Red Wine Polyphenols Prevent Acceleration of Neovascularization by Angiotensin II in the Ischemic Rat Hindlimb

Allison Walter, Nelly Etienne-Selloum, David Brasse, René Schleiffer, Virgile Bekarter, Paul M. Vanhoutte, Alain Beretz, and Valérie B. Schini-Kerth

Uniteé Mixte de Recherche 7175 Centre National de la Recherche Scientifique, Université Louis Pasteur, Département de Pharmacologie et Physicochimie, Faculté de Pharmacie, Illkirch, France (A.W., N.E.-S., R.S., P.M.V., A.B., V.B.S.-K.); Institut Pluridisciplinaire Hubert Curien, Uniteé Mixte de Recherche 7178 Centre National de la Recherche Scientifique-IN2P3, Université Louis Pasteur, Strasbourg, France (D.B., V.B.); and Department of Pharmacology, University of Hong Kong, Hong Kong (P.M.V.)

Received October 30, 2008; accepted February 2, 2009

ABSTRACT

Studies in both animals and humans indicate that angiogenesis is implicated in the development of atherosclerotic lesions. Thus, inhibition of angiogenesis may provide a novel therapeutic approach for the treatment of atherosclerosis. Because epidemiological studies have indicated an inverse relation between red wine intake and coronary disease, we determined the antiangiogenic potential of red wine polyphenols (RWPs) in the ischemic hindlimb model. Neovascularization was accelerated by the chronic infusion of angiotensin II (Ang II; 0.1 mg/kg/day). RWPs intake significantly prevented the angiogenic process, the formation of reactive oxygen species (ROS) and nitrated proteins, and the expression of hypoxia-inducible factor (HIF)/endothelial nitric-oxide synthase (eNOS), and vascular endothelial growth factor (VEGF). These findings indicate that RWPs have potent antiangiogenic properties in vivo by preventing the expression of proangiogenic factors, including VEGF and eNOS most likely by inhibiting oxidative stress. Thus, the antiangiogenic properties of red wine polyphenols might contribute to their protective effect against coronary disease.

Angiogenesis, the growth of new blood vessels from pre-existing blood vessels, is involved in both physiological and pathological processes, such as embryogenesis, wound healing, tumor growth and metastasis, diabetic retinopathy, and atherosclerosis, leading to ischemic diseases. The formation of new blood vessels is often initiated by hypoxia subsequent to an impaired perfusion of the tissues (Carmeliet and Collen, 2000). The stimulatory effect of hypoxia involves the stabilization of hypoxia-inducible factor (HIF)-1α, resulting in an increased expression of vascular endothelial growth factor (VEGF), a major proangiogenic factor (Carmeliet and Collen, 2000). VEGF, in turn, stimulates the formation of new blood vessels, at least in part, via activation of endothelial nitric-oxide synthase (eNOS) and the subsequent formation of nitric oxide (NO) (Tamarat et al., 2002a). The angiogenic response to VEGF is significantly reduced in mice deficient in the eNOS gene (Tamarat et al., 2002a). In addition, Ang II decreases the density of blood vessels in muscle in vivo (Tamarat et al., 2002a,b). The stimulatory effect of Ang II might be mediated in part via the up-regulation of VEGF because the peptide increased VEGF expression in retinal endothelial cells (Otani et al., 1998), the aortic

ABBREVIATIONS: HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor; eNOS, endothelial nitric-oxide synthase; NO, nitric oxide; Ang II, angiotensin II; RWP, red wine polyphenol; MMP-2, matrix metalloproteinase type II; DHE, dihydroethidine; ROS, reactive oxygen species.
wall (Walter et al., 2008), and ischemic hindlimbs (Tamarat et al., 2002a,b).

Epidemiological studies indicate that regular intake of moderate amounts of beverages rich in polyphenols, particularly those of red wine, is associated with a reduced risk of coronary disease (Renaud et al., 1998; Rimm et al., 1999). The protective effect of red wine in coronary arteries has been attributed, at least in part, to its ability to reduce the progression of early atherosclerotic lesions (Auger et al., 2002). Explanations for this beneficial effect of RWPs include the ability to prevent the oxidation of low-density lipoproteins (Lapointe et al., 2006), the aggregation and adhesion of platelets (Freedman et al., 2001), the migration and proliferation of smooth muscle cells (Iijima et al., 2002), and the enhancement of the endothelial formation of NO and the facilitation of endothelium-dependent hyperpolarizations, two major vasoprotective events (Fitzpatrick et al., 1993; Ndiaye et al., 2003). Alternatively, RWPs might also attenuate the progression of early atherosclerotic lesions by preventing their vasculatization. RWPs and polyphenolic compounds present in wine, such as delphinidin, inhibit the proliferation of endothelial cells (Martin et al., 2003). In addition, RWPs also prevent vascular smooth muscle cell migration and proliferation via the inhibition of the p38 mitogen-activated protein kinase and phosphatidylinositol 3-kinase pathways (Iijima et al., 2002). Moreover, RWPs inhibit the expression of VEGF and matrix metalloproteinase type II (MMP-2), two major proangiogenic factors, in vascular smooth muscle cells (Oak et al., 2003; 2004). Furthermore, RWPs prevent in vivo the Ang II-induced expression of VEGF and MMP-2 in the aortic wall (Walter et al., 2008). The aim of the present study was to evaluate the potential of RWPs to prevent ischemia-induced formation of new blood vessels and its potentiation by Ang II in the rat hindlimb and to characterize the underlying mechanisms.

### Materials and Methods

#### Preparations of RWPs

RWPs in dry powder from French red wine (Corbières A.O.C.) were provided by Dr. M. Moutounet (Institut National de la Recherche Agronomique, Montpellier, France) and analyzed by Dr. P. L. Teissedre (Faculté d’Oenologie de Bordeaux, Université Victor Ségalen, Bordeaux, France). The procedures used to prepare and analyze RWPs have been described previously (Carando et al., 1999). One liter of red wine produced 2.9 g of phenolic extract, which contained 471 mg/total phenolic compounds expressed as gallic acid.

#### Ischemic Model

The study conforms with the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Resources, 1996) and has been approved by the local ethics committee of animal experimentation (CREMEAS). Eleven-week-old male Wistar rats underwent surgery to induce unilateral hindlimb ischemia. The right femoral artery was occluded, 0.5 cm proximal to the bifurcation of the saphenous and popliteal arteries, under pentobarbital anesthesia (50 mg/kg i.p.). An osmotic minipump (Alzet 2004; Alza, Palo Alto, CA) was then implanted subcutaneously allowing the chronic infusion of a subhypertensive dose of Ang II (0.1 mg/kg/day) for 21 days. To study the effect of RWPs on ischemia- and Ang II-induced neovascularization, rats were divided into four groups: a control group receiving the solvent of RWPs (5% ethanol in the drinking water), an Ang II-treated group receiving the solvent of RWPs, a RWP (25 mg/kg/day)-treated group, and a group receiving the combination of Ang II plus RWPs. To study the effect of the antioxidant and NADPH oxidase inhibitor apocynin (Sigma-Aldrich, St. Louis, MO) on ischemia- and Ang II-induced neovascularization, rats were divided into four groups: a control group receiving water, an Ang II-treated group receiving water, an apocynin (25 mg/kg/day)-treated group, and a group receiving the combination of Ang II plus apocynin. RWPs and apocynin were given in the drinking water starting 7 days before the ligation of the femoral artery and the beginning of the Ang II infusion. The different treatments (RWPs and apocynin) did not affect the volume of water ingested by the rats per day (control group, 31.5 ± 2.2 ml; RWP group, 30.5 ± 2.3 ml; apocynin group, 29.8 ± 1.8 ml; Ang II group, 29.7 ± 2.1 ml; Ang II plus RWP group, 30.8 ± 1.4 ml; Ang II + apocynin group, 30.4 ± 1.9 ml; n = 5–10).

#### Quantification of Angiogenesis

**Microangiography.** Blood vessel density in the hindlimbs was evaluated using a high-definition microcomputed tomography system (Brasse et al., 2005) after the 28-day treatment period. Rats were anesthetized (50 mg/kg i.p. pentobarbital; Centravet, Velaine en Haye, France) and placed on a heating plate (37°C). A longitudinal laparotomy was performed to introduce a polyethylene catheter into the abdominal aorta for injection of a contrast agent (3 g/ml barium sulfite). Microangiography of hindlimbs was performed using the microcomputed tomography system in a two-dimensional dynamic mode with the acquisition of 2 images/s during the injection of the contrast agent. The blood vessel density was expressed as the microangiographic score (ratio of the percentage of pixels per image occupied by blood vessels in the quantification area of the ischemic and the nonischemic hindlimb). The quantification area was limited by the place of the ligation, the knee, the edge of the femur, and the external border of the leg.

**Microvessel Density.** The microvessel density was also assessed by microangiographic analysis. Ischemic and nonischemic gastrocnemius muscles were dissected and embedded in Tissue-Tek O.C.T. (Electron Microscopy Sciences, Hatfield, PA). Fixed cryosections (5 μm) were incubated with a mouse antibody directed against CD31 (BD Pharmingen, San Diego, CA). Capillaries were then counted in five randomly selected fields for each muscle section, and the mean value for each section was calculated (magnification, ×200).

#### Expression of Angiogenic Factors

**Immunostaining.** Fixed cryosections (5 μm) were incubated with antibodies directed against VEGF (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), eNOS (BD Pharmingen), 3-nitrotyrosine (Millipore, Billerica, MA), and HIF-2α (Novus Biologicals, Inc., Littleton, CO). Unfixed cryosections (25 μm) were incubated with the fluorescent dye dihydroethidine (DHE; 2.5 μM; Sigma-Aldrich) for the in situ detection of reactive oxygen species (ROS). The fluorescence intensity was quantified in eight randomly chosen fields for each muscle section, and the mean value for each section was calculated.

#### Statistical Analysis

Results are expressed as means ± S.E.M. Statistical evaluation was performed using one-way analysis of variance followed by Fisher’s protected least significant difference test where appropriate. A P
value less than 0.05 was considered to indicate statistically significant differences.

**Results**

**RWPs Prevent the Stimulatory Effect of Ang II on Hypoxia-Induced Neovascularization**

Three weeks after ligation of the femoral artery, perfusion of the ischemic hindlimb was restored as indicated by the microangiographic score (0.95 ± 0.15) and the ischemic/nonischemic leg capillary number (0.90 ± 0.07, Figs. 1A and B). Ang II significantly increased the hypoxia-induced angiogenesis in the hindlimb as indicated by average a 2.2-fold increase of the microangiographic score and a 2.3-fold increase of the ischemic/nonischemic leg capillary number (Fig. 1A and B). These stimulatory effects of Ang II were reduced significantly by the administration of RWPs (25 mg/kg/day) in the drinking water, whereas the treatment with RWPs alone affected only minimally ischemia-induced neovascularization (Fig. 1A and B). The different treatments did not affect the capillary density in the nonischemic hindlimb (Fig. 1B). Similar inhibitory effects on capillary density were also observed with 50 and 100 mg/kg/day RWPs (capillary density was reduced from 2.2 ± 0.2 to 1.15 ± 0.18 and 1.2 ± 0.2, respectively; n = 8) but not with 10 mg/kg/day (1.6 ± 0.5; n = 4).

**RWPs Abolish Ang II-Induced Proangiogenic Responses**

**In Situ Formation of Reactive Oxygen Species.** Because Ang II is a potent inducer of vascular oxidative stress (Landmesser et al., 2002), which stimulates proangiogenic responses such as the expression of VEGF (Bassus et al., 2001) and eNOS (Drummond et al., 2000), the for-

---

**Fig. 1.** A, intake of RWPs prevents the stimulatory effect of Ang II on the ischemia-induced neovascularization in the rat. The top panel shows representative microangiographies of the ischemic (I) and nonischemic (NI) hindlimbs observed 21 days after femoral artery ligation. Bottom, ischemic/nonischemic microangiographic score. Values are shown as mean ± S.E.M., n = 9. *, P < 0.05 versus control group; #, P < 0.05 versus Ang II group. B, intake of RWPs prevents the stimulatory effect of Ang II on the ischemia-induced formation of new blood vessels. Left, representative pictures of gastrocnemius muscle sections stained with an antibody directed against CD 31. Capillaries appear in white and myocytes in black. Right, ratio of the ischemic (I)/nonischemic (NI) number of capillaries. Values are shown as mean ± S.E.M., n = 8 to 12. *, P < 0.05 versus control group; #, P < 0.05 versus Ang II group. Magnification, ×20.
formation of ROS was assessed in the gastrocnemius muscles. In the nonischemic hindlimb, the basal formation of ROS was not affected significantly by the treatment with either Ang II or RWPs (Fig. 2A). In contrast, in the ischemic hindlimb, Ang II significantly increased (average 1.7-fold) the formation of ROS; this effect was abolished by the treatment with RWPs (Fig. 2A). The Ang II-induced formation of ROS was associated predominantly with capillaries and the conjunctive tissue surrounding muscular fibers (Fig. 2A).

**HIF-2α Expression.** A low basal level of HIF-2α, but not HIF-1α, was observed by immunostaining in both the nonischemic and the ischemic gastrocnemius muscles (Fig. 2C; data not shown). In the nonischemic gastrocnemius muscle, the basal level of HIF-2α was not affected by the treatment with either Ang II or RWPs (Fig. 2C). In contrast, Ang II significantly increased (by approximately 1.8-fold) the HIF-2α signal in the ischemic gastrocnemius muscle, and this effect was abolished by treatment with the RWPs (Fig. 2C). The latter given alone affected only minimally the HIF-2α signal in the ischemic hindlimb (Fig. 2C). The Ang II-induced HIF-2α signal was associated mainly with capillaries and surrounding muscular fibers and, also, to a lesser extent, with myocytes (Fig. 2C).

---

**Fig. 2.** Intake of RWPs prevents the stimulatory effect of Ang II on the ischemia-induced formation of ROS and ONOO⁻ and expression of HIF-2α. Representative pictures of gastrocnemius muscle sections from ischemic (I) and nonischemic (NI) hindlimbs incubated with DHE for the in situ detection of ROS (A) and stained with an antibody directed against either 3-nitrotyrosine (ONOO⁻, B) or HIF-2α (C). Right, corresponding cumulative data. Values are shown as mean ± S.E.M., n = 5. *, P < 0.05 versus control group; #, P < 0.05 versus Ang II group. Magnification, ×20.
**Regulation of VEGF Expression.** A basal level of VEGF expression (assessed both by immunostaining and Western blot analysis) was observed in the nonischemic and the ischemic gastrocnemius muscles (Fig. 3, A and B). Treatment with Ang II or RWPs did not significantly affect the VEGF signal in the nonischemic hindlimb (Fig. 3, A and B). In contrast, the Ang II treatment significantly induced a 1.3-fold increase in the VEGF fluorescent signal and a 1.9-fold increase in the VEGF protein level in the ischemic hindlimb (Fig. 3, A and B). The Ang II-induced VEGF signal in ischemic hindlimbs was associated predominantly with capillaries and the connective tissue surrounding muscular fibers and, also, to a lesser extent, with myocytes (Fig. 3A). The stimulatory effect of the Ang II treatment was prevented by the intake of RWPs (Fig. 3, A and B). Treatment with RWPs alone did not significantly modify the basal VEGF expression level in the ischemic hindlimbs (Fig. 3, A and B).

**eNOS Expression.** In the nonischemic hindlimb, a basal eNOS fluorescent signal was detected mainly in capillaries, and the intensity of the signal was not affected by treatment with either Ang II or the RWPs (Fig. 4A). Similar findings were observed by Western blot analysis (Fig. 4B). In contrast, in the ischemic hindlimb, the treatment with Ang II increased eNOS staining and the protein content of the enzyme by 1.8- and 1.75-fold, respectively (Fig. 4, A and B). The stimulatory effect of Ang II was prevented by RWPs, whereas RWPs alone had only minor effects (Fig. 4, A and B).

**Formation of Peroxynitrites.** Because the Ang II treatment significantly increased the formation of ROS and the expression of eNOS in the ischemic hindlimb, experiments

---

**Fig. 3.** Intake of RWPs prevents the stimulatory effect of Ang II on the ischemia-induced expression of VEGF. A, left, representative pictures of gastrocnemius muscle sections from ischemic (I) and nonischemic (NI) hindlimbs stained with an antibody directed against VEGF. Right, corresponding cumulative data. Magnification, ×20. B, representative Western blot showing the expression level of VEGF in ischemic and nonischemic muscles. Bottom, corresponding cumulative data of ischemic/nonischemic protein level ratios. Values are shown as mean ± S.E.M., n = 3 to 5. *, P < 0.05 versus control group; #, P < 0.05 versus Ang II group.
were performed to determine the level of peroxynitrites, a product derived from the chemical reaction of eNOS-derived NO and superoxide anions. The formation of peroxynitrites was assessed indirectly by the determination of the level of 3-nitrotyrosine. The treatment with Ang II increased by approximately 1.9-fold the level of nitrated proteins in the ischemic hindlimb, whereas no such effect was observed in the nonischemic hindlimb (Fig. 2B). The stimulatory effect of Ang II was prevented by the intake of RWPs (Fig. 2B). The treatment with either Ang II or RWPs alone did not modify the basal level of 3-nitrotyrosine formation observed in the nonischemic hindlimb (Fig. 2B). The Ang II-stimulated formation of peroxynitrites was associated mainly with capillaries and the connective tissue surrounding myofibers, two sites where an increased expression of eNOS and formation of ROS were observed (Fig. 2B).

Fig. 4. Intake of RWPs prevents the stimulatory effect of Ang II on the ischemia-induced expression of eNOS. A, left, representative pictures of gastrocnemius muscle sections from ischemic (I) and nonischemic (NI) hindlimbs stained with an antibody directed against eNOS. Right, corresponding cumulative data. Magnification, ×20. B, representative Western blot showing the expression level of eNOS in ischemic and nonischemic muscles. Bottom, corresponding cumulative data of ischemic/nonischemic protein level ratios. Values are shown as mean ± S.E.M., n = 3 to 5. *P < 0.05 versus control group; #, P < 0.05 versus Ang II group.

The Antioxidant and NADPH Oxidase Inhibitor Apocynin Prevents the Stimulatory Effect of Ang II on Ischemia-Induced Neovascularization and Expression of Proangiogenic Factors

Intake of apocynin (25 mg/kg/day) in the drinking water prevented the stimulatory effect of Ang II on the ischemia-induced neovascularization, the formation of ROS, and the expression of HIF-2α and VEGF in the ischemic hindlimb (Figs. 5 and 6). The treatment with apocynin alone had only minor effects (Figs. 5 and 6).

Discussion

The present study demonstrates that the accelerating effect of Ang II on ischemia-induced neovascularization and the induction that it causes of proangiogenic mechanisms
Ang II, at a dose that does not increase arterial blood pressure, significantly accelerates ischemia-induced neovascularization (assessed by microangiography and capillary density) and blood perfusion (assessed by laser-Doppler perfusion imaging) in ischemic hindlimbs (Tamarat et al., 2002a,b; present findings). This stimulatory effect of Ang II involves an up-regulation of VEGF expression, a key proangiogenic factor stimulating the migration and proliferation of endothelial cells and the formation of new blood vessels (Papapetropoulos et al., 1997; Tamarat et al., 2002a,b). Ang II is a potent inducer of VEGF expression in vascular smooth muscle and endothelial cells in vitro (Otani et al., 1998; Pagé et al., 2002), and VEGF-neutralizing antibodies prevent the Ang II-induced angiogenic effects in vivo (Tamarat et al., 2002b). NO produced by the endothelium may also contribute to the angiogenic response because both the Ang II and the VEGF-induced proangiogenic responses in ischemic hindlimbs are not observed in mice deficient in the gene encoding for eNOS (Tamarat et al., 2002a). VEGF induces the expression of the latter enzyme in native and cultured endothelial cells, and this results in an increased formation of NO (Bouloumié et al., 1999). The stimulatory effect of Ang II on ischemia-induced proangiogenic responses is mediated via activation of AT1 receptors because it is blunted by valsartan, a selective AT1 receptor inhibitor in rats (Tamarat et al., 2002b). Likewise, activation of AT1 receptors induces the expression of VEGF and the subsequent neoformation of blood vessels in the rabbit cornea (Fujiyama et al., 2001).

Ang II, by activating AT1 receptors, is a potent inducer of oxidative stress in the blood vessel wall. This is mainly due to augmentation of the expression and stimulation of the activity of NADPH oxidase (Mollnau et al., 2002; Sarr et al., 2006). Because ROS induce the expression of VEGF and eNOS in vascular cells (Bassus et al., 2001; Zhen et al., 2008), oxidative stress may be an early key mediator of the stimulatory effect of Ang II on the proangiogenic responses to ischemia. This hypothesis is supported by the present findings demonstrating that the treatment with Ang II markedly increases oxidative stress in ischemic hindlimbs. It is further supported by the fact that apocynin, an antioxidant and inhibitor of NADPH oxidase activity, abolished the response to the peptide in terms of ROS formation and VEGF and eNOS expression and neovascularization. ROS most likely stimulate proangiogenic responses via activation of transcription factors belonging to the hypoxia-inducible factor family, which control the expression of the VEGF and eNOS genes (Ema et al., 1997; Coulet et al., 2003). In line with these earlier studies, the present findings demonstrate that Ang II causes a pronounced up-regulation of HIF-2α in ischemic hindlimbs and that this effect is prevented by apocynin. Thrombin, platelet-derived growth factor-AB, and transforming growth factor also up-regulate HIF-1α via the NADPH oxidase-dependent formation of ROS in vascular smooth muscle cells (Görlich et al., 2001). NADPH oxidase-derived ROS contribute to the stabilization of both HIF-1α and HIF-2α in von Hippel-Lindau tumor suppressor gene-deficient cells (Block et al., 2007). Taken in conjunction, these findings suggest that NADPH oxidase-derived ROS, through activation of HIF-2α, mediate the stimulatory effect of Ang II on the expression of VEGF and eNOS in ischemic hindlimbs and the subsequent angiogenesis.

Vegetables and beverages, such as wine and tea, are rich in natural antioxidant molecules, including polyphenols, which have antiangiogenic properties. Thus, RWP s and anthocyanins contained in red wine inhibit the induction of VEGF expression in vascular smooth muscle cells by several growth factors (Oak et al., 2003, 2006). RWP s also prevent the activation by growth factors of MMP-2, a collagenase involved in the angiogenic process (Oak et al., 2004). Polyphenols also prevent the proliferation and migration of vascular cells, two major events in angiogenesis (Iijima et al., 2002; Martin et al., 2003). Likewise, green tea polyphenols and RWP s inhibit the formation of blood vessels in the chorioallantoic membrane of the chick embryo (Oak et al., 2005). The present findings indicate that chronic intake of RWP s prevents the stimulatory effect of Ang II on the ischemia-induced neovascularization and the accompanying augmented expression of proangiogenic factors, including HIF-2α, VEGF, and eNOS. In addition, Provinols, a polyphenolic extract isolated from red wine, also prevents ischemia-induced angiogenesis in the rat ischemic hindlimb (Baron-Menguy et al., 2007). The present findings demonstrate that this protective effect of RWP s is associated with the prevention of the Ang II-induced oxidative stress in the ischemic hindlimb, as indicated by the normalized level of ROS and the reduced formation of peroxynitrites. The beneficial effect of polyphenols may, as that

![Graph showing the effect of apocynin on blood perfusion](image)
of apocynin, be due to the ability to directly scavenge ROS such as hydroxyl radicals and superoxide anions (Miyagi et al., 1997). Alternatively, it could be due to the inhibition of the expression and activity of NADPH oxidase. Tea polyphenols down-regulated the expression of NADPH oxidase subunits p22phox and p67phox in endothelial cells while up-regulating that of catalase (Ying et al., 2003). Moreover, RWPs have been shown to directly inhibit the activity of NADPH oxidase in aortic smooth muscle cells (Jimenez et al., 2007). In addition, RWPs prevented the Ang II-induced hypertension and endothelial dysfunction by reducing the over-expression of the NADPH oxidase subunits p22phox and nox1 and, hence, the excessive oxidative stress in the aortic wall of rats (Sarr et al., 2006).

In conclusion, the present findings demonstrate that the chronic administration of RWPs prevents the potentiating effect of Ang II on ischemia-induced neovascularization. This effect is explained best by the ability of RWPs to prevent the excessive oxidative stress in the ischemic hindlimb, which in turn induces the expression of redox-sensitive genes such as HIF-2α, subsequently leading to that of major proangiogenic factors, including VEGF and eNOS. Thus, the present study...
implies that the antiangiogenic properties of polyphenols contribute to their beneficial effect on coronary artery disease.

Acknowledgments
We thank Professor Bernard Lévy and Dr. Jean-Sébastien Silvestre (Hôpital Lariboisière, Paris) for help with the ischemic model.

References
Collinson P, and d’Houtaud A (1998) Alcohol and mortality at ASPET Journals on May 17, 2017 jpet.aspetjournals.org Downloaded from

In Vivo Antiangiogenic Effect of Red Wine Polyphenols


Address correspondence to: Valérie Schini-Kerth, Unité Mixte de Recherche 7175 Centre National de la Recherche Scientifique, Département de Pharmacologie et Physiologie, Faculté de Pharmacie, BP60024, 67401 Illkirch, France. E-mail: valerie.schini-kerth@pharma.u-strasbg.fr