermore, it may be difficult to distinguish precisely between apoptosis and necrosis, two processes that can occur independently, or sequentially, as well as simultaneously (27). Necrosis and apoptosis may indeed represent morphological expressions of a shared biochemical network described as the "apoptosis–necrosis continuum" (28). Whether a cell dies by necrosis or apoptosis depends, in part, on the nature of the cell death signal, the tissue type, the developmental stage of the tissue and the physiological milieu (28, 29). Thus, further work needs to be done to clarify the signaling cascade FEHH4-1 may be involved in. Finally, we found that stimulation of Jurkat T-cells with PMA/PHA was blocked by resveratrol and FEHH4-1 in a dose-dependent manner. At non-cytotoxic concentrations, the efficacy of FEHH4-1 in inhibiting IL2 synthesis was significantly higher compared to that of resveratrol: about three fold, indicating that FEHH4-1 may be more stable under cell culture conditions and may have a higher affinity for Jurkat T-cells compared to resveratrol.

Conclusion

The novel resveratrol prodrug FEHH4-1 showed potent enhanced antiproliferative and pro-apoptotic activities against Jurkat leukemia $CD4^+$ T-cells compared to resveratrol. Notably, in strong contrast to resveratrol, FEHH4-1 did not induce apoptosis *via* the caspase-3 signaling cascade but *via* an undefined signaling pathway. Furthermore, FEHH4-1 blocked *IL2* gene expression in Jurkat T-cells at significantly lower concentrations than resveratrol. We suggest that FEHH4-1 could be a substitute for resveratrol in the future, and is worthy of study in other cell types and *in vivo* models.

References

1 Athar M, Back JH, Kopelovich L, Bickers DR and Kim AL: Multiple molecular targets of resveratrol: Anti-carcinogenic mechanisms. Arch Biochem Biophys *486*: 95-102, 2009.

2 Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S and Takada Y: Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer Res *24*: 2783-2840, 2004.

3 Joe AK, Liu H, Suzui M, Vural ME, Xiao D and Weinstein IB: Resveratrol induces growth inhibition, S-phase arrest, apoptosis and changes in biomarker expression in several human cancer cell lines. Clin Cancer Res *8*: 893-903, 2002.

4 Ge J, Liu Y, Li Q, Guo X, Gu L, Ma ZG and Zhu YP: Resveratrol induces apoptosis and autophagy in T-cell acute lymphoblastic leukemia cells by inhibiting AKT/mTOR and activating p38-MAPK. Biomed Environ Sci *26*: 902-911, 2013.

5 Banerjee S, Bueso-Ramos C and Aggarwal BB: Suppression of 7,12-dimethylbenzanthracene-induced mammary carcinogenesis in rats by resveratrol: role of nuclear factor-κB, cyclo-oxygenase 2, and matrix metalloprotease 9. Cancer Res *62*: 4945-4954, 2002.

6 Sengottuvelan M and Nalini N: Dietary supplementation of resveratrol suppresses colonic tumour incidence in 1,2-dimethylhydrazine-treated rats by modulating biotransforming enzymes and aberrant crypt foci development. Br J Nutr *96*: 145-153, 2006.

7 Luther DJ, Ohanyan V, Shamhart PE, Hodnichak CM, Sisakian H, Booth TD, Meszaros JG and Bishayee A: Chemopreventive doses of resveratrol do not produce cardiotoxicity in a rodent model of hepatocellular carcinoma. Invest New Drugs *29*: 380-391, 2011.
8 Harikumar KB, Kunnumakkara AB, Sethi G, Diagaradjane P, Anand P, Pandey MK, Gelovani J, Krishnan S, Guha S and Aggarwal BB: Resveratrol, a multitargeted agent, can enhance antitumor activity of gemcitabine *in vitro* and in orthotopic mouse model of human pancreatic cancer. Int J Cancer *127*: 257-68, 2010.
9 Harper CE, Patel BB, Wang J, Arabshahi A, Eltoum IA and Lamartiniere CA: Resveratrol suppresses prostate cancer progression in transgenic mice. Carcinogenesis *28*: 1946-1953, 2007.
10 Bove K, Lincoln DW, Tsan MF: Effect of resveratrol on growth of 4T1 breast cancer cells *in vitro* and *in vivo*. Biochem Biophys Res Commun *291*: 1001-1005, 2002.
11 Castillo-Pichardo L, Cubano LA and Dharmawardhane S: Dietary grape polypenol resveratrol increases mammary tumor growth and metastasis in immunocompromised mice. BMC Complement Altern Med *13*: 6, 2013.
12 Nguyen AV, Martinez M, Stamos MJ, Moyer MP, Planutis K, Hope C and Holcombe RF: Results of a phase I pilot clinical trial examining the effect of plant-derived resveratrol and grape powder on WNT pathway target gene expression in colonic mucosa and colon cancer. Cancer Manag Res *1*: 25-37, 2009.
13 Delmas D, Aires V, Limagne E, Dutartre P, Mazué F, Ghiringhelli F and Latruffe N: Transport, stability, and biological activity of resveratrol. Ann NY Acad Sci *1215*: 48-59, 2011.
14 Smoliga J and Blanchard O: Enhancing the delivery of resveratrol in humans: If low bioavailability is the problem, what is the solution? Molecules *19*: 17154-17172, 2014.
15 Pangeni R, Sahni JK, Ali J, Sharma S and Baboota S: Resveratrol: review on therapeutic potential and recent advances in drug delivery. Expert Opin Drug Deliv *11*: 1285-1298, 2014.
16 Kloesch B, Becker T, Dietersdorfer E, Kiener H and Steiner G: Anti-inflammatory and apoptotic effects of the polyphenol curcumin on human fibroblast-like synoviocytes. Int Immunopharmacol *15*: 400-405, 2013.
17 Tewari M, Quan LT, O'Rourke K, Desnoyers S, Zeng Z, Beidler DR, Poirier GG, Salvesen GS and Dixit VM: Yama/CPP32 beta, a mammalian homolog of CED-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-) polymerase. Cell *81*: 801-809, 1995.
18 Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CW, Fong HH, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC and Pezzuto JM: Cancer chemopreventive activity of resveratol, a natural product derived from grapes. Science *275*: 218-220, 1997.
19 Clement MV, Hirpara JL, Chawdhury SH and Pervaiz S: Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signaling-dependent apoptosis in human tumor cells. Blood *92*: 996-1002, 1998.
20 Ragione FD, Cucciolla V, Borriello A, Pietra VD, Racioppi L, Soldati G, Manna C, Galletti P and Zappia V: Resveratrol arrests the cell division cycle at S/G_2 phase transition. Biochem Biophys Res Commun *250*: 53-58, 1998.
21 Huang C, Ma WY, Goranson A and Dong Z: Resveratrol suppresses cell transformation and induces apoptosis through a p53-dependent pathway. Carcinogenesis *20*: 237-242, 1999.
22 Schneider Y, Vincent F, Duranton B, Badolo L, Gosse F, Bergmann C, Seiler N and Raul F: Antiproliferative effect of resveratrol, a natural component of grapes and wine, on human colonic cancer cells. Cancer Lett *158*: 85-91, 2000.
23 Ahmad N, Adhami VM, Afaq F, Feyes DK and Mukhtar H: Resveratrol causes WAF-1/p21-mediated G1-phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431 cells. Clin Cancer Res *7*: 1466-1473, 2001.
24 Formigli L, Papucci L, Tani A, Schiavone N, Tempestini A, Orlandini GE, Capaccioli S and Orlandini SZ: Aponecrosis: morphological and biochemical exploration of a syncretic process of cell death sharing apoptosis and necrosis. J Cell Physiol *182*: 41-49, 2000.
25 Sperandio S, de Belle I and Bredesen DE: An alternative, non-apoptotic form of programmed cell death. Proc Natl Acad Sci USA *97*: 14376-14381, 2000.
26 Debnath J, Baehrecke EH and Kroemer G: Does autophagy contribute to cell death? Autophagy *1*: 66-74, 2005.
27 Hirsch T, Marchetti P, Susin SA, Dallaporta B, Zamzami N, Marzo I, Geuskens M and Kroemer G: The apoptosis-necrosis paradox. Apoptogenic proteases activated after mitochondrial permeability transition determine the mode of cell death. Oncogene *15*: 1573-1581, 1997.
28 Zeiss CJ: The apoptosis-necrosis continuum: insights from genetically altered mice. Vet Pathol 40: 481-495, 2003.
29 Fiers W, Beyaert R, Declercq W and Vandenabeele P: More than one way to die: apoptosis, necrosis and reactive oxygen damage. Oncogene *18*: 7719-7730, 1999.

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