Suppression of angiogenesis, tumor growth, and wound healing by resveratrol, a natural compound in red wine and grapes

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ABSTRACT

Resveratrol (3,5,4’-trihydroxystilbene) is a natural compound found in several plants, including grapes, peanuts, and pines, and in their related products. Red wine is probably the most frequently consumed drink that is enriched in resveratrol. We investigated whether drinking resveratrol could suppress angiogenesis, a process of blood vessel growth involved in initiation, development, and progression of many diseases, including cancer, metastasis, and diabetic retinopathy. We found that resveratrol suppresses the growth of new blood vessels in animals. It directly inhibits capillary endothelial cell growth. It blocks both VEGF- and FGF-receptor-mediated angiogenic responses. In addition, resveratrol inhibits the phosphorylation of mitogen-activated kinase isoforms (MAPK\textsuperscript{p44}/MAPK\textsuperscript{p42}) induced by fibroblast growth factor-2 in proliferating endothelial cells in a dose-dependent manner. Oral administration of resveratrol significantly inhibits the growth of a murine fibrosarcoma in mice, and it significantly delays angiogenesis-dependent wound healing in mice. Our findings suggest that ingestion of resveratrol-enriched food could be beneficial for the prevention of cancer. However, its antiangiogenic effect could delay wound healing and possibly other angiogenesis-dependent processes under physiological conditions.

Key words: resveratrol • red wine • cancer • angiogenesis • MAP kinase

The health benefits of certain natural food products, including green tea, red wine, garlic, and fresh fruits, have been described for decades, and their roles in prevention of disease conditions have recently been confirmed by either epidemiological or molecular studies (1, 2). For example, it is known that catechins in green tea could have cancer-preventive effects for humans and animals (3, 4). Polyphenols in red wine have been linked to the reduction of the risk of cardiovascular diseases (5). These food products are available in markets and directly influence our daily diet habits. Although the beneficial effects of these natural products are unquestionable, the molecular mechanisms of their active ingredients in the body have to be elucidated.
We have recently discovered that epigallocatechin-3-gallate (EGCG), a polyphenol present in green tea, is an oral angiogenesis inhibitor (4). The molecular mechanism of the antiangiogenic activity could be due to its inhibitory effect on matrix metalloproteinase (27). This finding not only provides a mechanism for the cancer preventive effect of green tea, but also suggests that other polyphenols with structural similarities to EGCG could have antiangiogenic and possible antitumor activities. Resveratrol is a polyphenol compound enriched in red wine and other grape products. Red wine contains approximately 1.5–7.0 mg/l of resveratrol (6, 7). Resveratrol has been reported to have cancer chemopreventive activity in animals with both carcinogen-induced and implanted tumors (8). Its antitumor activity was observed in several tumor models and appears to be independent of cell type (2, 8). Thus, it is possible that resveratrol in red wine could be a novel angiogenesis inhibitor.

The growth and progression of all tumors, including leukemia, is dependent on angiogenesis, and disruption of blood vessel growth inhibits tumor expansion and its metastasis (9). In addition, physiological conditions, including embryonic development, reproductive cycles, and wound healing, are angiogenesis dependent (10, 11). Currently, more than 20 angiogenesis inhibitors are registered by the National Cancer Institute, including metalloproteinase inhibitors in early phases of clinical trials in the treatment of cancer (12). Although these angiogenesis inhibitors display antitumor effects in animals, they are often delivered to animals or patients by frequent systemic injections. For example, angiotatin and endostatin have to be produced as recombinant proteins and given by frequent systemic injections in order to achieve their antitumor effects (13, 14). This approach can be very costly and inconvenient for patients.

Thus, the discovery of oral angiogenesis inhibitors and understanding their molecular mechanisms will be of great advantage for use in therapeutic settings. We report on another polyphenol, resveratrol, found in grapes and red wine as a novel angiogenesis inhibitor. This inhibitor suppresses tumor growth and significantly delays wound healing in mice.

MATERIALS AND METHODS

Reagents, cells, and animals

Resveratrol (>99% pure) was purchased from Sigma (Stockholm, Sweden). Anti-phospho-MAP kinase antibody was provided by Dr. Carl-Henrik Heldin’s laboratory at the Ludwig Institute for Cancer Research, Uppsala, Sweden. Porcine aortic cell lines expressing PAE/VEGFR-2 and FGFR-1 (given by Dr. Lena Claesson-Welsh at the Department of Chemistry, Uppsala University) were maintained in F12/Ham + 10% fetal calf serum (FCS). Bovine capillary endothelial (BCE) cells were provided by Dr. Judah Folkman and maintained in Dulbecco’s modified Eagle’s medium (DMEM) with 10% heat-inactivated bovine calf serum (BCS), antibiotics, and 3 ng/ml recombinant human fibroblast growth factor-2 (FGF-2) (Scios Nova, Mountainview, CA). Recombinant human VEGF 165 was provided by Dr. Monica Tsang at the R&D Systems (Minneapolis, MN). Male and female C57Bl6/J mice (5–6 wk old) (Microbiology and Tumor biology Center, Karolinska Institute, Stockholm, Sweden) were acclimated and caged in groups of six or less. Animals were anaesthetized in a methoxyflurane chamber before all procedures and killed with a lethal dose of CO2. All animal studies were reviewed and approved by the animal care and use committee of the Stockholm Animal Board.

Endothelial cell proliferation assay
A 72-h BCE cell proliferation assay was performed as previously described (4, 15). BCE cells were maintained in DMEM containing 10% heat-inactivated BCS and 3 ng/ml of recombinant human FGF-2. Cells growing in gelatinized six-well plates were dispersed in 0.05% trypsin solution and resuspended with DMEM containing 5% BCS. Approximately 10,000 cells in 0.5 ml were added to each gelatinized well of 24-well plates and incubated at 37°C for 1 h. Samples in triplicates were added to each well. After 30 min incubation, FGF-2 was added to a final concentration of 1 ng/ml. After 72 h of incubation, cells were trypsinized, resuspended in Isoton II solution (Merch Eurolab, Stockholm, Sweden) and counted with a Coulter counter. P values were calculated according to the standard two-tailed Student t test. For PAE endothelial cell proliferation assay, PAE/VEGFR-2 and PAE/FGFR-1 cells (~10,000 cells/well) were seeded onto 24-well tissue culture plates and incubated in F12/Ham’s medium (1 ml/well) containing 1% FCS and antibiotics for 1 h. Samples in triplicate were then added to cells and incubated for 1 h before the addition of growth factors. VEGF at 100 ng/ml and FGF-2 at 5 ng/ml were added to VEGFR-2 and FGFR-1 cells, respectively. After 72 h incubation, cells were dispersed in trypsin, resuspended in Isoton solution, and counted by a Coulter counter.

**Endothelial cell chemotaxis assay**

The motility response of PAE/VEGFR-2 cells to VEGF was assayed by a modified Boyden chamber technique, as previously described, by using micropore nitrocellulose filters (8 mm thick, 8-µm pores) coated with 1.5% gelatin in phosphate-buffered saline (PBS) solution (16). Cells were trypsinized and resuspended in serum-free medium containing 0.25% BSA. The cells (25,000 cells/well) were placed in the upper chamber in serum-free medium containing 0.25% BSA and various concentrations of resveratrol samples with 50 ng/ml VEGF in the lower chamber. After about 6 h at 37°C, the medium was removed and cells attached to the filter were fixed in 99% methanol and stained with Giemsa solution. All experiments were performed in six replicates. The number of cells migrating through the filter was counted and plotted as the number of migrating cells per optic field (×32).

**Immunoblotting**

Cells were grown in 27-cm² dishes to 70–80% confluency in DMEM containing 10% BCS and 3 ng/ml FGF-2. Cells were starved in the same medium with 0.25% BCS. Samples were added to cells in a final 0.1% ethanol concentration in DMEM. After 16 h incubation, cells were stimulated for 5 min with 10 ng/ml FGF-2. Cells were washed twice with ice-cold PBS containing 1 mM NaVO₃ and lysed in 200 µl lysis buffer (1% Triton X-100; 137 mM NaCl; 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 10% glycerol; 1 mM NaVO₃; 1 mM phenylmethylsulfonyl fluoride). Cell lysates were centrifuged at 13,000 rpm for 10 min, and supernatants were separated by a NuPAGE 4–12% Bis-Tris Gel system. The separated proteins were transferred to a nitrocellulose membrane, which subsequently was blocked overnight in a blocking buffer (5% BSA in Tris-buffered saline containing 0.1% Tween-20). The membrane was incubated with a rabbit anti-phospho-MAP kinase antibody for 1 h at room temperature. The membrane was then washed with the blocking buffer without BSA. A secondary antibody, HRP-conjugated donkey anti-rabbit IgG, was incubated with the membrane for 1 h at room temperature. The membrane was washed several times and developed using ECL⁺ Plus system (Amersham Biotech, Uppsala, Sweden).
Chick embryo chorioallantoic membrane (CAM) assay

The CAM assay was performed as previously described (4, 15, 16, 20). Three-day-old fertilized white Leghorn eggs (OVA Production, Sorgarden, Sweden) were cracked, and chick embryos with intact yolks were carefully placed in 20- × 100-mm plastic petri dishes. After 48 h incubation in 4% CO₂ at 37°C, disks of methylcellulose containing various concentrations of resveratrol dried on a nylon mesh (4×4 mm) were implanted on the CAM of individual embryos. The nylon mesh disks were made by desiccation of 20 µl of 0.45% methylcellulose (in H₂O). After 48–72 h of incubation, we used a stereoscope to examine embryos and CAMs for the formation of avascular zones in the field of the implanted disks.

Mouse corneal micropocket assay

The mouse corneal assay was performed according to procedures previously described (4, 15, 16). Corneal micropockets were created with a modified von Graefe cataract knife in both eyes of each male 6- to 7-wk-old C57Bl6/J mouse. Micropellets (0.35×0.35 mm) of sucrose aluminum sulfate (Bukh Meditec, Copenhagen, Denmark) coated with hydron polymer type NCC (IFN Sciences, New Brunswick, NJ) containing approximately 80 ng of FGF-2 or 160 ng of VEGF were implanted in the corneal pockets. The pellet was positioned 0.8 mm from the corneal limbus. After implantation, erythromycin/ophthalmic ointment was applied to each eye. The resveratrol solution was prepared by addition of pure resveratrol in absolute ethanol into the drinking water. The final concentration of ethanol in the drinking water was 1%. Mice drank resveratrol from 3 days before growth factor implantation and throughout the experiment. The FGF-2- and VEGF-implanted animals received either resveratrol (n=5/group) as the sole drinking fluid or drank water (n=10) containing 1% ethanol as controls. The drinking fluid contained 0.4 µg/ml resveratrol and the final amount ingested was 1.2 µg/mouse/day (48 µg/kg). The amount of resveratrol in the drinking fluid for mice, which was equivalent to three glasses of red wine (about 150 ml per glass) for humans, was calculated as previously described (21). We chose the same brand of red wine that was used by other authors (6, 7). In those articles, 28 µg of resveratrol per 300 g rat was used. This amount is equivalent to approximately three to six glasses of red wine for a 50 kg human (21). In our experiments, we calculated according to both the body weight (25 g each mouse) and the volume of daily fluid intake of mice (~3 ml) and converted to the amount for mice. We should emphasize that we used only 50% of the previously reported amount of resveratrol per kilogram for our experiments. In this case, the amount of resveratrol in the drinking fluid for mice was equivalent to one to three glasses of red wine. We used a slit-lamp microscope to examine the corneal neovascularization of all animals on day 5 after pellet implantation.

Immunohistochemistry

The growth factor-implanted mouse eyes were enucleated and immediately frozen on dry ice in Tissue Tek (Histolab, Gothenburg, Sweden) and stored at −80°C before use. Tissue sections of 14 µm were dissected by a cryostat and were immersed in acetone for 10 min. Tissue slices were washed with PBS, blocked with 3% BSA in PBS for 20 min, and incubated for 1 h with a monoclonal rat anti-mouse antibody against CD31 antigen (PharMingen, San Diego, CA). After washing with PBS, a secondary FITC-conjugated rabbit anti-rat IgG (DakoPatts AB, Stockholm,
Sweden) was incubated with the tissue sections for 1 h. The immunostained signals were examined under a fluorescence microscope. Corneal microvessels were counted in at least six sections at 20× magnification.

Tumor assay

Male 5- to 6-wk-old C57Bl6/J mice were used for tumor studies. Approximately 0.5 × 10^6 murine T241 fibrosarcoma cells growing in log phase were harvested and resuspended in PBS. A single-cell suspension in a volume of 100 µl was implanted subcutaneously in the middle dorsum of each animal. The resveratrol solution was prepared by adding pure resveratrol in absolute ethanol to drinking water. The dose of resveratrol was 1 mg/kg/day. Mice drank resveratrol throughout the experiment. Six or seven mice were used in each treated and control group. Visible tumors were present after 72 h. Primary tumors were measured using digital calipers on the days indicated. Tumor volumes were calculated according to the formula: width^2 × length × 0.52, as previously reported (15).

Wound healing assay

Female 5- to 6-wk-old C57Bl6/J mice were used for wound healing studies. The animals were shaved, and full-thickness skin wounds (about 5 mm in diameter) were surgically created on their backs. No inflammation was observed during the experiment. Resveratrol was added to the drinking fluid at a concentration of 5.7 µg/ml in 1% ethanol 2 d before the operation and throughout the experiment. Diameters of wounds were measured daily. Statistical analysis was performed using two-tailed Student’s t test with INSTAT 1.1™ and Microsoft EXCEL 5™ programs.

RESULTS

Inhibition of endothelial cell growth and MAP kinase

To determine whether resveratrol, a phytoalexin and a polyphenol compound (Fig. 1A), could inhibit endothelial cell growth, we assayed resveratrol on BCE cells stimulated with FGF-2, as previously described (4, 15). As shown in Figure 1B, resveratrol inhibited capillary endothelial cell growth in a dose-dependent manner. The concentration of resveratrol needed to reach 50% inhibition (EC_{50}) on BCE cells seemed to be lower by 10-fold compared with several tumor cell lines, including murine B16 melanoma cells, T241 fibrosarcoma cells, and Lewis lung carcinoma cells. Our data are in agreement with a previous finding that resveratrol inhibits the growth of pulmonary endothelial cells (17).

Because resveratrol is able to suppress endothelial cell proliferation, the inhibitory effect could be mediated by inhibition of the phosphorylation of intracellular kinases in response to growth factors. MAP kinase is one of the key components involved in the signaling pathways of endothelial cell proliferation (25). We examined the effect of resveratrol on the FGF-2-induced activation of MAP kinases in BCE cells. An anti-phosho-MAP kinase specific antibody detected both MAPK^{p44} and MAPK^{p42} isoforms induced by FGF-2 in BCE cells (Fig. 1C). FGF-2 significantly stimulated the phosphorylation of MAPK^{p44} and MAPK^{p42} in BCE cells, whereas resveratrol inhibited the FGF-2-induced phosphorylation of these two proteins. Our data
demonstrate that resveratrol inhibits the phosphorylation of MAP kinases induced by growth factors.

To study whether resveratrol could inhibit FGF-2- and VEGF-receptor-mediated endothelial cell proliferation and migration, porcine aortic endothelial cell lines that stably express FGFR-1 and VEGFR-2 (PAE/FGFR-1 and PAE/VEGFR-2) were used. As shown in Figures 1D and E, resveratrol inhibited, in a dose-dependent manner, the FGF-2- and VEGF-induced proliferation of PAE/FGFR-1 and PAE/VEGFR-2 cells, respectively. To determine whether resveratrol could inhibit endothelial cell migration, a standard chemotaxis assay was carried out using a Boyden chamber. At a concentration of 1 µM, resveratrol significantly inhibited the VEGF-induced PAE/VEGFR-2 endothelial cell migration (Fig. 1F). These data demonstrate that resveratrol inhibits both FGF- and VEGF-receptor-mediated endothelial cell growth and chemotaxis.

**Inhibition of angiogenesis in developing embryos**

To study the antiangiogenic activity of resveratrol in vivo, we examined its inhibitory effect on angiogenesis in the CAM assay. In a concentration range of 1–100 µg per disc, resveratrol was able to induce avascular zones in the developing CAMs (Fig. 2B), and the observed inhibition was dose-dependent (Fig. 2C). No avascular zones were detected in the control CAMs implanted with PBS (Figs. 2A and C).

**Inhibition of FGF- and VEGF-induced angiogenesis**

To further investigate whether resveratrol could suppress angiogenesis in mammals, we prepared a drinking solution for mice, containing 0.4 µg/ml resveratrol in 1% ethanol, which was equivalent to the amount of resveratrol found in approximately three glasses of red wine, and assayed its inhibitory effect in the corneal neovascularization model (6). We also chose the two most potent angiogenic factors (FGF-2 and VEGF) (26) in this assay system. The amount of resveratrol in the drinking fluid for mice was calculated as described in “Materials and Methods.” This fluid significantly inhibited corneal neovascularization induced by VEGF (Fig. 3C) and FGF-2 (Fig. 3D) compared with control groups that drank 1% ethanol alone (Figs. 3A and B). The areas of corneal vascularization were significantly inhibited in the resveratrol-drinking group (Figs. 3G and H). In addition, the vessel density was significantly reduced in the resveratrol-drinking group (Figs. 3F and I) compared with the control group (Figs. 3E and I) in the FGF-2 implanted corneas.

**Suppression of tumor growth and wound healing**

The antiangiogenic effect of resveratrol led us to investigate whether this compound was able to inhibit the growth of a murine fibrosarcoma in mice. Oral administration of resveratrol at the concentration of 5.7 µg/ml, which corresponds to 25 µmol, significantly inhibited the growth of T241 fibrosarcoma in mice (treated vs. control=0.47) (Fig. 4A). This dose was used previously by others studying the antitumor effect of resveratrol in carcinogen-induced tumor models (8).

In addition to tumor growth, wound healing also requires angiogenesis. We tested the anti-wound healing effect of resveratrol in a mouse skin model. Oral administration of resveratrol significantly delayed wound healing in mice, as determined by measuring the sizes of wounds
and the percentage of animals with healed wounds (Figs. 4B–D). The measured sizes of wounds in the resveratrol-drinking group were significantly larger (P<0.05) from day 2 and throughout the experiment. These findings indicate that resveratrol is a novel oral angiogenesis inhibitor.

**DISCUSSION**

Our results for the first time demonstrate that resveratrol acts as an angiogenesis inhibitor when administrated orally. Consequently, it inhibits angiogenesis-dependent physiological and pathological processes, including wound healing and tumor growth. Its antiangiogenic mechanisms involve direct inhibition of capillary endothelial cell growth via suppression of the phosphorylation of the MAP kinase. This pathway appears to be common for two of the key growth factors, that is, FGF-2- and VEGF-induced angiogenesis, because resveratrol inhibits both FGF- and VEGF-receptor-mediated endothelial cell responses.

Several epidemiological studies suggest that moderate consumption of red wine for long-term periods reduces the risk of coronary heart disease mortality (5, 18). In red wine, resveratrol seems to be the key compound in the prevention of heart diseases because it inhibits blood coagulation and modulates lipoprotein metabolism (18). Although long-term consumption of red wine may also reduce cancer risks, such an epidemiological correlation has not been reported. Our present study is consistent with previous reports that resveratrol inhibits both tumor formation and the growth of tumor implants in animals (8). The growth of primary tumors and metastases depends on the degree of tumor neovascularization (9–11). This study provides compelling evidence that suppression of angiogenesis could be at least one of the mechanisms of the antitumor effect of resveratrol. Consumption of resveratrol could be beneficial in the prevention of angiogenesis-dependent diseases. However, we should emphasize that the antiangiogenic effect of resveratrol could be harmful in situations such as wound healing.

Recent studies suggest that antiangiogenic therapy has to be delivered for long-term periods in order to arrest a tumor at its dormant stage (9, 19). In clinical settings, most angiogenesis inhibitors have to be delivered to cancer patients by systemic injections for several years. Thus, there would be great advantages if oral angiogenesis inhibitors were available. Resveratrol, as a small molecule and an oral angiogenesis inhibitor found in natural food products, could well fulfill the criteria of long-term antiangiogenic therapy without injections. However, long-term consumption of resveratrol-enriched wine products may cause adverse health effects due to the alcohol content. Thus, there should be a caution not to encourage people to consume large amounts of wine, rather than moderate amounts of red wine (two to three glasses per day). Other food products and nonalcoholic beverages could be considered as alternative resveratrol sources.

The finding of resveratrol as an oral angiogenesis inhibitor may lead to the discovery of other angiogenesis inhibitors that share structural similarities with resveratrol. It may even be possible to make synthetic compounds that are structurally similar to resveratrol. In addition, these synthetic compounds could be more potent angiogenesis inhibitors than resveratrol. This seems to be a valid strategy for drug discovery (28). For example, based on the structure of fumagillin, a known angiostatic molecule, a synthetic analogue TNP-470 (AGM-1470), was found to be a potent and specific angiogenesis inhibitor, which is currently in phase 2 clinical trials (22) in the treatment of cancer.
Recent studies suggest that antiangiogenesis therapy in combination with other existing therapies, including chemotherapy and radiotherapy, produces synergistic efficacy against disease conditions (23). For example, angioatin and ionizing radiation synergistically suppress tumor growth in animals. The molecular basis of antitumor synergy is due to angiogenesis inhibitors targeting the newly formed blood vessels and radiation directly acting on the tumor compartment. Therefore, it is possible that in combination with other therapeutic strategies, resveratrol and its structurally related angiogenesis inhibitors could produce a similar synergistic efficacy in the treatment of angiogenesis-dependent diseases.

Taken together with our other recent finding that EGCG in green tea is an angiogenesis inhibitor (4), consumption of various plant products containing polyphenol-based compounds and adequate amounts of red wine may be beneficial in the prevention of cancer. Paradoxically, they might delay physiological processes in which angiogenesis is required.

ACKNOWLEDGMENTS

We thank Duojia Cao for technical assistance and Anna Eriksson and Niina Veitonmaki for reading the manuscript. This work is supported by the Human Frontier Science Program Organization, the Swedish Cancer Foundation, the Åke Wibergs Foundation, the Karolinska Institute Foundation, and the Magnus Bergvalls Foundation. R. Cao is supported by the David and Hagelens foundation.

REFERENCES


Received February 1, 2001; revised April 2, 2001.
Figure 1. Inhibition of endothelial cell growth by resveratrol. A) The chemical structure of trans-resveratrol. B) BCE cells were incubated with 1 ng/ml FGF-2 alone or together with various concentrations of resveratrol. After 72 h, cells were counted. Values represent means (±SE) of triplicate of each sample. C) Inhibition of FGF-2-stimulated phosphorylation of MAP kinases in BCE cells by resveratrol. Phosphorylated isoforms of MAPK\(^{44}\) and MAPK\(^{42}\) kinases in the presence and absence of resveratrol were detected by a specific antibody. D and E) Various concentrations of resveratrol were added to PAE/FGFR-1 and PAE/VEGFR-2 cells stimulated with 5 ng/ml FGF-2 and 100 ng/ml VEGF, respectively. After 72 h incubation, cells were counted. Values represent means (±SE) of triplicates of each sample. F) The motility response of PAE/VEGFR-2 cells to VEGF with or without resveratrol was assayed by a modified Boyden chamber technique. The number of cells migrating through the filter was counted. Values represent means (±SE) of six replicates of each sample.
Figure. 2. Various amounts of resveratrol immobilized on methylcellulose discs were implanted on chorioallantoic membranes (CAMs) of 6-day-old chick embryos. The formation of avascular zones was examined 2 days after implantation. A) A control CAM with a methylcellulose disc containing phosphate-buffered saline. B) A representative microphotograph of an example of the resveratrol-implanted CAM. Arrows point to the implanted disc. Restriction and regression of blood vessel growth can be observed in the area of the implanted disc. C) The number of avascular zones over the total number of CAMs tested at various concentrations of resveratrol is indicated above each bar.
Figure 3. Approximately 160 ng of VEGF or 80 ng of FGF-2 with sucrose aluminum sulfate and hydron was implanted into each corneal micropocket of mice. Corneas were photographed by a stereomicroscope on day 5 after growth factor implantation, and positions of implanted pellets are indicated by arrows in A–D. Corneas implanted with VEGF and FGF-2 of control mice drinking water with 1% ethanol (A and B). Examples of corneas of mice drinking resveratrol (C and D). Areas of VEGF and FGF-induced neovascularization (G and H) are presented as mean determinants (±SE) of 7–10 corneas in each group. Corneal sections were stained with an anti-CD31 antibody, followed by incubation with a FITC-conjugated secondary antibody. Corneal section of FGF-2 control group (E). Corneal section of resveratrol-FGF-2 group (F). Corneal microvessels are revealed in green color. Vessel counts (I) are presented as mean determinants (±SE) of 6–10 corneas in each group.
Figure 4. Antitumor and anti-wound healing effects. A) Oral administration of resveratrol at the dose of 1 mg/kg per day was given to C57Bl6/J mice implanted with a murine T241 fibrosarcoma. Tumor volumes were measured as previously reported (15). Tumor volumes represent mean determinants (±SE) of tumors of seven mice in each group. B–D) Full thickness skin wounds (approximately 5 mm in diameter) were created on C57Bl6/J mice by surgery. Resveratrol was added to the drinking fluid, and diameters of wounds were measured daily. Data are presented in D as mean determinants (±SE) of wounds of six mice in each group.