Antiangiogenic properties of natural polyphenols from red wine and green tea

Min-Ho Oak[a,b], Jasser El Bedoui[a], Valérie B. Schini-Kerth[a],*

[a]*Pharmacologie et Physico-Chimie des Interactions Cellulaires et Moléculaires, UMR CNRS 7034, Faculté de Pharmacie, Université Louis Pasteur de Strasbourg, F-67401 Illkirch, France*
[b]*Research and Development Center, Yangji Chemicals, An-San, South Korea*

Abstract

Epidemiological studies have indicated that regular consumption of red wine and green tea is associated with a reduced risk of coronary heart disease and tumor progression. The development of tumors and of atherosclerosis lesions to advanced plaques, which are prone to rupture, is accelerated by the formation of new blood vessels. These new blood vessels provide oxygen and nutrients to neighboring cells. Therefore, recent studies have examined whether red wine polyphenolic compounds (RWPCs) and green tea polyphenols (GTPs) have antiangiogenic properties.

In vitro investigations have indicated that RWPCs and GTPs are able to inhibit several key events of the angiogenic process such as proliferation and migration of endothelial cells and vascular smooth muscle cells and the expression of two major proangiogenic factors, vascular endothelial growth factor (VEGF) and matrix metalloproteinase-2, by both redox-sensitive and redox-insensitive mechanisms. Antiangiogenic properties of polyphenols have also been observed in the chick embryo chorioallantoic membrane since the local application of RWPCs and GTPs strongly inhibited the formation of new blood vessels. Moreover, intake of resveratrol or green tea has been shown to reduce corneal neovascularization induced by proangiogenic factors such as VEGF and fibroblast growth factor in mice.

The ability of RWPCs and GTPs to prevent the formation of new blood vessels contributes, at least in part, to explain their beneficial effect on coronary heart disease and cancer. This review focuses on the antiangiogenic properties of natural polyphenols and examines underlying mechanisms.

Keywords: Polyphenol; Angiogenesis; Atherosclerosis

1. Introduction

Epidemiological studies have suggested an inverse relation between regular consumption of natural polyphenols, particularly red wine and green tea, and the risk of coronary heart disease and cancer [1–7]. The beneficial effect of red wine and green tea on coronary diseases might be attributable, in part, to their ability to retard the progression of early atherosclerotic lesions, as observed in human coronary arteries at childhood, to advanced plaques, which are prone to rupture with superimposed thrombosis as suggested by studies with experimental models of atherosclerosis [8–12]. Since polyphenolic compounds from red wine and green tea such as quercetin and catechin were also able to prevent the progression of atherosclerotic lesions, polyphenols present in wine and green tea account, at least in part, for the protective effect of moderate wine and green tea consumption [8–11].

The formation and progression of atherosclerotic lesions are characterized by excessive vascular remodeling with accumulation of cells and lipids within the intimal layer of the pathologic artery [13]. Recent experimental and human studies have shown an increased number of adventitial vasa vasorum in advanced coronary atherosclerosis [14–17]. Moreover, a correlation between the extent of vasa vasorum and the severity of atherosclerotic plaques has been found in human coronary arteries [17]. Mechanical injury of the adventitial layer has also been shown to stimulate the formation of new blood vessels in injured arteries [18–20].

These observations have led to the proposition that adventitial vasa vasorum contribute to the development and progression of coronary atherosclerosis by providing oxygen and nutrients and possibly also of such complications as intimal hemorrhage and plaque rupture since these new blood vessels are fragile. Consistent with such a concept, two angiogenesis inhibitors, endostatin and TNP-470, reduced intimal neovascularization and plaque growth in apolipoprotein E-deficient mice [21].

During the angiogenic process, new blood vessels develop from the existing microvascular bed. The initial event involves dilatation of an existing blood vessel followed by an increase in vascular permeability and the degradation of extracellular matrices. Thereafter, endothelial cells (ECs) can migrate and proliferate, and these events are followed by maturation of new blood vessels. The angiogenic process is controlled by two major proangiogenic factors, matrix metalloproteinases (MMPs), which degrade extracellular matrices, and vascular endothelial growth factor (VEGF), which strongly stimulates EC migration and proliferation and the formation of new blood vessels.

Therefore, recent investigations have examined the possibility that natural polyphenols from red wine and green tea prevent the development of atherosclerotic lesions by inhibiting the formation of new blood vessels. Alternatively, the beneficial effect of natural polyphenols might also be related to their ability to up-regulate the level of high-density lipoprotein and to prevent oxidation of low-density lipoprotein [22,23], activation of platelets [24,25] and expression of prothrombotic and proatherosclerotic molecules such as monocyte chemoattractant-1 [26] and tissue factor [27] in vascular cells. This review focuses on recent in vitro and in vivo experimental evidence showing that natural polyphenols, particularly those from green tea and red wine, have antiangiogenic properties and discusses underlying mechanisms.

2. Polyphenols inhibit the expression of VEGF

Vascular endothelial growth factor, a major proangiogenic factor [28], has been suggested to contribute to intimal neovascularization in atherosclerosis. Indeed, VEGF is strongly expressed in human atherosclerotic plaques [29,30] and the number of VEGF-positive cells increases gradually with the progression of lesions [29]. The cellular sources of VEGF in human atherosclerotic plaques are predominantly vascular smooth muscle cells (VSMCs) and, to some extent, foamy macrophages [29]. In addition to inducing a proangiogenic response, VEGF also stimulates gene expression of several endothelial proteins involved in prothrombotic and proatherosclerotic responses including tissue factor [31], adhesion molecules [32] and monocyte chemoattractant protein-1 [33]. Moreover, VEGF induces monocyte procoagulant activity and promotes monocyte chemotaxis [31]. Thus, VEGF is likely to play an important role in the formation of new blood vessels and in the expression of proinflammatory and prothrombotic molecules in atherosclerotic plaques.

We have recently reported that red wine polyphenolic compounds (RWPCs) strongly inhibit growth factor-induced VEGF expression in VSMCs at concentrations that are likely to be achieved in blood after moderate consumption of red wine [34]. These investigations have indicated that the stimulatory effect of growth factors such as $PDGF_{AB}$ on VEGF expression is a redox-sensitive event (Fig. 1) [34,35].

The major enzymatic source of reactive oxygen species (ROS) in VSMCs in response to growth factors is the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. Indeed, exposure of VSMCs to thrombin or $PDGF_{AB}$ caused the generation of substantial amounts of ROS via activation of a p22phox-containing NADPH oxidase [35–37]. Moreover, prevention of the generation
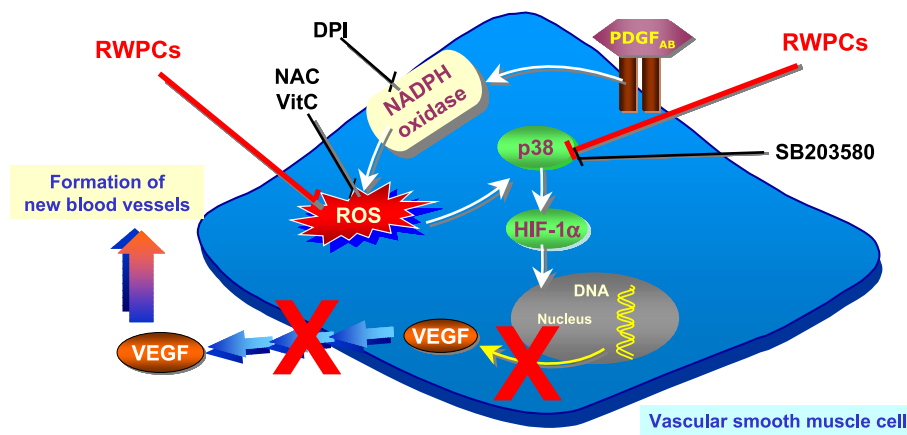

Fig. 1. Red wine polyphenolic compounds inhibit VEGF expression in VSMCs. Growth factors such as $PDGF_{AB}$ strongly stimulate the expression of VEGF via the formation of intracellular ROS. The formation of ROS by the NADPH oxidase mediates activation of the redox-sensitive p38 MAP kinase pathway, which, in turn, activates hypoxia-inducible factor-1 alpha, a key transcription factor regulating VEGF expression. Red wine polyphenolic compounds inhibit the stimulatory effect of $PDGF_{AB}$ most likely by preventing the formation of intracellular ROS and phosphorylation of p38 MAP kinase. DPI, diphenylene iodonium; NAC, N-acetyl cysteine; Vit C, vitamin C.

of ROS by either antioxidant treatments, diphenylene iodonium, a nonselective inhibitor of NADPH oxidase, or p22phox antisense attenuated the expression of VEGF in response to growth factors such as thrombin, $PDGF_{AB}$ and basic fibroblast growth factor (bFGF) [35,36,38]. Reactive oxygen species appear to control the expression of VEGF by activating the heterodimeric transcription factor hypoxia-inducible factor-1 [35].

Polyphenols from red wine and green tea have been shown to have antioxidant properties most likely due to their ability to directly scavenge ROS such as hydroxyl radical and superoxide anion [22,39,40] and to inhibit the expression of NADPH oxidase [41] and the activity of xanthine oxidase [42]. In addition, polyphenols can also increase the activity of catalase and glutathione peroxidase [43], which detoxify H_2O_2 by converting it to O_2 and H_2O.

Consistent with their antioxidant properties, the inhibitory effect of RWPCs on $PDGF_{AB}$-induced expression of VEGF was associated with the total prevention of the formation of ROS [34]. In addition, RWPCs also significantly reduced the H_2O_2-stimulated release of VEGF in VSMCs [34]. Altogether, these findings suggest that the inhibitory effect of RWPCs on the expression of VEGF involves, at least in part, their antioxidant properties.

However, although antioxidants such as vitamin C, N-acetylcysteine and diphenylene iodonium completely prevented the formation of ROS in VSMCs, they only partially reduced the $PDGF_{AB}$-induced expression of VEGF [34]. These findings suggest that the stimulatory effect of $PDGF_{AB}$ on VEGF expression involves both redox-sensitive and redox-insensitive mechanisms. Consistent with such an idea, RWPCs caused a sustained inhibition of $PDGF_{AB}$-induced VEGF expression that lasts several hours after their removal from the incubation medium, a condition that was not associated with an impaired formation of ROS [34]. Such a long-lasting effect of RWPCs might reflect their association with VSMCs and/or the possibility that RWPCs induce the production of one or several peptide(s)/protein(s), which, in turn, contribute(s) to prevent the expression of VEGF via redox-insensitive mechanisms.

Previous studies have shown that $PDGF_{AB}$ causes activation of several redox-sensitive protein kinases including members of the mitogen-activated protein kinases such as p38 MAPK, ERK1/2 and JNK and also PI3-kinase/Akt, which have all been involved in the up-regulation of VEGF expression in several cell types [44–46]. However, only the activation of the p38 MAPK pathway seems to play a major role in $PDGF_{AB}$-induced release of VEGF in VSMCs. Indeed, $PDGF_{AB}$-induced release of VEGF was abolished by SB203580, a specific inhibitor of p38 MAPK, slightly reduced by PD98059, an inhibitor of MEK, and not affected by wortmannin and JNKI, inhibitors of PI3-kinase and JNK, respectively [34]. Reactive oxygen species appear to mediate the stimulatory effect of $PDGF_{AB}$ on p38 MAPK activation since the response was prevented by several antioxidants and H_2O_2 is a strong activator of p38 MAPK [34]. Red wine

polyphenolic compounds strongly inhibited $PDGF_{AB}$-induced p38 MAPK phosphorylation without affecting that of ERK1/2, JNK and Akt in VSMCs. Although RWPCs have been shown to inhibit $PDGF_{BB}$ binding to the PDGF-β receptor and PDGF-β receptor phosphorylation [47], such an explanation is unlikely to account for the impaired expression of VEGF because the $PDGF_{AB}$-induced phosphorylation of ERK1/2, JNK and Akt was not affected by RWPCs. Taken together, RWPCs are potent inhibitors of growth factor-induced VEGF expression in VSMCs partly by the selective prevention of the redox-sensitive activation of the p38 MAPK pathway, which leads to VEGF gene expression.

In addition, green tea polyphenols (GTPs) and epigallo-catechin-3-gallate (EGCG) have also been shown to decrease VEGF production in head and breast carcinoma cells by inhibiting epidermal growth factor receptor-related pathways of signal transduction [48,49] such as the constitutive activation of Stat3 and NF-κB but not ERK1/2 or Akt [48].

3. Polyphenols inhibit MMP-2 expression and activity

The structural reorganization of the arterial wall during atherogenesis is controlled by MMPs, a family of structurally related zinc endopeptidases that are capable of degrading components of the extracellular matrix [50,51]. Recent findings have indicated that the gelatinases, MMP-2 and MMP-9, are dominant MMPs in vascular tissues and that they play an important role in the turnover of basement membrane type IV collagen during angiogenesis and formation of atherosclerotic plaques [52,53], leading to an increased risk of cardiovascular events. Besides MMP-2 and MMP-9, vascular cells also produce MMP-1, -3 and membrane type (MT) 1 MMP, which have all been involved in the destabilization and rupture of atherosclerotic plaques (see for review, Refs. [51,54]).

Matrix metalloproteinase-2 is expressed abundantly in atherosclerotic and restenotic lesions and has been suggested to play a key role in the degradation of the basement membrane, thereby promoting migration of ECs and VSMCs [55].

Matrix metalloproteinase-2 is unique among the MMPs in that it does not possess the propeptide sequence that is susceptible to proteolytic activation by other proteases such as plasmin or trypsin [53]. Activation of the precursor of MMP-2, pro-MMP-2, takes place predominantly at the cell surface and is mediated by MT1-MMP [50,53,54]. The following model of pro-MMP-2 activation by MT1-MMP and tissue inhibitor of metalloproteinase-2 (TIMP-2) has been proposed (see for review, Ref. [50]). MT1-MMP forms a dimer or multimers on the cell surface through interaction of the hemopexin domains. Pro-MMP-2 forms a tight complex with TIMP-2 through their C-terminal domains, therefore permitting the N-terminal inhibitory domain of TIMP-2 in the complex to bind to MT1-MMP

4

on the cell surface [50]. Binding of pro-MMP-2 to the MT1-MMP/TIMP-2 complex localizes the pro-MMP-2 on the cell surface, and activation is initiated by the proteolytic action of a second TIMP-2-free MT1-MMP molecule at the Asn^{37}–Leu^{38} bond of the MMP-2 propeptide [50]. Alternatively, MT1-MMP inhibited by TIMP-2 can act as a receptor of pro-MMP-2. It has been suggested that the maximum enhancement of pro-MMP-2 activation is observed at a TIMP-2/MT1-MMP ratio of 0.05, indicating that a large number of free MT1-MMPs may surround the ternary complex of pro-MMP-2–TIMP-2–MT1-MMP for effective pro-MMP-2 activation [56]. Pro-MMP-2 can be activated by other MT-MMPs in several cell types. These include MT2-MMP [57], MT3-MMP [58], MT5-MMP [59,60] and MT6-MMP [61]. Interestingly, pro-MMP-2 activation by MT2-MMP is direct and independent of TIMP-2 [62].

Recent in vitro and in vivo studies have revealed a major role for gelatinases in angiogenesis. When ECs are cultured on Matrigel, the formation of a tubular network is induced by the addition of recombinant MMP-2 in a dose-dependent manner and this response is inhibited by a neutralizing antibody directed against MMP-2 and by a tissue inhibitor of metalloproteinases, TIMP-2 [63]. Capillary ECs cultured on two-dimensional type I collagen gels produce low, constitutive levels of pro-MMP-2 with little endogenous activation of the protease. However, when placed in three-dimensional type I collagen gels, there is a marked increase in the total amount of MMP-2 mRNA and protein [64]. Matrix metalloproteinase-2 activity can also generate extracellular matrix degradation fragments, leading to

signals required for cell survival and migration [65]. In addition, the corneal neovascular area induced by bFGF in MMP-2-deficient mice is markedly reduced when compared with that of wild-type littermates [66].

Recent studies have indicated that RWPCs and GTPs strongly inhibit VSMC invasion induced by growth factors such as thrombin and $PDGF_{BB}$ [67,68]. The inhibitory effect of polyphenols is associated with a concentration-dependent inhibition of thrombin-induced MMP-2 activation (Fig. 2) [69,70]. In addition, although thrombin did not affect the expression level of MT1-MMP both at the mRNA and protein levels, the serine protease increased markedly the cell-associated MT1-MMP activity. Red wine polyphenolic compounds and GTPs inhibited MT1-MMP activity when added directly to the enzymatic assay. Altogether, these findings suggest that natural polyphenols from red wine and green tea are able to prevent activation of MMP-2 by directly inhibiting the activity of membrane-bound MT1-MMP. Consistent with such an idea, catechin, a polyphenolic compound, has been shown to prevent MT1-MMP-dependent activation of MMP-2 in cancer cells [71]. The inhibitory effect of RWPCs is not observed in VSMCs pretreated with RWPCs before the enzymatic determination of cell-associated MT1-MMP activity, indicating that RWPCs most likely inhibit the catalytic activity of MT1-MMP in a reversible manner [70]. At present, the active polyphenolic compounds of the red wine extract and the green tea extract still remain unclear. However, previous studies have shown that resveratrol and EGCG strongly inhibit MMP-2 expression and activity in different types of cells [67,72–74].
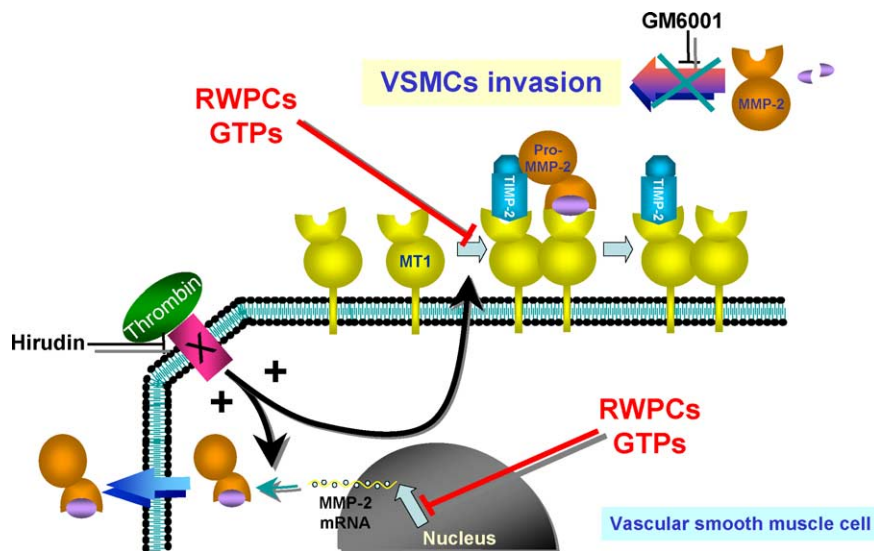

Fig. 2. Red wine polyphenolic compounds and GTPs inhibit MMP-2 expression and directly the activity of MT1-MMP. Thrombin stimulates the expression of cell-associated pro-MMP-2 protein and the activity of MT1-MMP via protease-activated receptor-independent mechanisms in VSMCs. These effects result in the formation of active MMP-2 and VSMC invasion. Red wine polyphenolic compounds and GTPs inhibit the expression of MMP-2 and the conversion of pro-MMP-2 into active MMP-2 by directly inhibiting MT1-MMP activity, thereby preventing VSMC invasion. MMP-2, matrix metalloproteinase-2; MT1, membrane type 1 matrix metalloproteinase; TIMP-2, tissue inhibitor of metalloproteinase-2; GM6001, a broad-spectrum MMP inhibitor; Hirudin, a potent and specific inhibitor of thrombin.

4. Polyphenols prevent migration and proliferation of ECs and VSMCs

Proliferation and migration of ECs and VSMCs are major events in the angiogenic process and are also hallmarks of numerous cardiovascular diseases including atherosclerosis and restenosis. Several studies have indicated that natural polyphenols are able to inhibit proliferation and migration of vascular cells [75,76].

Resveratrol has been shown to prevent the progression of ECs through the S and G_2 phases, and this effect is accompanied by an increased expression of the tumor suppressor gene protein p53 and an elevation of the level of the cyclin-dependent kinase inhibitor p21(WAF1/CIP1) [77]. Delphinidin strongly inhibited EC proliferation and migration in cyclin D1- and cyclin A-dependent pathways in response to VEGF [78,79]. Green tea polyphenols also significantly reduced EC proliferation in a dose-dependent manner and caused the accumulation of cells in the G_1 phase without affecting cell viability [80]. In addition, EGCG suppressed EC proliferation and migration by inducing apoptosis through mitochondrial depolarization, activation of caspase-3 and reduction of binding of VEGF to its receptors in human ECs [81,82].

Not only ECs but also proliferation and migration of VSMCs can be affected by RWPCs and GTPs. Iijima et al. [83] have reported that RWPCs strongly inhibit proliferation and migration of VSMCs; both these responses are associated with the down-regulation of cyclin A gene expression through the decreased expression of transcription factors CREB and ATF-1 and with the specific inhibition of p38 MAPK and PI3-kinase/Akt pathways. In addition, GTPs and EGCG also induced apoptosis of proliferating VSMCs in a p53- and NF-κB-dependent manner.

5. In vivo antiangiogenic properties of polyphenols

It is well established that neovascularization occurs in atherosclerotic plaques of humans [14,17] and also promotes the progression of tumors [84]. Although polyphenols from green tea and red wine showed strong antiangiogenic properties in several in vitro experiments, few studies have investigated their antiangiogenic properties in vivo. The local application of RWPCs and GTPs to the chick embryo chorioallantoic membrane (CAM) strongly inhibited angiogenesis as indicated by a marked reduction in the number and length of small blood vessels after a 48-h incubation period (Fig. 3) [85,86]. Moreover, previous studies have indicated that drinking resveratrol, a polyphenolic compound found in wine, significantly inhibited corneal neovascularization in mice induced by VEGF and bFGF [87]. Epigallocatechin-3-gallate from green tea also suppressed the proliferation of ECs and the formation of new blood vessels in the CAM assay [86]. In addition, drinking tea significantly prevented VEGF-induced corneal neovascularization in a mouse model [86]. It has been also reported that resveratrol can inhibit tumor growth and tumor-induced neovascularization in vivo [88].

6. Discussion and conclusion

The molecular mechanism of the in vivo antiangiogenic properties of red wine- and green tea-derived polyphenolic compounds remains unclear, but it may be due to their ability to inhibit several key events of the angiogenic process such as proliferation and migration of ECs and VSMCs and the expression of VEGF and activation of MMP-2.

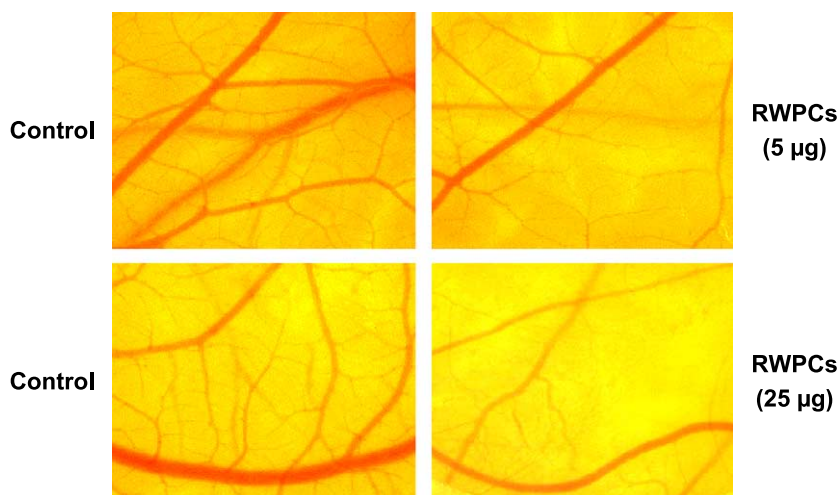The bioavailability of GTPs and RWPCs is an important determinant in understanding their biological activities.

Fig. 3. Antiangiogenic effects of RWPCs on the chick embryo CAM. The local application of RWPCs (5 and 25 μg) to the CAM strongly inhibits the formation of new blood vessels as compared with the control area after a 48-h incubation period. Similar observations were obtained in two additional experiments.

Lack of understanding on this issue has led to excessive claims regarding the in vivo biological activity of natural polyphenols based on extrapolation from in vitro studies. The absorption of only very few red wine compounds has been studied to date, and absorption of even fewer compounds has been studied within a wine matrix. Despite these limitations, flavanols, flavonols, anthocyanins and nonflavonoid stilbenes in red wine and green tea have been shown to be absorbed [89–93]. The inhibitory effect of RWPCs on proangiogenic responses is detected at concentrations as low as 3 μg/ml [34,83]. Although the concentration of RWPCs in blood after intake of red wine remains unknown, a previous study has indicated that intake of 100 ml of red wine by healthy volunteers caused an increase in plasma concentration of polyphenolic monomers of 2.5 μg/ml (gallic acid equivalents) [89]. The degree of availability of GTPs is still under debate. Recent studies have demonstrated that a small percentage of the per os-ingested green tea catechins appears in blood [90–93]. Lee et al. [90] have indicated that the mean peak plasma EGCG level is about 0.17 μM after drinking the equivalent of two cups of tea by human volunteers. Moreover, a receptor for EGCG has been identified in human cancer cells [94]. In this type of cells, the inhibitory effects of EGCG are mediated by the 67-kDa laminin receptor with a K_d value of about 40 nM.

The concentration of EGCG, which showed antiangiogenic effects in a mouse corneal model, was in the range of 0.1–0.3 μM [86]. Thus, the inhibitory effect of RWPCs and GTPs on proangiogenic responses is likely to be reached in blood after moderate consumption of red wine and green tea.

In conclusion, several recent studies have indicated that RWPCs and GTPs have in vitro and in vivo antiangiogenic properties by inhibiting the expression of two strong proangiogenic factors, VEGF and MMP-2, and also by preventing the proliferation and migration of ECs and VSMCs. The antiangiogenic properties of RWPCs and GTPs could contribute to explain the reduced risk of coronary heart diseases and cancer mortality following chronic consumption of moderate amounts of red wine and green tea.

Acknowledgment

References

[1] Renaud S, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 1992;339:1523–6.

[2] Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 1999; 319:1523–8.

[3] Nakachi K, Matsuyama S, Miyake S, Suganuma M, Imai K. Preventive effects of drinking green tea on cancer and cardiovascular disease: epidemiological evidence for multiple targeting prevention. Biofactors 2000;13:49–54.

[4] Hollman PC, Feskens EJ, Katan MB. Tea flavonols in cardiovascular disease and cancer epidemiology. Proc Soc Exp Biol Med 1999; 220:198–202.

[5] Sasazuki S, Kodama H, Yoshimasu K, et al. Relation between green tea consumption and the severity of coronary atherosclerosis among Japanese men and women. Ann Epidemiol 2000;10:401–8.

[6] Yang CS, Maliakal P, Meng X. Inhibition of carcinogenesis by tea. Annu Rev Pharmacol Toxicol 2002;42:25–54.

[7] Bianchini F, Vainio H. Wine and resveratrol: mechanisms of cancer prevention? Eur J Cancer Prev 2003;12:417–25.

[8] Hayek T, Fuhrman B, Vaya J, et al. Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. Arterioscler Thromb Vasc Biol 1997;17:2744–52.

[9] Vinson JA, Teufel K, Wu N. Red wine, dealcoholized red wine, and especially grape juice, inhibit atherosclerosis in a hamster model. Atherosclerosis 2001;156:67–72.

[10] da luz PL, Serrano Jr CV, Chacra AP, et al. The effect of red wine on experimental atherosclerosis: lipid-independent protection. Exp Mol Pathol 1999;65:150–9.

[11] Miura Y, Chiba T, Tomita I, et al. Tea catechins prevent the development of atherosclerosis in apoprotein E-deficient mice. J Nutr 2001;131:27–32.

[12] Tijburg LB, Wiseman SA, Meijer GW, Weststrate JA. Effects of green tea, black tea and dietary lipophilic antioxidants on LDL oxidizability and atherosclerosis in hypercholesterolaemic rabbits. Atherosclerosis 1997;135:37–47.

[13] Ross R, Agius L. The process of atherogenesis — cellular and molecular interaction: from experimental animal models to humans. Diabetologia 1992;35(Suppl 2):S34-S40.

[14] Barger AC, Beeuwkes III R, Lainey LL, Silverman KJ. Hypothesis: vasa vasorum and neovascularization of human coronary arteries. A possible role in the pathophysiology of atherosclerosis. N Engl J Med 1984;310:175–7.

[15] Zamir M, Silver MD. Vasculature in the walls of human coronary arteries. Arch Pathol Lab Med 1985;109:659–62.

[16] Williams JK, Armstrong ML, Heistad DD. Vasa vasorum in atherosclerotic coronary arteries: responses to vasoactive stimuli and regression of atherosclerosis. Circ Res 1988;62:515–23.

[17] Kumamoto M, Nakashima Y, Sueishi K. Intimal neovascularization in human coronary atherosclerosis: its origin and pathophysiological significance. Hum Pathol 1995;26:450–6.

[18] Booth RF, Martin JF, Honey AC, Hassall DG, Beesley JE, Moncada S. Rapid development of atherosclerotic lesions in the rabbit carotid artery induced by perivascular manipulation. Atherosclerosis 1989; 76:257–68.

[19] Martin JF, Booth RF, Moncada S. Arterial wall hypoxia following thrombosis of the vasa vasorum is an initial lesion in atherosclerosis. Eur J Clin Invest 1991;21:355–9.

[20] Barker SG, Talbert A, Cottam S, Baskerville PA, Martin JF. Arterial intimal hyperplasia after occlusion of the adventitial vasa vasorum in the pig. Arterioscler Thromb 1993;13:70–7.

[21] Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. Circulation 1999;99:1726–32.

[22] Frankel EN, Kanner J, German JB, Parks E, Kinsella JE. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. Lancet 1993;341:454–7.

[23] Miura Y, Chiba T, Miura S, et al. Green tea polyphenols (flavan 3-ols) prevent oxidative modification of low density lipoproteins: an ex vivo study in humans. J Nutr Biochem 2000;11:216–22.

[24] Sagesaka-Mitane Y, Miwa M, Okada S. Platelet aggregation inhibitors in hot water extract of green tea. Chem Pharm Bull (Tokyo) 1990; 38:790–3.

[25] Wang Z, Huang Y, Zou J, Cao K, Xu Y, Wu JM. Effects of red wine and wine polyphenol resveratrol on platelet aggregation in vivo and in vitro. Int J Mol Med 2002;9:77–9.

[26] Feng AN, Chen YL, Chen YT, Ding YZ, Lin SJ. Red wine inhibits monocyte chemotactic protein-1 expression and modestly reduces neointimal hyperplasia after balloon injury in cholesterol-fed rabbits. Circulation 1999;100:2254–9.

[27] Pendurthi UR, Williams JT, Rao LV. Resveratrol, a polyphenolic compound found in wine, inhibits tissue factor expression in vascular cells: a possible mechanism for the cardiovascular benefits associated with moderate consumption of wine. Arterioscler Thromb Vasc Biol 1999;19:419–26.

[28] Ferrara N, Davis-Smyth T. The biology of vascular endothelial growth factor. Endocr Rev 1997;18:4–25.

[29] Chen YX, Nakashima Y, Tanaka K, Shiraishi S, Nakagawa K, Sueishi K. Immunohistochemical expression of vascular endothelial growth factor/vascular permeability factor in atherosclerotic intimas of human coronary arteries. Arterioscler Thromb Vasc Biol 1999;19:131–9.

[30] Couffinhal T, Kearney M, Witzenbichler B, et al. Vascular endothelial growth factor/vascular permeability factor (VEGF/VPF) in normal and atherosclerotic human arteries. Am J Pathol 1997;150:1673–85.

[31] Clauss M, Gerlach M, Gerlach H, et al. Vascular permeability factor: a tumor-derived polypeptide that induces endothelial cell and monocyte procoagulant activity, and promotes monocyte migration. J Exp Med 1990;172:1535–45.

[32] Melder RJ, Koenig GC, Witwer BP, Safabakhsh N, Munn LL, Jain RK. During angiogenesis, vascular endothelial growth factor and basic fibroblast growth factor regulate natural killer cell adhesion to tumor endothelium. Nat Med 1996;2:992–7.

[33] Marumo T, Schini-Kerth VB, Busse R. Vascular endothelial growth factor activates nuclear factor-kappaB and induces monocyte chemo-attractant protein-1 in bovine retinal endothelial cells. Diabetes 1999; 48:1131–7.

[34] Oak MH, Chataigneau M, Keravis T, et al. Red wine polyphenolic compounds inhibit vascular endothelial growth factor expression in vascular smooth muscle cells by preventing the activation of the p38 mitogen-activated protein kinase pathway. Arterioscler Thromb Vasc Biol 2003;23:1001–7.

[35] Gorlach A, Diebold I, Schini-Kerth VB, et al. Thrombin activates the hypoxia-inducible factor-1 signaling pathway in vascular smooth muscle cells: role of the p22(phox)-containing NADPH oxidase. Circ Res 2001;89:47–54.

[36] Gorlach A, Brandes RP, Bassus S, et al. Oxidative stress and expression of p22phox are involved in the up-regulation of tissue factor in vascular smooth muscle cells in response to activated platelets. FASEB J 2000;14:1518–28.

[37] Brandes RP, Miller FJ, Beer S, et al. The vascular NADPH oxidase subunit p47phox is involved in redox-mediated gene expression. Free Radic Biol Med 2002;32:1116–22.

[38] Bassus S, Herkert O, Kronemann N, et al. Thrombin causes vascular endothelial growth factor expression in vascular smooth muscle cells: role of reactive oxygen species. Arterioscler Thromb Vasc Biol 2001; 21:1550–5.

[39] Hanasaki Y, Ogawa S, Fukui S. The correlation between active oxygens scavenging and antioxidative effects of flavonoids. Free Radic Biol Med 1994;16:845–50.

[40] Miyagi Y, Miwa K, Inoue H. Inhibition of human low-density lipoprotein oxidation by flavonoids in red wine and grape juice. Am J Cardiol 1997;80:1627–31.

[41] Ying CJ, Xu JW, Ikeda K, Takahashi K, Nara Y, Yamori Y. Tea polyphenols regulate nicotinamide adenine dinucleotide phosphate oxidase subunit expression and ameliorate angiotensin II-induced hyperpermeability in endothelial cells. Hypertens Res 2003; 26:823–8.

[42] Lin JK, Chen PC, Ho CT, Lin-Shiau SY. Inhibition of xanthine oxidase and suppression of intracellular reactive oxygen species in HL-60 cells by theaflavin-3,3′-digallate, (−)-epigallocatechin-3-gallate, and propyl gallate. J Agric Food Chem 2000;48: 2736–43.

[43] Khan SG, Katiyar SK, Agarwal R, Mukhtar H. Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: possible role in cancer chemoprevention. Cancer Res 1992;52:4050–2.

[44] Milanini J, Vinals F, Pouyssegur J, Pages G. p42/p44 MAP kinase module plays a key role in the transcriptional regulation of the vascular endothelial growth factor gene in fibroblasts. J Biol Chem 1998;273:18165–72.

[45] Jiang BH, Zheng JZ, Aoki M, Vogt PK. Phosphatidylinositol 3-kinase signaling mediates angiogenesis and expression of vascular endothe-lial growth factor in endothelial cells. Proc Natl Acad Sci U S A 2000;97:1749–53.

[46] Tanaka T, Kanai H, Sekiguchi K, et al. Induction of VEGF gene transcription by IL-1 beta is mediated through stress-activated MAP kinases and Sp1 sites in cardiac myocytes. J Mol Cell Cardiol 2000;32:1955–67.

[47] Rosenkranz S, Knirel D, Dietrich H, Flesch M, Erdmann E, Bohm M. Inhibition of the PDGF receptor by red wine flavonoids provides a molecular explanation for the "French paradox". FASEB J 2002;16:1958–60.

[48] Masuda M, Suzui M, Lim JT, Deguchi A, Soh JW, Weinstein IB. Epigallocatechin-3-gallate decreases VEGF production in head and neck and breast carcinoma cells by inhibiting EGFR-related pathways of signal transduction. J Exp Ther Oncol 2002;2:350–9.

[49] Sartippour MR, Shao ZM, Heber D, et al. Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells. J Nutr 2002;132:2307–11.

[50] Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 2003;92:827–39.

[51] Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. Circ Res 2002; 90:251–62.

[52] Pasterkamp G, Schoneveld AH, Hijnen DJ, et al. Atherosclerotic arterial remodeling and the localization of macrophages and matrix metalloproteases 1, 2 and 9 in the human coronary artery. Athero-sclerosis 2000;150:245–53.

[53] Nguyen M, Arkell J, Jackson CJ. Human endothelial gelatinases and angiogenesis. Int J Biochem Cell Biol 2001;33:960–70.

[54] Stetler-Stevenson WG. Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. J Clin Invest 1999; 103:1237–41.

[55] Zempo N, Kenagy RD, Au YP, et al. Matrix metalloproteinases of vascular wall cells are increased in balloon-injured rat carotid artery. J Vasc Surg 1994;20:209–17.

[56] Jo Y, Yeon J, Kim HJ, Lee ST. Analysis of tissue inhibitor of metalloproteinases-2 effect on pro-matrix metalloproteinase-2 activa-tion by membrane-type 1 matrix metalloproteinase using baculovirus/insect-cell expression system. Biochem J 2000;345(Pt 3):511–9.

[57] Butler GS, Will H, Atkinson SJ, Murphy G. Membrane-type-2 matrix metalloproteinase can initiate the processing of progelatinase A and is regulated by the tissue inhibitors of metalloproteinases. Eur J Biochem 1997;244:653–7.

[58] Takino T, Sato H, Shinagawa A, Seiki M. Identification of the second membrane-type matrix metalloproteinase (MT-MMP-2) gene from a human placenta cDNA library. MT-MMPs form a unique membrane-type subclass in the MMP family. J Biol Chem 1995; 270:23013–20.

[59] Llano E, Pendas AM, Freije JP, et al. Identification and characteriza-tion of human MT5-MMP, a new membrane-bound activator of progelatinase A overexpressed in brain tumors. Cancer Res 1999; 59:2570–6.

8

[60] Pei D. Identification and characterization of the fifth membrane-type matrix metalloproteinase MT5-MMP. J Biol Chem 1999; 274:8925–32.

[61] English WR, Puente XS, Freije JM, et al. Membrane type 4 matrix metalloproteinase (MMP17) has tumor necrosis factor-alpha convertase activity but does not activate pro-MMP2. J Biol Chem 2000;275:14046–55.

[62] Morrison CJ, Butler GS, Bigg HF, Roberts CR, Soloway PD, Overall CM. Cellular activation of MMP-2 (gelatinase A) by MT2-MMP occurs via a TIMP-2-independent pathway. J Biol Chem 2001; 276:47402–10.

[63] Schnaper HW, Grant DS, Stetler-Stevenson WG. Type IV collagenase(s) and TIMPs modulate endothelial cell morphogenesis in vitro. J Cell Physiol 1993;156:235–46.

[64] Haas TL, Davis SJ, Madri JA. Three-dimensional type I collagen lattices induce coordinate expression of matrix metalloproteinases MT1-MMP and MMP-2 in microvascular endothelial cells. J Biol Chem 1998;273:3604–10.

[65] Giannelli G, Falk-Marzillier J, Schiraldi O, Stetler-Stevenson WG, Quaranta V. Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5. Science 1997;277:225–8.

[66] Kato T, Kure T, Chang JH, et al. Diminished corneal angiogenesis in gelatinase A-deficient mice. FEBS Lett 2001;508:187–90.

[67] Maeda K, Kuzuya M, Cheng XW, et al. Green tea catechins inhibit the cultured smooth muscle cell invasion through the basement barrier. Atherosclerosis 2003;166:23–30.

[68] Oak MH, El Bedoui J, Schini-Kerth VB. Thrombin induces the expression of pro-MMP-2 and its conversion to MMP-2 in vascular smooth muscle cells: both responses are inhibited by red wine polyphenols. J Thromb Haemost 2003;(Suppl 1):682.

[69] El Bedoui J, Oak MH, Anglard P, Schini-Kerth VB. Green tea extract strongly prevents thrombin-induced pro-MMP-2 expression and its conversion to MMP-2 by directly inhibiting MT1-MMP in vascular smooth muscle cells. Fundam Clin Pharmacol 2004;18:243.

[70] Oak MH, Anglard P, El Bedoui J, Schini-Kerth VB. Red wine polyphenolic compounds strongly inhibit pro-MMP-2 activation in response to thrombin via direct inhibition of MT1-MMP in vascular smooth muscle cells. Circulation 2004;110:1861–7.

[71] Annabi B, Shedid D, Ghosn P, et al. Differential regulation of matrix metalloproteinase activities in abdominal aortic aneurysms. J Vasc Surg 2002;35:539–46.

[72] Demeule M, Brossard M, Page M, Gingras D, Beliveau R. Matrix metalloproteinase inhibition by green tea catechins. Biochim Biophys Acta 2000;1478:51–60.

[73] Oku N, Matsukawa M, Yamakawa S, et al. Inhibitory effect of green tea polyphenols on membrane-type 1 matrix metalloproteinase. MT1-MMP. Biol Pharm Bull 2003;26:1235–8.

[74] Godichaud S, Krisa S, Couronne B, et al. Deactivation of cultured human liver myofibroblasts by trans-resveratrol, a grapevine-derived polyphenol. Hepatology 2000;31:922–31.

[75] Fotsis T, Pepper MS, Aktas E, et al. Flavonoids, dietary-derived inhibitors of cell proliferation and in vitro angiogenesis. Cancer Res 1997;57:2916–21.

[76] Paper DH. Natural products as angiogenesis inhibitors. Planta Med 1998;64:686–95.

[77] Hsieh TC, Juan G, Darzynkiewicz Z, Wu JM. Resveratrol increases nitric oxide synthase, induces accumulation of p53 and p21 (WAF1/CIP1), and suppresses cultured bovine pulmonary artery endothelial cell proliferation by perturbing progression through S and G2. Cancer Res 1999;59:2596–601.

[78] Favot L, Martin S, Keravis T, Andriantsitohaina R, Lugnier C. Involvement of cyclin-dependent pathway in the inhibitory effect of delphinidin on angiogenesis. Cardiovasc Res 2003;59:479–87.

[79] Martin S, Favot L, Matz R, Lugnier C, Andriantsitohaina R. Delphinidin inhibits endothelial cell proliferation and cell cycle progression through a transient activation of ERK-1/-2. Biochem Pharmacol 2003;65:669–75.

[80] Kojima-Yuasa A, Hua JJ, Kennedy DO, Matsui-Yuasa I. Green tea extract inhibits angiogenesis of human umbilical vein endothelial cells through reduction of expression of VEGF receptors. Life Sci 2003; 73:1299–313.

[81] Yoo HG, Shin BA, Park JC, et al. Induction of apoptosis by the green tea flavonol (−)-epigallocatechin-3-gallate in human endothelial ECV 304 cells. Anticancer Res 2002;22:3373–8.

[82] Kondo T, Ohta T, Igura K, Hara Y, Kaji K. Tea catechins inhibit angiogenesis in vitro, measured by human endothelial cell growth, migration and tube formation, through inhibition of VEGF receptor binding. Cancer Lett 2002;180:139–44.

[83] Iijima K, Yoshizumi M, Hashimoto M, et al. Red wine polyphenols inhibit vascular smooth muscle cell migration through two distinct signaling pathways. Circulation 2002;105:2404–10.

[84] Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995;1:27–31.

[85] Maiti TK, Chatterjee J, Dasgupta S. Effect of green tea polyphenols on angiogenesis induced by an angiogenin-like protein. Biochem Biophys Res Commun 2003;308:64–7.

[86] Cao Y, Cao R. Angiogenesis inhibited by drinking tea. Nature 1999;398:381.

[87] Brakenhielm E, Cao R, Cao Y. Suppression of angiogenesis, tumor growth, and wound healing by resveratrol, a natural compound in red wine and grapes. FASEB J 2001;15:1798–800.

[88] Kimura Y, Okuda H. Resveratrol isolated from Polygonum cuspidatum root prevents tumor growth and metastasis to lung and tumor-induced neovascularization in Lewis lung carcinoma-bearing mice. J Nutr 2001;131:1844–9.

[89] Duthie GG, Pedersen MW, Gardner PT, et al. The effect of whisky and wine consumption on total phenol content and antioxidant capacity of plasma from healthy volunteers. Eur J Clin Nutr 1998;52:733–6.

[90] Lee MJ, Maliakal P, Chen L, et al. Pharmacokinetics of tea catechins after ingestion of green tea and (−)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. Cancer Epidemiol Biomarkers Prev 2002;11:1025–32.

[91] Yang CS, Chen L, Lee MJ, Balentine D, Kuo MC, Schantz SP. Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. Cancer Epidemiol Biomarkers Prev 1998;7:351–4.

[92] Chow HH, Cai Y, Alberts DS, et al. Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. Cancer Epidemiol Biomarkers Prev 2001;10:53–8.

[93] Chow HH, Cai Y, Hakim IA, et al. Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. Clin Cancer Res 2003;9:3312–9.

[94] Tachibana H, Koga K, Fujimura Y, Yamada K. A receptor for green tea polyphenol EGCG. Nat Struct Mol Biol 2004;11:380–1.