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Anti-Angiogenic and Anti-Inflammatory Effects of Statins: Relevance to Anti-Cancer Therapy

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Abstract

Angiogenesis is indispensable for the growth of solid tumors and angiogenic factors are also involved in the progression of hematological malignancies. Targeting the formation of blood vessels is therefore regarded as a promising strategy in cancer therapy. Interestingly, besides demonstration of some beneficial effects of novel anti-angiogenic compounds, recent data on the activity of already available drugs point to their potential application in anti-angiogenic therapy. Among these are the statins, the inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Statins are very efficient in the treatment of hypercholesterolemia in cardiovascular disorders; however, their effects are pleiotropic and some are not directly related to the inhibition of cholesterol synthesis. Some reports particularly highlight the pro-angiogenic effects of statins, which are caused by low, nanomolar concentrations and are regarded as beneficial for the treatment of cardiovascular diseases. On the other hand, the anti-angiogenic activities, observed at micromolar concentrations of statins, may be of special significance for cancer therapy. Those effects are caused by the inhibition of both proliferation and migration and induction of apoptosis in endothelial cells. Moreover, the statin-mediated inhibition of vascular endothelial growth factor synthesis, the major angiogenic mediator, may contribute to the attenuation of angiogenesis.

It has been suggested that the anti-cancer effect of statins can be potentially exploited for the cancer therapy. However, several clinical trials aimed at the inhibition of tumor growth by treatment with very high doses of statins did not provide conclusive data. Herein, the reasons for those outcomes are discussed and the rationale for further studies is presented.

Keywords

Vascular endothelial growth factor; 3-hydroxy-3-methylglutaryl-coenzyme A reductase; nitric oxide; heme oxygenase; apoptosis; endothelium; atherosclerosis; hypercholesterolemia

ABBREVIATIONS

bFGF = Basic fibroblast growth factor; EGF = Epidermal growth factor; eNOS = Endothelial nitric oxide synthase; EPCs = Endothelial progenitor cells; FPP = Farnesylpyrophosphate; GGPP = Geranylgeranyl pyrophosphate; HGM-CoA reductase = 3-hydroxy-3-methylglutaryl-coenzyme A reductase; HMEC-1 = Human microvascular endothelial cells-1; HUVEC = Human umbilical vein endothelial cells; HO-1 = Heme oxygenase-1; IL-8 = Interleukin 8; LPC = Lysophosphatidylcholine; MAPK = Mitogen activated protein kinase; MCP-1 = Monocyte chemotactic protein-1; MMPs = Metalloproteinases; oxLDL = Oxidized low density lipoprotein; PDGF-BB = Platelet-derived growth factor-BB; PI 3-K = Phosphatidylinositol -3-kinase; TGF =

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Transforming growth factor; TNF = Tumor necrosis factor; UPA = Urokinase plasminogen activator; VEGF = Vascular endothelial growth factor

INTRODUCTION

Mediators of Physiological and Pathological Angiogenesis

Tumor growth is strongly dependent on the formation of new blood vessels, which infiltrate the growing mass of tumor cells, providing the oxygen, nutrients and removing metabolites. Without the supply of new blood vessels the size of a tumor can only reach a volume of about 2 mm³ [for a review see: 1] {Heymans, Luttun, *et al.* 1999 #620} as diffusion of oxygen can occur at the distance of only 100–200 mm. The decreasing oxygen tension in the growing tumor leads to hypoxia, one of the strongest stimuli for the expression of mediators of neovascularization.

Blood vessels are formed in three different ways, namely vasculogenesis, angiogenesis and arteriogenesis [2]. The first one, vasculogenesis, relies on *de novo* establishment of capillaries from endothelial progenitor cells [3,4]. This mode of growth is predominant during embryonic development, though it occurs also in the adult organism. It has been demonstrated that circulating endothelial progenitor cells contribute to the formation of neovessels in ischemic tissues, in the ovary during ovulation and corpus luteum formation, or during wound healing and atherosclerotic plaque growth. Some data also indicate *de novo* formation of blood vessels in growing tumors [1, 5].

Angiogenesis is the formation of new capillaries from preexisting blood vessels and this is the main way in which blood vessels are created [for a review see: [2]. Angiogenesis is thus distinct from vasculogenesis. The process is initiated by the dissolution of endothelial basement membrane by proteinases. Their action weakens the tight contact of endothelial cells with the basement membrane and underlying mural cells, thus changing the phenotype of the endothelial cells, which become permissive to the activity of growth factors.

Among the latter, the most important in tumor angiogenesis appears to be vascular endothelial growth factor A (VEGF-A), which is also indispensable for physiological and reparative angiogenesis. Its expression is significantly enhanced or induced by numerous mediators, including hypoxia, inflammatory cytokines, other growth factors, such as basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), transforming growth factor (TGF), platelet-derived growth factor-BB (PDGF-BB), as well as such mediators as nitric oxide, reactive oxygen species and prostaglandins [for reviews see: 6, 7]. Finally, the development of mature blood vessels from capillaries occurs in the process of arteriogenesis [for a review see: 2].

Angiogenesis in Tumors

The idea of blocking tumor growth by the inhibition of angiogenesis was put forward in the early 70's by Judah Folkman [8]. The feasibility of this attractive, although initially criticized approach was finally confirmed by demonstration of the efficacy of anti-angiogenic strategy in several experimental models. The discovery of endostatin, angiostatin and other endogenous inhibitors of angiogenesis has surged the hope for the potential application of those compounds in the therapy of cancer in humans. Unfortunately, although several trials of anti-angiogenic approach have been initiated so far (Table 1), the expectations have not yet been fulfilled [for a review see: [9]. Nevertheless, recent randomized clinical trials have demonstrated a significant, although still modest prolongation in the survival rate of patients with colon and kidney cancers who received Avastin, a humanized anti-VEGF monoclonal antibody [10–12]. These promising results

represent only a portion of different approaches aimed to block the growth of tumor blood vessels. Thus, further studies are warranted to elucidate both the background of tumor resistance to this type of treatment and to find new targets for anti-angiogenic therapy. Interestingly, a recent demonstration that several statins, inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, can influence angiogenesis and inhibit experimental tumor growth has suggested their possible application in anti-cancer therapy.

WHAT ARE STATINS

Statins are the most widely prescribed drugs, used for the treatment of hypercholesterolemia and resulting cardiovascular diseases. The potency of statins has been demonstrated in several large randomized clinical trials, and recently those drugs are the first choice for the reduction of cholesterol level [for reviews see: 13, 14]. Statins are competitive inhibitors of HMG-CoA reductase, the enzyme which catalyses the rate-limiting step in cholesterol synthesis, a four-electron reductive deacylation of HMG-CoA to CoA and mevalonate. Statins inhibit HMG-CoA reductase reversibly by binding to the enzyme's active site. The K_M for statins is in the nanomolar range, and they effectively displace HMG-CoA, the natural substrate, which binds at micromolar concentrations [15].

The inhibition of mevalonate synthesis leads to dramatic reductions in both cholesterol and its isoprenoid intermediates: farnesylpyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP) (Fig. (1)). Additionally, statins may influence the level of cholesterol by the upregulation of hepatic LDL expression and, thereby, enhanced cholesterol clearance [for a review see: 13].

A decrease in cholesterol level is undoubtedly beneficial for patients with cardiovascular diseases, but a vast accumulation of evidence indicates that the effects of statins extend beyond their cholesterol lowering properties.

Non-Cholesterol Dependent Effects of Statins

The reduction in FPP and GGPP decreases isoprenoid formation, affecting in this way the processes of posttranslational protein isoprenylation. It is estimated that about 0.5–1% of protein can be modified in that way [cited after [16], thus the effects of statins may be much broader than could be expected only from the decrease in cholesterol level.

Isoprenoid intermediates serve as important lipid moieties for the post-translational modification of a variety of proteins. Members of the Ras and Rho GTPase family are major substrates for the post-translational modification by prenylation. The others include heme-A, nuclear lamins, the gamma-subunit of heterodimeric G-proteins and other small guanosine triphosphate (GTP)-binding Ras-like proteins, such as Rab, Rac, Ral and Rap [for a review see: 13].

The main targets of statins, Ras and Rho, are small GTP-binding proteins, which cycle between the inactive GDP-bound and active GTP-bound conformational states. In endothelial cells, Ras translocation from the cytoplasm to plasma membrane is dependent on farnesylation, whereas geranylgeranylation is required for Rho translocation. By inhibiting both Ras and Rho isoprenylation, statins cause accumulation of inactive forms of both those proteins in cytoplasm [13].

Interestingly, it has been observed that some beneficial effects of statins, i.e. improvement in cardiovascular functions, have occurred much earlier than the level of cholesterol was decreased [17, 18]. It is supposed that those effects are to a significant extent dependent on the enhancement of endothelial nitric oxide synthase (eNOS) expression and/or activity,

which results in a decrease in platelet activation, attenuation of adhesion molecules expression, decrease in inflammatory cytokines production and an increase in reactive oxygen species generation [for a review see: 19].

Indeed, small G-proteins Rho and Rac influence eNOS expression and NO availability. Rho negatively regulates eNOS expression and Rac contributes to NAD(P)H-oxidase activation and superoxide production, what inactivates NO. Statins inhibit both Rho and Rac GTPase activity via inhibition of geranylgeranylation, leading to eNOS upregulation [for a review see: 20]. Such an effect on eNOS can be mimicked by hydroxyfasudil, a Rho-kinase inhibitor or by *Clostridium botulinum* C3 transferase [21], which confirm the role of geranylgeranylation in modulation of eNOS expression [22].

Pharmacokinetics Properties of Statins

Nine statins have been tested for their clinical applications. The prototype is mevastatin, which was first isolated from *Penicillium citrinum*. Lovastatin is another natural statin produced by *Aspergillus terreus*. Pravastatin and simvastatin are chemically modified derivatives of lovastatin, while fully synthetic statins include atorvastatin, fluvastatin, cerivastatin, pitavastatin and rosuvastatin, and they are much more potent than the mevastatin derivatives.

Currently, treatment protocols employ lovastatin (Mevacor, Merck Frosst), simvastatin (Zocor, Merck Frost), atorvastatin (Lipitor, Pfizer), pravastatin (Pravachol, Bristol Meyer-Squibb), fluvastatin (Lescol, Novartis), rosuvastatin (Crestor, Astra Zeneca) and pitavastatin (Livalo, Kowa Company). Cerivastatin has been withdrawn from the market due to fatal cases of rhabdomyolysis. Also, along with license expiration the generic drugs have been introduced. Statins are prescribed at various doses, usually in the range from 10–80 mg/day, depending on the type of statin [16] (Table 2). Only cerivastatin, due to its high potency has been prescribed in lower doses, 0.2–0.4 mg per day, what, however, did not prevent its toxicity in some patients.

Statins differ in their tissue permeability and metabolism. Lipophilic statins, such as simvastatin, are considered to enter endothelial cells by passive diffusion more efficiently than hydrophilic statins, such as pravastatin and rosuvastatin, which are primarily targeted to the liver. Moreover, statins are bound by serum proteins and their bioavailability is limited. The plasma concentrations of statins in patients treated for lipid disorders are in the nanomolar range. Serum levels of statins ranged between 2 and 50 nmol/l for cerivastatin (when it was given at doses of 0.2 to 0.8 mg/day) [23, 24] and between 2 and 200 nmol/l for atorvastatin, which is prescribed at doses of 10–80 mg/day [25]. Such nanomolar concentrations of several statins demonstrated significant biological effects, also *in vitro*, indicating that despite limited bioavailability, the drugs are effective at such low concentrations. Meanwhile, in numerous *in vitro* studies the effects of very high, above 10 μ M, concentrations of statins have been tested. This raises the questions of the physiological relevance of such experiments, as the concentrations above 10 μ M are not attained *in vivo* in patients treated for lipid disorders. On the other hand, in experimental trials aimed at demonstrating the anti-cancer effect of statins much higher doses of those drugs have been given, exceeding even 100 times those used for treatment of hypercholesterolemia. However, even in such situations the plasma concentrations of statins did not exceed a few micromoles per litre [26].

POTENTIAL ANTI-TUMOR EFFECTS OF STATINS

It has recently been suggested that extra hepatic effects of statins might play a potentially beneficial role in cancer therapy. The presumption is based on experimental data obtained

both *in vitro* and *in vivo* [for reviews see: 16, 27], [28] and in some clinical observations. Regarding the latter, reduced number of deaths from cancer occurred among patients involved in several large trials of statins for prevention of cardiovascular events [29] (Table 3). Recent follow-up studies in patients 5 years after closure of 4S study (Scandinavian Simvastatin Survival Study), demonstrated no difference in the mortality from incidences of cancer between the original simvastatin group and placebo group [30]. However, during the 10 year follow-up, mortality from cancer, forming the largest proportion of non-cardiovascular deaths, appeared lower in the original simvastatin group than in the original placebo group, although this difference was not significant [30]. Also, recent analysis of database containing of more than 3 000 statins users and almost 17 000 matched controls revealed that the usage of statins was associated with 20% reduction of cancer risk and the data suggest that statins are protective when used longer than 4 year [31].

The majority of other large trials with statins for the treatment of hypercholesterolemia did not demonstrate significant differences in the incidence of cancer in comparison with placebo groups (Table 3). However, in a PROSPER study incidences of newly diagnosed cancers were more frequent in patients taking pravastatin as compared with non-treated subjects [32]. Of note, in that study the average age of participants was 75 years at baseline, which was unique in comparison to other studies.

Some additional information on the influence of statins in cancer derives also from smaller studies. For example, a potential beneficial effect of statins on melanoma has been suggested [33]. Another randomized study demonstrated that pravastatin at 40 mg/day, which is a standard dose for lipid lowering therapy, prolonged the survival of patients with advanced hepatocellular carcinoma [34], while a non-randomized prospective study showed that incidence of breast cancer was almost two times lower among statin users than among non-users [35]. Also Blais *et al.* [36] reported that probability of having any cancer was 28% lower in patients treated with statins than in users of bile acid binding resins. Finally, of particular interest, the recent retrospective analysis of 1953 patients with colorectal cancer and 2015 control patients without cancer showed that use of statins for at least five years was associated with a 47 percent relative reduction in the risk of cancer [37]. However, the number of patients taking statins in this study was small (6.1% of patients with cancer and 11.6% of control group). Therefore, further investigation of the overall benefits of statins is necessary before any conclusive recommendations of statins as chemopreventive agents against colorectal cancer.

On the other hand an increased risk of cancer has been associated with pronounced cholesterol lowering [37, 38]. Those data parallel the results of some animal experiments, which demonstrated that statins can increase the frequency of certain cancers in rodents, especially when used at high doses [for a review see: 27]. In those studies a rise in hepatocellular tumors, pulmonary adenomas, liver adenomas and carcinomas, thyroid neoplasms in rats, and forestomach squamous papillomas and several other tumors in mice and rats have been observed [27].

Importantly, the clinical response to lovastatin in treatment of cancers, if occurred, required very high doses, 20–35 mg/kg/day [26, 39, 40]. Such doses, even if given for a short time, usually a week followed by a two week break, may increase the risk of complications associated with statin therapy, such as rhabdomyolysis. Indeed, one study, in which 10–20 mg/kg/day of lovastatin was administered to acute myelogenous leukemia patients had to be halted due to the drug-related toxicities [41]. As rhabdomyolysis has occurred in about 40 patients who died after taking cerivastatin, the safety of application of very high doses of statins has to be taken into consideration.

In sum, the majority of clinical trials aimed to demonstrate antitumoral effect of statins did not provide conclusive results (Table 4) [for a review see also: 27] and in our view should be considered as negative.

Molecular Basis for Potential Effect of Statins on Tumor Growth

The effect of statins on Ras and Rho protein prenylation explains the effect on tumor cells [for reviews see: 16, 42]. The Rho family of small GTPases coordinates many aspects of cell motility through the reorganization of actin cytoskeleton and changes in gene transcription [43]. Additionally, Ras activation due to Ras mutations has been detected in approximately 30% of human cancers [44], thus by interfering with Ras farnesylation, statins may influence tumor cell growth and differentiation [for a review see: 28].

In vitro studies have shown that statins can inhibit proliferation and trigger apoptosis of tumor cells [for reviews and references see: 16, 42]. Such an effect has been observed in acute myelogenous leukemia cells (AML), juvenile myelomonocytic leukemia, squamous carcinoma of the cervix, rhabdomyosarcoma, medulloblastoma, mesothelioma, astrocytoma, pancreatic tumor, neuroblastoma and several other tumors [for reviews see: [16, 42]. The growth arrest appears to be p53-independent and is mediated by down-regulation of cyclin-dependent kinase-2 (CDK-2) activity with concomitant up-regulation of CDK inhibitors p²¹Cip1 and/or p²⁷Kip1 [45]. However, such an activity is attained only at high micromolar concentrations of statins, which inhibit the proteasome functions [45, 46]. It has been suggested that this effect is independent of HMG-CoA reductase inhibition, as it can be mediated even by a closed ring, pro-form of lovastatin or simvastatin [46]. However, the data are not clear and the mechanism remains poorly defined [for discussion and references see: 16].

Experimental evidences indicate that cells must proliferate to be sensitive to statin-induced apoptosis [16]. However, there are tumor cells, such as breast and prostate cancer cells, which even at the proliferating phase were not sensitive to statin-induced apoptosis [47, 48]. The mechanisms of induction of apoptosis may involve down regulation of bcl-2, caspase activation and PARP cleavage, as has been shown for statin-sensitive AML and colon cells [49–52]. The results also suggest that statins trigger apoptosis by modulating several signaling pathways, including the Raf/MEK/ERK cascade [53].

Statins trigger apoptosis by blocking protein geranylgeranylation [28, 54]. The isoprenylation is also essential for Rho-mediated invasion of various tumors [55], including pancreatic, colon and gastric cancer cells [55–57], melanoma cells [58] and breast cancer cells [59]. Accordingly, it has been shown that atorvastatin (3 μ M) inhibited Rho activation and reverted the metastatic phenotype of human melanoma cells *in vitro* [60]. Also other studies on pancreatic cancer, melanoma and mammary carcinoma cells demonstrated that statin treatment interferes with RhoA membrane localization [61, 62].

Only GGPP was able to revert the pro-apoptotic effect of statins [reviewed in: 16], while FPP had little or no effect in a variety of cell systems. In agreement with the role of geranylgeranylation also the specific blockers of geranylgeranyltransferase have demonstrated anti-tumor effect. Thus, GGTI-298 mimicked the effect of lovastatin in human AML cells, while the farnesyl transferase inhibitor (FTI 277) was much less effective in triggering apoptosis in AML cells [48]. However, recent studies have also suggested that the inhibition of Ras farnesylation by specific farnesyltransferase blockers may be considered for anti-cancer therapies [for a review see: [28].

It has to be emphasized, however, that in the studies determining the effects of statins on tumor cell apoptosis very high concentrations of drugs have been often used. In one study

the concentrations of 10 μM of fluvastatin, atorvastatin, lovastatin, pravastatin and simvastatin have been reported to inhibit the proliferation of breast cancer cells by only 15–25% [63]. Meanwhile, the potent inhibitory effect of those statins (except for pravastatin) on proliferation of MCF-7 breast cancer cells has been reported in another recent study, but 90% attenuation has been attained at 50 μM concentration [64]. Similarly, proliferation and migration in primary cultured human glioblastoma cells has been reduced about 30–40% in cells treated with 100 μM or higher concentrations of statins [65]. Many other studies reported the inhibitory effect of lower, but still micromolar concentrations of statins in the case of various tumors [for reviews see: 16, 27]. Such levels of statins may not be attainable *in vivo*. On the other hand, in one recent study low, i.e. equal or less than 1 μM concentration of lovastatin, attenuated TNF-induced colon carcinoma cell invasion *in vitro* [66]. Also atorvastatin at relatively low, 3 μM concentrations diminished migration of melanoma cells [60]. A cytostatic effect of nanomolar concentrations of lovastatin has been also observed in one of the earliest studies [67]. Thus, it is possible that low concentrations of statins which are attained in the plasma of patients could have some anti-tumor effects.

As mentioned, the plasma concentrations of statins in patients receiving those drugs because of hypercholesterolemia are much lower than usually used in experimental studies. On the other hand, it is conceivable that anti-cancer therapy may require much higher doses. However, prolonged administration of lovastatin at the maximum recommended dose, i.e. 80 mg/kd/day, resulted in a serum steady-state concentration of an active drug at the level of 0.15–0.3 μM , while the peak serum concentration reached 3.92 μM [26]. Unfortunately, despite such large doses, the effect of statins on tumor growth was minimal.

Effects of Statins on Angiogenesis

Besides inhibiting tumor growth by direct action on tumor cells, drugs that would affect angiogenesis might be particularly useful in the treatment of cancer. Interestingly, biphasic effects of statins on angiogenesis have been observed and these have been ascribed to opposite actions of low (nanomolar) versus high (low micromolar, i.e. 1–10 μM) drug concentrations [68].

Pro-angiogenic activities of statins are due to their effects on both mature endothelial cells and endothelial progenitor cells, which are protected from senescence and apoptosis even by low nanomolar concentrations of statins [69, 70]. At the molecular level those protective activities of statins are mostly ascribed to the stimulation of the PI3-Akt kinase pathway, resulting in the phosphorylation of eNOS, a critical mediator of angiogenic and anti-apoptotic activity in endothelial cells [71, 72]. The phosphorylation of Ser 1177 of eNOS by Akt is dependent on statin-mediated recruitment of Akt to the eNOS complex by hsp90 chaperone protein [73–75]. On the other hand, higher, micromolar concentrations of statins may exert weak or no effect on Akt kinase phosphorylation [72], although Kureishi *et al.* noted that a 1 μM concentration of simvastatin enhanced Akt phosphorylation in HUVEC cells, the effect was claimed to be responsible for inhibition of apoptosis [76]. In accordance with the involvement of eNOS in pro-angiogenic and anti-apoptotic activities of statins, are recent data demonstrating that these effects are abolished in eNOS knockout mice [77].

Statins have also been reported to enhance the expression of eNOS, thereby preventing in this way downregulation of eNOS mRNA content induced by hypoxia [78], TNF [78, 79] or oxidized LDL (oxLDL) [80]. Interestingly, the concentrations of statins that enhance eNOS expression appears to be already anti-angiogenic [81]. The biological significance of such an induction of eNOS is not clear, but it may represent the protective mechanisms that are activated in the endothelial cells in the presence of high statin concentrations.

In a recent study, the effects of various statins on HUVEC proliferation induced by LPC and oxLDL were compared. In that work cerivastatin, simvastatin and fluvastatin caused a dose-dependent inhibition of endothelial cell growth [82]. The strongest inhibition of HUVEC proliferation was achieved at statin concentrations of 0.1 $\mu\text{mol/l}$ (cerivastatin), 2.5 $\mu\text{mol/l}$ (simvastatin) and 1 $\mu\text{mol/l}$ (fluvastatin). Interestingly, cell proliferation induced by oxLDL (10 $\mu\text{g/ml}$) and LPC (20 $\mu\text{mol/l}$) could be effectively prevented using even lower statins concentrations, i.e. between 0.01 and 0.1 $\mu\text{mol/l}$ (cerivastatin), 1 and 2.5 $\mu\text{mol/l}$ (simvastatin), and 0.25 and 1 $\mu\text{mol/l}$ (fluvastatin). Similar effects have been observed by others [83,84]. This study also parallels a previous one, which demonstrated that simvastatin enhanced oxLDL induced cytotoxicity in endothelial cells [85].

In other experiments, the antiproliferative effects of four statins on human endothelial cells were compared and all four compounds tested, i.e. atorvastatin, fluvastatin, lovastatin, and simvastatin, inhibited cell proliferation [86]. Nearly complete growth blockade was achieved at the concentration of 10 μM [86]. The authors were able to demonstrate that the antiproliferative effect of statins is not due to induction of necrosis but rather induction of apoptosis, which appears to be mediated by enhanced caspase activity [86].

The effects of several statins on the viability of cultured rat pulmonary vein endothelial cells were also recently tested [87]. It has been shown that hydrophobic lovastatin, simvastatin, atorvastatin, fluvastatin and cerivastatin, but not hydrophilic pravastatin, markedly reduced viability of cultured rat pulmonary vein endothelial cells leading to apoptosis, as suggested by DNA fragmentation, DNA laddering, and activation of caspase-3 [87]. This effect was associated with the activation of apoptosis-related intracellular signal transduction systems: attenuation of localization of RhoA to the membrane, induction of Rac1, and an increase in phosphorylation of c-Jun N-terminal kinase and c-Jun [87].

In another recent study, Feng and co-workers [88] observed that atorvastatin at a 10 μM concentration decreased the number of viable HUVECs by approximately 50% after 24 hours of incubation. Microvascular cells appeared more resistant, although cytotoxicity has been observed after prolonged, 48-hour, incubation. Interestingly, the authors did not observe any effect of 10 μM atorvastatin on the growth of primary or NIH 3T3 fibroblasts and, moreover, atorvastatin failed to induce cell death in head and neck squamous carcinoma cells [88].

In some studies, even lower concentrations of statins seemed to induce apoptosis in endothelial cells. For example, fluvastatin was proapoptotic for EAhy926 endothelial cells already at the concentration of just 1–2 μM [89], while enhanced apoptosis of HUVEC has been obtained in the presence of 1 μM atorvastatin [90]. Our data indicate that a concentration of atorvastatin above 1 μM is pro-apoptotic for microvascular endothelial cells (unpublished). Thus, one can suggest that the anti-angiogenic effect of statins is to a significant extent related to the induction of endothelial cell apoptosis. Nevertheless, although widely investigated, the field is far from clarity. For example, the anti-apoptotic effect of simvastatin on differentiated endothelial cells (HUVEC) has been claimed by some researches to occur at 1 μM concentration [76], whereas others reported that the same low micromolar concentration has pro-apoptotic activity [72, 90].

The influence of statins on vascular cells appears to be also cell type dependent, as reflected in various effects on Akt kinase activity. Activation of Akt has been observed in macrovascular endothelial cells [90, 91], whereas an inhibition has been noted in microvascular endothelial cells [73] and vascular smooth muscle cells [92, 93]. Atorvastatin has been shown to enhance eNOS phosphorylation in HUVEC but not in microvascular

endothelial cells, and, consequently, this statin promoted tube formation in macro- but not microvascular endothelial cells [73].

Endothelial cells seem to be quite sensitive to statin-induced apoptosis. However, clinical data do not confirm such an influence in patients with cardiovascular diseases. Rather the improvement in endothelial functions is a common feature, leading to the conclusion that real effect of statins are exerted at nanomolar concentrations. If micromolar concentrations would be effective in cancer treatment, then one should be concerned about the potential damage to the endothelium and the risk of cardiovascular complications.

Effects of Statins on Progenitor Endothelial Cell –Relevance to Cancer Therapy

Bone marrow-derived endothelial progenitor cells (EPC) constitute the source of cells which by initiating the formation of new capillaries, may influence the vasculature in tumors [94, 95], atherosclerotic plaques, epidermal wounds, ischemic tissues, as well as stimulate regeneration of endothelium in vessels injured by balloon angioplasty. Therapy with statins increased the numbers of EPC [69] and might have induced the formation of new blood vessels by promoting the proliferation, migration and survival of circulating EPCs [71]. Statins rapidly mobilize EPCs from the bone marrow and accelerate vascular structure formation via activation of PI3/Akt and eNOS, thus the mechanisms of pro-vasculogenic actions of statins appears in this case to be similar to stimulation of mature endothelial cells [76, 96].

Statins have been shown to prevent the deterioration of endothelial progenitor cells in atherosclerotic settings. Thus, 1 μ M atorvastatin protected against oxLDL-mediated impairment of EPC differentiation. Additionally, it prevented the oxLDL induced senescence of EPCs [97].

Nevertheless, so far, no convincing effects of statins on EPC in tumors have been demonstrated. On the other hand, some studies suggested that peripheral blood “EPC” are derived from monocyte/macrophages [98]. Therefore, inhibition of MCP-1 production by cerivastatin, as observed in a study by Zhu *et al.* [99] might also influence the pro-angiogenic effects on EPC. In that study, second hand smoking significantly enhanced tumor angiogenesis and the number of circulating progenitor cells, while cerivastatin (2.5 mg/kg/day) demonstrated some inhibitory effects. Further investigations are, however, necessary.

Effect of Statins on VEGF and Other Angiogenic Mediators

VEGF is a major angiogenic mediator involved in the growth of majority, if not all, tumors [for review see: 7]. Therefore, inhibition of VEGF production and/or its effects on endothelial cells is considered as one of the main target for anti-angiogenic therapy.

The downregulation of VEGF synthesis by statins has been shown recently in different cell types, including tumor cells [100], endothelial cells [101] and vascular smooth muscle cells [81, 102, 103]. In studies using ras transformed NIH 3T3 tumor cells high concentrations of lovastatin (12 and 24 μ M) were used [100]. Similar doses of mevastatin (25 μ M) completely blocked bFGF-induced VEGF expression in cultured rat primary endothelial cells [101]. Also, cerivastatin at high concentration (0.5 μ M) diminished VEGF synthesis in primary human dermal microvascular endothelial cells [68]. Likewise, we have shown that basal and induced synthesis of VEGF in vascular smooth muscle cells and human microvascular endothelial cells (HMEC-1) were significantly decreased by micromolar concentrations of statins [81]. Furthermore, in HMEC-1 the moderate inhibitory effect on VEGF production can be attained at 0.1–1 μ M of atorvastatin, which is within the range or close to concentrations measured in human plasma (our unpublished data).

Interestingly, as we [102] and others [104] have demonstrated, statin therapy may also decrease the circulating VEGF levels. Moreover, when blood plasma collected from patients before and two months after atorvastatin therapy was added to HUVEC, the stimulation of endothelial cells proliferation by plasma after therapy was lower, indicating the potential anti-angiogenic effect of statins used at doses relevant to cardiovascular diseases [102]. However, there are also reports demonstrating the stimulatory effect of statins on VEGF production. The induction of VEGF generation has been shown in osteoblasts, what is of potential relevance to the protective influence of statins on bone growth. Maeda and co-workers [105,106] have observed that a 100 nM concentration of simvastatin or 10 nM cerivastatin markedly increased VEGF mRNA expression in a nontransformed osteoblastic cell line (MC3T3-E1). Pretreating MC3T3-E1 cells with mevalonate or geranylgeranyl pyrophosphate abolished simvastatin-induced VEGF mRNA expression, while manumycin A, a protein prenylation inhibitor, mimicked statin effects on VEGF expression [106]. Higher, i.e. micromolar concentrations of simvastatin also augmented VEGF production in a dose-dependent manner. The activity of simvastatin was blocked by mevalonate or by pretreatment with wortmannin and LY294002, specific phosphatidylinositol-3 kinase inhibitors [106]. Thus, statins stimulate VEGF expression in osteoblasts via reduced protein prenylation and by activation of the phosphatidylinositol-3 kinase pathway, promoting osteoblastic differentiation. This may hold promise for the treatment of osteoporosis in the future.

In contrast to ours [81] and others [68] data demonstrating decreased VEGF production by statin-treated vascular smooth muscle cells, Takenaka *et al.* [107] observed that simvastatin significantly stimulated VEGF release in a dose-dependent manner in A10 rat vascular smooth muscle cells. The stimulation was, however, visible, at high, micromolar concentrations of the drug. Also, in another recent study, simvastatin enhanced VEGF expression in hearts of apoE knockout mice, improving vascularization [108]. Phosphorylation of anti-apoptotic Akt is lower, whereas phosphorylation of proapoptotic p38 mitogen-activated protein kinase (MAPK) is higher in the ApoE^{-/-} mice compared with controls. Simvastatin did not change the lipid profile but blocked p38 MAPK phosphorylation in the ApoE^{-/-} myocardium. Concomitantly, enhanced expression of VEGF and its receptor VEGFR-2 mRNAs as well as increased production of eNOS have been observed in those mice [108].

In another recent study, human dermal microvascular endothelial cells (HDMECs) were stimulated with fluvastatin [109]. The experiments demonstrated that fluvastatin increased the expression of Id1 protein (inhibitor of differentiation), which is involved in the control of cell cycle progression, and thereby could delay cellular differentiation and senescence. No effect on VEGF production has been observed, and fluvastatin treatment neither led to phosphorylation of Akt kinase nor regulated p21 and p27, and only slightly affected p53 [109].

In summary, stimulatory effect of statins on VEGF production in macrovascular endothelial cells (HUVEC) [81], vascular smooth muscle cells [107], or osteoblasts [105, 106] has been observed mostly under high concentrations of statins. The significance of such an induction by statins at concentrations, which might already be pro-apoptotic, is unknown. It might be speculated that the stimulatory effects of statins on VEGF synthesis under such conditions reflects a form of nonspecific stress response. Cell type-specific effects have to also be taken into consideration.

Besides influencing VEGF synthesis the anti-angiogenic effects of statins at micromolar concentrations may arise from interference with other angiogenic mediators. In our hands, atorvastatin at 0.1–1 μ M decreased urokinase plasminogen activator (uPA) synthesis and

interleukin-8 (IL-8) production (our unpublished results). As uPA activity is required for the VEGF- and bFGF-induced angiogenesis [110], and since in animals devoid of the *uPA* gene neovascularization was significantly impaired in comparison to the wild type counterparts [111], one may speculate that decrease in this angiogenic mediator by statins may add to their inhibitory effect on tumor growth. Similarly, attenuation of IL-8 synthesis may have an anti-angiogenic outcome. It is also possible that by reducing T lymphocyte activation [112, 113], statins may interfere with pro-angiogenic actions mediated by inflammatory reactions. Other anti-angiogenic effects of statins may include the inhibition of the expression of monocytes/macrophage chemoattractant protein-1 (MCP-1) [114] and metalloproteinase-1 [115–117] by endothelial cells.

Matrix metalloproteinases (MMPs) degrade the extracellular matrix component. They are implicated in a wide variety of pathological process, including atherosclerosis and cancer [for a review see: 118]. Modulation of MMPs production and activity can also be a part of the pleiotropic effects of statins. Indeed, they inhibited the synthesis of MMP-1, -2, -3 and -9 in vascular smooth muscle cells [119] and MMP-1 in endothelial cells [115]. The production of MMP-1 [120] as well as MMP-9 [121] was also decreased in macrophages. Some studies also indicate that statins can influence the release of metalloproteinases by cancer cells. The production of MMP-9 by fibrosarcoma cells [122] and v-Ha-Ras transformed NIH 3T3 fibroblasts [123] was diminished by simvastatin and lovastatin, respectively. Also, the production of MMP-9 and uPA was attenuated by cerivastatin in highly invasive MDA-MB-231 breast cancer cells [124]. Thus, inhibitory effects of statins on MMP secretion by tumor cells may impede tumor metastasis, while the same effect in endothelial cells may attenuate their angiogenic activity.

Anti-Inflammatory Effects of Statins

According to current knowledge, the attenuation of inflammatory response in vascular the wall and diminishing the inflammatory properties of circulating leukocytes constitute a significant part of the beneficial pleiotropic effects of statins. The decrease in synthesis of inflammatory cytokines and pro-adhesive molecules present on endothelial cells [125] as well as the attenuation of proatherogenic Th1 response [126, 127] may comprise the mechanism for the beneficial pleiotropic effects of statins. However, one of the most important anti-inflammatory actions of statins is related to the enhancement of the expression of eNOS.

The effect of statins on the expression of endothelial NO synthase has been demonstrated for the first time by Laufs [80]. Thus, as mentioned earlier, statins increase endothelial NO production by upregulating eNOS. The effect is mediated by enhancement of the eNOS mRNA stability but not eNOS transcription [78]. Interestingly, the effect of statins on eNOS is reversed by GGPP but not by FPP, which is consistent with a non-cholesterol lowering effect of statins and suggests that inhibition of Rho by statins mediates the increase in eNOS expression [for a review see: [13].

Statins can also enhance eNOS availability by influencing the caveolin-1, the protein that binds to eNOS in caveole, negatively regulating its activity [128]. The enhancement of NOS activity may be pro-angiogenic, as NO can stimulate the production of VEGF [for a review see: 6] and is required for the pro-angiogenic effect of VEGF in endothelial cells [for reviews see: [129, 130]. However, if such an effect of statins would operate in tumor endothelial cells, then statins could potentially enhance tumor growth. Fortunately, the clinical data obtained from analysis of patients undergoing statin therapy did not provide evidence for such a risk.

Recently, a number of studies have demonstrated the significant role of heme oxygenase-1 (HO-1) in protecting vascular wall cells from oxidative injury [for reviews see: [131, 132]. HO-1 is a stress-inducible enzyme degrading heme to carbon monoxide, iron and biliverdin, which appear to be more than merely by-products of heme degradation. Indeed, at least part of HO-1 activities are very similar to the vasculoprotective role of NO [for reviews see: [133, 134]. Moreover, we [132, 135–136, 137] and others [138–141] have shown that HO-1 is involved in the augmentation of VEGF synthesis and angiogenesis. Other evidence also suggests that overexpression of HO-1 can augment tumor angiogenesis and protect tumor cells from oxidative injury including that induced by cytotoxic drugs [142–145]. Therefore, it can be hypothesized that specific targeting of HO-1 in tumors or tumor endothelial cells could be regarded as new type of anti-angiogenic strategy. Interestingly, recent studies suggested that statins can upregulate HO-1. For example, Lee *et al.* [146] have demonstrated that simvastatin at concentrations of 1–10 μM induced HO-1 expression in human vascular smooth muscle cells, but, interestingly, neither in human macrophages nor in human endothelial cells. On the other hand, Grosser *et al.* [147, 148] have demonstrated the induction of HO-1 by statins in ECV304 cells, which they claimed to be endothelial cells but which are in fact identical to a bladder cancer cell line, T24/83 [149]. Moreover, in the latter studies, extremely high concentrations of statins (even above 100 μM) have been applied, raising the concern of physiological relevance. Thus, the data on the effect of statins on HO-1 expression in tumor cells are still incomplete and it remains to be established whether statin treatment may have any effect on HO-1 expression in neoplastic cells and whether this may influence tumor growth and the response to therapy.

VARIOUS EFFECTS OF STATINS ON ANGIOGENESIS IN ISCHEMIC TISSUES AND TUMORS - POTENTIAL IMPLICATIONS FOR THERAPY

Evidence available so far demonstrates the conflicting and potentially opposing effects of statins on angiogenesis and tumor growth, suggesting on one hand the stimulation of angiogenesis in ischemic cardiovascular complications while on the other, the inhibition of tumor growth. In an angiogenesis disc system, used by Weis *et al.* [68] inflammation-induced neovascularization in normocholesterolemic mice was enhanced with low dose of cerivastatin (0.5 mg/kg/day), but was significantly inhibited by high concentrations of either cerivastatin or atorvastatin (2.5 mg/kg/day). Weis and co-workers have also observed that high dose of cerivastatin decreased Lewis tumor growth in C57Bl/6 mice and attenuated tumor vascularization. Also Vincent *et al.* [116] demonstrated that cerivastatin at 2.5 mg/kg/day inhibited endothelial cell proliferation and *in vivo* angiogenesis in matrigel and in chick chorioallantoic membrane. Additionally, Zhu *et al.* shown recently that cerivastatin suppressed by approximately two-thirds the stimulatory effect of second hand smoke on tumor size and capillary density [99] via inhibition of MCP-1. Interestingly, cerivastatin had no effect on tumor angiogenesis in mice breathing clean room air [99].

Another recent study performed by Sata and co-workers [150] adds more to the understanding of the diverse effect of statins on tumor growth and angiogenesis. In mice inoculated with syngeneic colon cancer cells and concomitantly treated with cerivastatin (6 mg/kg per day), the tumors were significantly smaller than those in mice treated with saline. However, cerivastatin did not affect blood flow or capillary density in the tumor. On the other hand, in the same specimens that harbored the tumor, cerivastatin significantly augmented blood flow recovery after resection of the right femoral artery. The capillary density in the ischemic leg tended to be increased by cerivastatin, but this increase did not reach statistical significance. In turn, cerivastatin markedly inhibited the progression of atherosclerosis in ApoE knockout mice, and decreased the number of vessels in atherosclerotic lesions at the aortic root. Importantly, in their previous study, Sata *et al.*

[151] demonstrated that the pro-angiogenic effect of cerivastatin was abrogated in eNOS deficient mice.

Thus, in contrast to work of Weis and co-workers [68] who observed an angiostatic effect of a high dose of cerivastatin (2.5 mg/kg per day), the study of Sata *et al.* [150] suggests that cerivastatin can be pro-angiogenic even at higher doses (6 mg/kg per day), which is about 1000-fold that for human use in the treatment of hypercholesterolemia. The authors suggest that the pro- or anti-angiogenic effects of statins might also depend on distinct mechanisms of angiogenesis associated with cancer, tissue ischemia, or inflammation. However, such a hypothesis requires verification in additional studies.

CONCLUDING REMARKS

Cell culture experiments and experimental anti-tumor therapy in animal models have demonstrated that statins may inhibit proliferation of cancer cells, induce their apoptosis and decrease tumor growth as single agents or in combination with other anti-cancer drugs [16]. Recent data also suggest that statins may influence the tumor cells indirectly, acting as inhibitors of angiogenesis.

All those effects are, however, obtained only when high concentrations of statins are applied. Such doses are not used for treatment of hypercholesterolemia. Nevertheless, a treatment of life threatening cancer may warrant the applications of high doses of drugs, as in the case of chemotherapeutics. However, in such situations the risk of side effects has to be seriously considered.

Three years ago, cerivastatin was withdrawn from clinical use due to serious side effects, including several fatal cases. Cerivastatin is a very potent statin and experimental data indicate its potential for anti-cancer therapy. Nevertheless, the toxicity of cerivastatin, even when it was applied in low doses for treatment of hypercholesterolemia represents a warning signal. It is of course possible that benefits for patients with cancer may significantly outweigh the potential risk. However, we have to admit that the trials in which statins have been particularly tested for the treatment of cancer have not provided conclusive data. Moreover, even in those trials, in which very high doses of statins have been administered to patients (e.g. 30 mg/kg/day), the concentration of the drug in plasma reached maximally a few micromoles per liter, far from those required to induce apoptosis of cancer cells.

Statins appear to induce apoptosis in endothelial cells at high micromolar concentrations. Fortunately, there is no evidence for endothelial injury after application of doses that are used for treatment of hypercholesterolemia. Again, however, if anti-angiogenic effects of statins were to be considered for anti-cancer therapy, the risk of significant endothelial dysfunctions and cardiovascular complications has to also be taken into consideration.

On the other hand, the pharmacological concentrations of statins in the plasma of patients taking those drugs for hyperlipidemic disorders appear to be at levels that preserve the functions of endothelial progenitor cells and inhibit their apoptosis. If the initial tumor blood vessel formation is dependent on endothelial progenitor cells, i.e. occurs through vasculogenesis, then statins may potentially enhance tumor growth. This requires careful investigation.

Also, as discussed, apparent cell type-dependent effects of statins may give different outcomes in their activity. Moreover, the mechanisms of blood vessel formation, which may be either enhanced by statins in an ischemic skeletal and heart muscle or inhibited in the atherosclerotic plaque and tumors, appear to be site-dependent, and thus may constitute the

basis for the observed differences [150]. Finally, the interpretation of data is additionally complicated by the various concentrations of the drugs used *in vitro*.

One may suggest that recently observed anti-angiogenic effects of statins could, in combination with their known pro-apoptotic effect on tumor cells constitute the rationale for more detailed, randomized clinical studies in oncological patients. It could also be foreseen that statins will be more effective when given in combination with other drugs [for reviews see: 27, 28]. However, until further data are available, increased lipid levels are the primary target of statin therapy. Further basic research is required, to clarify whether the pleiotropic actions of statins, particularly their anti-inflammatory effects may influence the response of tumor cells to already available therapy. Although many studies have supported the potential application of statins as anti-angiogenic and anti-cancer drugs, we are still far from complete understanding of the underlying mechanisms.

Therefore, in our opinion, despite the epidemiological data suggesting that the usage of statins is safe in regard to the risk of cancer, the potential applications of those drugs for anti-cancer therapy is not yet sufficiently warranted. It appears necessary to demonstrate that tolerable and non-toxic concentrations of statins can inhibit tumor growth and/or tumor angiogenesis without causing serious side effects.

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Fig. (1).
Overview of the mevalonate pathway.

Table 1

Examples of Angiogenic Inhibitors in Clinical Trials

Inhibitor	Mechanism of action	Stage of clinical trial	Type of tumor treated	References
Avastin	Binds VEGF and prevents activation of endothelial cells	III	Colorectal cancer Renal carcinoma	[10,11,152]
Endostatin	Unknown? Inhibition of nitric oxide dependent pathway	I/II	Refractory solid tumors	[153]
Angiostatin	Not fully known (ATP synthase inhibitor?; changes eNOS activity?)	I/II	Solid malignant	[156]
Metalloproteinase inhibitors (e.g. marimastat)	Block the proteolytic activity	I-III	Colorectal cancer, glioma, melanoma, breast others	[157–159]
Tyrosine kinase inhibitors	Inhibit phosphorylation of VEGF receptors	II/III	Colorectal cancer Gastrointestinal tumors	[9]
VEGF trap	High affinity decoy receptor, binds VEGF	I	Non-Hodgkin lymphoma, solid tumors	[9]
Anti-VEGF receptor antibody	Inhibits VEGF-R2 activity	I	Colorectal cancer	[160]
Thalidomide	Unknown? Inhibition of bFGF induced angiogenesis	II	Colorectal cancer; melanoma, glioblastoma	[161]

Table 2

Features of Most Commonly Used Statins [Based on: [16, 162]

Statin	Source	Lipophilic	Form	Bioavailability (%)	Relative lipid lowering potency	Plasma elimination (half-life in hours)	Dosage used for cholesterol lowering (mg/day)
Lovastatin	Fungi	Yes	Lactone	<5	3	2	20–80
Simvastatin	Fungi	Yes	Lactone	<5	6	3	10–80
Pravastatin	Fungi (modified)	No	Acid	17	2	2	10–40
Fluvastatin	Synthetic	Yes	Acid	24	1	2	20–80
Atorvastatin	Synthetic	Yes	Acid	14	12	15	10–80
Cerivastatin	Synthetic	Yes	Acid	60	200	3	0.2–0.8
Rosuvastatin	Synthetic	No	Acid	NA	NA	NA	5–80
Pitavastatin	Synthetic	Yes	Acid	NA	NA	NA	4

NA – not available

Note: doses used for treatment of cancer are usually in mg/kg/day, thus they are about 100 higher than those used for lipid lowering (see table 4).

Table 3
Effects of Statins Therapy in Patients with Cardiovascular Diseases on Incidence of Cancer or Cancer Deaths

Study	Statin	Duration (years)	Incidence of cancer	Source
4S (Scandinavian Simvastatin WOSCOPS)	Simvastatin	7–10	Slightly lower in placebo group [29] or no difference in recent study [30]	[29,30]
	Pravastatin	5	No difference	[163]
AFTEX	Lovastatin	5	No difference in incidence of breast cancer	[164]
	Pravastatin	6	Significantly less melanoma	[165]
LIPID	Pravastatin	5	No difference	[33]
CARE	Pravastatin	5	No difference	[166]
ALLHAT-LLT	Pravastatin	3	Higher incidence of cancer in patients receiving statin ($p=0.02$)	[32]

Note: patients have been treated with statins at doses suggested for cardiovascular diseases.

Follow up analysis were performed usually several years after the study have been finished due to demonstration of the beneficial effect of statins on cardiovascular mortality.

Table 4**Summary of Clinical Trials with Statins for Anti-Cancer Therapy**

Statin	Dosage	Tumor types	Effect on cancer	Major Side effect	Reference
Lovastatin	2–45 mg/kg/day for one week, then 3 weeks off	Prostate cancer, astrocytoma, glioblastoma, breast, colorectal, ovary, sarcoma, lung	Only in one out of 88 patients a reduction of 45% of astrocytoma size	Gastrointestinal dysfunction, myalgia and muscle weakness	[26]
Lovastatin	30 mg/kg/day for one week, then 3 weeks off	Glioma	In 7 out of 18 patients some minor or partial response observed, but disease progressed	No significant	[39]
Lovastatin	35 mg/kg/day for one week, then 3 weeks off	Gastric adenocarcinoma	Partial stabilization in one out of 16 patients	Gastrointestinal dysfunction, myalgia and muscle weakness	[40]
Pravastatin	40 mg/kg/day	Hepatocellular carcinoma	Prolonged survival (18 vs 9 months; $p=0.005$). Patients received simultaneously 5-fluorouracil	No observed	[34]
Simvastatin	40 mg daily for 12 weeks	Chronic lymphocytic leukemia	No progression during treatment; progression afterwards	No significant	[167]
Fluvastatin	2–8 mg/kg/day for 2 weeks every 4 weeks	Osteosarcoma, Ewing's sarcoma, astrocytoma, medulloblastoma, lung carcinoma	6 partial responses (out of 12 patients)	Insomnia, nausea, vomiting, myalgia	[168]

Table 5

Summary of the Pro- and Anti-Angiogenic Effects of Statins

Pro-angiogenic effects:	
<i>Concentration:</i>	0.001–0.1 μ M (lower for cerivastatin)
<i>Mechanisms:</i>	Activation of PI3-Kinase Activation (phosphorylation) of eNOS and enhancement of eNOS stability Involves inhibition of RhoA geranylgeranylation Mobilization of progenitor cells Protection of endothelial cells from apoptosis
<i>Model systems:</i>	<i>in vitro</i> – Matrigel assay Enhanced migration <i>In vivo</i> : enhanced blood flow recovery in the ischemic hind limb Disc angiogenesis assay
Anti-angiogenic effects	
<i>Concentration:</i>	above 0.1 μ M (lower for cerivastatin)
Potential mechanisms:	Induction of apoptosis of endothelial cells : activation of p21 and p27 Enhancement of caspase activity Inhibition of proliferation Decreased activation of transcription factors: NF- κ B, AP-1, HIF-1 Inhibition of the synthesis of pro-angiogenic mediators: VEGF, IL-8 Inhibition of MCP-1 Inhibition of uPA Induction of β 4-integrin
<i>Model systems:</i>	<i>in vitro</i> – Matrigel assay <i>In vivo</i> : Inhibition of tumor angiogenesis Inhibition of angiogenesis in chick chorioallantoic membrane Inhibition of the growth of atherosclerotic plaque