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Minireview paper

Perindopril: possible use in cancer therapy

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Since angiogenesis is essential for the growth of any solid tumor, emerging efforts are being made to develop antiangiogenic therapy. To date, however, no antiangiogenic agent has become widely available for the clinical setting. Angiotensin I-converting enzyme (ACE) inhibitors are commonly used as antihypertensive agents and it has recently been suggested that they decrease the risk of cancer. Studies have found that an ACE inhibitor, perindopril, is a potent inhibitor of experimental tumor development and angiogenesis at a clinically comparable dose. The potent angiogenic factor, vascular endothelial growth factor (VEGF), is significantly suppressed by perindopril and also inhibits VEGF-induced tumor growth. *In vitro* studies showed that perindopril is not cytotoxic to either tumor cells or endothelial cells. Since perindopril is already in widespread clinical use without serious side effects, it may represent a potential new strategy for anticancer therapy. [© 2002 Lippincott Williams & Wilkins.]

Key words: Angiogenesis, angiotensin II, perindopril, vascular endothelial growth factor.

Angiogenesis and cancer

It is now widely recognized that angiogenesis plays a pivotal role in the development of solid tumors.[1–9] Any tumor mass in excess of a few cubic millimeters totally depends on the formation of a vascular network that provides the growing tumor with oxygen and essential nutrients. Therapies aimed at destroying tumor vasculature can achieve rapid regression of experimental tumors and it has been shown that tumor cell apoptosis is significantly increased by treatment with antiangiogenic agents.[1,5,10] It has been shown that antiangiogenic therapy showed less drug resistance than conventional chemotherapy. With regard to conventional chemotherapy, drug resistance is encountered in about 30% of all cancer patients.[11] Tumor cells readily acquired drug resistance because of their genetic instability, heterogeneity and high mutation rate of the tumor cells, whereas the endothelial cells are genetically stable and acquire much less drug resistance. Recently, striking experimental results have been reported.[5,12] This report revealed that successive cycles of therapy using the conventional chemotherapeutic drug led to acquired drug resistance as a result of selection of drug-resistant tumor cells. On the contrary, repeated cycles of antiangiogenic therapy are followed by prolonged tumor dormancy without for their therapy. Thus, once genuine antiangiogenic therapy is shown to effective in a clinical trial, it could become a major or even the sole anticancer therapy. Accordingly, antiangiogenic therapy is under investigation around the world, including the use of gene therapy, antiangiogenic recombinant proteins, monoclonal antibodies and various drugs (Table 1). Although some of these agents are now undergoing phase I, II and III clinical trials at the certain institutes, no agent can be widely available at this time in clinical practice.

Because of the concept of antiangiogenesis therapy, long-term administration is required to examine compound toxicity. Furthermore, it is difficult to simply compare the therapeutic effect of antiangiogenesis therapy and conventional chemotherapy. The antiangiogenesis compound has a cytostatic effect on the cancer cell, whereas conventional chemotherapy directly injures the cancer cells. These difficulties in assessing the antitumor activity of such cytostatic drugs in-patients have also been discussed previously.[13–15] It appears that time will be required before antiangiogenic compounds under current use can be applied widely in clinical practice. Alternative

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Table 1. Angiogenesis inhibitors in clinical trials

Drug	Mechanism	Trial (phase)
Drugs that block matrix breakdown		
COL-3	synthetic MMP inhibitor; tetracycline derivative	II
marimastat	synthetic inhibitor of MMPs	III
neovastat	naturally occurring MMP inhibitor	III
BMS-275291	synthetic MMP inhibitor	III
Drugs that inhibit endothelial cells directly		
endostatin	inhibition of endothelial cells	I
squalamine	extract from dogfish shark liver; inhibits sodium–hydrogen exchanger, NHE3	II
thalidomide	unknown	III
Drugs that block activators of angiogenesis		
SU6668	blocks VEGF, fibroblast growth factor and platelet-derived growth factor receptor signaling	I
anti-VEGF Antibody	monoclonal antibody to VEGF	II
interferon-α	inhibition of bFGF and VEGF production	III
SU5416	blocks VEGF receptor signaling	III
Drugs that inhibit endothelial-specific integrin/survival signalling		
EMD 121974	small molecule blocker of integrin present on endothelial cell surface	II
Drugs with non-specific mechanism of action		
CAI	inhibitor of calcium influx	II
interleukin-12	up-regulation of intergeron gamma and IP-10	II
IM862	Unknown mechanism	II

From NCI database: http://cancertrials.nci.nih.gov/news/angio/table.html (updated 08/01/01).

approach might be to find a clinically available compound that also shows antiangiogenic activity until these new drugs become widely available.

Renin–angiotensin system and angiogenesis

The renin–angiotensin system is a known determinant of vascular fluid homeostasis and blood pressure regulation.[16,17] Angiotensin II (AT-II), which is an octapeptide produced by the enzymatic cleavage of angiotensin I by angiotensin I-converting enzyme (ACE), exerts a large number of physiological effects, including vascular tone, hormone secretion, tissue growth and neuronal activities[16] (Figure 1). Recently, it was reported that a retrospective cohort study of 5207 patients receiving ACE inhibitor or other hypertensive drugs with a 10-year follow-up showed that ACE inhibitor decreased incident cancer and fetal cancer (Glasgow study).[18] Other antihypertensive drugs, calcium channels blockers, diuretics and β-blockers have no apparent effect on the risk of cancer. *In vitro*, ACE inhibitor retarded the growth of cultured cancer cells, and some ACE inhibitors inhibited angiogenesis and the growth of induced tumor in rats. AT-II has been shown to induce neovascularization and enhance vessel density in experimental systems.[19–21] It has been shown that AT-II selectively increased the blood vessel flow and ACE inhibitor decreased the intratumoral blood flow without affecting the blood flow in normal organ.[22]

To date, many angiogenic factors have been identified. Vascular endothelial growth factor (VEGF) is one of the most potent of these and is known to play a pivotal role in angiogenesis.[23–28] VEGF is composed of a group of six glycoproteins that originate from alternative mRNA splicings (in humans VEGF 121, 145, 165, 183, 189 and 206). In contrast to other angiogenic factors, VEGF acts almost exclusively on endothelial cells (EC), which have its high affinity type-III tyrosine kinase receptors, flt-1 and KDR/Flk-1. In addition to activity as a potent angiogenic factor, VEGF is known to be a survival factor on newly formed EC.[29,30] Without VEGF, apoptosis is rapidly induced in EC. VEGF was originally identified as a vascular permeability factor (VPF).[31] It has been shown to increase the permeability of microvessels 50 000 times the level induced by histamine. As such, it stimulates the extravasation of plasma proteins, such as fibrins. When these proteins are deposited in the extracellular matrix, they may serve as a foundation for the formation of the tumor stroma and a new capillary network.

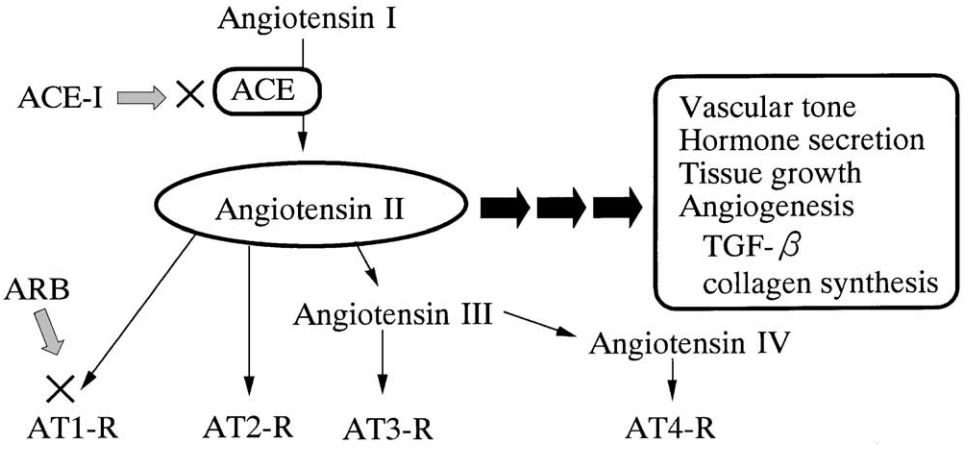

Figure 1. Schema of the renin–angiotensin system. AT-II is an octapeptide produced by the enzymatic cleavage of AT-I by ACE. It exerts a large number of physiological effects, including vascular tone, hormone secretion, tissue growth and neuronal activities. The level of AT-II was decreased by ACE-I, whereas its level was not altered by ARB. AT-II exerts its biological activities via AT1-R to AT4-R. ACE-I, ACE inhibitor; ARB, AT-II type 1 receptor blocker; AT1-R–AT4-R, AT-II type 1–4 receptors.

Actually, it has been shown that VEGF expression is increased in human surgical specimens in several types of tumors, and correlated with aggressive behavior and a poor prognosis. In animal models, overexpression of VEGF has been found to enhance tumor growth, whereas the suppression of VEGF reduced tumor growth.[32–38] VEGF gene expression has been induced by several types of cytokines and recent studies have shown that AT-II also induced VEGF in several types of cells, including tumor cells in a dose-dependent fashion.[39–41] AT-II also induces KDR/Flk-1 expression in EC, which has been shown to play a major role in VEGF-mediated angiogenesis both *in vitro* and *in vivo*.[25,42,43]

Other than cancer, angiogenesis has been shown to play an important role in several pathological diseases, such as ocular neovascularization, arterial plaque formation, psoriasis, gastrointestinal ulcers and rheumatoid arthritis.[11] The results of the EUCID study have highlighted the importance of the renin–angiotensin system in the pathogenesis of diabetic retinopathy.[44] This study suggested that ACE inhibitor lisinopril may slow the progression of the diabetic retinopathy of type 2 diabetes patients. Several clinical and experimental studies have also suggested a close relation between the renin–angiotensin system and diabetic retinopathy. The vitreal VEGF concentrations are higher in patients with diabetic retinopathy and the expression level of retinal VEGF mRNA was increased in streptzotocin-induced diabetes rats.[45–48] Furthermore, the treatment of ACE inhibitors, ramipril and perindopril, significantly reduced diabetes-associated changes in VEGF gene expression and vascular permeability.[49]

Recently, it has been shown that antiangiogenic compounds prevent the development of experimental liver fibrosis development.[50,51] AT-II induced the contraction of proliferation of hepatic stellate cells thereby playing a pivotal role in liver fibrogenesis,[52] and it increased the transforming growth factor (TGF)-β and collagen I gene, which is the major extracellular matrix component.[53,54] We recently reported that perindopril had a significant inhibitory effect on the experimental liver fibrosis development, associated with the suppression of hepatic stellate cell activation.[51]

On the other hand, several ACE inhibitors have been shown to stimulate angiogenesis under certain conditions. The ACE inhibitor, quanaprilat, promotes angiogenesis in rabbits with hindlimb ischemia.[55] Perindopril also has been shown to promote angiogenesis in an ischemia reperfusion experimental model.[56] A clinical study showed that ACE inhibitor treatment restores hepatocyte growth factor (HGF), which is one of the potent angiogenic factors,[57] produced in patients with congestive heart failure.[58] As shown in Table 2, ACE inhibitors showed a variety of biological activities in angiogenesis. It is difficult to explain precisely why ACE inhibitors exert such diverse effects in angiogenesis. It has been reported that tumor neovascular endothelial cells showed a different phenotype from normal endothelial cells.[59] It is possible that physiological angiogenesis of the cardiovascular system and pathological angiogenesis of the tumor may have different molecular mechanisms. Further studies are required to elucidate these issues before clinically prescribing an ACE inhibitor as an antiangiogenic agent.

Table 2. Multifunctional activities of ACE inhibitors

ACE inhibitor	Function	Reference
Perindopril	tumor growth and angiogenesis inhibition	62
	retinal angiogenesis inhibition	49
	liver fibrosis inhibition	54
	promotes ischemia reperfusion angiogenesis	56
	reduces the risk of stroke	86
Captoril	tumor growth and angiogenesis inhibition	20, 62
	angiostatin production	61
Lisinopril	slow progression of diabetic retinopathy	44
Quanaprilat	promotes ischemia reperfusion angiogenesis	55
Combind	reduces the risk of cancer	18
	HGF production	57

ACE inhibitor and cancer

It has been shown that one of the ACE inhibitors, captopril, inhibited the growth of experimental tumors and angiogenesis.[20,21] However, these reports suggested that the inhibitory effect of captopril was not mediated by ACE inhibition, but was the result of suppression of the matrix metalloproteinases (MMPs) or caused by the production of angiostatin, a fragment of plasminogen as an endogenous inhibitor of neovascularization.[60] Captopril possesses a free thiol group in its structure and has been shown to be involved in anti-oxidative stress or angiostatin production.[61] It is possible that the tumor suppressive effect of captopril can be partly attributed to these biological characters.

Recently, we found that the ACE inhibitors, captopril, temocapril and perindopril significantly suppressed tumor growth of a murine hepatocellular carcinoma (HCC) experimental model.[62] Among them, perindopril showed a more potent inhibitory effect than the other two compounds. To date, several other clinically available agents have also been shown to inhibit tumor development and angiogenesis in animal experiments;[63,64] however, most have been tested at very high doses when compared to the clinical dose range. Therefore, clinical use of these agents does not seem to be feasible. Noteworthy, perindopril showed a tumor growth inhibitory effect at a low dose comparable to the human clinical dose. Perindopril also had a significant inhibitory effect on tumor growth even after the tumor was fully established. Neovascularization in the tumor was markedly suppressed by perindopril treatment. It was found that perindoprilat, which is a metabolized active form of perindopril, did not influence the *in vitro* proliferation of tumor cells or endothelial cells, suggesting that the inhibitory effect of perindopril on tumor development was not related to cytotoxicity. Perindoprilat, however, markedly inhibited VEGF-induced endothelial cell migration and tubular formation *in vitro*. Perindopril also significantly suppressed VEGF mRNA expression in tumor cells and endothelial cells *in vitro*, and also significantly decreased VEGF mRNA expression in the tumor. The degree of ACE level, which corresponded to ACE inhibitory activity, was closely related to VEGF suppression in the tumor. The tetracycline-regulated gene expression system (Tet system) is a recently developed method of switching a target gene on and off *in vivo*.[65] By using this system, we assessed the contribution of VEGF expression to tumor growth.[66] When compared with the LacZ-transduced control tumors, the tumors overexpressing VEGF showed a marked increase in growth *in vivo*. After VEGF expression was terminated by adding tetracycline to the drinking water (1 mg/ml), the tumor growth rate decreased significantly and became similar to that in the control group. This indicates that the tumor augmentation in this system was exclusively mediated by the overexpression of VEGF. Perindopril at a low clinically comparable dose markedly suppressed the VEGF-induced augmentation of tumor growth. This suggests that, unlike captopril, perindopril inhibits tumor development and angiogenesis via AT-II inhibition leading to suppression of VEGF.

Interestingly, we did not find an inhibitory effect of AT-II type 1 receptor (AT1-R) antagonists in clinical use, losartan and candesartan comparable to that of ACE inhibitors in our experiment. To date, several types of AT-II receptors have been identified. Among them, AT1-R mediates most of the biological effects of AT-II, including an increase in the intracellular Ca^{2+} concentration, cell contraction, and proliferation.[67] As shown in Figure 1, the most striking biological difference between ACE inhibitor and AT1-R antagonist treatment is the AT-II level, which has been shown to stimulate angiogenesis.[19–21] The AT-II level is decreased by ACE inhibitor, whereas the level does not change with an AT1-R antagonist. Other than AT1-R, AT2-R has been identified to exert a distinct biological function from that of AT1-R.[67] In addition to these two receptors, it has been suggested that the other types of receptors, AT3-R and AT4-R, mediate the biological activity of AT-II, although sequence cloning of these receptors has not yet been performed. It has been shown that AT4-R induced the DNA synthesis of endothelial cells.[68] It may be possible that AT-II utilizes other types of

receptors besides AT1-R in the tumor development. Alternatively, the surrounding stroma plays an important role in producing VEGF in addition to tumor cell-derived VEGF, since strong VEGF promoter activity has been found in the stroma.[69] ACE inhibitors, including perindopril, are reported to inhibit the synthesis of stromal components, such as type IV collagen and TGF-β.[70] This inhibitory effect is not mediated by AT1-R activation.[71] Since we found that the relative stromal volume was decreased in perindopril-treated tumors, it is possible that there is some interaction between perindopril and stromal VEGF, which cannot be evaluated by *in vitro* methods. We found that captopril, temocapril and perindopril all inhibited liver tumor development and angiogenesis. Among these, perindopril had the most potent inhibitory activity and was even effective at a clinical dose. Perindopril is a hydrophilic compound, whereas the other two ACE inhibitors are more lipophilic. The liver has a specific organic anion transporter that allows hydrophilic compounds to cross the cell membrane more efficiently than by simple diffusion, which is the route for lipophilic compounds. Perindopril is metabolized to an organic anion after administration and may show a higher affinity for liver cells than the other two drugs, thus producing higher concentrations in HCC tumors.

Until recently, it had been believed that angiogenesis starts at the relatively late stage of the tumor at a size of several hundred microns to 1 mm in diameter or when the tumor contains roughly 10^5–10^6 cells.[3] Recently it was demonstrated that angiogenesis has already begun at a very early stage when the tumor contains just 100–300 cells.[72] As described, it has been suggested that angiogenesis is involved in the early carcinogenesis step.[73–75] A recent study of endothelial cell markers in dysplastic lesions of the liver suggested that alterations in the hepatic microcirculation already occur at a very early stage of liver carcinogenesis.[76] Accordingly, we examined the effect of perindopril on the exogenous and endogenous models of rat liver carcinogenesis, respectively, using diethylnitrosamine and a choline-deficient, L-amino acid-defined (CDAA) diet to determine whether or not perindopril also affects the carcinogenesis step. In both models, perindopril significantly suppressed hepatocarcinogenesis at a low clinically comparable dose.[77]

HCC is one of the most common malignancies in the world with an estimated annual incidence of greater than 1 million new cases per year.[78] Since most cases of HCC develop from patients with chronic liver disease, such as liver cirrhosis, only the minority of the patients can undergo a radical operation due to their limited hepatic reserves. Consequently, several alternative therapies have been employed, such as a transarterial embolization and percutaneous intratumoral ethanol injection. However, there is still no satisfactory prognosis improvement of HCC to date. One of the reasons for the poor prognosis in HCC is the high rate of recurrence. It has been shown that this high recurrence rate, even after curative therapy, is due to intrahepatic metastasis or multicentric development of each respective neoplasm clone.[78,79] Since the high-risk group of HCC development seems to be clearer than the other types of tumor, it is likely that a primary or secondary chemopreventive agent would be beneficial in improving the prognosis of HCC. Several agents, such as interferon and acylic retinoid, have been shown to prevent secondary HCC recurrence; however, there are still problems for common clinical application with its high cost and long-term toxicity, respectively.[80,81] Some of the clinically available compounds, such as thalidomide and penicillamine, have been shown to possess anti-angiogenic activity, and are currently under clinical trials.[63,64] Long-term application, however, of these agents sometimes leads to a severe side-effects, such as bone marrow suppression. On the contrary, ACE inhibitors are used currently for the treatment of hypertension and congestive heart failure without causing serious side effects, such as myelosuppression, in more than 100 countries. Taken together, it is possible that the ACE inhibitor, perindopril, can be utilized as a chemopreventive and therapeutic agent against HCC in the future.

Conclusions and future perspectives

As described above, the ACE inhibitor, perindopril, shows a significantly inhibitory effect on tumor growth and even on the carcinogenesis step at a low clinically comparable dose. These biological effects are possibly due to the antiangiogenic activity. It has been shown that a combination of antiangiogenic therapy with cytotoxic therapy, such as chemotherapy and radiation, increased the therapeutic curative effect in tumor-bearing animals, for which either agent alone showed only an inhibitory effect.[82–85] We also observed that the combination of perindopril and interferon-β showed a greater tumor growth suppressive effect than perindopril alone (unpublished data). For future clinical applications of perindopril, it would be preferable to employ these combination therapies to improve

effectiveness. It should be noted, however, that ACE inhibitors including perindopril also show pro-angiogenic activity under certain conditions as described.[55,56,58] Furthermore, it has been shown that there is a significant relationship between AT-II polymorphisms and disease progression of chronic hepatitis.[86] This suggests means that the effectiveness of the renin–angiotensin system inhibition by ACE inhibitors may be markedly varied in each clinical case. However, ACE inhibitor is already used widely in clinical practice without serious side effects compared to conventional chemotherapeutic drugs, such as bone marrow suppression. Furthermore, it has been reported that perindopril reduced the risk of stroke among both hypertensive and non-hypertensive patients with a history of stroke or transient ischemic attack (PROGRESS study).[87] Considering that considerable time is needed to develop new antiangiogenesis drugs for widespread clinical use and since the safety of ACE inhibitors has been demonstrated, ACE inhibitors such as perindopril may provide a new strategy for cancer therapy.

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